Heart rate variability in relation to the menstrual cycle in trained and untrained women

Inaugural dissertation to attain the academic degree

Submitted

At the 26th of August 2004

At the Philosophical Faculty IV

Institute of Sports Science of the Humboldt University of Berlin

By

Spielmann, Nadine

Date of Birth: 3rd of May 1975

Native place: Niedererlinsbach, Switzerland

President of the Humboldt University of Berlin

Prof. Dr. Mlynek, Jürgen

Dean of the Philosophical Faculty

Prof. Dr. phil. Benner, Dietrich

Experts:

Prof. Dr. med. Roland Wolff

Prof. Dr. med. Niklaus Friederich

PD Dr. med. Andreas Patzak

Date of viva voce:

16th of December 2004

……dedicated to my father.

List of contents

List of figures

List of spectra

List of tables

1 Introduction

The term heart rate variability (HRV) conventionally describes the beat-to-beat fluctuations in the heart rate or the variations in consecutive RR intervals. The HRV is mainly caused by efferent modulations of the sinus node which is considered as the pacemaker of the heart rate.

Casting a retrospective glance, the existence of physiological rhythms in the beat-to-beat heart rate signal was attended twenty years prior to the first clinical application of the HRV which was appreciated in 1965 by Hon and Lee [32]. Twelve years later, Wolf et al. [96] found associations between a higher risk of post infarction mortality and reduced HRV. For many years, the HRV has only been expressed in mean values and standard deviations as a representation of the time domain analysis until Akselrod et al. [1] described the relation between quantitative evaluations of the beat-to-beat cardiovascular control by the power spectral analysis of the heart rate fluctuations.

Nowadays, the frequency domain analysis obtained by mathematical processing of the RR intervals is well accepted to assess the neural mechanisms controlling the heart rate. Thereby two main spectral components which are considered as markers of the sympathetic and parasympathetic control of the heart have been discriminated: a high frequency component (HF) which ranges from 0.4-0.15 Hz and a low frequency one (LF) ranging from 0.15-0.04 Hz. Furthermore, the HRV measure is apparently easy to derivate because of the availability of new, digital, high-frequency, long- and short-term multichannel electrocardiogram (ECG) recordings. Due to this, the HRV is considered as a useful method for both clinical and research studies to examine the autonomic nervous modulation of the heart. Especially specific experimental conditions as awake

and sleeping situations, different body positions, physical training as well as pathological conditions provide a good insight into the vegetative control of the heart. Nevertheless the susceptibility of the autonomic nervous system by factors such as respiration, internal and external influences have to be considered. To avoid affected HRV results, standardized measurements conditions in accordance to the guidelines of the Task Force [84] have to be established.

In animal model researches, dogs with acute myocardial ischemia were observed to show an increased HRV after 6 weeks of exercise training [84]. Based on these findings, exercise training was thought to accelerate the recovery of the physiological sympathovagal interaction and to decrease cardiovascular mortality and sudden cardiac death. Moreover, endurance trained athletes have been noted to show profound bradycardia (lower resting heart rate) compared with sedentary control which implicated enhanced vagal and/or diminished sympathetic activity [13, 46, 51, 57, 70, 74, 85, 89]. Thus, physical activity was thought to affect positively the indexes of the HRV in healthy humans as well as in patients of specific pathologies.

Still conflicting results exist in literature concerning the effects of aerobic training on the HRV. Some studies have reported an increase in the magnitude of the HRV in the time and the frequency domain while others have reported absence of modifications of the sympathovagal balance in the sinus node. Most studies involve only male subjects whereas studies comparing trained with untrained females remain still inconsistent. Furthermore, few authors supposed modulation of the vegetative control of the heart in relation to the menstrual cycle in women whereas others could not find any affected HRV by endogenous female hormones [30, 50, 76, 75, 97].

On the basis of these considerations, one purpose of the present study was to investigate the effects of long term endurance training on the efferent autonomic cardiac control of the heart at rest in male and female athletes compared with sedentary controls. In addition another aim was to assess the possible influence of endogenous hormones on the vegetative control of the heart in endurance trained and untrained females. Therefore, the HRV was investigated throughout the menstrual cycle in women.

2 Theory

2.1 Physiology

2.1.1 The autonomic nervous system

The vegetative nervous system innervates the smooth musculature, the heart and the glands. Its function serves for the neuronal control of the internal environment, the so-called homeostasis, and the adaptation of the internal environment to external stimuli (e.g. physical activity). Organs and organ systems without direct relation to the homeostasis as the neuronal control of the sexual organs and the inner eye muscles are also controlled vegetatively. Due to the involuntary control, the vegetative nervous system is also known as the autonomic nervous system (ANS). The ANS works together with the voluntarily controlled somatic nervous system which controls the afferent and efferent interaction with the environment.

The ANS consists of three different parts: the sympathicus, the parasympathicus and the intestinal nervous system. The intestinal nervous system, a special nervous system of the gastrointestinal tract, functions without extrinsic influence of the spinal cord and the brain stem.

The terminal neurons of sympathicus and parasympathicus are located outside the central nervous system. The anatomical part of the sympathicus, the thoracal lumbar system, originates from the thoracic medulla, the second and third segment of the lumbar medulla. The target organs of the sympathicus are the smooth vessels of every organ, the heart and the glands. The parasympathicus originates from the brain stem and the sacral medulla. The preganglionic parasympathetic fibres to the organs of the

abdominal and thoracal cavity originate from the nervous vagus. Therefore the expressions “nervous vagus” and “vagal” instead of “parasympathetic nerve” and “parasympathetic” are also known. The smooth musculature, the glands of the gastrointestinal tract, the excretion, the sexual organs, the salivary, the lacrimal glands, the inner eye musculature and the lungs are innervated by the parasympathetic activity beside the neuronal control of the heart.

The sympathetic and the parasympathetic nerves react in an interaction of different nervous activity levels. Due to this, the expression sympathovagal or sympathetic-parasympathetic balance is used. The quantification of the contribution of the two nervous branches remains difficult because both parts are permanently active.

The ANS supervises the internal environment of the body by receptors i.e. baroreceptors, chemo- and mechanoreceptors which compare constantly the actual values with set points in a closed control loop to keep the homeostasis as constant as possible. Internal and external influences such as physical activity, food uptake and thermic changes are immediately balanced by specific adaptation i.e. modulated respiration, heart rate and/or blood pressure induced by the ANS.

The autonomic nerves have a pivotal role in the regulation of the cardiovascular system. The study of the cardiovascular variability is mainly assumed to access the activity of the sympathetic and parasympathetic nervous activity. The heart rate variability (HRV) analysis may examine autonomic fluctuations under different physiological circumstances (e.g. work-load, body position change). The vegetative control of the heart in relation to endogenous hormonal fluctuations is part of the focus of this study. Thus, the following chapter will only focus on the cardiovascular system and the autonomic (vegetative) control of the heart.

2.1.2 The cardiovascular system

The cardiovascular system constitutes the junction of the blood vessels including arteries, capillaries and veins with the heart. The most important assignments of this transport system are the supply of cells with nutrients and oxygen as well as the removal of metabolic products. The blood circulation system comprises the systemic and the pulmonary circulation illustrated by the following figure (figure 2-1).

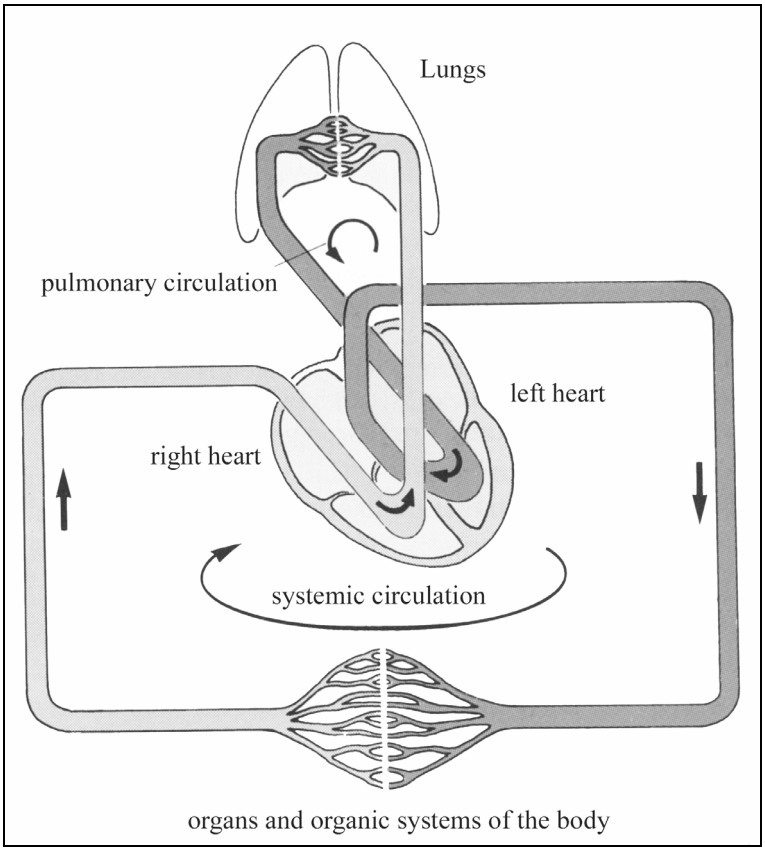


Figure 2-1: *Illustration of the pulmonary and the systemic circulation (data modified from [77])*

The circulation of the blood through the body only functions by the pump function of the heart. The right part of the heart consisting of atrium and chamber takes up the venous blood which is poor in oxygen and supplies it to the lungs where it will be enriched (arterialisation) with oxygen. After the arterialisation the blood arrives at the left part of the heart. Afterwards it will be transported to the different organs. The pump effect of the heart is caused by rhythmic series of relaxations (diastole) and contractions (systole) of the heart chambers. The cardiac muscle (myocardium) fibres are excitable structures with resting and action potentials. A stimulation of the ventricles (chambers) is spread out over all fibres of the heart; the heart reacts on stimulation completely or not. The rhythmic pulsations of the heart happen by stimulations originating in the heart itself: the so-called auto rhythm.

The sinus node generates the impulse for a heart beat. The stimulation spreads out the atrium muscles. The atrioventricular node conducts the stimulation with a delay to the bundle of His (atrioventricular bundle), to the left and the right bundle branch and the Purkinje fibers.

Finally the stimulation reaches the chamber muscles throughout the ends of the Purkinje fibers (see figure 2-2).

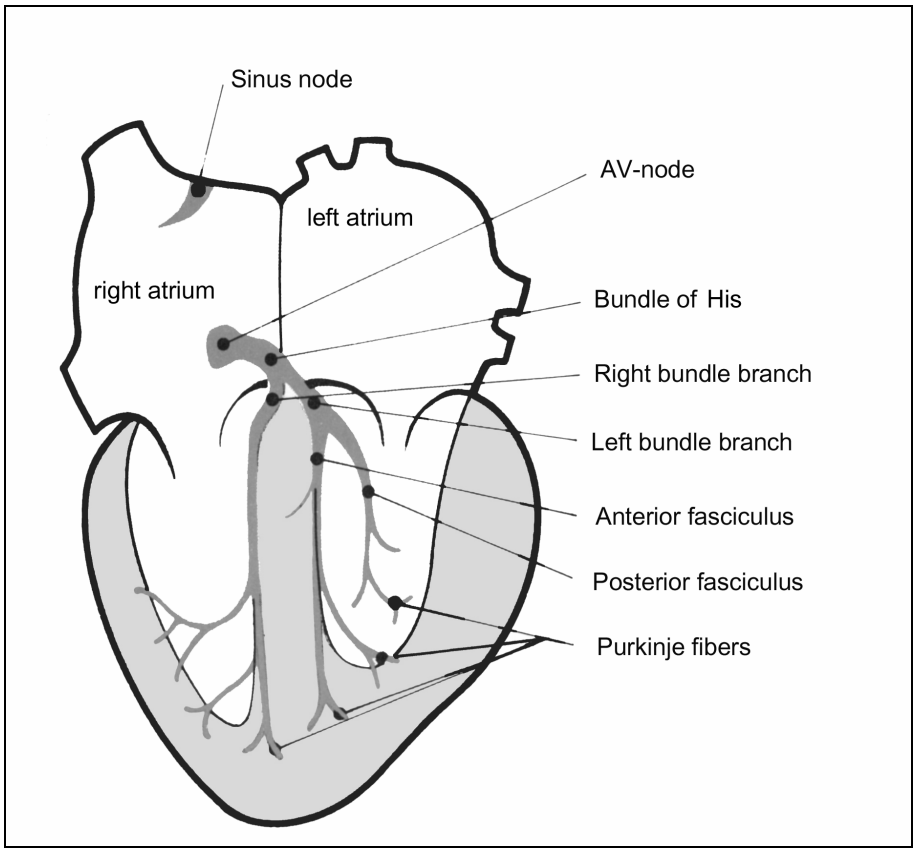


Figure 2-2: *Conduction system of the heart (frontal tomogram modified from [77])*

Due to the highest frequency, the sinus node is the primary pacemaker of the heart. The stimulation originated at the sinus node spreads out and stimulates the whole heart; the so-called sinus rhythm.

2.1.3 Electrocardiogram

An electrical field measurable at the body surface, results from the expansion and involution of the excitation (stimulation) of the heart. The electrocardiogram (ECG) represents the potential difference in relation to the time as an expression of the excitation of the heart. Changing of potential differences illustrates the time dependent changes of the direction and size of this electrical field; measurable with electrodes attached at different parts of the body.

The ECG is classified into parts characterised by letters which stand for certain segments of the excitation of the heart (figure 2-3).

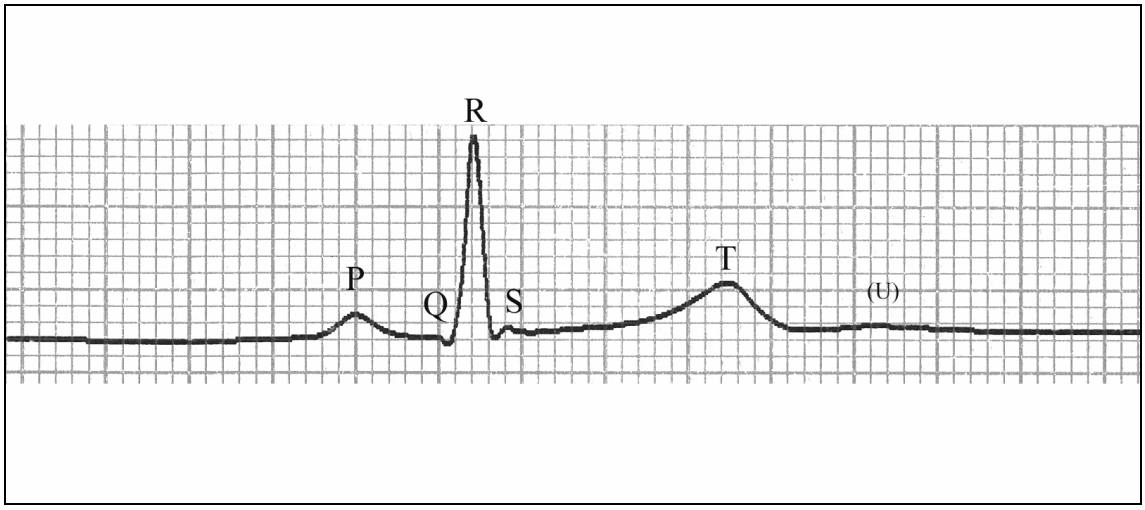


Figure 2-3: *Part of an ECG recording of our study which presents the single segments classified by letters*

The stimulation of the atriums starts with the P-wave implying the spread out stimulation over both atriums. The PQ-segment illustrates the completed excitation of the atriums. The stimulation expansion of the chambers starts at Q and ends at the beginning of T. The QRS-segment stands for the expansion of the ventricles and the T-wave for the ventricular involution of the stimulation. Beside this, the ST-segment illustrates the total stimulation of the ventricles. Sometimes an U-wave close to the T-wave is visible; but its meaning remains unclear.

2.1.4 The vegetative control of the heart

The vegetative heart nerves of the parasympathicus and the sympathicus have a direct influence on the action of the heart. The frequency, the systolic strength formation, the contraction velocity and the velocity of the atrioventricular conduction are affected. The effect of the vegetative nerves is chemically transmitted by acetylcholine (parasympathicus) and noradrenaline (sympathicus). The permanent stimulation of the vegetative nerves is called vagal and sympathetic tone. The sympathetic and parasympathetic nervous activity still interacts in addition to this tone.

The contraction strength of the atrium myocardium diminishes with the parasympathetic influence. This results in a shortened duration of the contraction curve (from foot till top) which reduces the duration of the action potential. The sympathetic influence increases the contraction power in the atrium as well as in the chamber myocardium. Thus, the sympathicus accelerates the atrioventricular conduction and shortens the conduction between atrium and chamber action whereas the parasympathetic influence

decelerates the atrioventricular conduction. Thereto the vagal and sympathetic activities constantly interact; this interaction is assumed to be reciprocal.

The control of the arterial blood pressure, the cardiac output and the blood flow distribution take place in the lower brain stem. Sympathetic and parasympathetic axons are the efferences whereas the arterial baro-, chemo- and mechanoreceptors of the atriums and the ventricles of the heart are the afferences to the cardiovascular effectors. The higher brain stem and the hypothalamus supervise the medullary self-control. The control of the medulla takes place by the neuronal connections of the hypothalamus and medullary cardiovascular centre and by the direct neuronal connections from the hypothalamus to the preganglionic neurons. The hypothalamus adapts the higher neuronal control of the cardiovascular system during complex vegetative functions i.e. thermoregulation, control of food intake and of the physical activity.

The function of the circulation is permanently controlled by receptors at different locations of the cardiovascular system. The afferent impulses of theses receptors are directed to the medulla oblongata. From there, impulses of efferent fibres proceed to the effectors in the heart and the vascular system of the central nervous system. The total peripheral resistance and the cardiac output are important adaptations of the circulation system as well as the vascular capacity and the distribution of the blood volume. The adaptations are separated into short, medium and long term regulation mechanisms. Pressoreceptor- (baroreceptors), chemo receptor- and ischemia reactions of the central nervous system are short term regulation mechanisms because of their fast (reaction) effect. Hormonal influences of adrenaline, noradrenaline and adiuretine supply the vasomotor effects and diminish the conduction. The increase of afferent impulses of the pressoreceptors cause an inhibition of sympathetic and an enhanced stimulation of the parasympathetic structures in the medulla oblongata; thereto the tonic activity of the sympathetic fibres, the heart rate, the strength and velocity of the myocardial contraction are reduced. The decrement of the total peripheral resistance results in a capacity increment of the capacitance vessels and a decrement of the systolic blood pressure. Reduced stimulation of the pressoreceptors results in the opposite reaction. Acute arterial pressure variation provokes a changing of the arterial pressoreceptors which concerns the flow resistance and the cardiac output. The aim of this reaction is the fast approach to the set point; a kind of homeostatic (self) regulation mechanism of the circulation. Due to internal and external influences on the homeostasis, specific adaptations supervised by the ANS aim to hold the set point as constant as possible.

Enhanced excitation of the pressoreceptors also inhibits the inspiratory and expiratory neurons of the respiratory centre and diminishes the respiratory frequency and the ventilation volume. The inspiration and expiration cause different pressures in the thorax which influence the blood circulation especially in the pulmonary circulation. This pressure gradient determines the volume and the velocity of the blood which thereby influences the vegetative control of the heart. The inspiration correlates with an increasing heart rate and the expiration with a decrease; this phenomenon is known as the respiratory sinus arrhythmia (RSA) due to its similarity to a sinus curve.

In summary, biological rhythms found in the vegetative nerves and conducted to the heart may also result in periodic fluctuation of the heart rate [16]. Despite of this, the sinus node remains the primary pacemaker of the heart whereas its rhythm is constantly modulated by the sympathetic and parasympathetic activity of the ANS; this activity is rhythmically modulated. Thereby a direct reflection of the autonomic activity can be expressed by the time-dependence of hemodynamic variables and therefore an insight into the cardiovascular control can be gained [2].

2.2 Physiology of the rhythm derivation

The sinus node is the pacemaker of the heart. Its rhythm is modulated by the sympathetic and parasympathetic activity. These rhythms are related to the activity of the pressoreceptors (baroreflex), the respiratory and the thermoregulatory related reflexes. Receptors are part of a closed feedback control system. They are sensitive for a certain stimulus from which they constantly compare the actual with the set point value. Finally the receptors interact with the sensory cell of a specific organ, the so-called control organ, to keep the set point as constant as possible. The feedback systems consist of non-linear elements which are mainly responsible for the development of systemic changes of the efferent nerve activity to the heart.

The pressoreceptors also known as baroreceptors control the arterial blood pressure in the arterial vessel walls. The set point in this closed control loop is the mean blood pressure in the arterial vessels. Alterations of the dilatation in the vessel walls (actual value) stimulate the pressoreceptors in the aortic arch and the carotid sinus which leads to nervous impulses to the higher brain stem (control organ). Thereby the heart rate is modulated as well.

The respiration is supervised by respiratory neurons which control the respiratory frequency and the tidal volume. The inspiration and expiration neurons which regulate the central breathing rhythm are located in the medulla oblongata. The inspiration neurons activate the inspiration muscles while the expiration neurons are simultaneously blocked by blocking neurons. The expiration starts when the effect of the blocking neurons is neutralised and the inspiration neurons diminished. Inspiration and expiration neurons permanently interact by the inhibition of each other which leads to a central rhythm of the breathing. This rhythm is additionally stabilized by peripheral influences of the stretch receptors which regulate the tidal volume of the breathing. Stretch receptors in thorax and lungs control the stretch stimulus of the inspiration and expiration. Alteration of the stimulus leads to adequate nervous impulses to the respiratory centre. This reflex control of the respiratory centre to the respiration musculature is also known as "Hering-Breuer-reflex". The breathing rhythm is additionally modulated by the chemical breathing control. Peripheral and central chemoreceptors thereby control the CO_2 and the O_2 partial pressure and the pH of the arterial blood. An increasing CO_2 partial pressure leads to nervous impulses to the respiratory centre which activates the respiratory musculature to enhance the tidal volume and in part the breathing frequency. The increased tidal volume finally diminishes the CO_2 partial pressure to the set point value. The stretch- and the chemoreceptors modify the central breathing rhythm to stabilise the homeostasis of the breathing. The respiratory neurons and nerves for the cardiovascular control are in the medulla oblongata of the brainstem. The respiratory rhythms are mediated to the cardiorespiratory nerves, too. This is one of the sources for the modulations of the sympathetic and parasympathetic activity to the heart.

The thermoregulation in the hypothalamus keeps the core temperature at the set point by balancing the fluctuations in the heat regulation. This regulation also affects the vegetative control of the heart. Increased heat in the internal environment as enhanced core temperature, results in a dilatation of the vessels and perspiration whereas coldness provokes a constriction of the vessels and an enhanced muscle tone. The temperature is permanently controlled by cold and warm receptors in the skin which react on adapted temperature stimulus by nervous impulses to the hypothalamus.

These short term vegetative rhythms in the vegetative activity modulate the heart rate. A relation between the vegetative control system and the vegetative rhythms with rhythmic modulations are supposed to correlate with main frequencies in the HRV.

Main frequencies for the control systems of the pressoreceptors, of the respiratory as well as of the thermo related functions were found.

Fluctuations of the heart rate can be presented as a function of power density in relation to its frequency in a spectrum. As with the main fluctuation, the control system may be related to short term variability or long term variability which are defined by high (HF) and low frequency (LF) bands; HF 0.4-0.15 Hz and LF 0.15-0.04 Hz. HF stands for parasympathetic and LF for sympathetic activity of the vegetative control of the heart. Thus, the above mentioned control systems could be mirrored in parasympathetic or sympathetic nervous activity by its main frequency in the HRV spectrum.

The control system of the baroreflex (pressoreceptor) showed a main frequency around 0.1 Hz in the long term variability (LF band) and thereby could have a relation to the sympathetic nervous activity. The main respiratory related function was found in the short term variability (HF band) and thus could have a strong relation with the parasympathetic nervous activity which is also known as “respiratory sinus arrhythmia”. Provoked stimulations with coldness and heat indicated a thermo regulated function which fluctuated in long term variability (LF band) i.e. in the sympathetic nervous activity bands of the spectrum. Nevertheless, data concerning the thermo related control system and its frequency are inconsistent and should be handled with care [84].

In summary, the respiratory sinus arrhythmia is thought to contribute to HF component i.e. to the parasympathetic nervous activity whereas the baroreflex and thermoregulation related control systems seemed to be related to the LF component i.e. to the sympathetic nervous activity of the power spectral analysis of the HRV [16].

2.3 Measurement of HRV

The basic source of the HRV is a high quality ECG recording which requires standardized conditions to minimize artefacts and high sampling rate to avoid interferences. ECG recordings and HRV calculations should only be carried out in accordance to the guidelines of the Task Force [84].

Also, the QRS complex should have sufficient amplitude and a stable baseline. The ECG signal is first analog recorded and then digitally converted. Afterwards a stable reference point has to be located by algorithms in accordance to Friesen et al. [26] to define the RR interval series. The proper interpolation of ectopic beats, arrhythmic events, missing data and noise-effects of the RR interval results in the NN interval

series (NN means normal to normal) [84]. The HRV parameters are now calculated by different mathematical operations. Alongside the two main HRV analyses in the time and the frequency domain, new analysis and parameters have been also investigated. The power spectral density (PSD) analysis, also known as the frequency domain analysis, provides the basic information on how power as an expression of variance distributes in the function of frequency. This can be illustrated for example by a spectrum where the power density is presented over the frequency [84]. The validity of the HRV parameters is related to the recording duration whereas only HRV segments of identical length are can be compared.

Figure 2-4 presents the step by step derivation of the time and the frequency domain analysis of the HRV.

1st Step presents a typical ECG recording.

2nd Step shows the time-event series consisting of the NN intervals. Slow and fast fluctuations can be noted by the different interval lengths.

3rd a Step presents selected parameters of the time domain analysis which are calculated by simple mathematical operations as the mean (meanNN) or the standard deviation of all NN intervals (SDNN).

3rd b Step shows a spectrum of the frequency domain analysis which presents the variability as a function of power density in relation to the frequency.

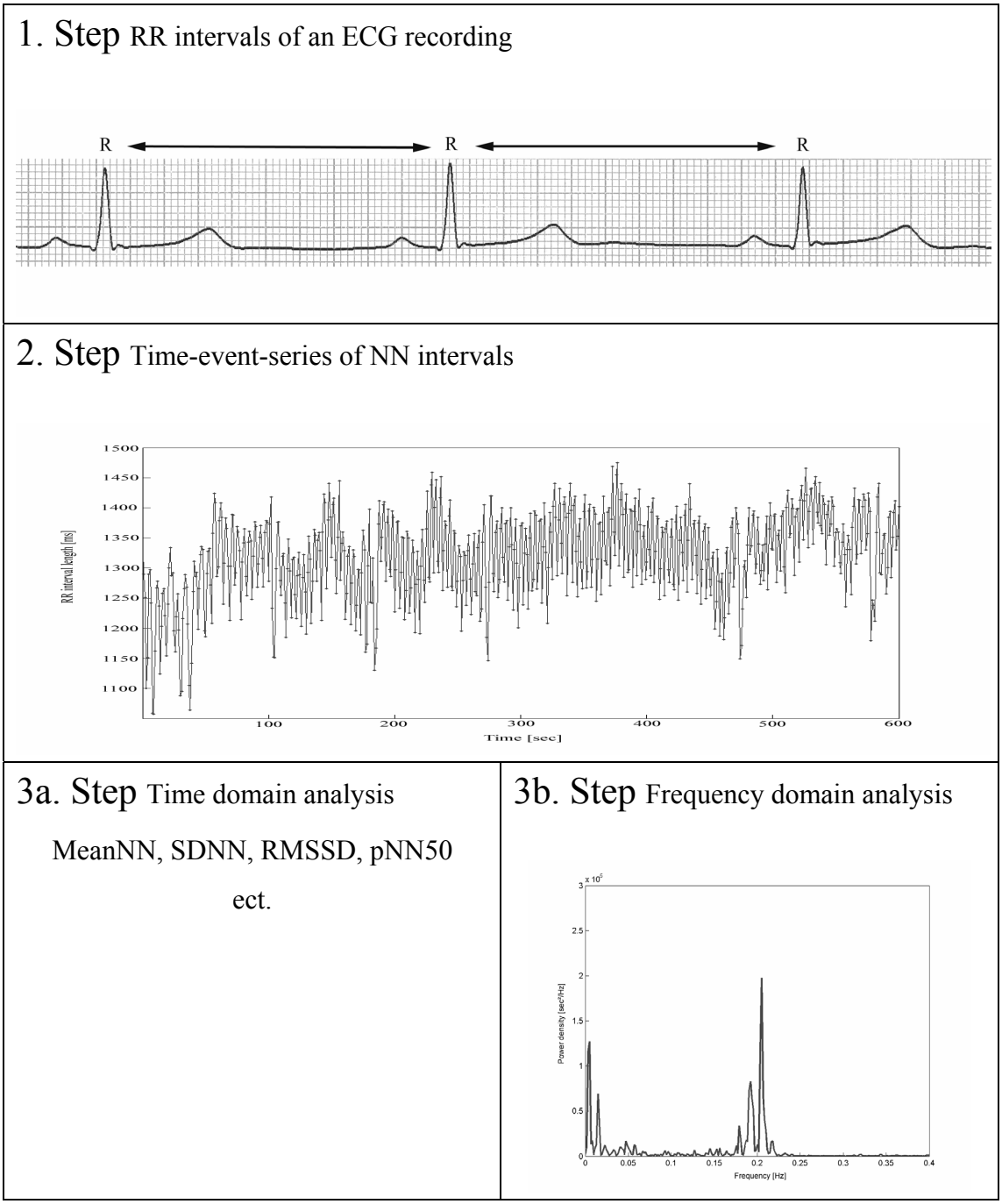


Figure 2-4: Steps in the analysis of the HRV

2.3.1 Time Domain

In the time domain analysis the intervals between successive NN interval series or at any point in time are determined. Thus the calculation of the time as well as of the

frequency domain parameters is based on the NN interval series or the instantaneous heart rate which leads to different results due to the non-linear relation between the fluctuation of the NN intervals and the heart rate.

The main time domain variables for short term recordings include the mean of all consecutive NN intervals (meanNN), the standard deviation of the NN intervals (SDNN), the square root of the mean squared differences of the successive NN intervals (RMSSD) and the number of intervals greater than 50 ms (NN50) and the proportion which derives by dividing NN50 by the total number of NN intervals (pNN50). The meanNN can be used as a marker of the heart rate and the SDNN as an expression of the total variability of the heart rate. Thus, the high correlation of the RMSSD, NN50 and pNN50 estimates a relation with the short term variation i.e. the HF component of the heart rate [62, 84]. Still the time domain analysis gives only information about the amount of variability without regarding the period length of the oscillations.

2.3.2 Frequency Domain

The spectral analysis of the frequency domain decomposes the series of continuous NN intervals or instantaneous heart rates into its sum of sinusoidal functions which differ in the amplitudes and the frequencies. Thus, the main advantage of this analysis is that not only the amount of variability but also the frequency specific oscillation can be obtained [16]. The power of each component is plotted as a function of its frequency and is computed in defined frequency bands; ergo the magnitude of variability is implied as a function of frequency. Therefore the power spectral density (PSD) provides basic information of how power (variance) distributes as a function of frequency [84].

In relation to the data evaluation, the PDS can be calculated by nonparametric or parametric methods which both provide comparable results. In most cases the Fast Fourier Transform (FFT) is used because of the algorithm simplicity, the high processing speed and its stability [26]. However, in accordance to the Task Force [84], the use of nonparametric methods, e.g. FFT, is only suggested with a regularly sampled interpolation of the discrete event series (DES). Standards for the nonparametric methods (e.g. the FFT) include the formula of the DES, the frequency of the sampling DES interpolation, the number of samples used for the spectrum and the spectral window used. Hann, Hamming and the triangular window are the most frequently used spectral windows [84].

The FFT decomposes the series of sequential NN intervals into a sum of sinusoidal functions resulting in the magnitude of variability as a function of frequency. The power density in relation to the frequency can be shown best by spectral analysis which reflects the amplitude of the heart rate fluctuations at different oscillation frequencies [16].

2.3.2.1 Frequency domain parameters in absolute values

The total variability of the spectral power analysis is expressed by the total power [ms²] whereas further parameters depend on the recording time. Short-term recordings only include three main spectral components; the very low frequency (VLF), the low frequency (LF) and the high frequency (HF) power in [ms²]. These components express the absolute value of the power [ms²] in a defined frequency band. Therefore the expression HF, LF and VLF power is used. The Task Force of European Society of Cardiology and the North American Society of Pacing and Electrophysiology laid down the bands of the specific frequencies in a special report in 1996 [84]. The three main frequencies of the short term recordings are defined by the following bands:

VLF ranges from **<0.04 Hz to 0 Hz**
(Including the ultra low frequency (ULF) which is only accepted for long term recordings <0.003 – 0 Hz)

LF ranges from **0.04-0.15 Hz** with central frequency around 0.1 Hz

HF from **0.15-0.4 Hz** with central frequency at respiratory rate around 0.25 Hz

The distribution of the LF and HF power and its central frequencies are modulated by fluctuations of the cardiovascular system. Therefore main periodic fluctuations of the respiratory sinus arrhythmia (RSA), the baroreflex (pressoreceptor) and thermoregulation related reflexes were mirrored in the spectra. Due to their main frequencies, strong relations between the HF power and the RSA and the LF power and the baroreflex were found. Whereas the relation between the thermoregulation and the VLF remains disputable and specific physiological process attributable are still in doubt.

2.3.2.2 Frequency domain parameters in normalized units

HF and LF may also be expressed in normalized units which represent the relative value of each power component in proportion to the total power minus the VLF component. To avoid a mix-up of the parameters, the HF and LF in normalized units are expressed as HFnu and LFnu. The representation of HFnu and LFnu illustrates more clearly the control and the balance of the two nervous branches, i.e. the sympathetic and the parasympathetic nerves of the autonomic nervous control. The normalization of the HF and LF also tends to diminish the effect of the total power on the values of the HF and LF power. Nevertheless, the Task Force [84] recommends a quotation of the HFnu and LFnu with the absolute values of the HF and LF power in order to describe completely the distribution of power in spectral components.

2.4 Physiological interpretation of HRV

Each cardiac cycle is modulated by the integrated efferent sympathetic and vagal activities which are directed to the sinus node. Additionally, frequent small adjustments in the heart rate are made by periodic modulations of this activity, which are related to cardiovascular control mechanisms i.e. the respiratory sinus arrhythmia, the baroreflex (pressoreceptor) and the thermoregulation related HRV. Fluctuations can be illustrated in a spectrum which may help to find physiological interpretations related to the vegetative control of the heart.

Strong relations between the HF and the LF component and the sympathetic and the parasympathetic nervous activity were established by clinical and experimental observations of autonomic manoeuvres, e.g. electrical stimulation, pharmacological receptor blockades and animal research. Nevertheless the autonomic control of the heart and its relation to the VLF remains disputable. Thus, only the physiological interpretation of the HF and the LF component are described in the following chapters.

2.4.1 HF power and parasympathetic activity

The respiratory frequency approximates to 0.2-0.4 Hz at rest in general and a respiratory related modulation of the heart rate has been found. This phenomenon is known as the respiratory sinus arrhythmia (RSA). The RSA is highly correlated with the HF component of the HRV. In a spectrum, the peak of the respiratory related modulation can be found at the respiratory frequency. Controlled respiration i.e. metronome

breathing at different frequencies showed a higher increase of the RSA with approaching respiratory frequency to the intrinsic baroreflex related heart rate fluctuations. The maximum was found at 0.1 Hz (i.e. 6 breaths/min) whereas the RSA greater than 0.1 Hz negatively correlated with the amount of RSA [16, 43]. This implies a positive correlation of tidal volume and the amplitude of the respiratory related heart rate oscillations [62].

Beside the controlled respiration, the following investigations also showed a relation between the vagal activity (the HF component) and the RSA. Pharmacological blockades by high doses of atropine abolished the short term variability and the RSA [16]. Subjects with paraplegia, i.e. completely interrupted sympathetic nervous innervations, but with preserved nervous vagus, showed a decreased LF component but normal fluctuations in the respiratory related frequencies i.e. the HF component. Finally a nervous vagus cut in animal research models reduced the vagal activity and the RSA which illustrated a strong correlation of the vagal activity and the respiratory related heart rate fluctuations [62]. This findings support, that the parasympathetic activity is the major marker of the HF component and responsible for the respiration linked oscillation of the HRV [2]. Additionally, the RMSSA and pNN50 are also found to highly correlate with the HF component which demonstrates that both parameters of the time domain are vagally mediated [84].

2.4.2 LF power and sympathetic activity

The LF component is mediated by both branches of the vegetative control of the heart, which can be demonstrated in several conditions. High doses of atropine which block the vagal activity abolished the short term variability (HF component) and also diminished the long term variability (LF component). Beside this, propranolol, which blocks the sympathetic nervous activity attenuated, the LF without affecting the HF component. Patients with paraplegia of totally interrupted sympathetic innervations of the heart showed unaffected high fluctuations and reduced but still measurable low fluctuations of the HRV. Thus, the LF component is considered not only as marker of the sympathetic but also as a marker of the parasympathetic nervous activity [42, 62, 65, 84]. Ergo, the LF component is mediated by two parts; the sympathetic and vagal nerves with a predominant sympathetic activity. Still the precise quantification of vagal and sympathetic parts is conflicting due to the unknown distribution of the activity. Additionally, correlation between the fluctuations in the peripheral vascular resistance i.e. fluctuations of the blood pressure (baroreflex) and the LF component were found.

This implies that the baroreflex related fluctuations with a spectral peak around 0.1 Hz are mediated by the sympathetic nervous activity [16]. Furthermore, the LF component was noted to be increased in certain conditions as 90° tilt, standing, mental stress and moderate exercise [84].

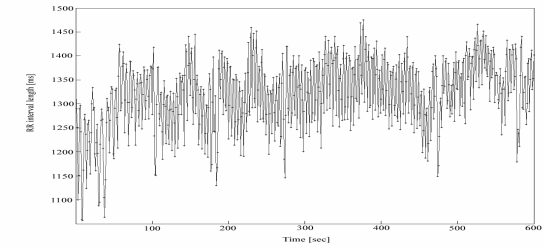
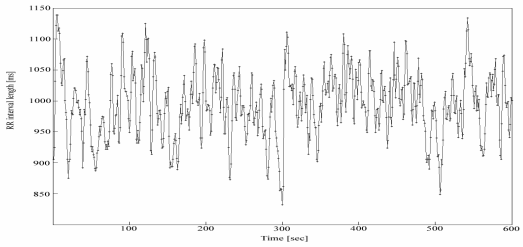
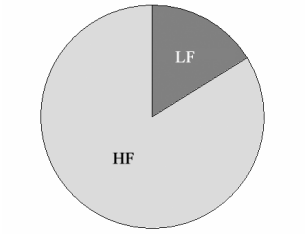
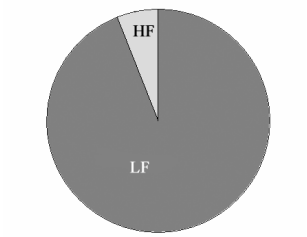
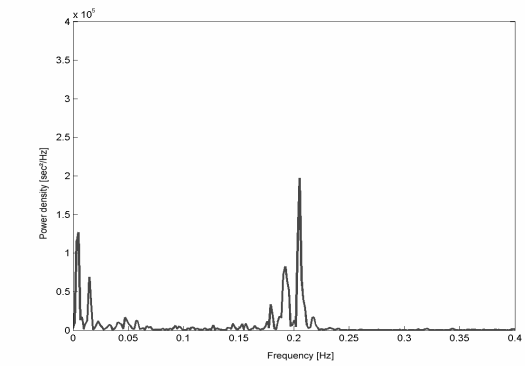
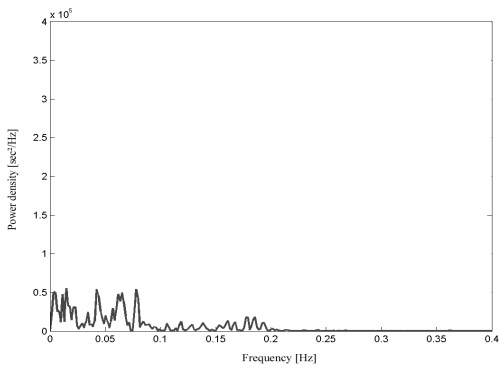
2.4.3 Orthostatic test

The orthostatic test is the best known test to provoke an increase in the sympathetic activity. The active and the passive (i.e. tilt up table) posture change from supine to standing causes a shift of the blood volume induced by the hydrostatic pressure change. This results in reduced arterial blood and central venous pressure. Thereby, the diminished venous supply to the heart leads to a decrease of the arterial blood pressure. The activation of the sympathicus induced by the stretch- and pressoreceptors of the ANS causes a constriction of the vessels, an increase of the heart rate as well as a normalization of the arterial blood pressure.

In supine position, the vagal activity is predominant whereas a shift towards the LF component leads to an increase of the sympathetic nervous activity while standing [44, 84, 90]. The following table illustrates the physiological difference of the vegetative control of the heart between supine and standing position with the help of three different illustrations. Still, no difference was observed by Ryan et al. [73] while lying in left or right lateral position compared with the supine position.

Furthermore, the LF/HF ratio which consists of the LFnu and HFnu is used to illustrate the interaction of the sympathetic and parasympathetic activity and can be seen as a mirror of the sympathovagal balance [84]. During the orthostatic test, this balance is shifted towards the sympathetic predominance because of the LFnu increase and the HFnu decrease which directly interact. In accordance to the Task Force [84], the LF/HF ratio and the normalized units minimize the effect of the total power changes and therefore must be quoted with absolute values to describe completely the spectral power components.

Table 2-1: *The orthostatic test presented in three different analysis steps based on data of the present study*

Supine position	Standing position
	
The time-event series imply the range of the NN interval lengths and its fluctuations time. In supine position the range between the shortest and the longest interval ranges from 1000-1450 ms whereas while standing this range is decreased from 850-1150. In supine position components of fast fluctuations are more pronounced compared with standing.	
	
HFnu and LFnu illustrate best the difference between supine and standing position. While standing the sympathetic activity increased (LFnu) compared with the supine position. The sympathovagal balance was affected and the orthostatic test induced a shift of the vagal towards the sympathetic predominance.	
	
In supine position a spectral peak of the HF component (0.2 Hz) can be noted. While standing, this peak is strongly diminished whereas several peaks in the LF band (0.1-0.04 Hz) can be noted. These peaks consist of vagal and sympathetic activity.	

2.4.4 Influence of respiration

Respiratory rate and tidal volume are known to exert major influences on RR interval or heart rate fluctuations. The spontaneous breathing is individually different and is modulated by physiological, psychological and external influences. For instance, women are known to have increased breathing frequencies with increasing progesterone level during the menstrual cycle whereas endurance trained athletes are known to have slower breathing frequencies than sedentary men. Therefore, ways to minimize the physiological differences and the external influences during the HRV measurements are needed. Thus, the controlled breathing to the rhythm of an auditory signal at a fixed rate was thought to be a valuable tool to standardize the respiratory influences on HRV. Different studies [6, 9, 53] investigated the difference between the spontaneous and the controlled breathing on the vegetative control of the heart.

Bloomfield et al. [6] compared spontaneous breathing with different metronome-set breathing rates. The HF component was determined during each respiratory cycle. They discovered that the HF power during spontaneous compared to metronome-guided breathing was significantly different at the same breathing rate. Mental concentration and subjects discomfort tended to decrease the HF power. The metronome breathing caused disturbances of the ANS and thereby the HRV was affected. Increasing sympathetic activation was noted when the manoeuvre of the metronome breathing tended to be stressful; ergo the physiological process was affected.

However, controlled respiration at frequencies within the range of resting physiological range provided to be a convenient tool to provoke an enhancement of the sympathetic modulation of the heart period [53].

In addition, Brown et al. [9] compared the influence of different metronome breathing rates on the RR interval power. Breathing rates (BR) of about 10 breaths per min. or less yielded the maximum RR interval power. BR of more than 10 breaths/min reduced the level of RR interval power. That means that the RR interval variability decreased with increasing breathing frequency (BF). At the slowest BR, maximum RR intervals were longer and minimum RR intervals shorter which also provoked major increases of LF power. Ergo, the respiratory frequency overlaps in the LF band which resulted in an increase of the LF component due to the slow breathing. Based on these findings, Brown et al. [9] supposed that slow BR only augments the LF power by a shift of the

power without affecting the vegetative control of the heart. Therefore, slow BR does not reflect an increased sympathetic neural outflow.

Nevertheless the best way to measure HRV under standardised conditions and without any respiratory induced influences has not yet been found. Not only metronome breathing which modifies the ANS but also the spontaneous breathing causes problems with the evaluation of the HRV. It still remains essential to control the respiration to have a feedback of the respiratory induced influence on the HRV [62, 44, 2, 43]. The way of the respiratory control only depends on the investigation of the physiological phenomena modulating the HRV.

2.5 Effect of physical activity on HRV

The effect of physical activity on the autonomic nervous control of the heart was investigated in animal models. Dogs with documented higher risks of acute myocardial ischemia were trained by running for six weeks. After the training intervention they showed an enhancement of the HRV of 74% [84]. These findings indicated the positive physiological effect of training especially endurance training on the cardiovascular system. Based on these findings the modification of the autonomic balance by physical activity was more and more investigated.

2.5.1 Physical activity affecting the cardiovascular system

Physical activity is associated with hemodynamic changes and alterations of the loading conditions of the heart [72]. The cardiovascular responses to physical activity vary in dependence of the type, the intensity and the volume load of the training. Endurance training leads to the most adaptive changes of the cardiovascular function due to the increased volume load compared with strength trainings. Based on the subject of the thesis, only the cardiovascular responses in relation to endurance training are described in this chapter.

The “athlete’s heart” is a well known phenomenon which describes the increase of the left ventricular diastolic cavity dimensions, the wall thickness and the mass after long term athletic exercise [2]. The lower heart rate leads to longer periods of the diastole which enhances the stroke volume. The higher stroke volume is induced by the larger blood volume of athletes and the Frank Starling Mechanism (FSM). The FSM describes that a prolongation of the diastole results in an enhanced cardiac work load. Endurance

training leads to an improvement of the heart's ability to pump blood by an increased stroke volume which is possibly due to an increase in the end-diastolic volume and a slight enhancement of the left ventricular mass. This results in a more efficient pressure to time relationship i.e. the stroke volume increases and the heart rate decreases. At rest and at sub maximal exercise intensity, the metabolic load on the heart is thereby decreased. Increased heart rate (>100 beats/min) is called tachycardia which can occur either by neural stimulation or by an elevation in circulating catecholamines. While exercising, the increasing heart rate raises the cardiac output [2] whereas the increased heart volume and enhanced contractility lead to higher stroke volume at rest and during exercise. In addition to these responses, endurance training reduces the resting and sub maximal systolic, diastolic and mean arterial blood pressure.

The cardiovascular control of the heart is modulated by physical activity and thereby the vegetative control of the heart may also be affected. HRV measurements are used to investigate the difference of the sympathetic and parasympathetic activity in sedentary subjects and athletes. Studies involving female athletes remain rarely and further investigations concerning gender differences are needed.

2.5.2 Exercise induced HRV changes

Athletes have lower resting heart rate (HR) compared with sedentary people. This bradycardia leads to longer average RR interval length in athletes [74] because of the enhanced diastole. A lower resting heart rate can be induced by higher vagal activity and/or diminished sympathetic activity. Thus, exercise training is thought to modulate the sympathovagal control of the heart resulting in a predominance of the vagal activity by an increased vagal and/or a decrease sympathetic nervous activity. This modulation is supposed to lead to an enhancement of the vegetative control of the heart which has been quantified by an increased HRV. Selected studies investigating the difference between trained and untrained subjects illustrate the difficulties to prove the relation between bradycardia and increased HRV.

2.5.2.1 HRV in sedentary subjects and athletes

Sacknoff et al. [74] compared the HRV (15 min supine) of aerobic trained (3h/week) with sedentary subjects in the time and the frequency domain. In the time domain, the trained subjects showed significantly increased meanNN, SDNN and pNN50. Therefore a resting bradycardia i.e. lower resting heart rate was noted in athletes whereas the LF, the HF and the total power were significantly lower. The results of the time domain

indicate an increased HRV which could not be proven in the frequency domain. Unfortunately Sacknoff et al. [74] failed to control the respiration of the subjects which could have affected the results of the power spectral analysis of the HRV.

Perini et al. [63] compared parameters of the frequency domain of high endurance trained cyclists and inactive men in sitting position by a short time recording. The cyclists showed similar HF and reduced LF power and thus significantly lower LF/HF ratio than their sedentary counterparts. Similar results were also found by Perini et al. [63] comparing tri-athletes and untrained men in the same way. In neither study was the respiration controlled.

Kouidi et al. [46] investigated the resting HRV (24h recording) of endurance (A), sprint (B) and weight lifting (C) trained athletes compared to sedentary (D) subjects. Parameters of the time domain were evaluated including the HRV triangular index (HRVI), which is the integral of the density distribution. All results had the following ranking, starting with the best results according to the HRV: Group A, group B, group C and finally group D. Therefore, group A showed the most increased meanNN, SDNN and HRVI and the lowest resting heart rate. A relation between the maximal oxygen uptake and the HRVI was only found in group A. Still, athletes showed enhanced HRV compared to their sedentary counterparts. The fact that the endurance trained athletes had the most significantly increased HRV indicates that the exercise training pattern contributes significantly to the modulation of the HRV.

Goldsmith et al. [29] investigated the HRV (24h recording) in aerobically trained and untrained young men in the frequency domain. Athletes had significantly higher HF power during the day, the night and over the entire 24h recording duration. Based on this finding they concluded a substantially greater parasympathetic activity in athletes.

Melanson et al. [57] compared the HRV by short time recordings (10 min) at rest in low (L), moderate (M) and high (H) endurance trained men. The classification of the groups was related to a self reported habitual physical activity level of the subjects. While recording the ECG, volunteers had to match their breathing to an auditory signal (metronome) set to 10 breaths/min. Parameters of the time domain were significantly but similarly increased in M and H compared to L. Ergo, the heart rate was lower in M and H than in L. In the frequency domain, L had lower LF, HF and total power compared with M and H. Still, no difference was found between moderately (M) and highly (H) active men in the HRV. Although the time and frequency domain measures of HRV were greater in active than sedentary (L) individuals, Melanson et al. [57] failed

to demonstrate a dose-dependent manner of HRV with increasing level of physical activity.

Finally, Rennie et al. [70] investigated the effects of moderate and vigorous activity on the HRV (5 min at rest) in more than 3000 British Civil Servants during two years. Subjects were classified into 4 groups in accordance to their metabolic rate based on the self reported activity level: inactive (1), light (2), moderate (3) and vigorous (4) active group. The resting heart rate was lowest in group 4 and highest in group 1 whereas the SDNN was highest in group 4 and lowest in group 1. No gender differences were noted in the time but the frequency domain. LF and HF power were highest in group 4 and lowest in group 1 considering the males whereas the females did not have any differences between groups 1 to 4. Rennie et al. [70] still concluded that vigorous activity is associated with higher HRV. Unfortunately, neither respiration nor menstrual cycle related modulations of the vegetative control of the heart which are known to affect the HRV were controlled.

2.5.3 Training interventions and HRV

Endurance training is assumed to modulate strongest the cardiovascular system compared with other types of training [46]. Nevertheless the exact mechanism induced by physical activity which affects the vegetative control of the heart, remains unknown. Therefore, training interventions of short (5-9 weeks) and long time (3-6 months) duration have been carried out to investigate the direct effect of training on the cardiovascular system and its adaptation due to the autonomic nervous system. Trained and untrained men and women were included in different training intervention programs, which are presented exemplary separated into short and long time interventions, to illustrate the difficulties of the HRV measurements and its physiological interpretation.

2.5.3.1 Short time interventions

Pigozzi et al. [64] investigated the effect of 5-weeks exercise training on the autonomic regulation of the heart in untrained women under daily conditions. The sedentary control group was daily active (e.g. housekeeping and cleaning) whereas the intervention group completed a 5-week endurance training of 1h duration 3 times per week. No significant difference was found in the time and in the frequency domain after the training intervention. Therefore, Pigozzi et al. [64] concluded that the training intervention might have been too short to modulate the cardiovascular control of the heart. Besides, nor respiratory neither menstrual cycle related modulations were controlled in this study.

The aim of Uusitalo et al. [85] was to examine the HRV under influence of heavy (70-90% of VO_{2max} and 130% volume) and moderate (<70% VO_{2max} and 5-10% volume) exercise intensity according to the subjects´ individual anaerobic threshold in endurance trained women. The first HRV measurement (20 min supine) took place after 4 weeks and the second after 6-9 weeks of training intervention. The average RR interval length significantly increased and the resting heart rate decreased in the heavy training group whereas the blood pressure remained unaffected in both groups. Nevertheless the SDNN, the LF and the total power significantly decreased despite of increased average RR interval length in the heavy trained group. Uusitalo et al. [85] attributed these findings to a possible sign of impending fatigue due to the heavy training intervention. Additionally, they failed to take account of to control the menstrual cycle and its hormonal fluctuations in athletes which might have been affected by the heavy endurance training intervention in this study.

2.5.3.2 Long time interventions

Catai et al. [13] investigated the effects of a 3-months walking and jogging intervention in young (mean 21 years) and middle-aged (mean 53 years) men on the HRV (24h recording). The average RR interval length was significantly increased and the resting heart rate decreased in both groups after the training intervention. The SDNN, the HF and the total power remained unaffected in both groups whereas only the young men showed a significantly increased LF power. Catai et al. [13] missed to demonstrate a HRV change after 3-month of aerobic exercise training which increased the aerobic capacity in both groups. Thus they explained the increased LF power in young men with the age dependent changing of the autonomic nervous system.

Loimaala et al. [51] compared two exercise regimes of varying intensities on HRV and the baroreflex sensitivity (BRS) in middle-aged men. Thereby an inactive control group was compared with exercise 1 (E1), which had to walk or run 4-6 times a week at an intensity of 55% of VO_{2max}, and exercise 2 (E2) group which trained similar but at an intensity of 75% of VO_{2max}. The resting heart rate decreased in E2, but remained similar in E1. No significant changes occurred in the time or the frequency domain measures of the HRV or the BRS in either of the exercise groups. Loimaala et al. [51] finally concluded that their intervention training program despite enhanced VO_{2max} was not able to modify the cardiac vagal outflow in middle-aged men.

Furthermore, Wayne et al. [89] investigated the effect of 6-month endurance training on the HRV (2 min supine) at rest in older and younger men. The training program consisted of walking, jogging and bicycling 4-5 times a week with increasing work-load (from 50 to 85%) according to the subjects' maximal heart rate. The VO_2max increased in both groups after the intervention. Additionally, the resting heart rate significantly decreased whereas the SDNN significantly increased in both groups. No other HRV parameter was evaluated in this study. Still Wayne et al. [89] concluded that exercise training increases the parasympathetic activity and the HRV which was expressed by the lowered resting heart rate and the enhanced SDNN.

2.5.4 Summary

Most studies [13, 46, 51, 57, 70, 74, 85, 89] noted a lower resting heart rate (bradycardia) in athletes and in sedentary individuals after the training interventions whereas two studies [13, 85] additionally noted an increased average RR interval length. These findings are thought to be based on enhanced vagal activity due to the training. Still, uniform HRV results in the frequency domain in relation to physical activity could not be demonstrated because of missing methodological standards which might have affected the results.

Further studies must be in accordance with the guidelines of the Task Force [84], which include the selection of the subjects, training intervention programs and the control of respiration related modulations on the HRV. Additionally, enhanced HRV in athletes has to be quoted not only by the lower resting heart rate and the increased average RR interval but by the SDNN and the LF, HF and total power to describe in detail and to prove completely the training induced effect on the vegetative control of the heart.

Studies including females should also observe hormonal fluctuations and physiological differences in relation to the menstrual cycle.

2.6 Physiological differences of genders

Physiological differences between males and females are based on gender, i.e. sexual characteristics with different internal and external reproductive organs. Gender specific hormonal concentrations, receptor sensitivity and fluctuation pattern result in different modulation of the body. Women are known to have a fluctuation of the endogenous hormones related to the menstrual cycle whereas men do not have any cycle related hormonal fluctuations.

2.6.1 Menstrual cycle

The menstrual cycle starts with the first day of bleeding. The cycle length varies from 21 to 32 days with a mean time of 28 days. The menstrual cycle is a feedback control system of the hypothalamus, the pituitary glands and the ovary. Neurons in hypothalamus produce the hypothalamic releasing hormone (LHRH) known also as gonadotrophic releasing hormone (GnRH). LHRH, a decapeptide, stimulates the secretion of gonadotrophic hormones; the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH). Furthermore FSH stimulates the maturation of several follicles of which only one follicle will get to the end of maturation.

The maturing follicle produces a rising amount of natural estradiol, which consists of estrone, estriol and the predominantly estradiol-17β (E2). Natural estradiol mostly consists of E2 and therefore only E2 is described acting for the natural estradiol.

E2 affects the proliferation of the endometrium. The feedback of E2 to the pituitary glands and the hypothalamus provokes a midcyclic enhanced LHRH production and a stronger sensitivity of hypophysis cells to LHRH.

LH stimulates the ovulation and the luteinizing of follicular granulose cells (corpus luteum). The granulose cells start the production and secretion of progesterone (P). The feedback of E2 and P to the pituitary glands and the hypothalamus reduces the FSH and LH secretion. This results in a reduction of the P level, which leads to start of the luteolisis. At least the menstruation bleeding results from an abrupt P withdrawal (i.e. the withdrawal bleeding) and with it a new menstrual cycle starts again.

The following figure (figure 2-5) illustrates the feedback control system and its hormonal interaction including the maturation of the follicle during one menstrual cycle.

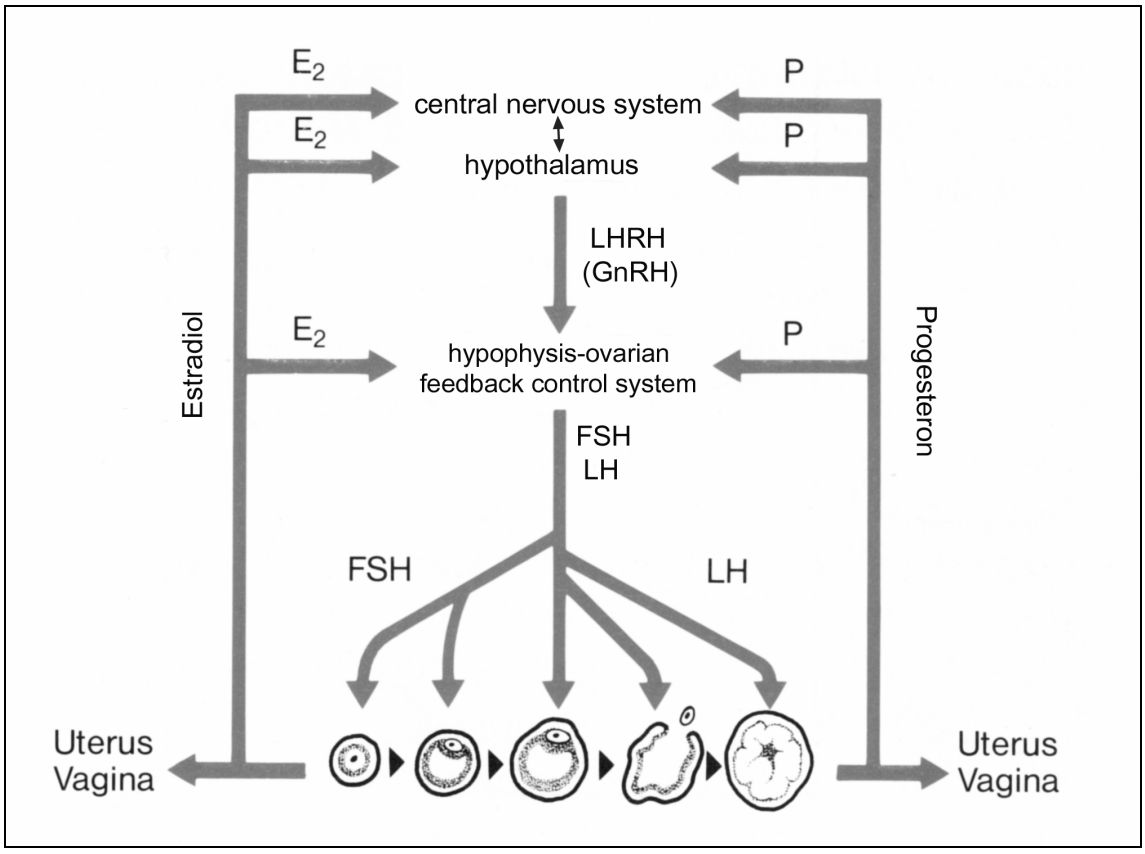


Figure 2-5: Feedback control system of the hypothalamus, the hypophysis and the ovary (modified from [77])

2.6.2 Basal body temperature

The basal body temperature (BBT) changes rhythmically throughout the menstrual cycle. The BBT rises 0.5 °C up after ovulation and remains elevated during the luteal phase till the onset of the menstrual bleeding. Faber et al. [24] found strong evidence in individual regulation of body temperature at different set points in women. The rate of heat production was positively related to the temperature of the body. Additionally the elevated BBT was associated with the increase of the P level which was observed during the ovulatory cycle and the pregnancy in women. Today an increase of the thermoregulatory set point is suggested to elevate the BBT during the luteal phase in women. Furthermore, Janse de Jorge [34] supposes that all thresholds for all

thermoregulatory effector responses are shifted in a similar direction during the luteal phase.

In animal models the P administration decreased the activity of warm sensitive and increased the activity of cold sensitive neurons in pre-optic area [60]. On the other hand, E2 administration increased the activity of warm sensitive neurons and decreased the body temperature. But there was no indication of a strong specificity between thermo sensitive and steroid-sensitive neurons [79]. So the increase of the thermoregulatory set point is suggested to be related to the ratio between E2 and P in women [12].

2.6.3 Respiratory response

Research on animals has suggested that progesterone increases the ventilation (V_E) by means of a central effect in the brain stem. This respiratory response to P is likewise modulated by estrogen (E2). In addition the V_E has been shown to be affected by the body temperature [34]. Thus, elevated P level and core temperature during the luteal phase of the menstrual cycle suggest an increased V_E as shown by White et al [92].

Supposing a training induced influence, Schoene et al. [78] compared the respiratory response of trained and untrained women at rest and during exercise. The ventilation drive at rest and the exercise ventilation were significantly increased in the luteal phase in both groups. In spite of this increase, no decrease in the exercise performance was noted. The same results were found by Beidleman et al. [3] who compared the V_E at rest and during exercise at sea level and at altitude in trained women. The V_E at rest as well as the exercise V_E were increased with increasing P level during the menstrual cycle. Nevertheless, the menstrual cycle could not affect the maximal and the sub maximal exercise performance at sea level as well as at altitude in women.

Menstrual cycle induced influences on the respiratory response were also thought to affect the balance between the CO_2 controlling system and the CO_2 exchange. Therefore, Dutton et al. [22] did a CO_2 rebreathing test based on Rebuck's outlines [69] at different phases of the menstrual cycle. They noted that the sensitivity of the respiratory centre to CO_2 was significantly increased in the luteal compared to follicular phase; still a significant decrease of the CO_2 threshold was not found. The same results concerning the CO_2 sensitivity were found by Schoene et al. [78].

2.6.4 Hormonal fluctuation in men

Men have a feedback control system consisting of the hypothalamus, the anterior lobe of pituitary gland and testicles. The LHRH neurons of hypothalamus secrete a decapeptide into the portal system every 2 to 4 hours. The LHRH pulses as well as the LH and FSH pulses mostly occur at night and in the early morning hours.

LH stimulates the Leydig cells, which results in an augmentation of the androgen production. The Leydig cells give feedback to the hypothalamus and the anterior lobe of pituitary gland, which closes the feedback control loop. Testosterone (T) of which only soluble T in plasma has a biological effect, is the most important testicular androgen. It reaches the anterior lobe of pituitary gland and the central nervous system via the bloodstream and has a negative feedback to the LH and LHRH production. Moreover, T is coupled to the protein sexual hormone binding globulin (SHBG).

In men, the influences of FSH as well as the intra testicular androgen result in the spermatogenesis. Androgens are additionally known to stimulate the protein synthesis, the bone and muscle growth and the masculine hair growth. Men have diurnal fluctuation of the hormones whereas the LH and T production depends on sleeping habits, and not on the time of day.

The BBT of men only shows small fluctuation and is more or less stable throughout the month.

2.7 Effect of menstrual cycle on HRV

The menstrual cycle is characterized by phases with different endogenous hormones and consequently by different neurotransmitter concentrations. The cyclic changes in estradiol and progesterone levels modulate physiological functions including e.g. the basal body temperature and the respiration of women. However the relation between the menstrual cycle and the vegetative control of the heart remains disputable due to the lack of studies. Four studies exist which investigated the HRV in relation to endogenous hormonal fluctuations in sedentary women [30, 50, 76, 97] whereas one study [75] investigated the reflex control of autonomic function induced by an orthostatic test during the menstrual cycle. These studies are described detailed in the following chapter. The menstruation phases are verified in days or described in phases according to the authors' data to compare the study intervention phases/days. Therefore the first day of bleeding means the first day of the menstrual cycle, e.g. day 5 stands for the 5th day of the menstrual cycle whereas the cycle length is generally between 28-32 days.

2.7.1 HRV in relation to the menstrual cycle

Guasti et al. [30] compared the HRV (25 min supine) of the follicular (day 5±1) with the luteal phase (day 23±3) in 13 women. The blood pressure and the heart rate remained similar throughout the menstrual cycle. In the frequency domain, the LF and the HF power were not significantly different whereas the LFnu was significantly increased and the HFnu decreased in the luteal phase. Consequently, the LF/HF ratio was significantly increased in the luteal compared to the follicular phase. Guasti et al. [30] suggested an increased sympathetic activity in the luteal phase.

Yildirir et al. [97] investigated the HRV (5 min supine) during the follicular (day 11±1) and luteal phase (day 21±2) in 43 women. The heart rate, the LF and HF power were similar in both phases. A significant increase was only noted in the LFnu and consequently in the LF/HF ratio in the luteal phase whereas the HFnu was not significantly different. Based on these findings, Yildirir et al. [97] still concluded that the sympathetic activity was enhanced in the luteal compared with the follicular phase.

Sato et al. [76] compared the HRV (20 min supine, 10 min sitting) of the follicular (day 7-10) and the luteal phase (3-7 days prior to the next bleeding) at 3 consecutive days in 20 females. Each day of the study was repeated in the following month and thus, the

study lasted for two menstrual cycles. The resting heart rate and the blood pressure remained similar. Still, the LFnu significantly increased, the HFnu decreased and the LF/HF ratio consequently increased in the luteal compared with the follicular phase. The absolute values of the LF and HF were not mentioned. Sato et al. [76] suggested a predominant sympathetic activity in the luteal phase.

Saeki et al. [75] investigated the autonomic reflex control by an orthostatic test (5 min supine, 5 min sitting) in 5 different phases which are abbreviated by the first letter of the phase: menstruation (day 1-3), follicular (between M and O), ovulation (3 prior and 4 days after O), luteal (between O and P) and the premenstrual phase (7 days prior to next bleeding). Subjects´ (n=10) breathing frequency was controlled by a metronome (15 breaths/min) during the orthostatic test. In supine and sitting position, the HF power was significantly higher in F than in M. The LF/HF ratio was similar in both positions whereas the LF power was not calculated. The LF/HF ratio increased significantly by changing the position from supine to sitting in M, F and P. Saeki et al. [75] concluded an increased vagal activity in F at rest and an enhanced sympathetic activity in M, F and P during the orthostatic test. Based on these findings they supposed a different modulation of the vegetative control of the heart at rest and at the orthostatic reflex control during the menstrual cycle.

At least, Leicht et al. [50] examined the HRV (20 min supine) of 10 women in 3 different phases M (day 3.8±0.5), O (day 15.8±0.7) and L (day 22.1±0.4). The resting heart rate was significantly greater at O compared with M and L whereas the LF, HF and total power as well as the LFnu, HFnu and the LF/HF ratio remained similar. Leicht et al. [50] concluded that there was no association between the cyclic variation of the endogenous hormones and the cardiac autonomic control.

In summary, Guasti et al. [30], Sato et al. [76], and Yildirir et al. [97] suggested an enhanced sympathetic activity in the luteal compared with the follicular phase. Their findings are based on the LFnu, HFnu and the LF/HF ratio whereas the absolute power values of LF and HF were not evaluated [76] or remained unaffected [30, 97] during the menstrual cycle. In accordance to the guidelines of the Task Force [84] the normalized units have to be quoted with the absolute values of LF and HF power in order to describe completely the distribution of power in spectral components. However the authors [30, 76, 97] did not follow these guidelines. Saeki et al. [75] concluded an enhanced vagal activity in the follicular phase at rest and an increased sympathetic

activity during the orthostatic test in M, F and P based on the results of the HF power and the LF/HF ratio. They supposed an enhanced sympathetic activity in M, F and P due to an increased LH/HF ratio, but they failed to validate these findings by using the absolute power values of LF as demanded by the Task Force [84].

Solely Leicht et al. [50] could not find any modulations of the vegetative control of the heart in relation to the menstrual cycle or in the time or the frequency domain of the HRV. Based on this finding and due to the shortcomings of the above mentioned studies, further investigation is needed to clarify the cardiac autonomic control during the menstrual cycle in women.

Further studies on female subjects should only be done in consideration of special guidelines, which are not yet been laid down. Until then, at least the following points should be considered in HRV studies in relation to the menstrual cycle:

1. Monitoring of the menstrual cycle by hormonal analysis.
2. HRV measurements in accordance to the guidelines of the Task Force [84], which include the respiration control.
3. Control of the mental changes, e.g. individual symptoms and feelings during the menstrual cycle with a daily questionnaire.

2.8 Mood state control

2.8.1 Mood state during the menstrual cycle

During the menstrual cycle women mentioned individual different symptoms and feelings, which might affect the mood state. The changes of the cardiovascular control could be modulated by mental fluctuations in women. Therefore, the profile of mood state (POMS) might be a useful tool to investigate the subjects' well-being during the intervention days of the study.

2.8.2 Profile of mood state (POMS)

Profile of mood states (POMS) is a self measured confidential scale which records individual change in mood states. The original version created by McNair et al. [56] consists of 65 items; the short version of 35 items. Items are adjectives characterizing the mood state in an answer scale of 5 to 7 possibilities, ranging from "absolutely not" to "very strong". The original scale was classified into six and the short scale into the following four subscales: depression, fatigue, vigor and anger. Each subscale includes certain adjectives expressing the mood state. These adjectives are awarded points based on the value of the subscale on which the evaluation of POMS is based. Reliability of POMS was proved by a coefficient of internal consistency.

The mood response to exercise has been investigated. Agreement exists that exercise has a positive influence of the individual mood state. But the effects of maximal exercise differ from sub maximal exercise in sports. Differences are also related to the level of physical fitness in human beings [82]. The main findings of Pronk et al. [67] indicated acute increase of fatigue and depression as well as a decrease of anger and vigor after maximal exercise. This implies that the mental well-being of individuals can be influenced by physical activity. Differences between men and women are suggested because of the menstrual cycle.

In addition to this, the POMS questionnaire is considered as a valuable tool to predict overtraining and staleness in elite sports. Coker et al. [15] compared female starters and non-starters of a softball team prior to playing. Significant mean differences were found between starters and non-starters on constructs of anger, confusion, tension and depression; prior to the play non-starters presented higher fatigue. The author suggested

that in spite of the same training conditions the non-starters did not share the same psychological profile as their starting peers.

The mood state measured by POMS and the resting salivary Cortisol levels were examined in 14 female college swimmers in 1989 by O'Connor et al. [61]. Significant alterations in tension, depression, anger, vigor, fatigue and global mood across the training season were noted in swimmers compared to controls. A correlation between salivary Cortisol level and a depressed mood during overtraining could be found. Swimmers classified as stale had significantly higher global mood expressed by the overall score, enhanced depression and increased salivary Cortisol levels than swimmers without performance decrements. The valence of salivary Cortisol is disputed and thus these findings have to be considered with care.

Morgan et al. [59] collected data of mood states during a ten year research effort in 400 male and female competitive swimmers. The results indicated that mood disturbances increased in a dose response manner as training stimulus increased. These mood disturbances fell back to baseline levels with a reduction of the training load.

The study of Raglin et al. [68] compared successful and unsuccessful women's rowing teams. In baseline mood state, no difference was noted and mood disturbances increased in both groups during the training season. At the end of the season, the mood of the successful rowers returned to baseline whereas the mood of the unsuccessful females still showed significantly elevated mood disturbances.

Williams et al. [94] noted in moderately trained runners that more economical values of running were associated with more positive mental health profiles. The graphical representation of the POMS scores resulted in a mountain profile or in an inverted one; thereby the expression Iceberg profile was used to mention the positive POMS results (less negative feelings, better mood state), whereas the inverted Iceberg profiles illustrated the negative POMS scores (enhanced negative feelings, worse mood state) [59]. Soccer players also presented iceberg profiles during successful performance and with a high percentage of winning in a study of Filaire et al. [25] whereas decreased performance resulted in a decrease in vigor and an increase in tension and depression.

Based on these findings the notion is, that successful elite athletes tended to produce so-called iceberg profiles of mood but less successful ones not. Thereto mood could be considered as an emotional state influenced by personality and environmental factors [14].

2.9 Derivation of the question

The measurements of the HRV, which are non-invasive and easily handled, could be a valuable tool for sports medicine, because of the insight into the autonomic nervous system (ANS) and its status. Training causes short and long term disturbances in the ANS, which are rarely described. The physiological reactions to different training interventions, which cause autonomic disturbances and acute or chronically adaptations could be investigated by the HRV. Thereby the diagnoses of approaching overstrain and overtraining in athletes is of interest which could be investigated using the orthostatic test, i.e. reflex control of the ANS. Still data of athletes, particularly female athletes, are inconsistent and the physiological significance of the HRV is not yet practicable in relation to training interventions. Due to this, the first purpose of this study was to investigate whether the short time recording of the HRV including the orthostatic test could be a valuable tool for male and female athletes who were involved in individual training patterns during the study. The guidelines of the Task Force [84] were observed in this study.

The influence of the hormonal fluctuations (P, E2, LH and FSH) during the menstrual cycle on the vegetative control of the heart has been investigated in several studies whereas the difference between sedentary and physically active women, i.e. highly endurance trained females is missing. Acute or chronic adaptations induced by training could affect the modulation of estrogen and progesterone on the HRV. Such training induced adaptations do not have to go along with menstrual cycle disturbances and dysfunctions. Female athletes may have a normal ovulatory cycle. Based on this, the second aim of this study was to investigate the HRV at rest and during the orthostatic test in relation to the fluctuations of P, E2, LH and FSH in course of the menstrual cycle in trained (i.e. highly endurance trained) and untrained (i.e. sedentary or moderately active) women.

Progesterone is known to affect the breathing during the menstrual cycle in sedentary women. The oxygen uptake, the respiratory frequency and the tidal volume are individually modulated by the progesterone fluctuations, but data concerning female athletes are missing. The respiratory frequency and the tidal volume are known to affect the HRV by afferent reflexes in the baroreceptors and the pulmonary stretch receptors. Ergo the modulated breathing is expected to affect the HRV in the second part of the

menstrual cycle with increasing progesterone level. However the respiratory related modulations of the HRV in endurance trained women has not yet been investigated. The investigation of the respiration on the HRV requires spontaneous breathing instead of metronome controlled breathing. Therefore the fourth aim of the study was to investigate the influence of the spontaneous breathing which might be modulated by progesterone on the HRV in trained and untrained women.

Men do not have any cycle related hormonal fluctuations but individually different levels of testosterone. Therefore, the influence of testosterone especially free testosterone on the vegetative control of the heart was investigated by the HRV in athletes and sedentary men. Additionally, male athletes served as controls for the athletic women and untrained men for the sedentary females. Finally the repeated HRV measurements in the time and frequency domain in the male groups served as a test for the reliability of our study.

Furthermore, the blood glucose and the insulin concentration are supposed to have an influence on the vegetative control of the heart based on data of diabetic patients. The relation of the blood glucose and the insulin on the HRV in healthy and/or trained individuals as well as gender differences are missing. Thus, an additional aim of this study was to investigate the relation between the HRV and the metabolic supply by the blood glucose and insulin concentration in trained and untrained men and women.

2.9.1 Control parameters

Due to the high sensitivity of the ANS to internal and external influences, several parameters were controlled during the study i.e. at each study day.

The conduction and the impulse transmission of the nerves and the muscle cells depend on the homeostasis of sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}) and chloride (Cl^-) concentration. An affected homeostasis of the electrolytes may lead to an affection of the conduction system of the heart which would modulate the HRV. Due to this, the electrolytes Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^- were controlled in the blood serum during the study.

Haemoglobin concentration was controlled because of anaemia which is often diagnosed in highly endurance trained athletes whereas the hematocrit level served to ensure the balance of the fluid.

At least, the POMS questionnaire was filled in daily by the subjects to evaluate the mood state during the study which might have been affected by the menstrual cycle, the

individual training interventions or other influences. Additionally the alcohol uptake, the basal body temperature, the resting heart rate, the sleep quality and the training (duration, intensity and type) were noted daily by the volunteers.

3 Methods

The study was approved by the Human Ethics Committee of the Medical Faculty Charité of the Humboldt University of Berlin in accordance with the declaration of Helsinki.

3.1 Experimental design

The influence of the menstrual cycle on the heart rate variability (HRV) was investigated in five different phases in trained and untrained women in this study. In addition, the influence of testosterone on the HRV was examined in five study days in trained and untrained men. The HRV was investigated at spontaneous breathing for 20 min at rest and during the orthostatic test, which consisted of three parts: 20 min supine, 10 min standing and 20 min. supine. The change of the body position was done actively by the subjects. The breathing frequency was controlled by respiration belts. The HRV evaluation was done in the time and the frequency domain including those parameters which were allowed for short time measurements in accordance to the Task Force [84].

The five menstrual cycle phases were individually determined based on the daily measurement of the basal body temperature (BBT) one month prior to the study. The BBT was orally determined by a digital thermometer in the morning before getting up. During this month, females also had to fill in a daily questionnaire to the mood state, the alcohol intake and the activity i.e. training pattern. Additionally, several blood borne parameters (INS, BG, WBC, RBC, Hc, Hb, Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^-) were analysed for each study day. This included the analysis of the endogenous hormones (P, E2, LH,

FSH) in women which served to control the menstrual cycle phases, along with the daily measured BBT. The study days of men were set at the same time interval as the menstrual cycle phases of women and thereby men served as control group. Additionally, the reliability of the method and procedure was veryfied by the five study days of men due to the repeated measurements. The blood borne parameters (INS, BG, WBC, RBC, Hc, Hb, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} and Cl^{-}) were analysed including the testosterone and the SHBG concentration in men for each study day. The subjects also had to fill in a daily questionnaire which served to control of the mood state, the alcohol intake and the activity i.e. training pattern during the study. Based on this, the preceding month and the test month of women were compared with each other and with the men's month to ensure stable conditions.

60 volunteers, consisting of 30 men and women in the age of 25-38 years were included in this study. Subjects were without any kind of infection, arterial hypertension, and diabetes mellitus as well as without any psychological and/or psychosocial overstrain situations (e.g. overtraining in athletes) in the last 12 months. The male and female groups were separated into long term (≥2 years of training) endurance trained athletes and sedentary subjects. The athletes, consisting of marathon runners, cyclists and triathletes had to train ≥5h per week whereas the sedentary subjects were only included in moderate but not regular daily activity which was <2h per week. The maximal oxygen uptake in relation to the body weight was determined by a maximal effort test to establish the endurance capacity (EC) of athletes and sedentary subjects. The maximal effort test was done by bicycle for the sedentary subjects. Athletes who did not reach the maximal oxygen uptake on bicycle were additionally tested on a treadmill. The EC was set for male athletes at ≥55ml/min/kg, for female athletes at ≥50ml/min/kg, for sedentary men at <55ml/min/kg and for the untrained women at <45ml/min/kg.

Moreover, subjects were non-obese, non-smokers and did not take any kind of medications and/or drugs. The female subjects had regular menstrual cycles for at least 6 months prior to the study. They did not have any pre menstrual tension syndromes (PMS) before the menstrual bleeding and no woman was actually pregnant. Furthermore, none of the female subjects used oral contraceptives or any form of hormonal replacement therapy. Female athletes did not have menstrual dysfunctions or any other menstrual disturbances during high intensity training periods. Prior to the study, all subjects completed a health history questionnaire and a medical examination.

The subjects, who were finally included in the study, are described in details in the following chapter.

3.2 Subjects

Sixty subjects were tested in this study and forty nine male and female volunteers were included in the final study. Each subject was familiarized with the testing equipment and procedures used in the laboratory. They provided a written informed consent prior to participation. The use of tobacco products was forbidden and alcohol intake was restricted during the study. The athletes were allowed to train to their individual training pattern. The untrained subjects were moderate daily active, but never involved in regular exercise training. Every kind of physical activity was noted in the questionnaire during the study and thus all groups have a training anamnesis.

The study population was first separated into a female and male group and secondly by the relative oxygen uptake (VO_{2rel}) and the training anamnesis into a trained and untrained group.

Training anamnesis consisted of training age in years (T_{Age}), training sessions per week (TS_{pweek}) and of the training units in hours per week (TU_{pweek}). The following 4 subgroups resulted: 13 trained (MT), 14 untrained men (MUT), 11 trained (WT) and 11 untrained women (WUT). Descriptive characteristics of the volunteers are presented in table 3-1 and data concerning training anamnesis in table 3-2.

Due to non-normal distribution, all data are presented in median, first and third quartile.

Table 3-1: *Anthropometrical data of volunteers separated into 4 subgroups*

	MT (n=13)	**MUT** (n=14)	**WT** (n=11)	**WUT** (n=11)
Height [cm]	180.0 (167.7/192.5)	183.4 (175/194)	167.0 (155/178)	172.0 (158/184)
Weight [kg]	67.5 (60.3/90.5)	72.8 (62.8/95)	55.0 (45/73)	62.2 (56/74)
BMI	22.2 (19.7/24.4)	22.3 (19/26.3)	20.5 (18.3/23)	20.8 (18.4/24.8)
Age [years]	26.7 (22.3/32.6)	25.6 (21.5/30.9)	33.8 (21.4/37.2)	28.9 (22.9/33.5)
VO_{2rel} [ml/min/kg]	62.8 (55.6/80.0)	49.5 (38.2/55.9)	50.9 (49.6/68.3)	40.8 (38.6/45.6)

Table 3-2: *Anamnesis of training data including training age in years (T_{Age}), training sessions per week (TS_{pweek}) and training units per week in hours (TU_{pweek})*

	MT (n=13)	**MUT** (n=14)	**WT** (n=11)	**WUT** (n=11)
T_{Age} [years]	5 (2/20)	2 (0/10)	6 (2/15)	4 (0/20)
TS_{pweek} [units]	4 (3/7)	1 (0/3)	4 (3/7)	1(0/3)
TU_{pweek} [hours]	8 (5/12)	1 (0/3.5)	6.5 (5/13)	1 (0/4)

3.3 Study days of men and women

Female volunteers measured their basal body temperature (BBT) each morning and recorded the day of menses to verify a recent history of menstrual cycle regularity one month before entering the study and throughout the one month experimental period. The BBT was orally measured before getting up in the morning by the digital thermometer KD-132 (K-Jump Health Co., Lot-No: 07/02, Ltd. Taiwan Imp., SCALA Electro GmbH, Stahnsdorf, Germany). Thereby a temperature curve based on the individual determined BBT was evaluated for every woman. The menstruation (bleeding phase) and the ovulation with the characteristic enhanced BBT were chosen to determine these five phases individually; the menstruation, the middle of the follicular phase, the ovulation phase, the middle of the luteal phase and the pre menstruation phase. Females´ first experimental day was M and the last PreM. Women were involved in the study for two successive months starting with the preceding month i.e. BBT recording followed directly by the test month; ergo every woman had 5 study days.

The phases were verified as follows:

Menstruation phase (**M**): second or third day of bleeding

Middle of follicular phase (**MidF**): between M and O

Ovulation phase (**O**): ovulation ±1 day

Middle of luteal phase (**MidL**): between O and next M

Pre menstruation phase (**PreM**): 1 or 2 days prior to the next menstrual bleeding

Men could start the series of experimental investigations at any time. Their experimental period varied from 24 to 32 days similar to the women's cycle length. In this period they had five experimental days every four to five days during one month. Although men do not have any cycle related changes during one month, they had to take part of five days including the daily BBT recording. Equal study conditions should thereby lead to comparable results between men and women.

3.4 Procedure

Volunteers had to fill in a POMS questionnaire every morning including the measurement of the resting heart rate and the basal body temperature before getting up. The alcohol consumption and sports activity of the previous day as well as the duration and the quality of sleep were required.

Subjects arrived at the laboratory at the individually equal time each morning, i.e. between 07.00 and 11.00 am. All of them were requested to refrain from eating and drinking for at least 8 hours before the experiment. They also had to avoid stress and exercise in the morning to be in a relaxed condition and quiet mood when arriving. Each of the five sessions were identical and were scheduled for the same time of day. The sessions were organized into groups of one to four persons.

The temperature in the laboratory was between 20 to 24°C and the humidity between 40 to 48%. The room was darkened and without acoustic disturbance. Volunteers had to feel comfortable during the experimental phase. They were instructed to be as relaxed as possible and to breathe spontaneously at their own rate.

After a resting period, the subjects rested 20 minutes in supine position, ten minutes in upright standing position and another 20 minutes in supine position. Volunteers stayed afterwards in supine position for the venous collecting of blood samples which was done by a doctor or a medical technical assistant (MTA).

3.5 Analysis

The analysis of the blood borne parameters was done by the “Laboratory 28” whereas the hormones and the lactate values were analysed in our laboratory.

3.5.1 Blood borne parameters and electrolytes

The analysis of the haemoglobin (Hb), hematocrit (Hc), leukocyte (WBC), erythrocyte (RBC), blood glucose (BG) and electrolytes (Na^+, Ca^{2+}, K^+, Mg^{2+} and Cl^-) was carried out by the “Labor 28” (Labor 28, Berlin, Germany). Hb, Hc, WBC, RBC and BG were analysed from EDTA fresh blood. BG was stored in a sodium-fluoride tube whereas Na^+, Ca^{2+}, K^+, Mg^{2+} and Cl^- were determined in the blood serum. The blood sample rested for 30 min before it was centrifuged with 2000 revolutions/min for 15 min by the centrifuge EBA 8 (Centrifuge EBA 8, A. Hettich, Tuttlingen, Germany). Then, the

blood serum was separated by a pipette from the blood clot, filled in primary tubes and stored at 4-8°C till analysis at the same day. The analyses of the electrolytes and the blood borne parameters were different and thus several methods are described in the following part.

3.5.1.1 Analysis of the electrolytes

Na^+, K^+ and Cl^- were determined in mmol/l by the indirect potentiometer (Hitachi 747-400, Tokyo, Japan) with the following test principle: Electrodes of selective ions provoke an electrometrical power which is related to the activity of the ions in hydrous solution. The electrometrical power can be converted into mmol/l by a formula. The specification of the analysis of Na^+ was 80 mmol/l, K^+ 1.5 mmol/l and of Cl^- 60 mmol/l.

Ca^{2+} was analysed by the colour test with end-point and blank value determination (Hitachi 747-400, Tokyo, Japan). Ca^{2+} forms with Kresolphthalein-complexion a violet complex in alkalise solution. The intensity of the violet colour is direct proportional to the concentration of Ca^{2+} which results in the calculation of Ca^{2+} in mmol/l with a precision of 2.12 mmol/l intra- and 2.1 mmol/l interassays.

Mg^{2+} was analysed by the atom absorption spectrometer with flame technique which is based on the proportion of the element's concentration and its absorbed radiation after atomising (Beer's Law). The measurement of the element's concentration at specific wavelength happens after atomising.

BG [mmol/l] was determined by the glucose dehydrogenise (B-D-Glucose) which catalyses the oxidation of blood glucose (BG) to the following equation:

$$\text{B-D-Glucose} + \text{NADH} \rightarrow \text{D-Gluconolacton} + \mathbf{NADH} + H^+$$

The amount of **NADH** which results from this equation is proportional to the glucose concentration.

Finally, the analysis of the WBC [G/l], RBC [G/l], Hc [%] and Hb [mmol/l] concentrations has been done by the analysis system of Sysmex 9500 which consists of different measurement methods.

WBS are analysed by the galvanic current and resistance principle, the RBC by the galvanic current with hydrodynamic focussing, the Hb with sheaths flow and photometric measurement whereas the Hc value is calculated by the cumulative summation of the RBC impulses. These analysis methods are all based on the principle that the resistance between the electrodes changes by suspension of the blood cells which produces electrical impulses. These impulses which are proportional to the cell

size are counted and imply a distribution which reflects the cell size. Additional high frequency resistance to the galvanic current results in impulses which reflect the specific gravity. Based on these data the cells are counted with the help of a scatter plot. The sheaths flow enhances the suspension and thus, the counting gets more specific for the Hb concentration. WBC has an intra- and an interassay of 1.15% and 3.45 %, RBC of 0.89% and 0.55 %, Hc 0.96% and 0.79% and finally Hb 0.32% and 0%.

3.5.2 Hormonal analysis

Hormone status of progesterone (P), estradiol-17β (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone (tT), insulin (INS) and sex-hormone binding globulin (SHBG) was determined from blood serum which was frozen till analysis. The shelf-life of the hormones was 2 months frozen at -20°C. The menstrual cycle was monitored by five analyses of the P, E2, LH and FSH levels whereas tT and SHBG were analysed for men and finally the INS level for both genders.

3.5.2.1 Analysis of the hormones

The hormonal analysis was done in our laboratory by the Immunoassay Analyser Immulite 2000 of the Diagnostic Products Corporation DPC (DPC Cirrus Inc., Los Angeles, USA; German agency DPC Biermann GmbH, Bad Nauheim, Germany). Immulite 2000 is a continuous Random Access System to manage chemiluminescent immunoassays automatically. Immulite uses test tubes, reagents and substrate to analyse the hormonal levels. A synthetic globe with parameter specific antigens is inside the test tubes. The immunoreactions, the incubation, the washing steps and the development of the signal take place in the test tubes. The reagents which are identified by barcodes are marked by an alkalised phosphatase. At least the substrate used is a chemiluminescent enzyme substrate.

In this study, commercial kits of DPC Biermann were used with the following quality numbers: P 267, 277; E2 241, 249; LH 254, 259; FSH 267, 272; fT 157, 158, 163; INS 126, 126A, 130; SHBG 237, 238, 241. Additionally, twice distilled water mixed with a washing module (quality number 063, 064, 069) in the ratio of 1:9 i.e. 100 ml washing module added to 900 ml distilled water was used beside the chemiluminescent enzyme substrate with the quality numbers 179, 181, 190. The serum of the patients was filled in probe tubes which were identified by numbers.

Prior to the analysis, the calibration, the daily routine and the analysis of the controls had to be done. The calibration included a high and low calibration level, which was determined four times to calculate the slope (max. 10% variation) and the intercept (max. 2% variation) in accordance to the master standard curve of DPC Biermann. The control serums CON4, CON5 and CON6 (quality number 019) and the special SHBG control (quality number 017) were used to measure the controls which were done twice (high and low level). The analysis was only started with approved calibrations and controls, which had to be renewed after the analysis of 500 test tubes.

The run of the automatically Immulite analysis is now described step by step:

1. The patient's serum and the reagents are incubated in Immulite test tubes.
2. Then, the incubation time, which varies from 30-60 min starts at 37°C.
3. After the incubation, water is added to the test tubes to wash the globe. Then, the test tubes are rotated around the vertical axis to separate serum, water and reagent liquid from the globe. Finally the globe is free of unbound markers and liquids.
4. Then the bound marker is quantized by a luminescent Dioxetane-substrate.
5. Finally, the photo multiplier measures the emitted light and the software calculates the results of each probe.

After the analysis, the syringes had to be cleaned, waste liquids had to be taken away and data printed or stored on the disc. Immulite remained in idle to keep the accumulated data and the analysis instrument ready.

3.5.2.2 Intra- and interassay variance

Intra- and interassay variance was calculated in accordance to the General Medical Council of Germany (December 2003) and to IMMULITE reference values: LH (4.9% / 8.9%), FSH (5.7% / 7.4%), E2 (8.4% / 8.8%), P (7.6% / 8.6%), SHBG (6.2 %/ 9.8%), tT (7.2% / 7.2%), INS (4.8% / 6.0%).

3.6 Recording of electrocardiograms

Different data were recorded during the study including the ECG, the respiration frequency and the spiroergometric tests on bicycle and treadmill. The procedure of each recording is described in the following chapters.

3.6.1 Electrocardiogram

Subjects arrived in the laboratory. Three ARBO (H124) electrodes (Kendall, Medizinische Erzeugnisse GmbH, Neustadt/Donau, Germany) were attached according to the second derivation of Nebh A (anterior). The first electrode was mounted to the fourth intercostal space and the second to the apex of the heart on the left side of the midclavicular line. The third electrode was attached at the right collarbone as earthing. Electrode diameter was 55 mm and contact surface 20 mm. Men with significant body hair were shaved before mounting the electrodes. Electrodes were connected with an electrocardiogram amplifier (ECG-Amplifier) from Biovision (Biovision, Bleichstrasse 6a, 61273 Wehrheim, Germany).

An elongation cable of 2 metres conducted the electrocardiogram (ECG) signal with an entry impedance of 10 gigaΩ to the input box from Biovision (Biovision, Wehrheim, Germany). The input box had 16 channels, of which a maximum of eight were used. A wide-bend cable connected the input box to the A/D converter DAQ card (DAQ card 700, National Instruments, München, Germany). The sample rate was set to 1000 Hz and the resolution to 12-bit. The ECG recordings were made using a commercial computer running the Daisylab software (Daisylab version 6.0, National Instruments, München, Germany).

The ECG recordings were detected and corrected by hand for ectopic, electrical disturbances and missed beats by the automatic detection algorithm implemented in RASCHlab (RASCHlab-mfc version 0.1, using LibRASCH version 0.3.1 of Ralph Schneider, Technical University of Munich, Germany). After shortening the ECG values to ten minutes segments, RR interval distances not classified as sinus rhythms and outside the range of 27 to 200 beats per minute were eliminated; no interpolation was done. Sequences with more than 5 eliminated beats per minute were not taken for the evaluation.

The time domain parameters meanNN, SDNN, RMSDD and pNN50 were calculated. Afterwards the heart rate time series was band-pass filtered by a Hamming window as an apodization function in order to reduce aliasing. Next a Fast Fourier Transformation (Source: Proc. Algorithms in Matlab, Disk., 1996) was performed on the filtered signal; each heart rate time series was expressed by a sequence of amplitudes describing the time-dependent magnitude of the oscillations.

The main frequency bands LF, HF and VLF were defined at the following bands according to the Task Force [84]: LF (0.04-0.15 Hz), HF (0.15-0.4 Hz) and VLF

(0.0033-0.04 Hz). Due to short time records, ULF was not evaluated. TP consisted of LF, HF and VLF power.

3.6.2 Breathing frequency

The spontaneous breathing frequency was recorded by the respiratory belt Effort Sensor of Viasys (Viasys Health Care GmbH, order number 706428, Höchberg, Germany). Piezoelectric parts in the respiration belt register the tension in the belt produced by inspiration and expiration, i.e. the rise and the sink of the abdominal wall. Different tension results in different electrical signals which were conducted by an elongation cable of 2 metres to the input box, the A/D converter and finally the computer.

The belt was fixed around the abdomen between belly and costal arch. Volunteers were not disturbed by the belt and felt comfortable. In supine position the respiration is more abdominal than thoracal and thus the use of only one belt was sufficient to record the abdominal breathing. The inspiration and the expiration as an expression of the breathing rhythm i.e. the respiratory frequency were measured at rest and during the orthostatic test. The evaluation of the breathing excursions per unit of time was registered at the screen.

3.6.3 Spiroergometric tests

All volunteers underwent a bicycle maximal-effort test to measure the maximal (VO_{2max}) and the relative (VO_{2rel}) oxygen uptake as a measure of cardio respiratory endurance capacity. The VO_{2max} is the maximum uptake of oxygen in millilitres per minute (ml/min). The VO_{2rel} is the maximum uptake of oxygen relative to body weight in millilitres per minute per kilo body weight. Spirometric data were recorded breath by breath. Men could carry out the bicycle test at any time around the experimental period. Women were tested in the middle of the luteal phase (MidL) because of missing premenstrual tension syndrome before menstruation. The sedentary subjects were not familiar with a maximal effort test and therefore the bicycle instead of the treadmill was chosen for the test. The athletes which consisted of marathon runners, cyclists and triathletes were tested on bicycle and on treadmill. The VO_{2rel} was determined by the results of the bicycle and the treadmill test to have comparable results for the three kinds of athletes.

One day prior to the test hard training had to be restricted; on the day of the assessment exertion, exercise and caffeine had to be avoided. The procedure of the bicycle and

treadmill test as well as the ventilation recording was similar. The protocols of both tests are described separately in the following chapters whereas the ventilation recording was described only once. The maximal ergometric tests ended with subject's exhaustion.

3.6.3.1 Protocol of bicycle ergometric test

The bicycle exercise test was done by an Ergometrics 800 S bike (Ergo Line, Medical Measurement Systems, Binz, Germany). Ergometrics 800 S was a computer-controlled high performance bicycle. Prior to the bicycle test, a 12-lead standard ECG recording (ECG-recorder Quinton, model Q 7/0 sx, serial 00334-165-0107, Quinton instrument co., Seattle, Wa., USA) was obtained at rest in supine position.

The protocol started with a warm-up period consisting of three minutes of cycling with the minimum of 60 revolutions per minute (60 rpm) at a workload of 0 watt. The increase in workload was 50 W every three minutes without any breaks. The rotation frequency of 60 rpm had to be maintained throughout the test.

3.6.3.2 Treadmill testing

Subjects were monitored using the 12-lead ECG recording during the test. Volunteers wore a security belt of Saturn on treadmill (Saturn, HP Cosmos, Sports Equipment GmbH, Nussdorf/Traunstein, Germany) during the test. The protocol started with a warm-up period consisting of three minutes walking at a speed of 1.5 metres per second (m/s). The increase of the speed was 0.5 m/s every three minutes. Interruptions of 30 seconds after each level served for the measurement of blood pressure. The treadmill test was also a maximal-effort test.

The exercise test ended when subjects were exhausted. The treadmill test could be stopped at any time with a delay within five seconds.

3.6.3.3 Ventilation recording

The ventilation, oxygen uptake, carbon dioxide excretion and derived parameters changing with increasing workload were measured by Oxycon Champion Record manufactured by Viasys (Viasys Health Care GmbH, Höchberg, Germany). After a running up time (adjustment of the measuring instruments) of 30 min, volume and gas had to be confirmed by calibration. The calibration of volume was done by a 2 litre calibration pump. Gas calibration was done by twice solidified calibration gas. 15 percent by volume of oxygen, 5 percent by volume of carbon dioxide and the reminder of nitrogen were the components of the gas mixture (Calibration gas of Messer

Griesheim GmbH, bottle number A022941, Krefeld, Germany). The result of both calibrations was a correction factor calculated by computer to correct the measurement. Dry atmospheric air was the reference for the oxygen analyser. The ergometric data were analysed by the software Lab Manager, version 4.5.2.

Volunteers had to wear a synthetic basic face mask of Viasys (Viasys Health Care GmbH, Höchberg, Germany) connected to a Triple V Volume Sensor of Viasys. The analysis of oxygen was based on the differential paramagnetic principle and the analysis of carbon dioxide was based on infra-red absorption principle. The inspiration and expiration air flow was analysed during the bicycle and treadmill test. Subjects were monitored using the 12-lead ECG during the test. The Borg Scale was used to measure the subjects' exhaustion at each level. Blood pressure was measured at each level and five minutes after the test.

3.7 Statistical evaluation

Statistical evaluation was performed using the STATISTICA 1999 Edition Kernel-Version 5.5 A (Stat Soft Inc., 2300 East 14th Street, Tulsa, OK 74104, USA).

3.7.1 Profile of mood states

The Brown-Forsythe-Test was used to compare the homogeneity of variances of all female profiles between the preceding and the test month. The Brown-Forsythe-Test was also used to compare the results between the groups i.e. men versus women and trained versus untrained in the test month. Significance levels were statistically determined at p-values of <0.05 (significant), <0.01 (very significant) and <0.001 (highly significant) in accordance to Hartung et al. [31].

Kruskal-Wallis ANOVA with repeated measurements was used to compare the difference of the POMS profiles between men and women and also between trained and untrained men and women in the 5 different phases of menstrual cycle.

3.7.2 Heart rate variability

Due to non-normal distribution and heterogeneity of the variance of variables, non parametric tests were selected for the statistical analysis and data are reported as medians and quartiles 1st and 3rd). Inter- and intragroup comparisons were done by the

Mann-Whitney and the Wilcoxon test. The significance level was adjusted to Bonferroni because of multiple testing and repeating measurement designs.

Kruskal-Wallis ANOVA with measurements repeated five times was used for inter- and intragroup comparison in the menstrual cycle trend. 2x2 MANOVA with measurements repeated five times was used to compare men versus women and trained versus untrained subjects. 2x2x3 MANOVA with measurements repeated five times was used to compare men versus women and trained versus untrained during the orthostatic test. Correlation was tested by Spearman's rank correlation.

3.7.3 Reliability

The reliability of the study was controlled by the intragroup correlations coefficient (ICC), the coefficient of variation and the Bland-Altman plots [5].

4 Results

4.1 Profile of mood state

No significant difference was noted between the profile of mood (POMS) questionnaire of the pre (the month prior to the study) and the test month for all women in the profiles during the different menstrual phases. The time course of the profiles including the sub scales and the overall score showed no differences during the months (figure 4-1).

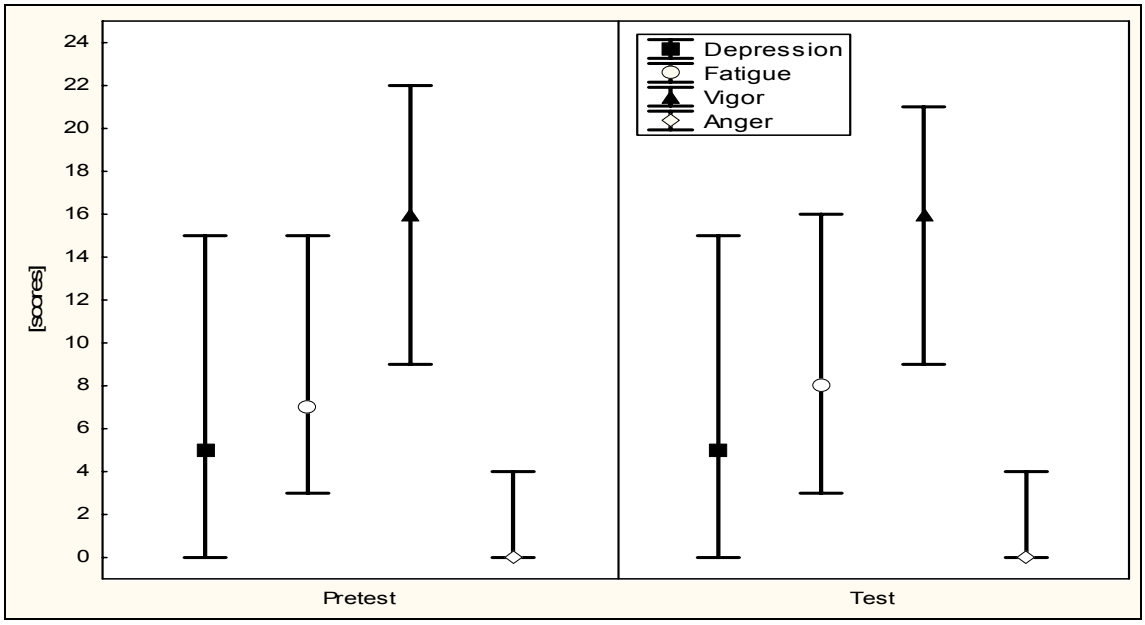


Figure 4-1: *Similar profiles of mood state (POMS) separated into depression, fatigue, vigor and anger (subscales of the POMS) in the pre and the test month of women; no significance was noted*

There was no significant gender difference in the overall score of all subjects (trained and untrained men and women) of the study month (figure 4-2) whereas differences between trained and untrained subjects and differences between male and female athletes and sedentary subjects were noted.

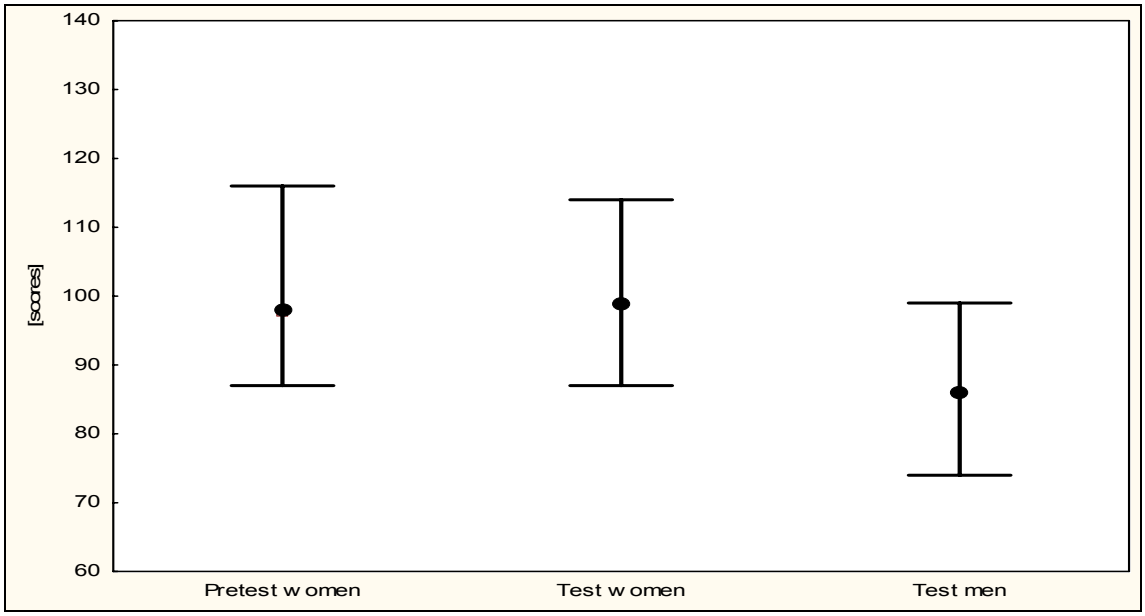


Figure 4-2: *Overall score of POMS in pretest and test month of women compared with men; overall scores were not significantly different in men and women*

Trained compared with untrained men had significantly higher sub scores in depression ($p<0.01$) and significantly higher sub scores in fatigue ($p<0.05$). Still the overall score was similar in trained and untrained men. Trained and untrained women were similar in sub scores and the overall score.

In the trained group, women had significantly higher sub scores in depression, fatigue and anger compared with the male athletes ($p<0.001$); still the overall score was similar. In the sedentary group, women had significantly higher sub scores in depression and fatigue than the untrained men but with comparable overall scores ($p<0.01$).

4.2 Blood borne parameters

The results of the blood borne parameters which include the hormonal fluctuations in women, the comparison of blood glucose and insulin in trained and untrained subjects and the electrolytes are presented in the following chapters.

4.2.1 Monitoring of the menstrual cycle

All women had a normal ovulatory cycle. No difference between trained and untrained women was noted. Female athletes did not show affected menstrual cycle phases and/or modulated hormonal concentrations compared with the sedentary females. Despite typical hormonal fluctuations, some women had levels of LH and FSH which were constantly below the 95% range. The reference ranges of LH, FSH, E2 and P was generated by DPC Biermann. The hormonal concentrations were significantly different during the five days of the menstrual cycle ($p<0.001$).

The menstrual cycle monitoring is based on hormonal levels measured at M (menstruation), MidF (middle of follicular phase), O (ovulation), MidL (middle of luteal phase) and PreM (pre menstruation phase) in figure 4-3 and 4-4.

4.2.2 Monitoring of hormonal fluctuation in men

Total testosterone (tT) and SHBG were analysed whereas the free androgen index (fAi) and the percentage of free testosterone (fT) were calculated based on tT value. No significant differences in tT, SHBG, fAi and fT was found during the study month. The hormonal levels of trained and untrained men were individually different but similar during the study month. Therefore, men did not have any hormonal fluctuations in the course of one month.

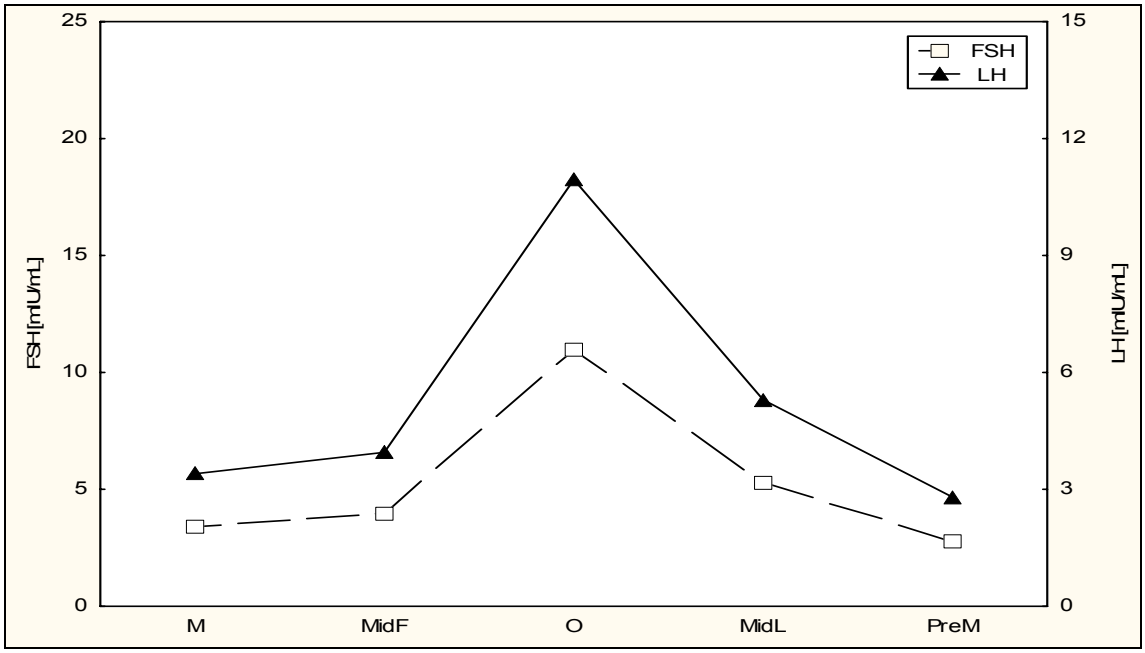


Figure 4-3: *LH and FSH concentration all women; LH and FSH peaks are noted around the ovulation day (O)*

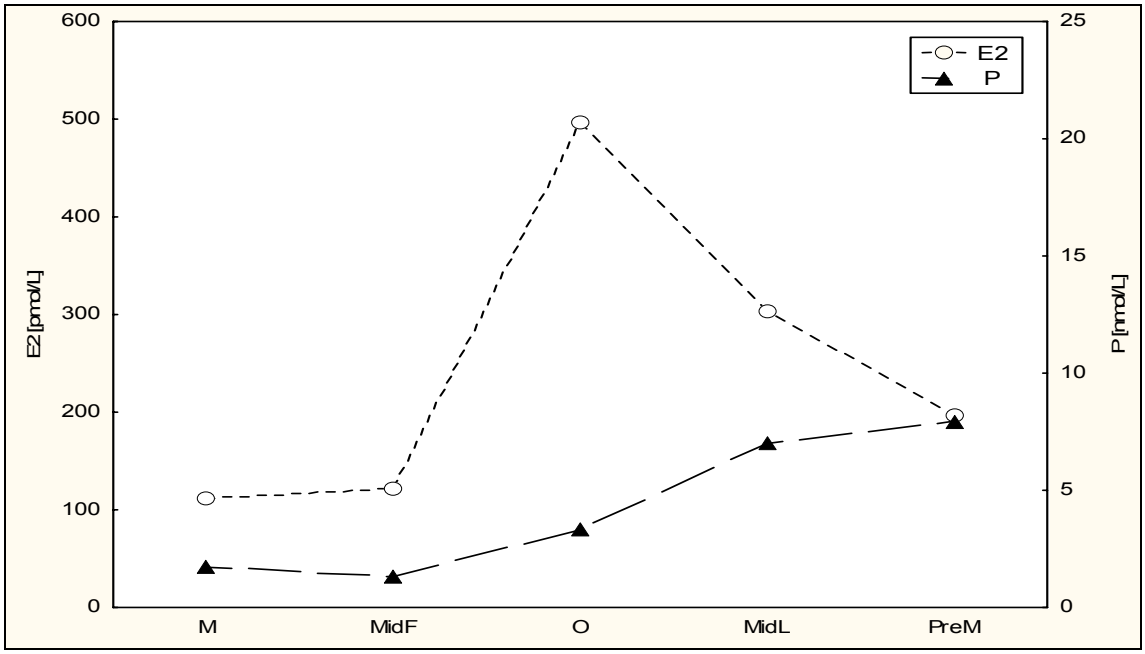


Figure 4-4*: P and E2 concentration of all women during five phases of the menstrual cycle; E2 peak around ovulation (O) combined with a constant increase of P*

4.2.3 Glucose and insulin concentration

Insulin (INS) levels were similar between the sedentary subjects and the athletes without gender differences. Nevertheless trained individuals had lower INS levels than untrained subjects whereas the INS levels were stable in course of the study month in men and women (figure 4-5).

In contrast to INS, the blood glucose (BG) was similar in trained and untrained subjects. No fluctuation of BG was found in course of the study month in men and women (figure 4-6).

The five menstrual cycle phases of women are identical with the five study days of men characterized as follows; M=1, MidF=2, O=3, MidL=4, PreM=5.

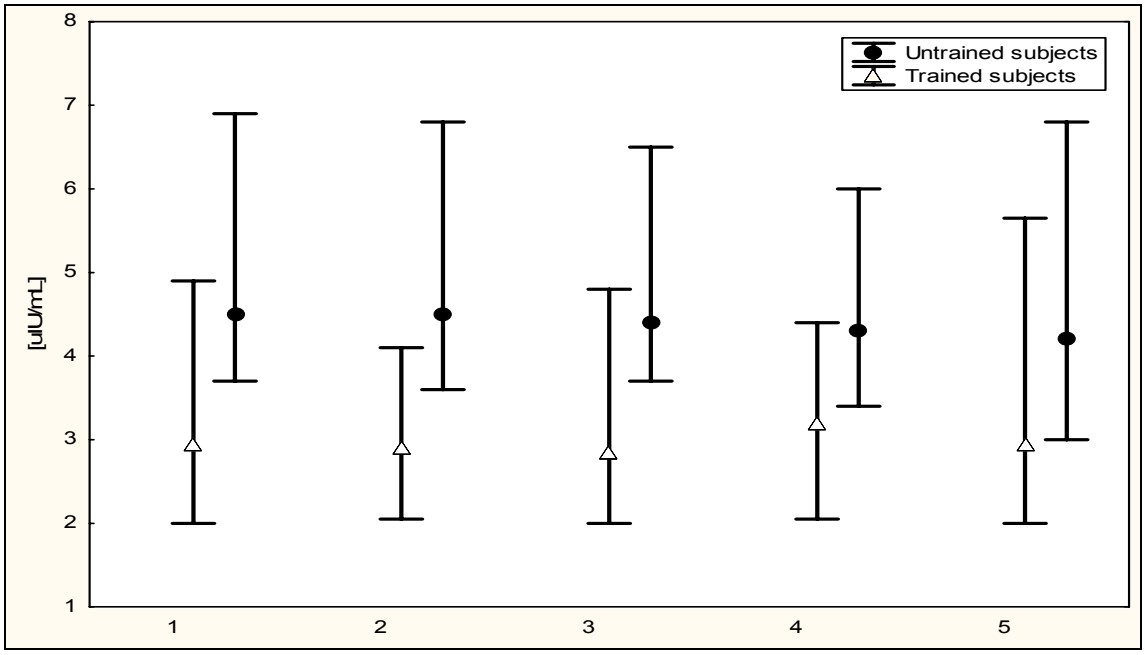


Figure 4-5: *INS concentrations of trained and untrained men and women during the study; lower INS levels in trained and higher ones in untrained subjects were noted*

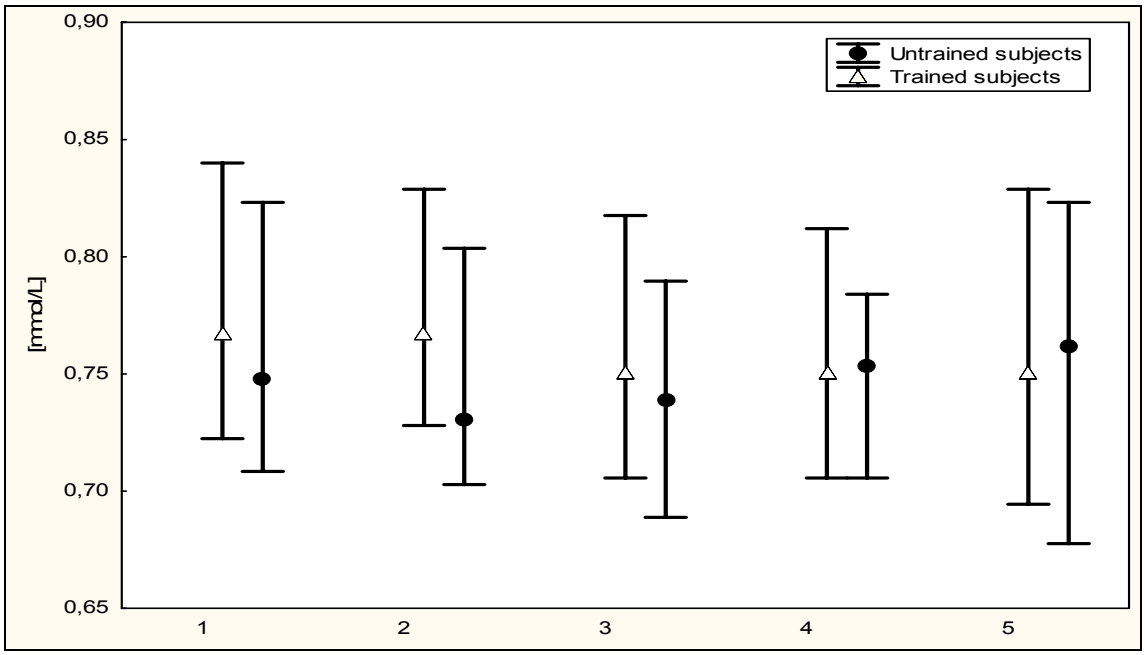


Figure 4-6: *BG of athletes and sedentary men and women during the study; similar levels were noted in athletes and sedentary subjects*

4.2.4 Electrolyte concentration and blood count

Electrolytes Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-, the white (WBC) and red blood cells (RBC), haemoglobin (Hb) and hematocrit (Hc) were in the normal range and did not fluctuate in course of the study month. No difference was noted between athletes and sedentary subjects and between men and women with exception of the hematocrit which was lower in women than in men as presented in figure 4-7. Figure 4-8 till 4-12 present the course of Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- during the study and between men and women.

As mentioned above, the five menstrual cycle phases of women are identical with the five study days of men characterized as follows; M=1, MidF=2, O=3, MidL=4, PreM=5.

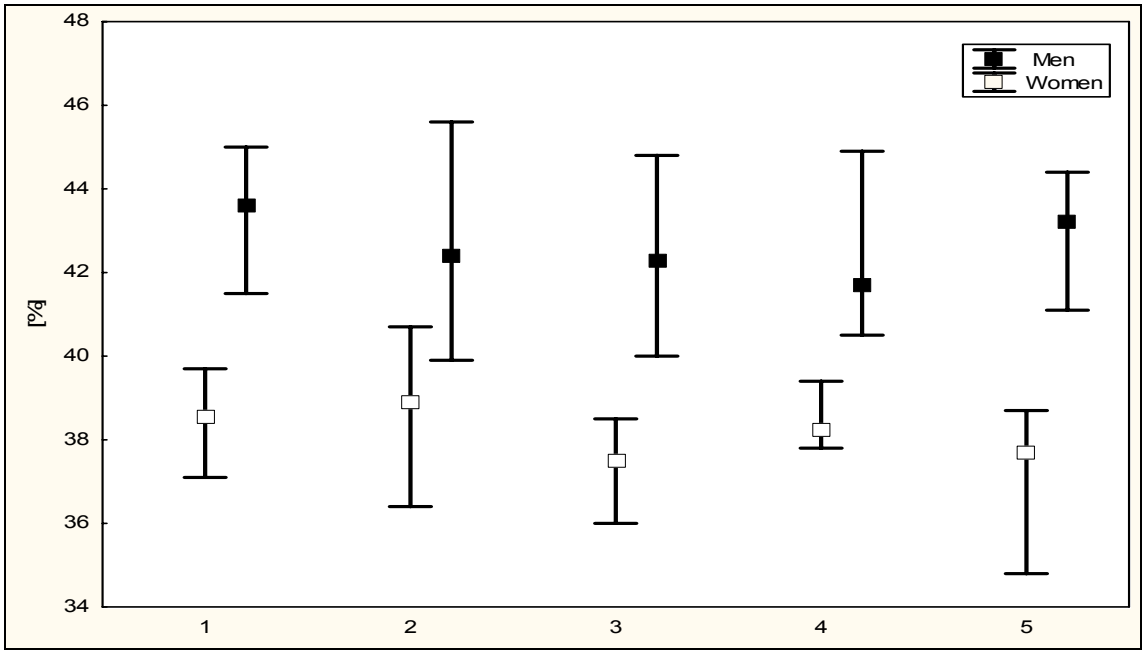


Figure 4-7: *The stable course of the **Hc** of men and women in % during one month i.e. one menstrual cycle; women have higher Hc (lower %) than men*

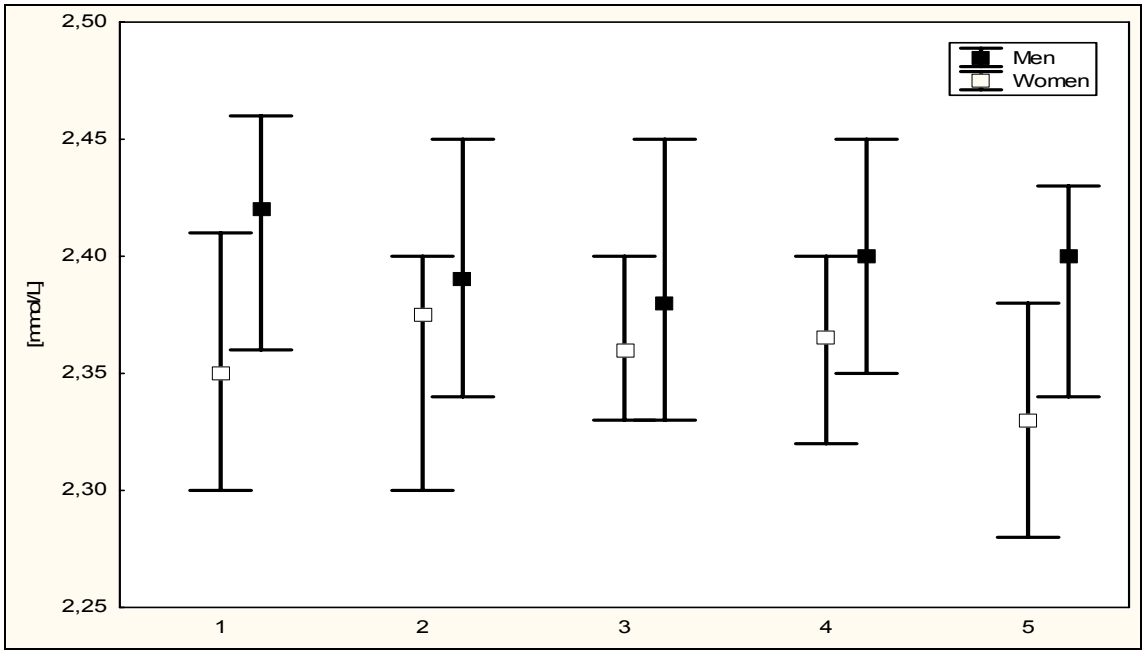


Figure 4-8: *Similar **Ca^{+}** levels in men and women during one month; no menstrual cycle related fluctuations were noted*

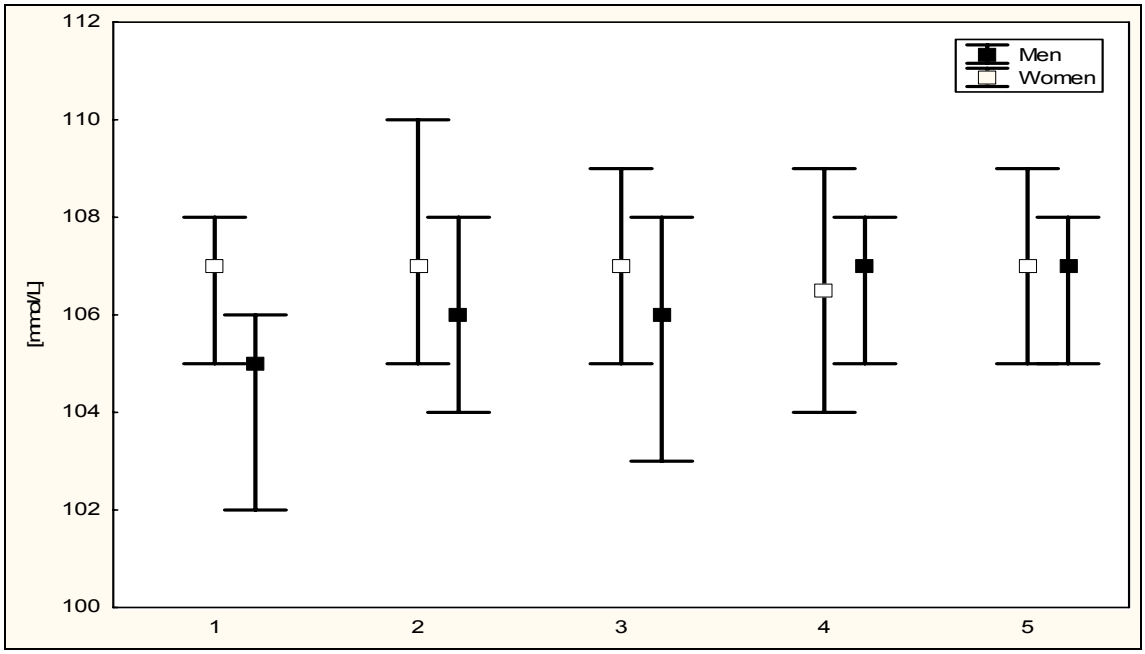


Figure 4-9: *Cl^- levels of men and women in course of one menstrual cycle; no differences were noted*

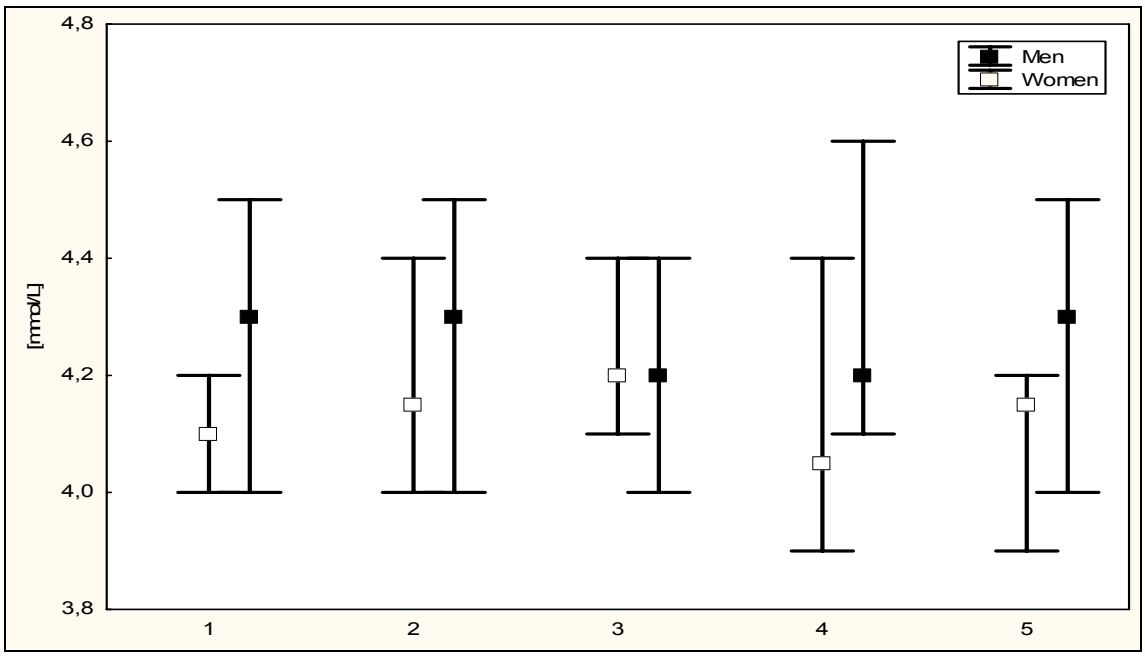


Figure 4-10: *Similar K^+ concentrations of male and female subjects during five study days*

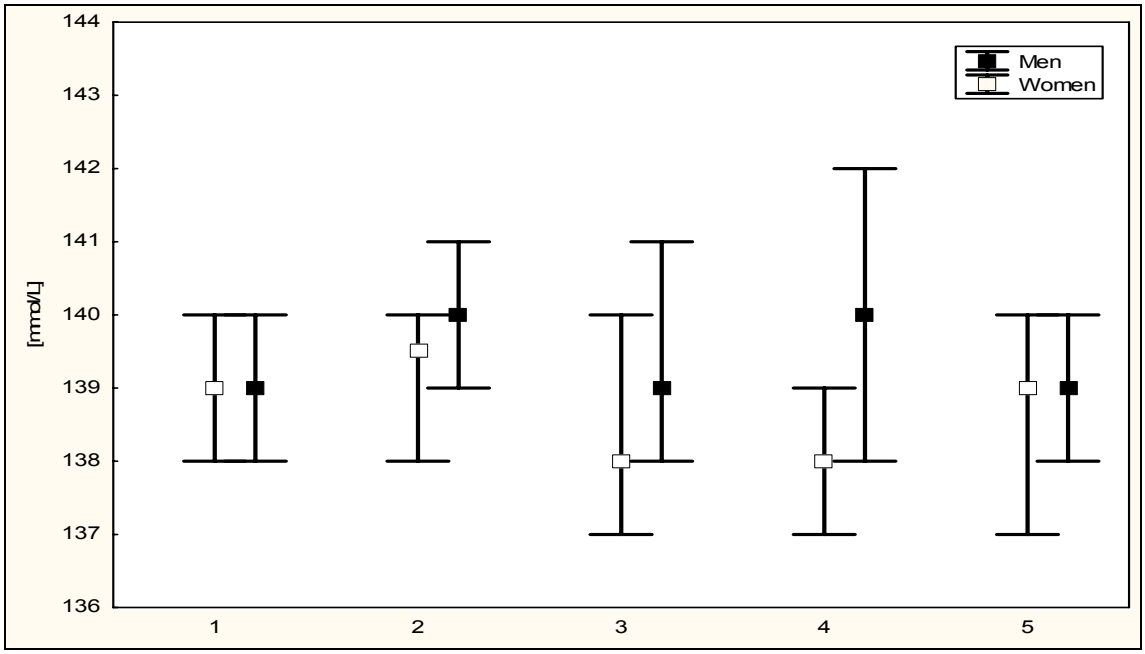


Figure 4-11: *No significant difference was noted in* Na^+ *levels between men and women and during the study*

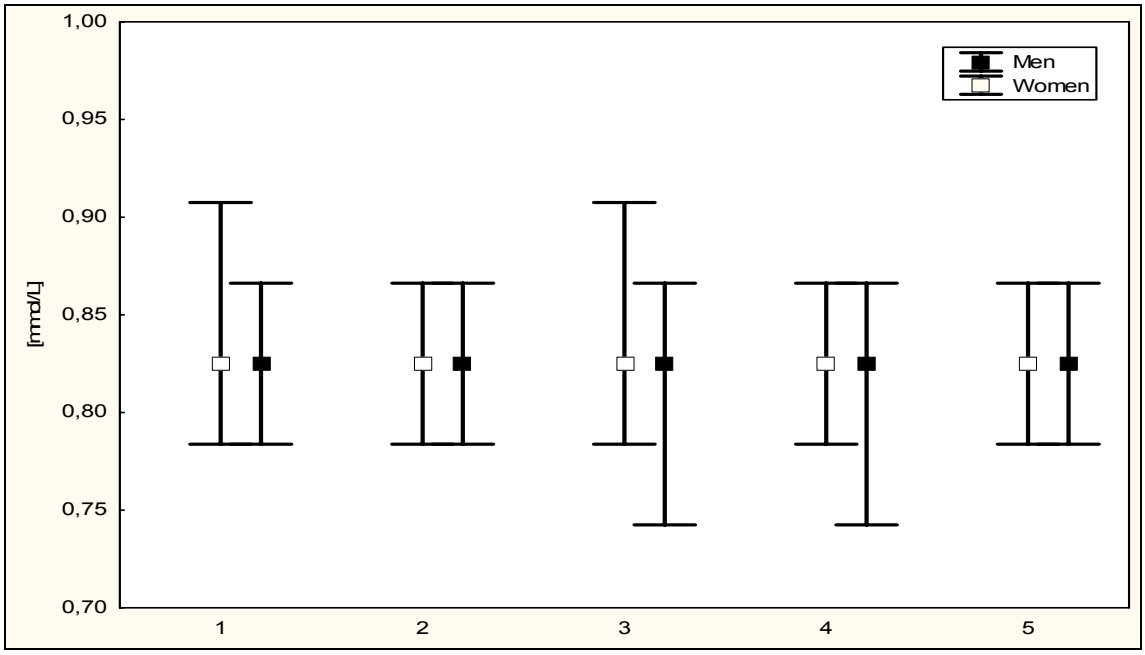


Figure 4-12: Mg^{2+} *concentration in men and women without any significant differences between genders and in course of the study month*

4.3 Breathing frequency

The breathing frequency was different within the groups and during the intervention days. Breathing frequency (BF) of men was significantly lower than the BF of women ($p<0.01$).

Male athletes had a significantly lower BF than the sedentary ones ($p<0.001$), but the BF were stable throughout the five study days (table 4-1 and figure 4-13).

Table 4-1: *Breathing frequency (BF) of men and women*

	Trained men (n=13)	**Untrained men** (n=14)	**Trained women** (n=11)	**Untrained women** (n=11)
BF	10.7 (7.6/15.9)	13.1 (11.3/14.6)	15.4 (10.3/17.2)	12.5 (9.3/15)

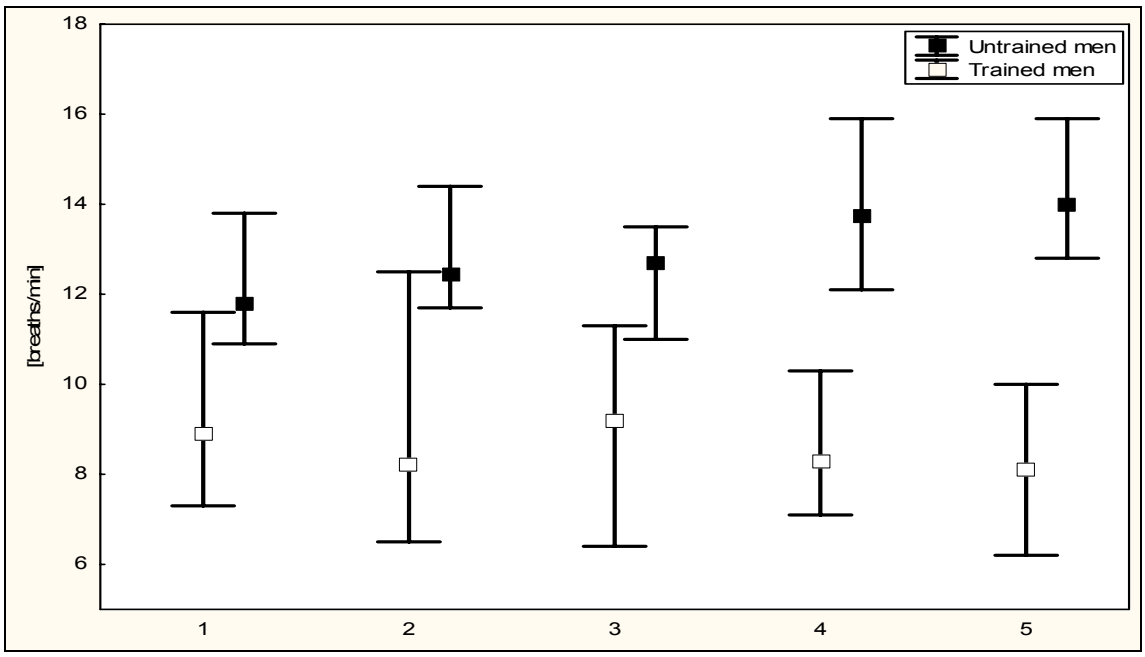


Figure 4-13: *Breathing frequency was significantly lower in trained than in untrained men ($p<0.001$) without any significance in course of the study*

Women showed the opposite; untrained women had significantly lower BF than trained women ($p<0.01$); results are presented in table 4-1. Nevertheless, the BF of trained women reacted different during the menstrual cycle whereas the BF of the sedentary females was stable in course of the study. Table 4-2 illustrates the BF throughout the five menstrual cycle phases in trained and untrained women.

Female athletes had significantly higher BF in MidL ($p<0.05$) and in PreM ($p<0.05$) compared with the M which is demonstrated in the following table and illustrated in figure 4-14.

Table 4-2: *Breathing frequency in course of the menstrual cycle in trained and untrained women*

	Trained women (n=11)	**Untrained women** (n=11)
M	11.0 (8.4/16.6)	11.7 (8.7/14.4)
MidF	13.4 (9.8/16.6)	13.7 (7.9/15.3)
O	15.0 (12.5/16.5)	11.7 (9.9/13.6)
MidL	15.8 (12.4/17.7)	13.5 (11/15.1)
PreM	16.3 (12.3/18.1)	14.3 (9.3/15)
Significance	MidL and PreM vs. M ($p<0.05$)	n.s. in course of the menstrual cycle

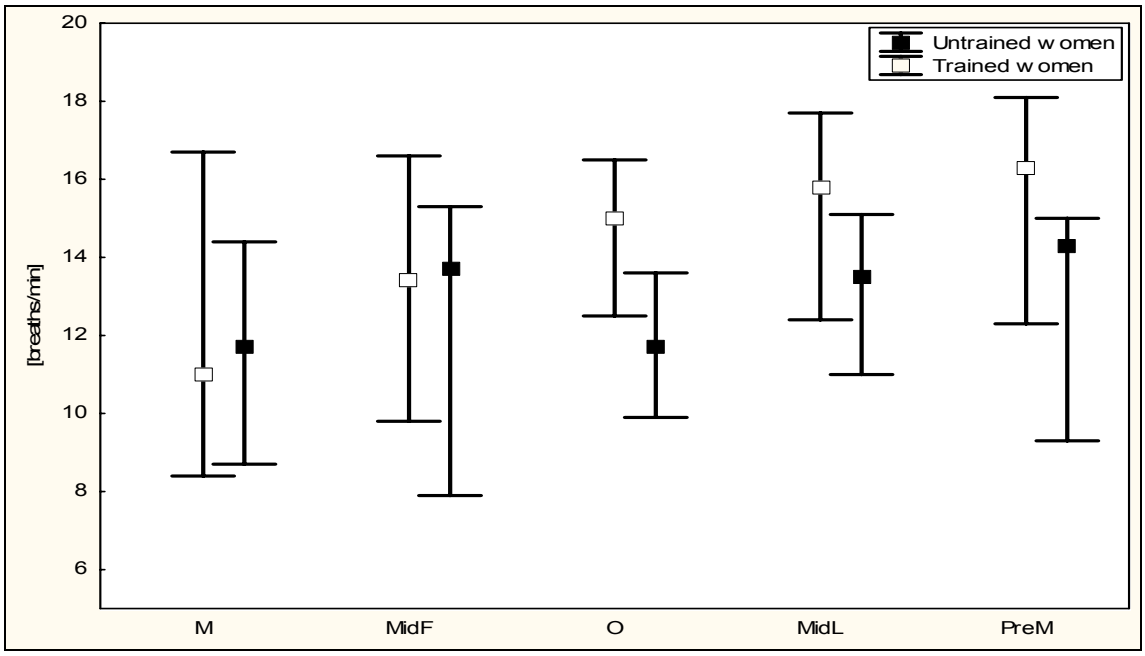


Figure 4-14: *The median of the breathing frequency of untrained women was significantly lower compared with trained women ($p<0.01$); still the athletes showed significantly enhanced BF in MidL ($p<0.05$) a in PreM ($p<0.05$) compared to M whereas the untrained women did not show any significance in course of the menstrual cycle*

4.4 Heart rate variability

The results of the heart rate variability (HRV) recordings are presented in the time and the frequency domain at rest, during the menstrual cycle and during the orthostatic test. Finally, the orthostatic test is presented in the five menstrual cycle phases.

4.4.1 Heart rate variability at rest

The HRV at rest was different in athletes compared with the sedentary subjects. Differences between male and female athletes were noted whereas the sedentary men and women were similar. The results are presented first in the time and second in the frequency domain.

4.4.1.1 Time Domain

The meanNN was significantly higher in men than in women (for athletes $p<0.01$, for sedentary subjects $p<0.001$). Male and female athletes had significantly higher meanNN than the sedentary men and women (for males $p<0.05$, for females $p<0.001$).

The SDNN, RMSSD and pNN50 were significantly higher in trained men and women compared with the untrained subjects (SDNN, RMSSD for men $p<0.01$, pNN50 for men $p<0.05$ and SDNN, RMSSD, pNN50 for women $p<0.01$).

The results (median, $1^{st}/3^{rd}$ quartile) are also presented in details in table 4-3 for men and in table 4-4 for women.

Table 4-3: *Time domain parameters of trained and untrained men with its significance level (p-value)*

	Trained men (n=13)	**Untrained men** (n=14)	**p-value**
meanNN [ms]	1076.0 (814.1/1947.3)	1035.0 (614.1/1289.6)	p<0.05
SDNN [ms]	83.4 (32.8/237.1)	67.6 (28.2/160)	p<0.01
RMSSD [ms]	72.4 (23.1/256.1)	49.4 (14.9/173.9)	p<0.01
pNN50 [%]	42.3 (2.5/81.8)	31.3 (0/78.9)	p<0.05

Table 4-4: *Time domain parameters of trained and untrained women with its significance level (p-value)*

	Trained women (n=11)	**Untrained women** (n=11)	**p-value**
meanNN [ms]	1052.0 (791.2/1884.3)	953.8 (716.7/1129.7)	p<0.001
SDNN [ms]	86.3 (37.6/232.8)	71.0 (21.6/133.4)	p<0.01
RMSSD [ms]	79.8 (22.5/263.5)	54.6 (10.1/137.6)	p<0.01
pNN50 [%]	50.2 (1.8/76.7)	33.9 (0/74.9)	p<0.01

4.4.1.2 Frequency Domain

The TP and LF power were significantly higher in trained compared with the sedentary subjects (for men p<0.01-0.001, for women p<0.05).

The VLF was significantly higher in male athletes compared with untrained men (p<0.01) whereas trained and untrained women were similar.

The absolute HF power was similar (n.s.) in the athletic and the sedentary groups.

Table 4-5 presents the detailed results of the male and table 4-6 of the female group.

Table 4-5: *Frequency domain parameters of trained and untrained men with its significance level (p-value)*

	Trained men (n=13)	**Untrained men** (n=14)	**p-value**
TP [ms²]	5564.3 (770.3/43882.9)	3496.1 (758/20648.4)	p<0.01
VLF [ms²]	1533.5 (107.3/13548.4)	959.3 (187.1/11693)	p<0.01
LF [ms²]	2064.6 (297.7/27244)	1127.4 (144.5/9024.5)	p<0.001
HF [ms²]	913.1 (41.9/8668.5)	687.5 (68.7/6531.8)	n.s.

Table 4-6: *Frequency domain parameters of trained and untrained women with its significance level (p-value)*

	Trained women (n=11)	**Untrained women** (n=11)	**p-value**
TP [ms²]	5758.8 (785.7/44507.7)	3827.3 (357.5/14957.5)	p<0.05
VLF [ms²]	1200.3 (236.6/9635.9)	1067.6 (81.2/5199.3)	n.s.
LF [ms²]	1803.0 (137.8/25196.3)	1099.0 (89.4/10284.8)	p<0.05
HF [ms²]	1209.2 (102.9/15458.3)	928.9 (37.7/4487.3)	n.s.

The LFnu, HFnu as well as the LF/HF ratio were significantly higher in trained compared with untrained men (p<0.01). Still, no difference was noted in LFnu, HFnu as well as LF/HF ratio between trained and untrained females.

Male athletes had significantly higher LFnu and LF/HF ratio than female athletes (p<0.05) whereas trained women had significantly increased HFnu compared to trained men (p<0.05).

Table 4-7 and 4-8 present these results in detail.

Table 4-7: *LFnu, HFnu and LF/HF ratio in trained and untrained men and its significance level (p-value)*

	Trained men (n=13)	**Untrained men** (n=14)	**p-value**
LFnu	71.7 (16.2/97.7)	59.2 (19.7/91)	p<0.01
HFnu	28.3 (2.3/83.8)	40.8 (9/80.3)	p<0.01
LF/HF ratio	2.5 (0.2/43)	1.5 (0.2/10.1)	p<0.01

Table 4-8: *LFnu, HFnu and LF/HF ratio in trained and untrained women*

	Trained women (n=11)	**Untrained women** (n=11)	**p-value**
LFnu	57.6 (20.6/92.8)	53.5 (20/91)	n.s.
HFnu	42.4 (8/79.4)	46.5 (9/80)	n.s.
LF/HF ratio	1.4 (0.3/11.4)	1.2 (0.3/10.1)	n.s.

4.4.2 Heart rate variability during the menstrual cycle

The results of the heart rate variability (HRV) in relation to the menstrual cycle were compared between trained and untrained women. Data are presented in five different menstruation phases, which were individually specified for each woman. The focus of interest was the HRV in course of one menstrual cycle in two different female groups (athletes and sedentary women) whereas only the course of the HRV in five phases is considered.

4.4.2.1 Time domain

No difference was found in the HRV of the time domain between trained and untrained women in course of one menstrual cycle. Both female groups reacted similarly throughout the five menstruation phases and no significance was noted in the time domain parameters as presented in the following figures (figure 4-15 to 4-19).

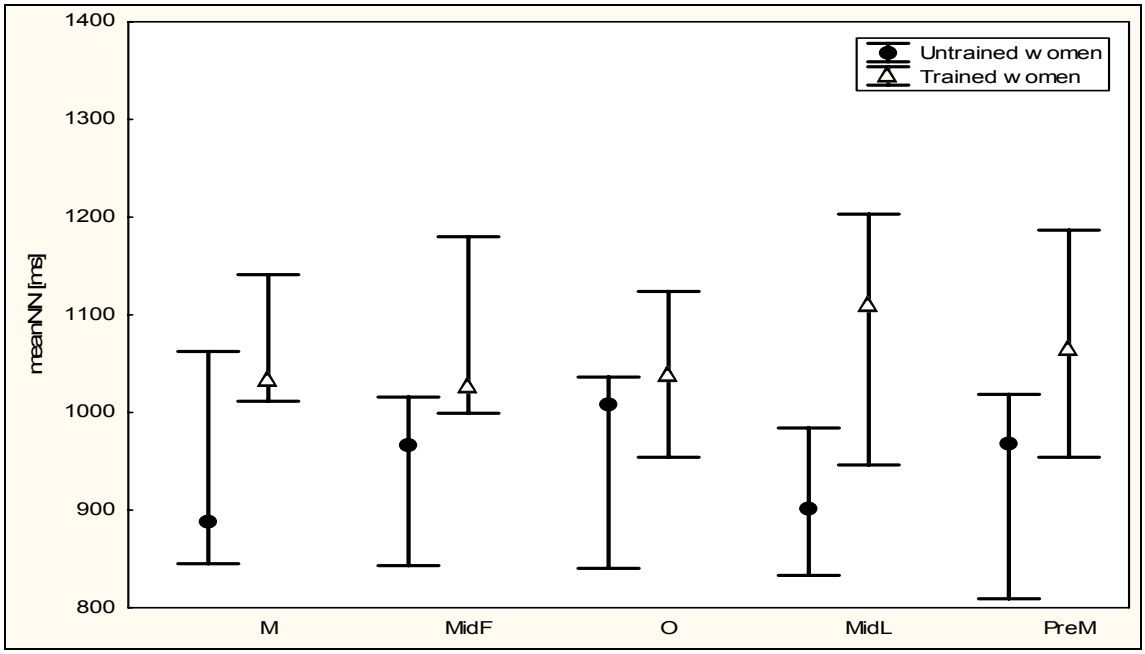


Figure 4-15: *The course of the meanNN during the menstrual cycle was similar in both groups; no significance was noted*

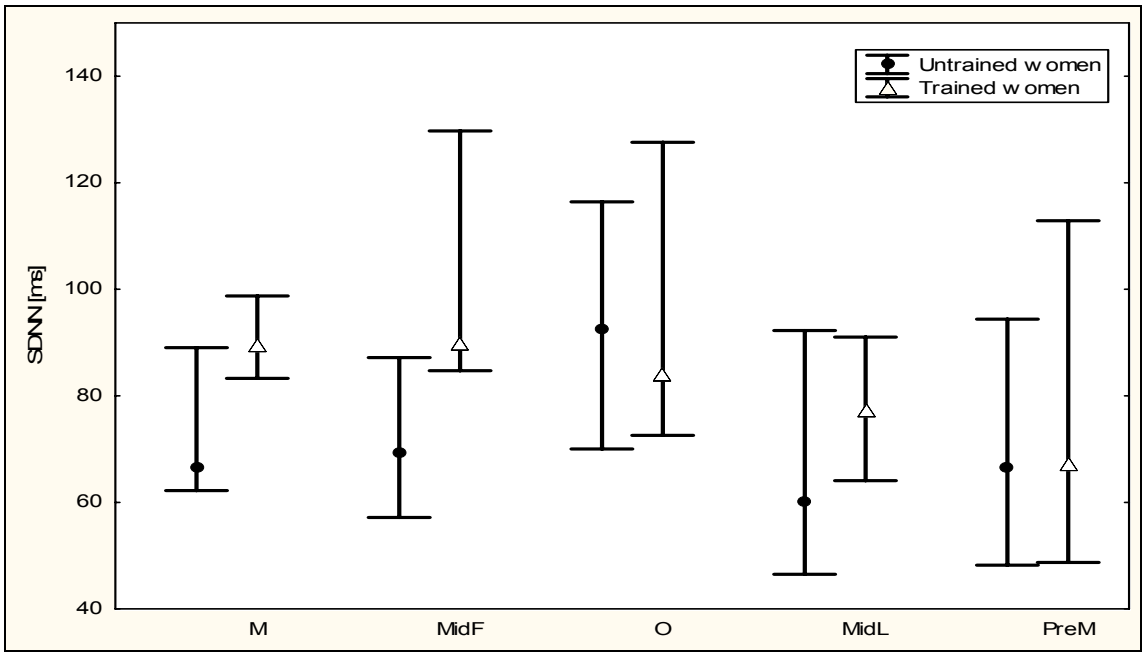


Figure 4-16: *The course of the SDNN was not significantly different between athletic and sedentary females during the five menstruation phases*

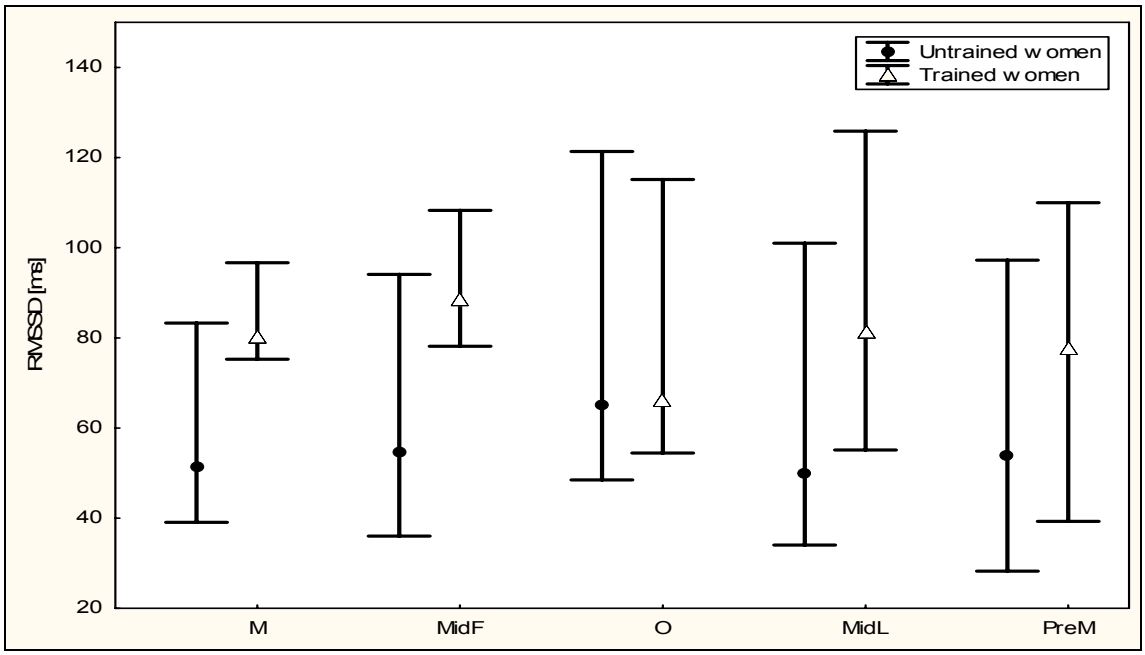


Figure 4-17: *The course of the RMSSD was similar in both groups; no significance was noted*

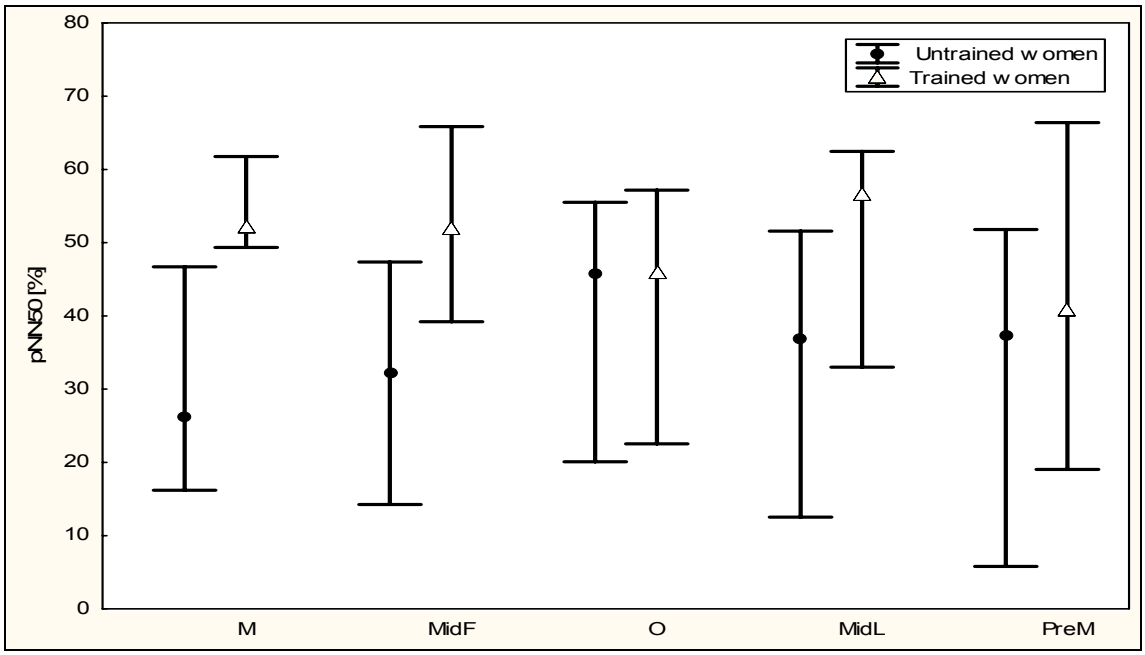


Figure 4-18: *The course of pNN50 was not significantly different in trained and untrained women during the menstrual cycle*

4.4.2.2 Frequency domain

There was no significant difference in the HRV of the frequency domain between trained and untrained women in course of the menstrual cycle. The course of the single parameters was the same in both female groups. Thus, athletic and sedentary women reacted similar throughout the five menstruation phases as presented in the figures 4-19 to 4-25.

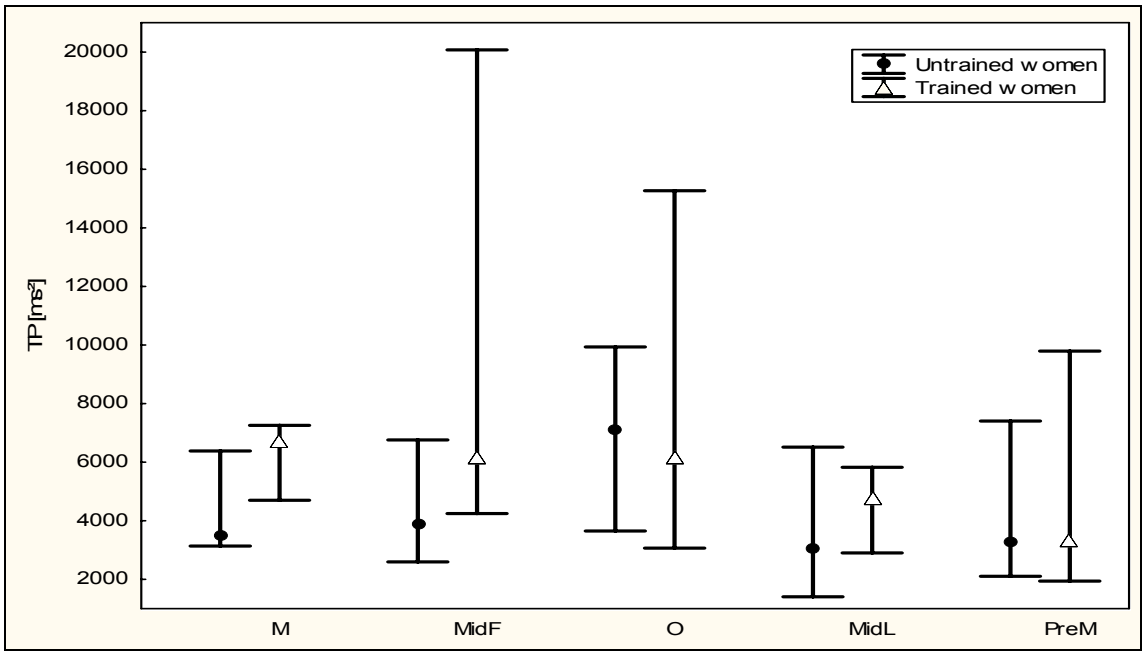


Figure 4-19: *The course of the TP was not significantly different in trained and untrained women during the menstrual cycle*

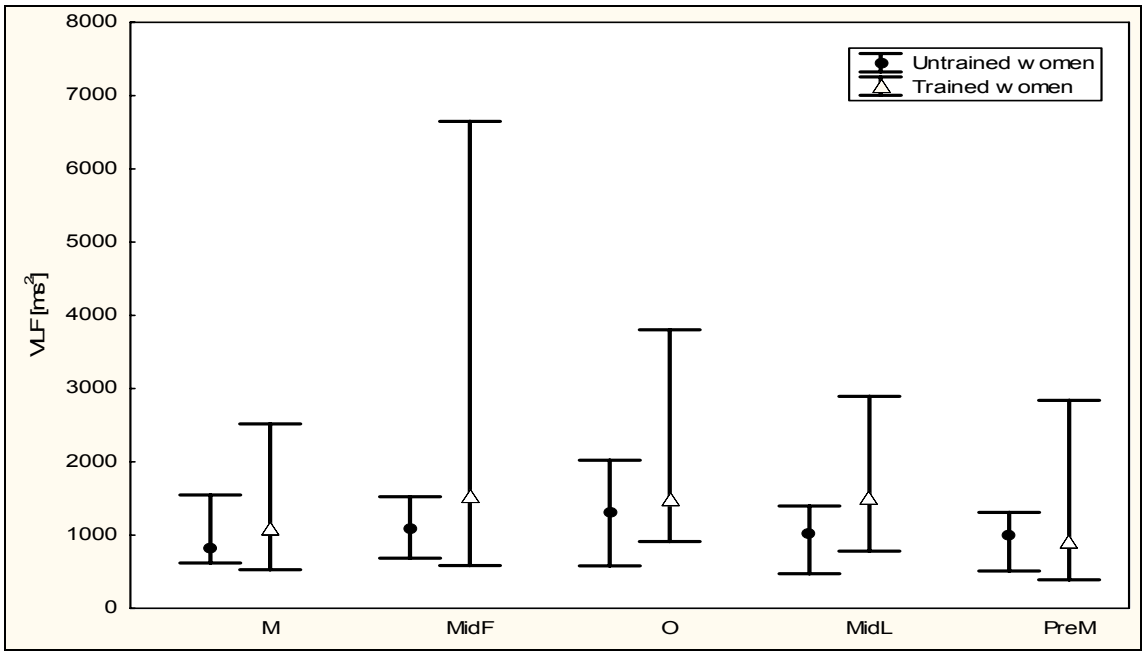


Figure 4-20: *The course of the VLF was similar in both groups during the five menstruation phases*

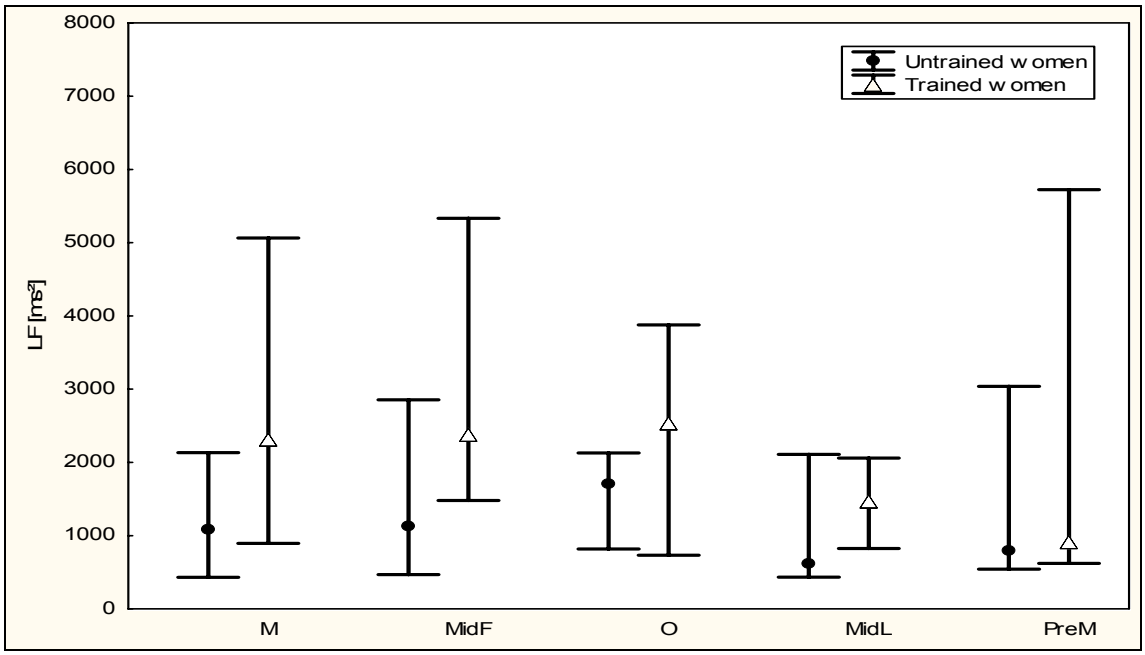


Figure 4-21: *The course of the LF power was the same in both groups; no significance was noted during one menstrual cycle*

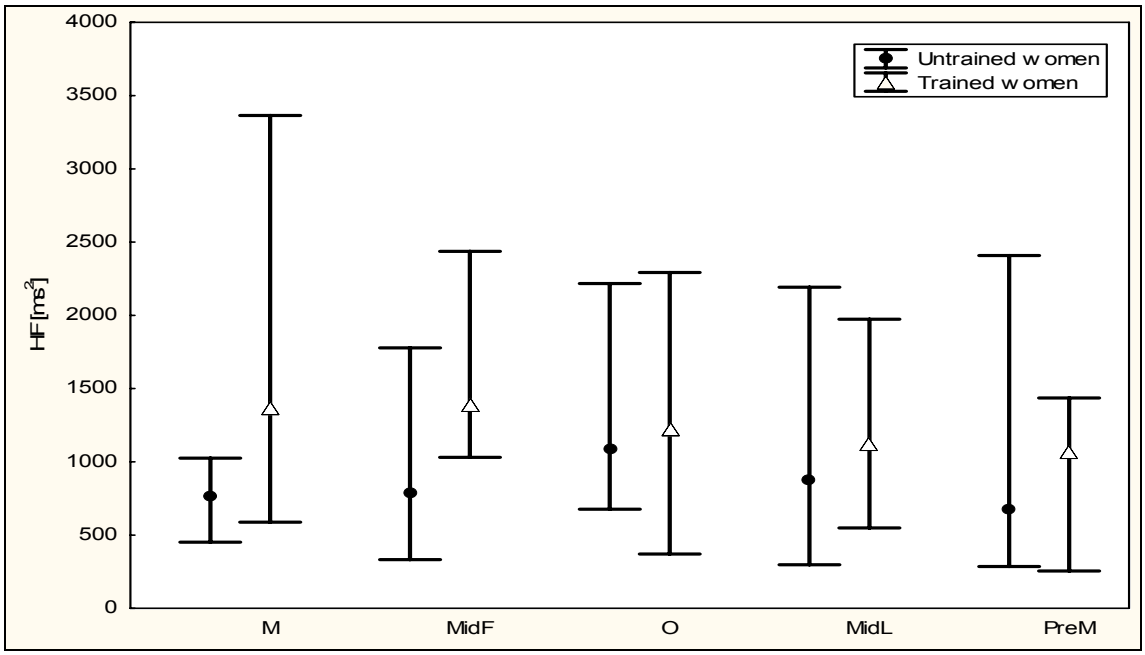


Figure 4-22: *The course of the HF power was not significantly different in athletic and sedentary women throughout the five menstruation phases*

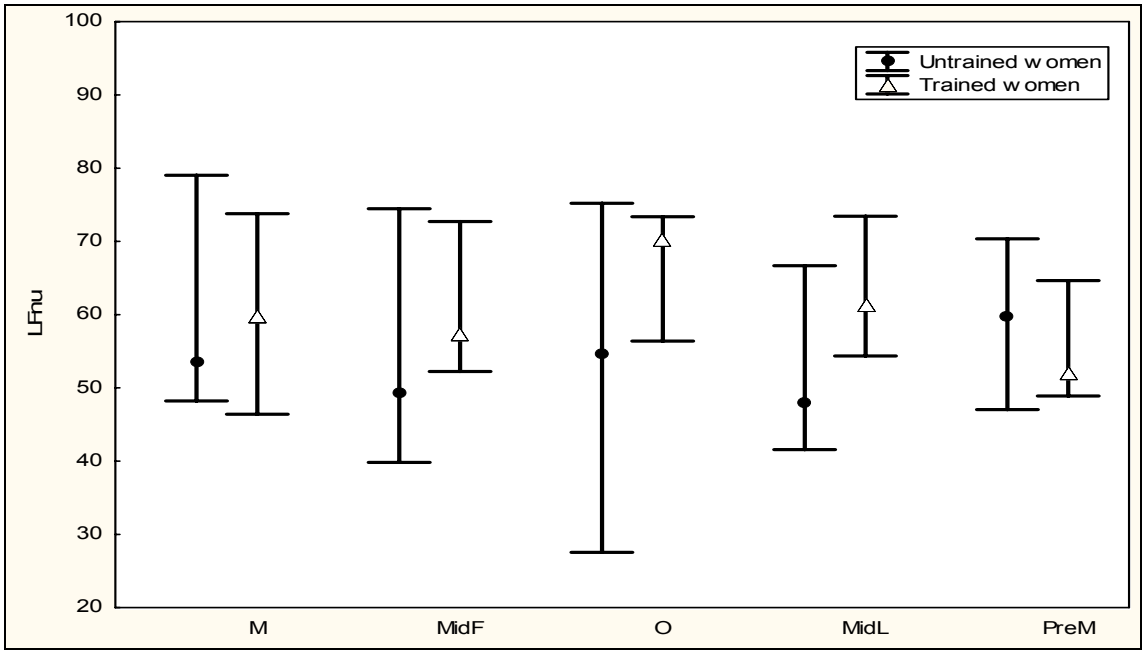


Figure 4-23: *The course of the LFnu was similar in both female groups; no significance was noted during one menstrual cycle*

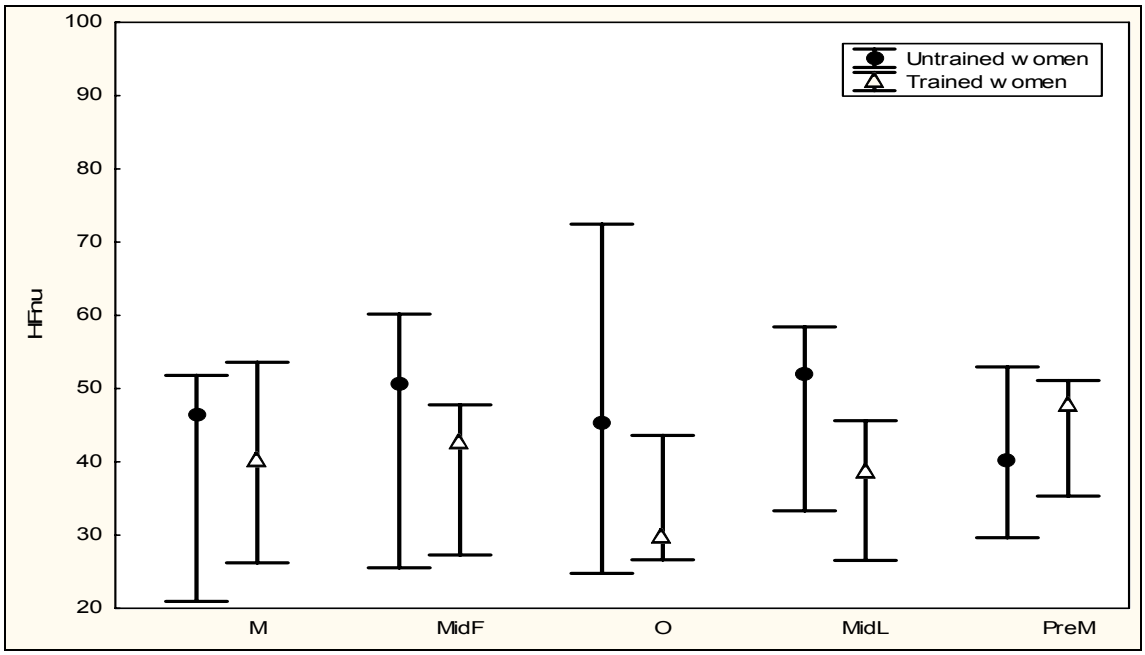


Figure 4-24: *The course of the HFnu was not significantly different in trained and untrained women during the menstrual cycle*

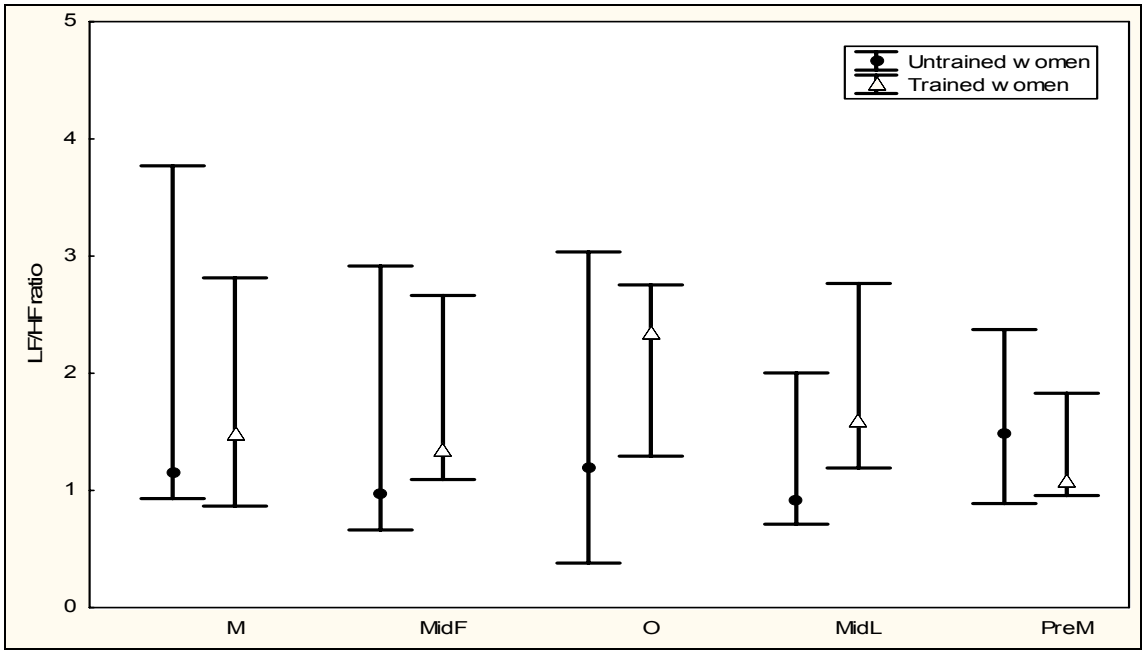


Figure 4-25: *The course of the LF/HF ratio was similar in athletic and sedentary females; no significance was noted during the five menstruation phases*

4.4.3 Heart rate variability during the orthostatic test

Differences in the provocation of the orthostatic test induced by an active body position change were investigated in the four subgroups of all study days. Therefore trained and untrained men and women were compared in the HRV parameters of the time and the frequency domain in five orthostatic tests during the study.

4.4.3.1 Time Domain

The HRV parameters of the time domain reacted similar in all groups. No significance was noted between genders and between trained and untrained subjects. In supine position every single HRV parameter was increased compared with standing whereas while standing the opposite was noted. Still the five orthostatic tests provoked the same reaction in all groups as presented in the following figures (figure 4-26 to 4-30).

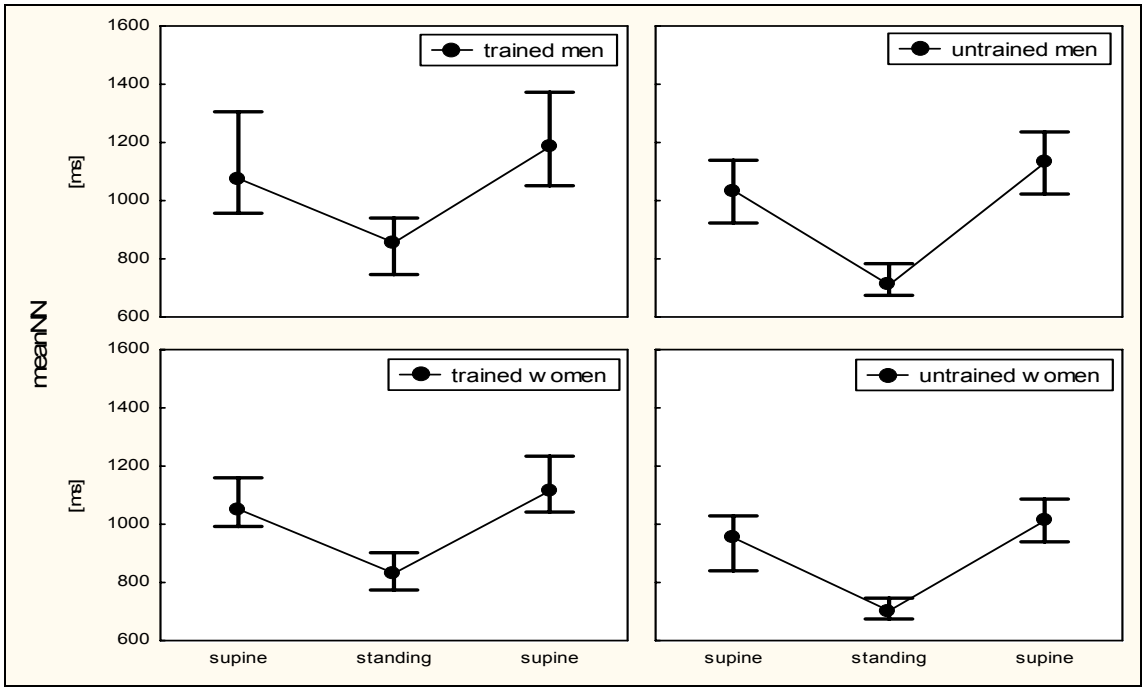


Figure 4-26: *MeanNN during the orthostatic tests in trained and untrained men and women; no significance was noted between the groups*

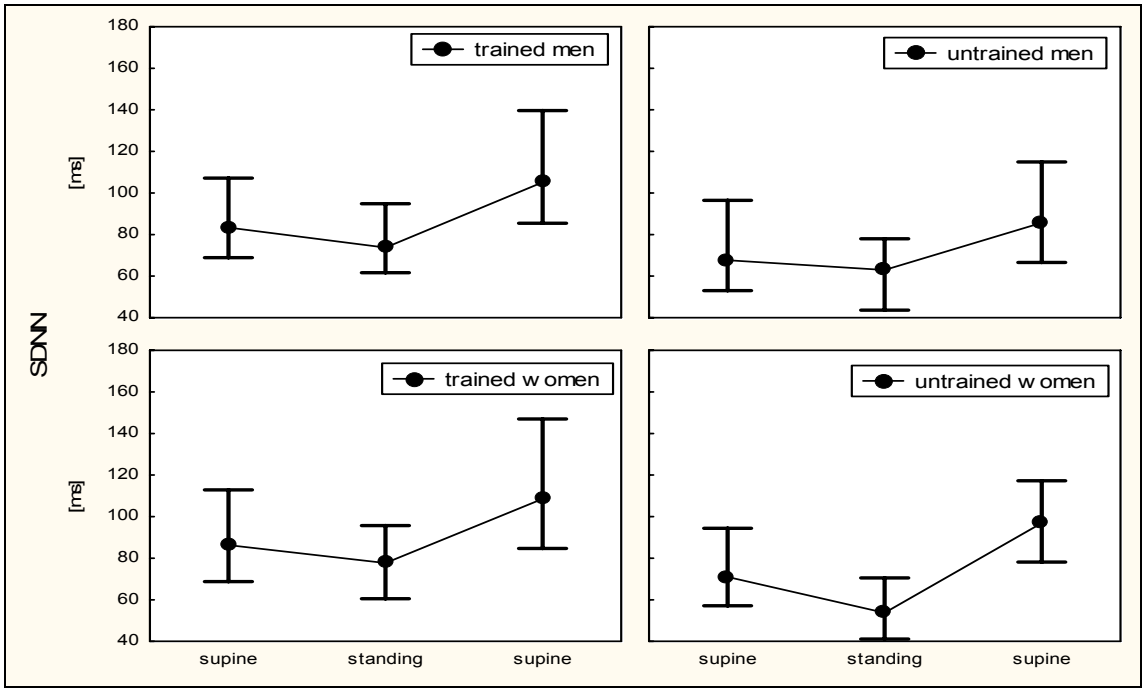


Figure 4-27: *SDNN during the orthostatic tests was not significantly different in athletic and sedentary men and women*

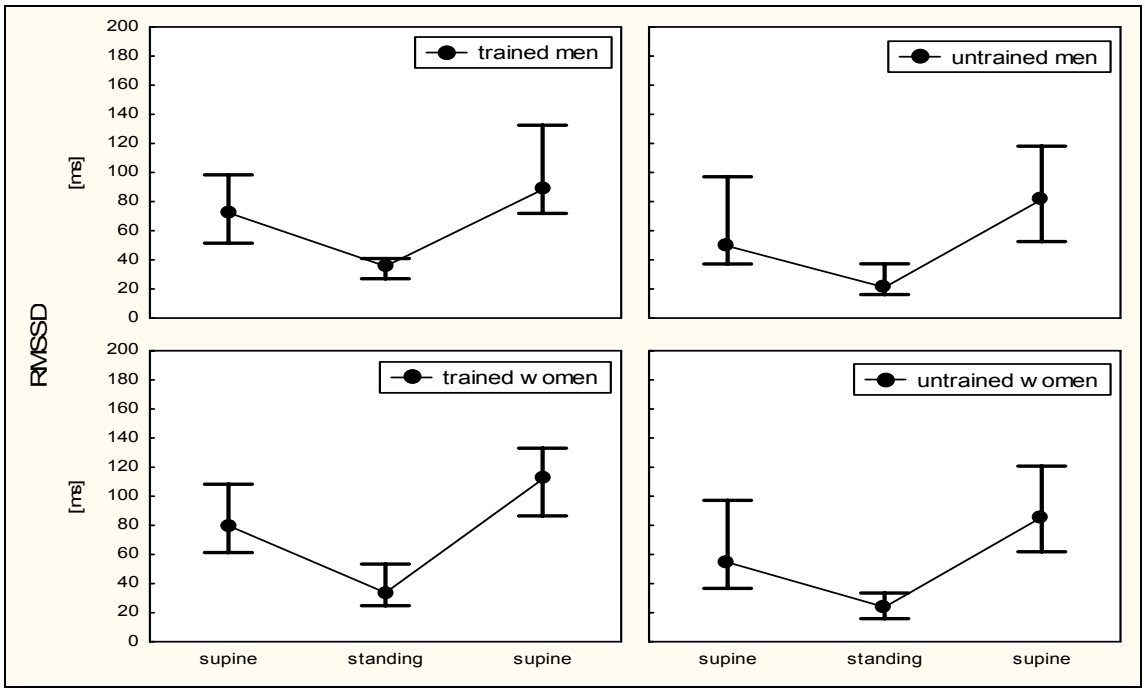


Figure 4-28: *RMSSD during the orthostatic tests was similar in all groups; no significance was noted*

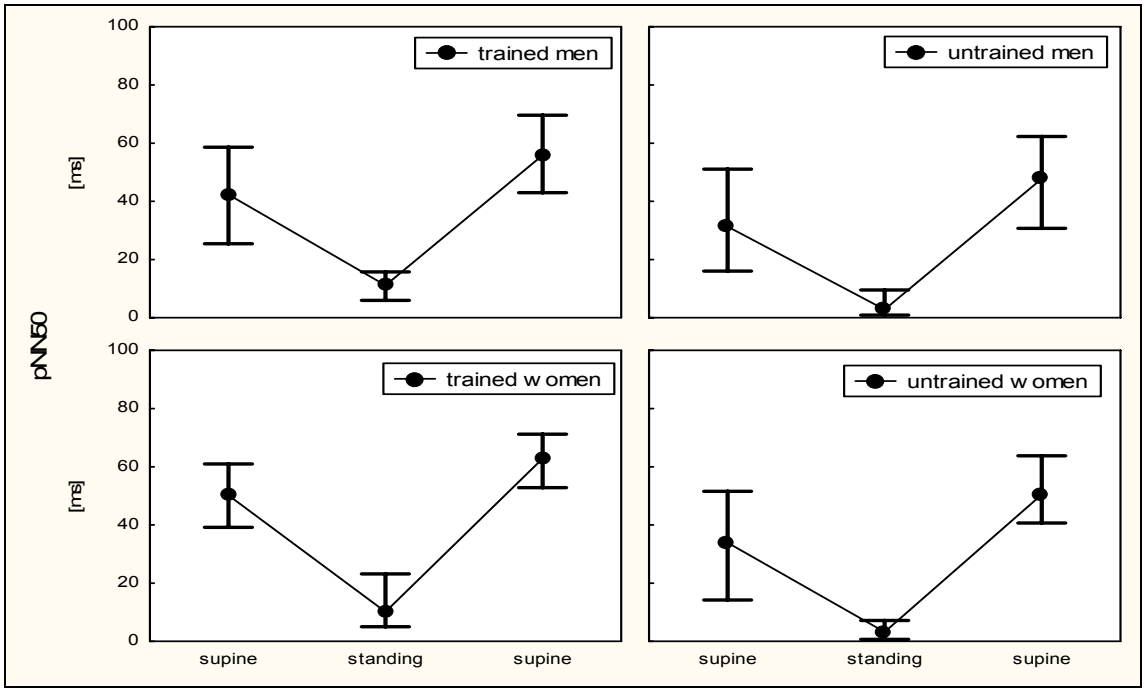


Figure 4-29: *pNN50 during the orthostatic tests in trained and untrained men and women without any significant difference between the groups*

4.4.3.2 Frequency Domain

The HRV parameter of the frequency domain reacted different between trained and untrained men and women during the orthostatic tests. Thereby male and female athletes were compared as well as sedentary men and women. Different results were noted between the groups which are described in details below the corresponding figures (figures 4-30 to 4-36).

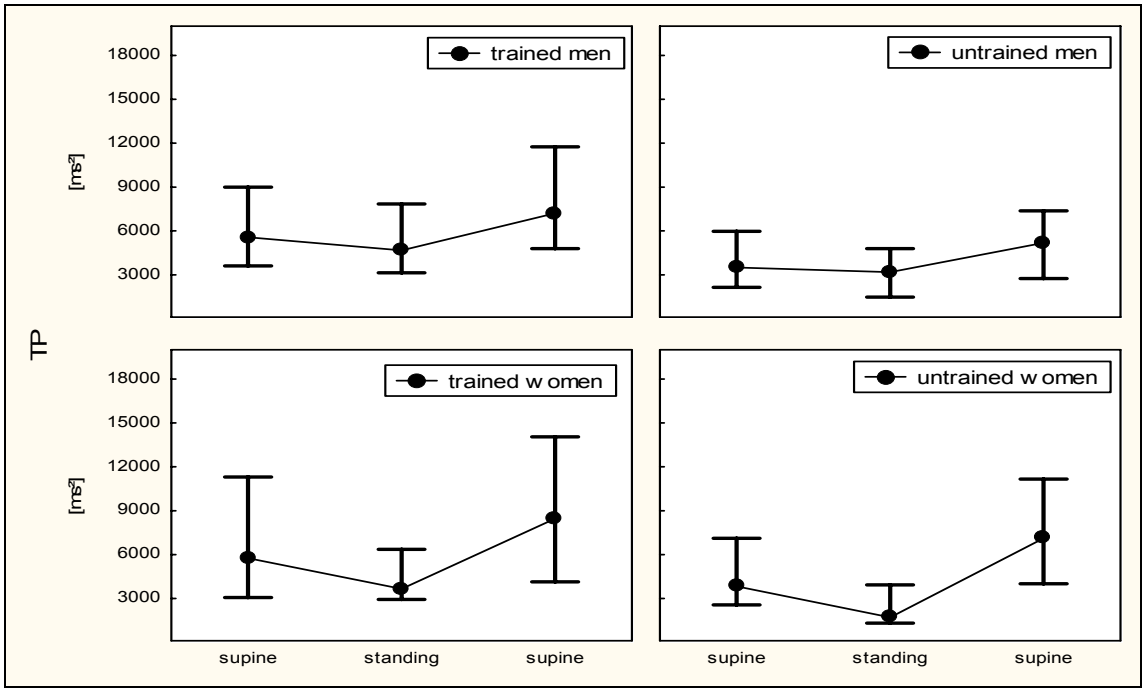


Figure 4-30: *The TP during the orthostatic tests decreased while standing and increased in supine position in all groups whereas the TP of trained and untrained women decreased and increased more significantly compared with men (for athletes $p<0.05$, for sedentary subjects $p<0.01$)*

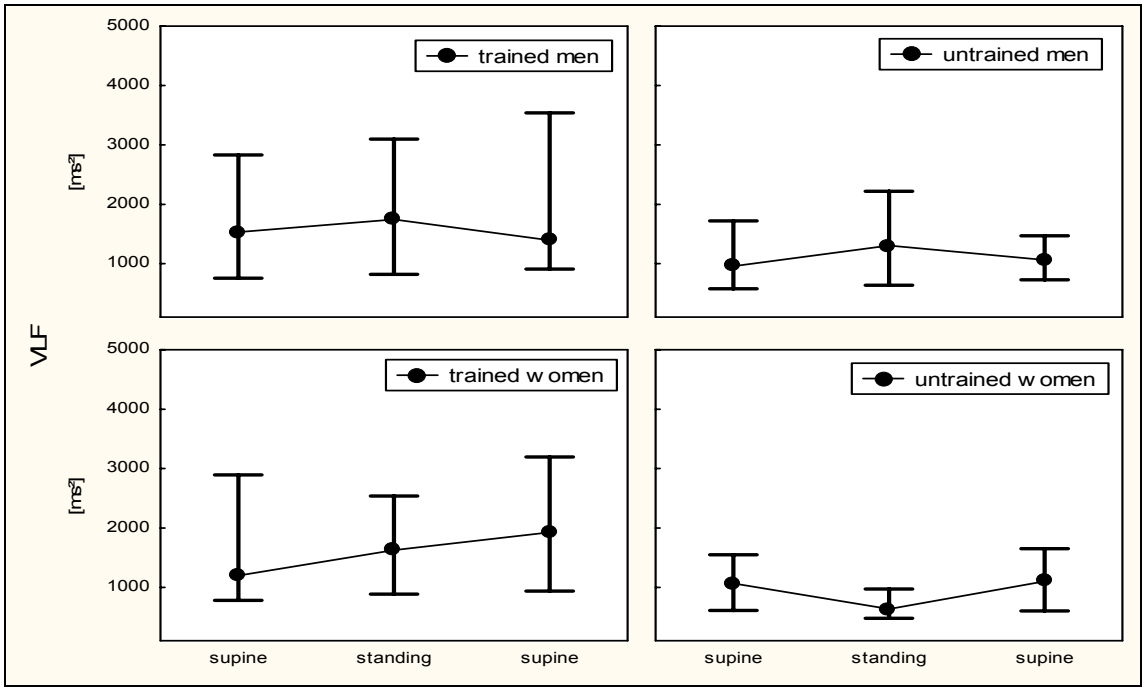


***Figure 4-31**: The VLF was not significantly different between male and female athletes whereas the VLF reacted opposite in the sedentary group; while standing the VLF increased in untrained men and decreased in women which resulted in a significantly different course of VLF ($p<0.05$)*

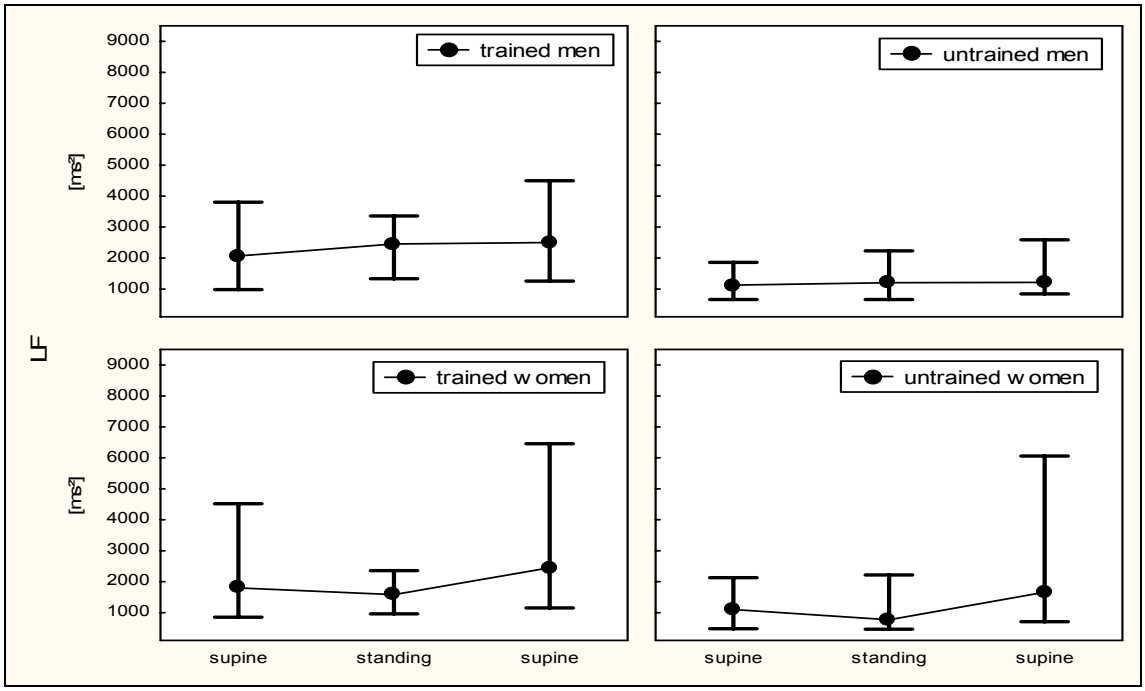


***Figure 4-32:** The LF power was increased while standing and remained enhanced in supine position in athletic and sedentary men whereas the LF power of women decreased while standing and increased in supine position; thus the LF power was significantly different in athletes ($p<0.001$) and in sedentary subjects ($p<0.001$).*

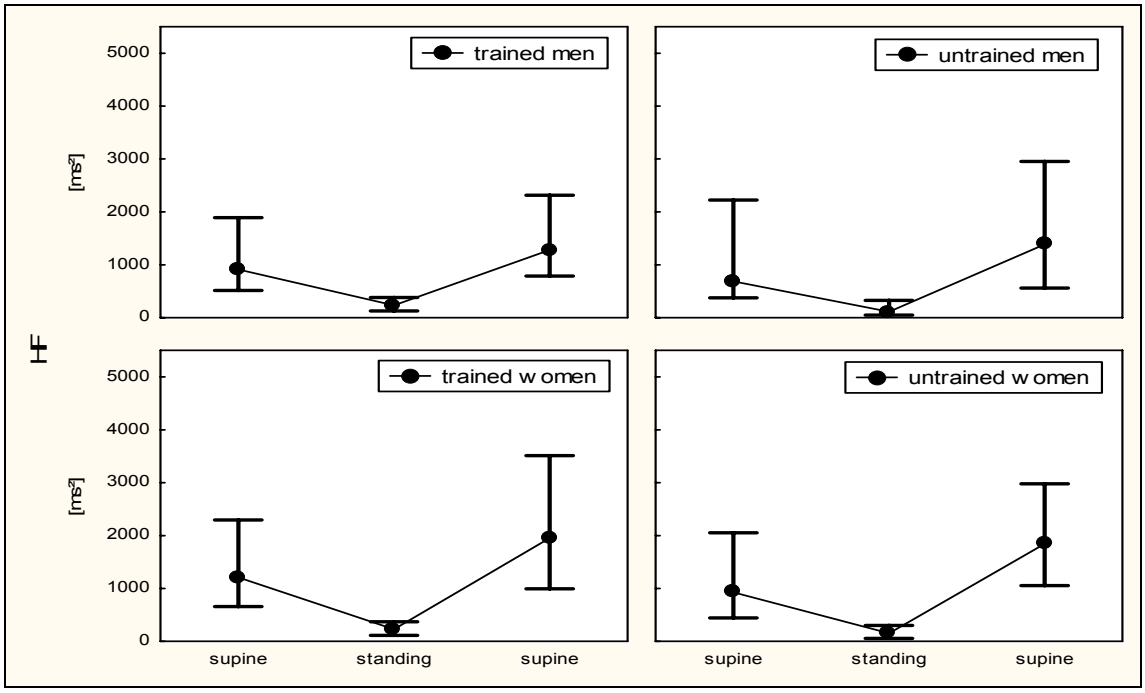


Figure 4-33: *The HF power was similar in all groups with a decrease while standing and an increase in supine position; no significant difference was noted*

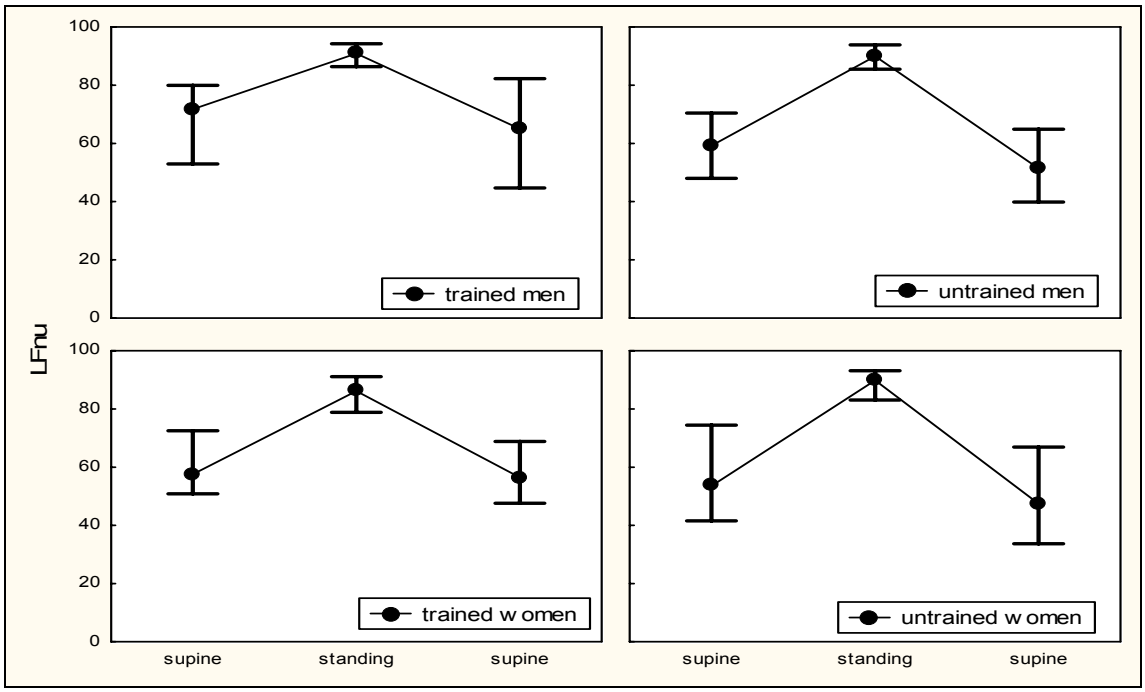


Figure 4-34: *The LFnu was not significantly different in athletes and sedentary men and women; while standing the LFnu increased and decreased in supine position*

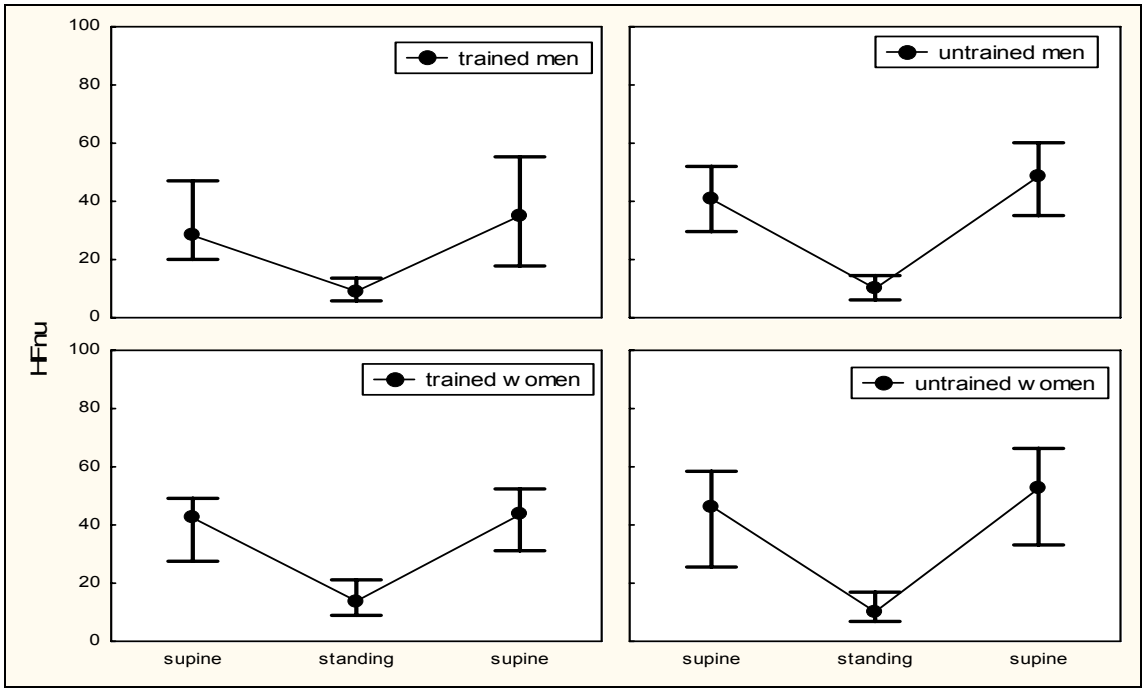


Figure 4-35: *The HFnu reacted in the opposite way and was increased in supine position and decreased while standing in all groups; no significant difference was noted*

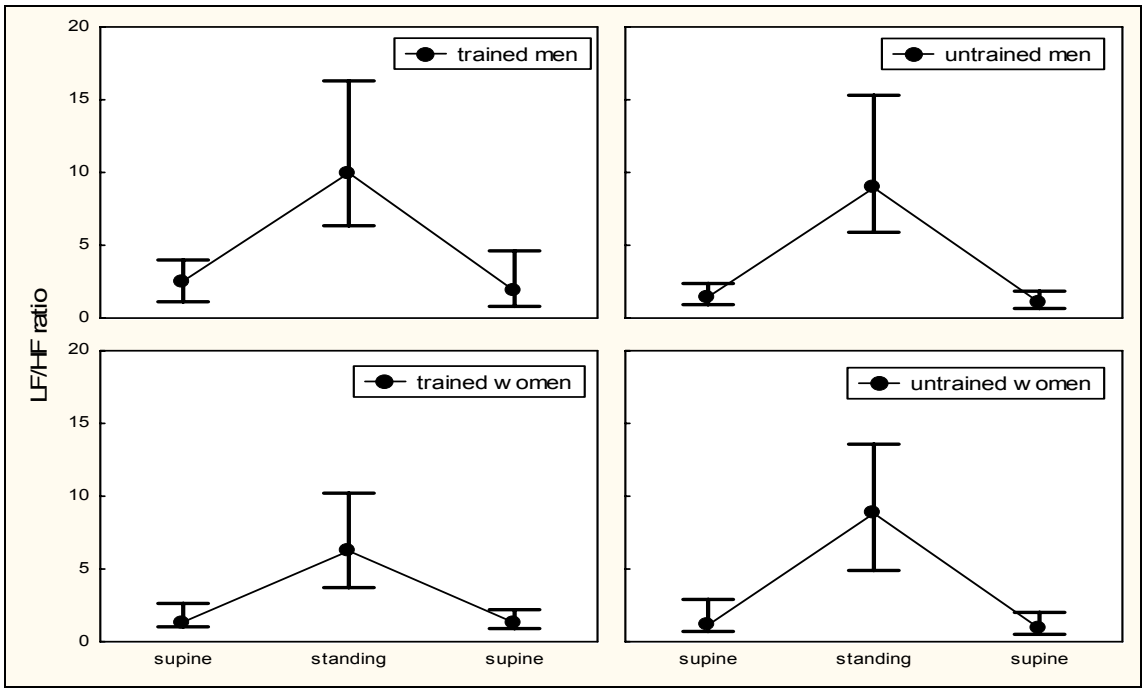


Figure 4-36: *The LF/HF ratio decreased while standing and increased in supine position whereas the male athletes had a significantly higher increase and decrease compared with the female athletes ($p<0.05$); the sedentary groups were not significantly different*

4.4.4 Orthostatic test during the menstrual cycle

The orthostatic test was done five times during the study in women. The main interest was the effect of the menstrual cycle on the orthostatic provocation in women, which was investigated in five different menstrual cycle phases. No difference was noted in the time and frequency domain of the HRV in trained and untrained women. The orthostatic provocation reacted similarly five times during the course of one menstrual cycle. No significance was noted between the orthostatic provocation in athletic and sedentary females. Thereby, the HRV results of the time and frequency domain are presented in one female group.

4.4.4.1 Time domain

In the time domain the HRV parameters reacted similarly throughout the menstrual cycle phases which are illustrated by the following figures (figure 4-37 to 4-40). No significance was noted between the five phases in women.

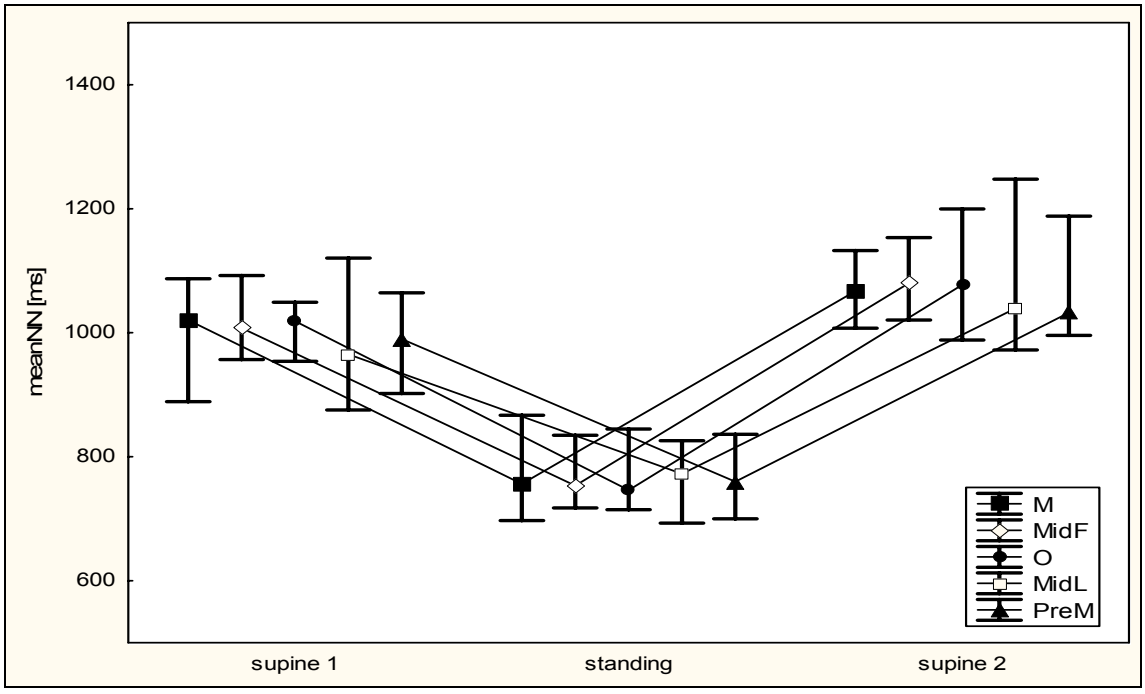


Figure 4-37: *The meanNN during the orthostatic test was similar five times; no significant difference was noted during the menstrual cycle in women*

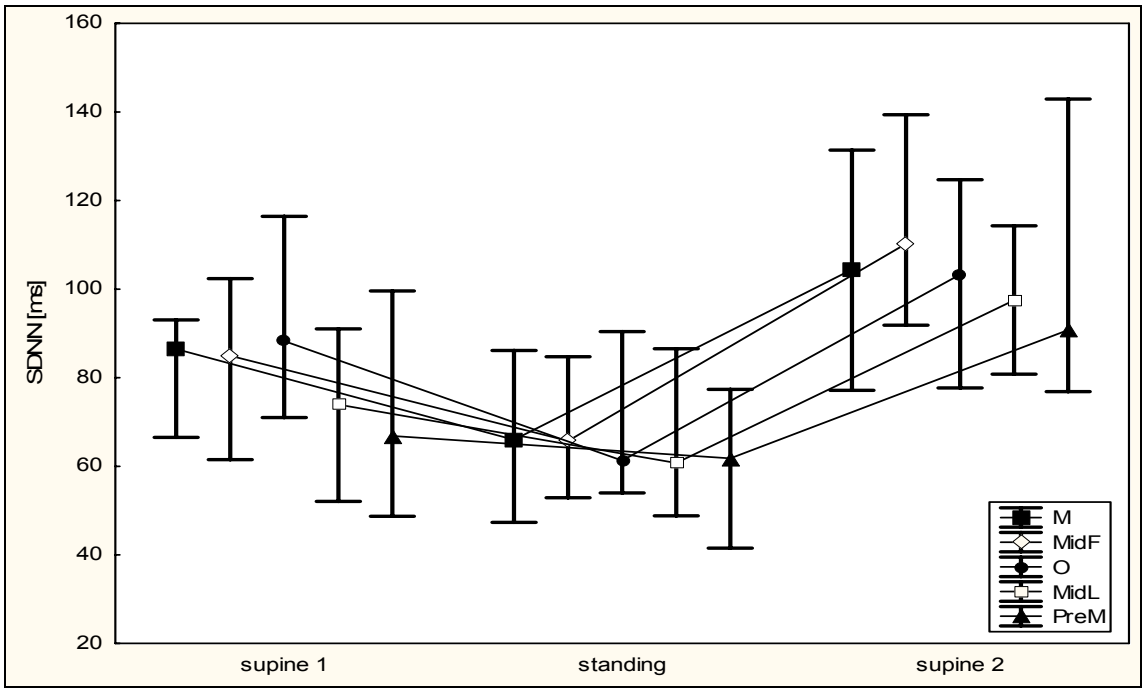


Figure 4-38: *The SDNN during the orthostatic test was not significantly different in course of the menstrual cycle in women*

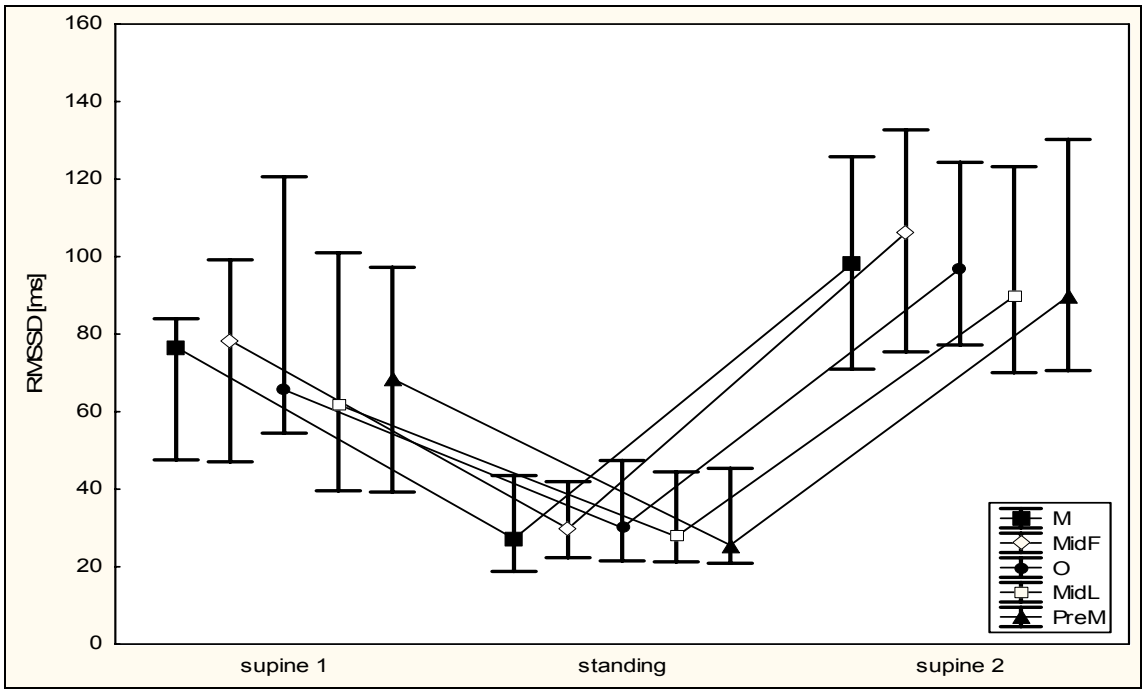


Figure 4-39: *The RMSSD during the orthostatic test reacted similarly during the five menstrual cycle phases in women; no significant difference was noted*

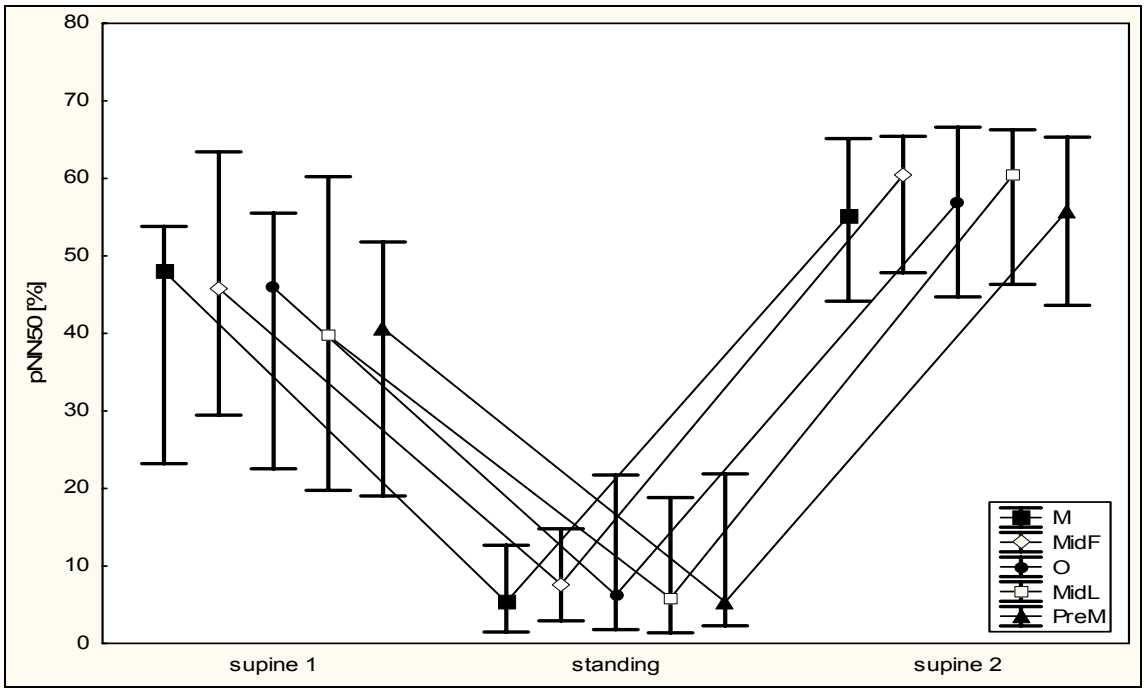


Figure 4-40: *The pNN50 was not significantly different throughout the five menstrual cycle phases in women*

4.4.4.2 Frequency domain

The HRV parameters of the frequency domain were not significantly different in the five orthostatic tests. Throughout one menstrual cycle, the results of the orthostatic test were similar in women. The orthostatic provocation of each HRV parameters in course of the menstrual cycle is presented in the following figures (figure 4-41 to 4-47).

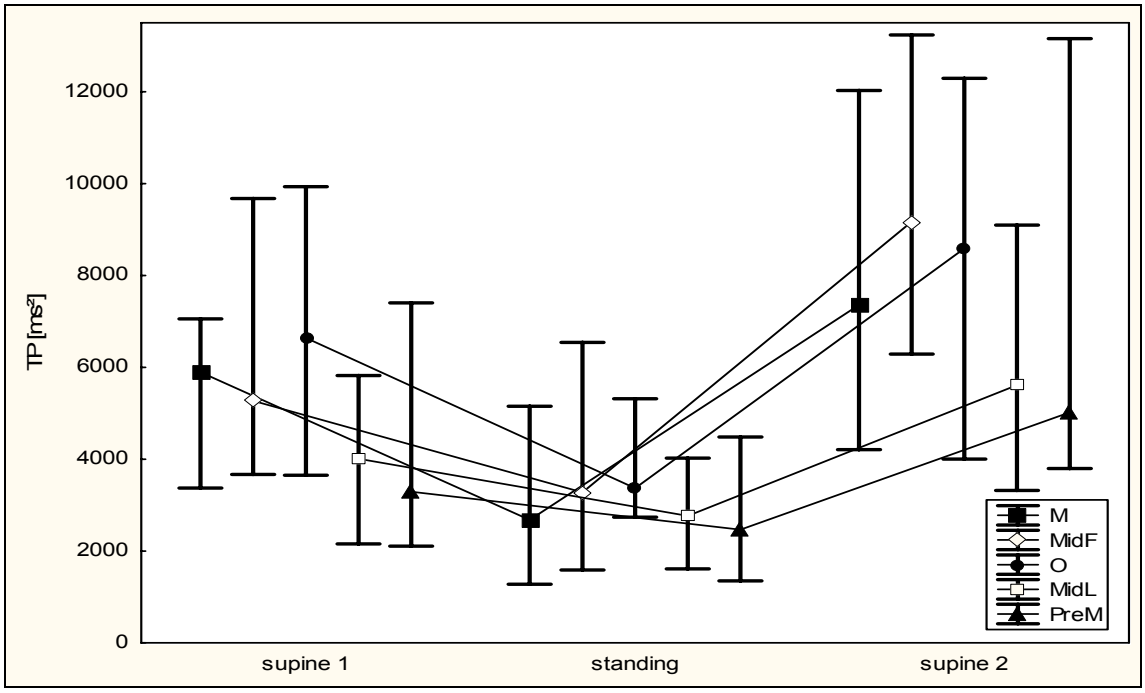


Figure 4-41: *The TP during the orthostatic test was similar five times in women; no significant difference was noted*

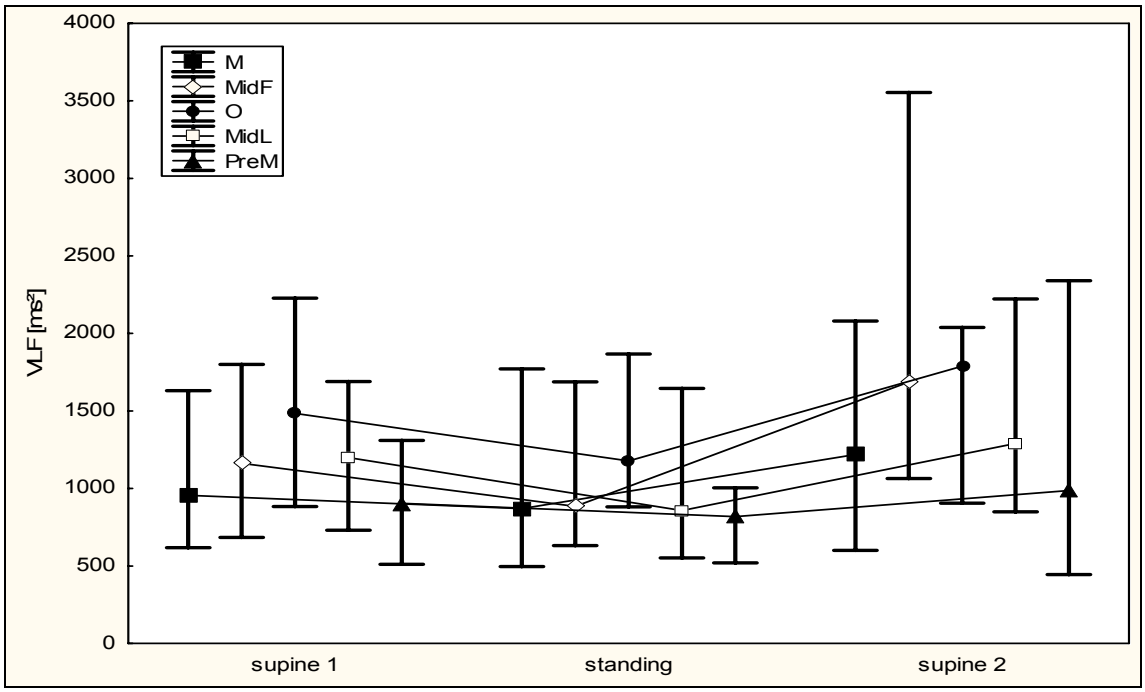


Figure 4-42: *The VLF during the orthostatic test was not significantly different throughout one menstrual cycle in women*

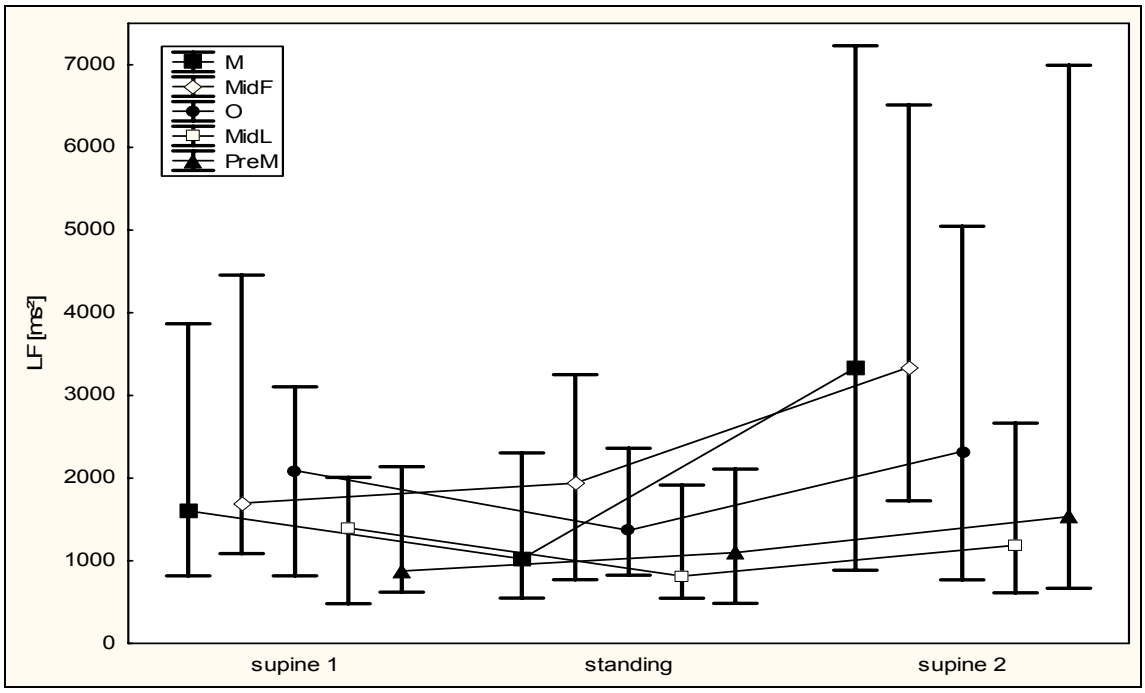


Figure 4-43: *The LF power during the orthostatic test was similar in all menstrual cycle phases; the results were not significantly different between the five days*

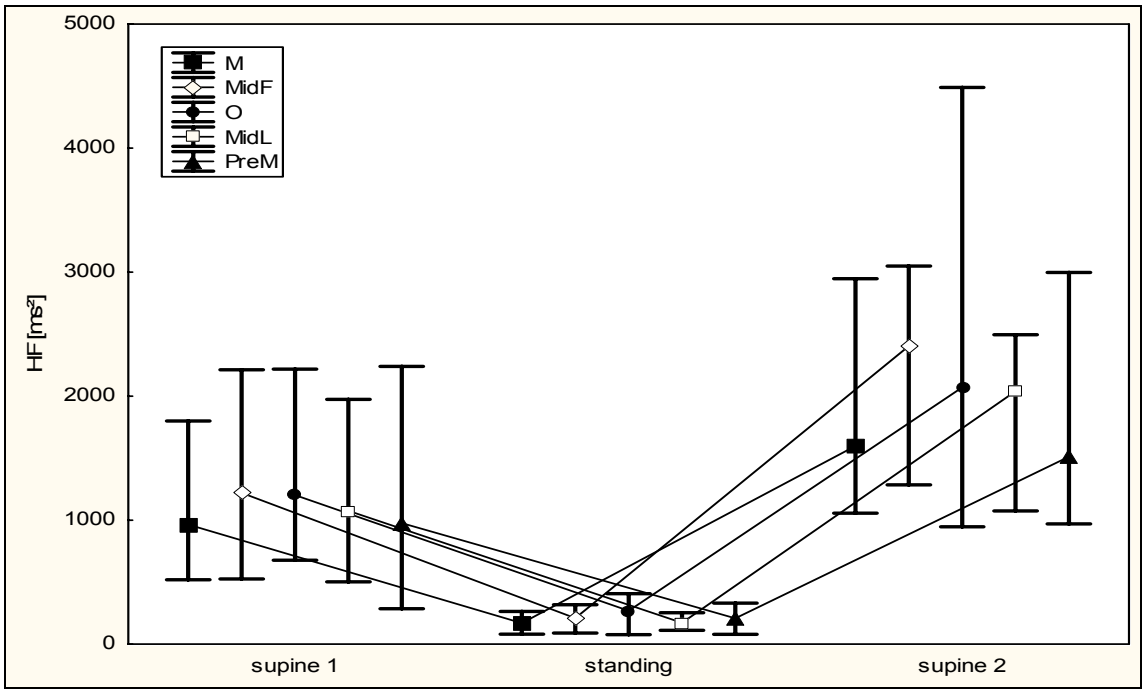


Figure 4-44: *The HF power during the orthostatic test was not significantly different in five menstruation phases in women*

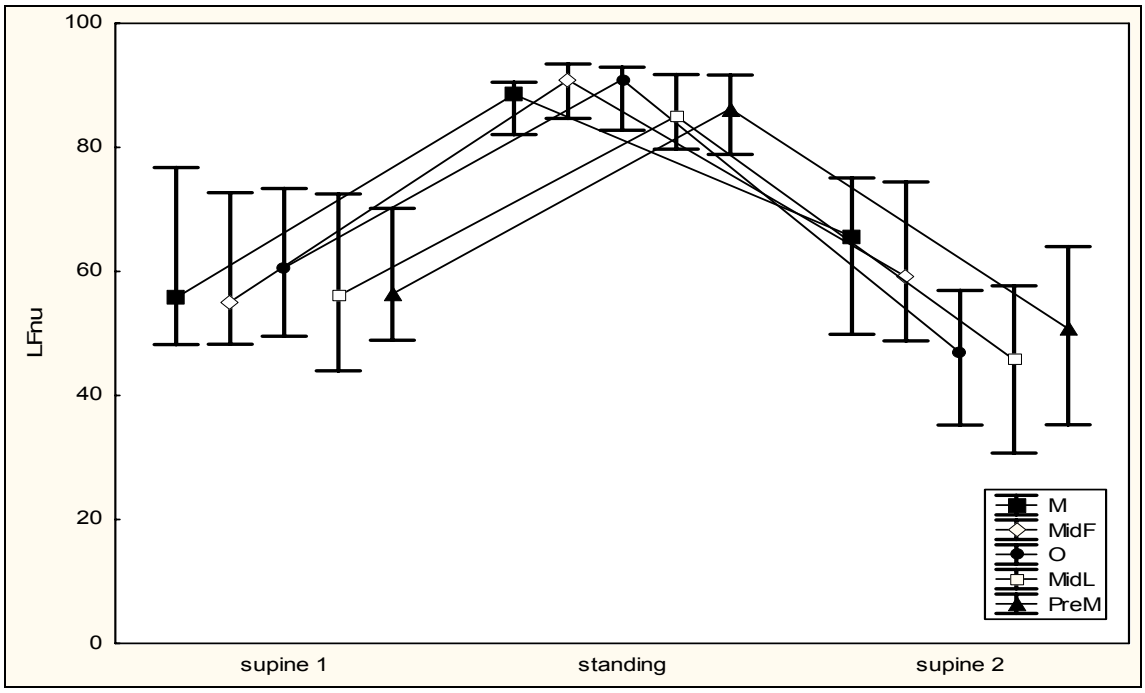


Figure 4-45: *The LFnu during the orthostatic test was similar in course of the menstrual cycle; no significant difference was noted between the five study days in women*

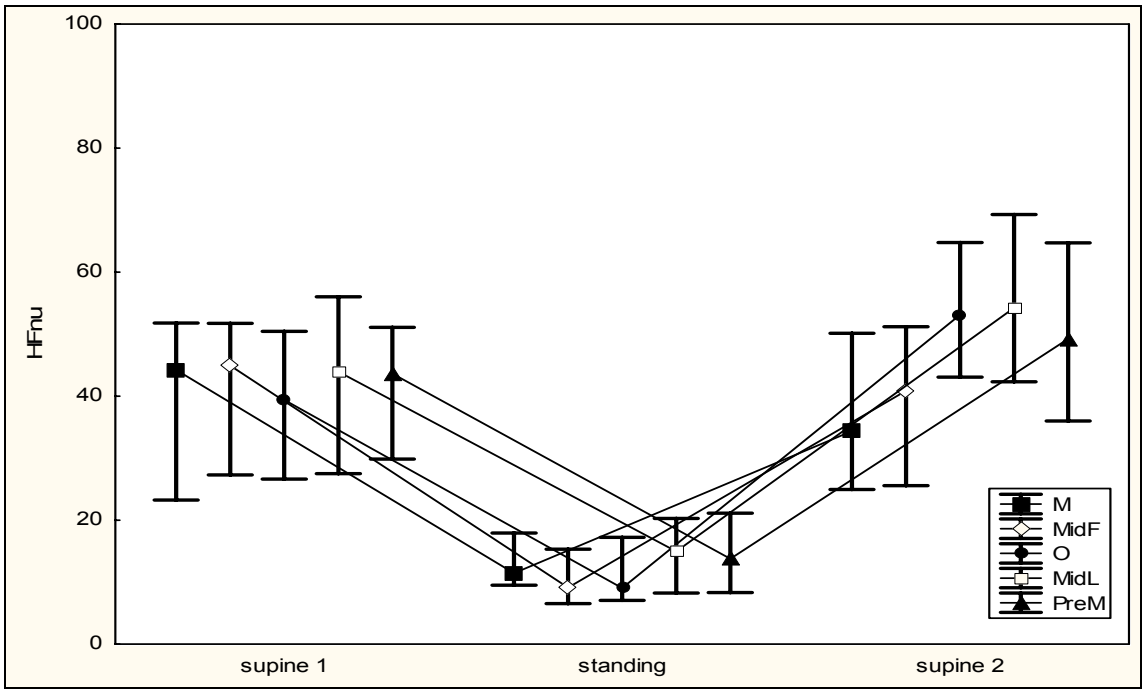


Figure 4-46: *The HFnu during the orthostatic test was not significantly different throughout one menstrual cycle in women*

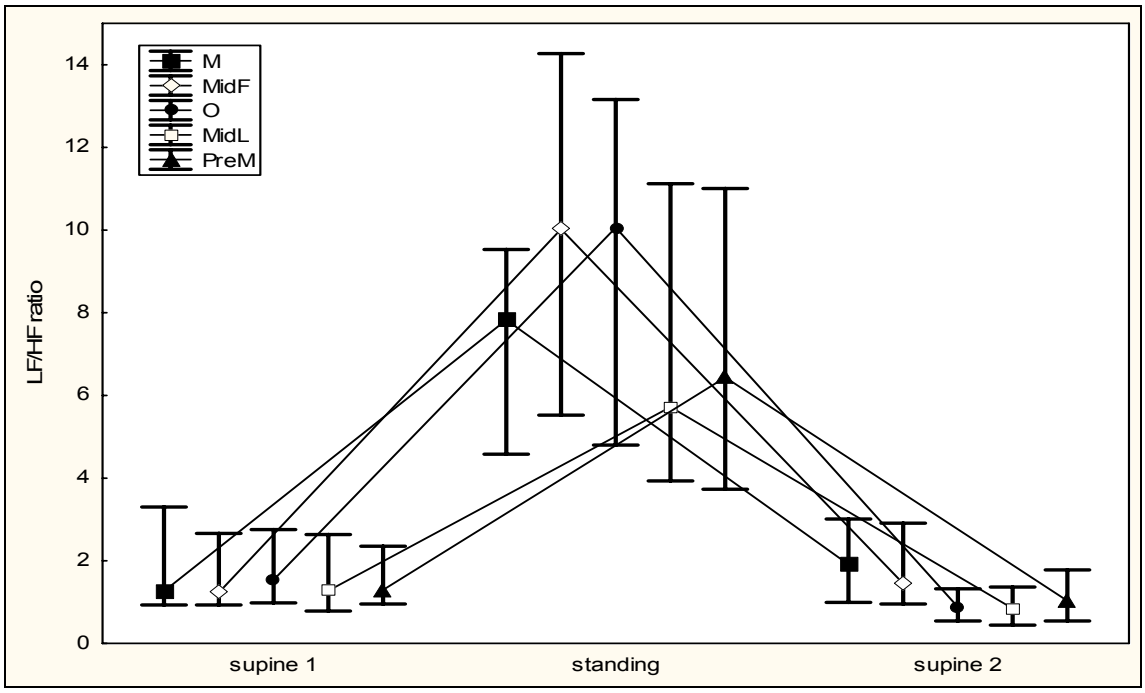


Figure 4-47: *The LF/HF ratio during the orthostatic test was not significantly different in five study days in course of the menstrual cycle in women*

4.5 Regression and correlation

No correlation or regression could be found between any parameter and the HRV parameters.

The POMS, the blood borne parameters, the ergometric data and the respiration did not correlate with any of the parameters of the HRV; also no regression was found. No menstrual cycle phase correlated with the parameters of the HRV and no regression was noted. Additionally no difference was found between men and women.

Finally no correlation and regression could be found during the orthostatic provocation in trained and untrained men and women.

4.6 Reliability

The reliability of the repeated measurements has been controlled by the intragroup correlation coefficient (ICC), the coefficient of variation (CV) and additionally by Bland-Altman plots. The ICC is presented by the mean, the minimum and the maximum in the following table (table 4-9) whereas the CV evaluated in percentage can be noted in table 4-10. The more detailed results of the ICC can be found in the appendix. No universal standards exist in classifying the ICC in the HRV measurements. In accordance to Lee et al. the ICC was considered poor if ICC<0.40, acceptable if ICC ranged from 0.41-0.60, good if ICC ranged between 0.61-0.80 and excellent if ICC>0.81.

The Bland-Altman plots illustrated a dispersion of the meanNN, the SDNN, the RMSSD and the pNN50 within ±2 standard deviations. Nevertheless the dispersions of the frequency domain parameters were not acceptable considering the Bland-Altman plots due to the unacceptable limits of agreements (mean ± 2 standard deviations) which are not related to the original scale of measurement. Exemplary two Bland-Altman plots illustrate an acceptable and an unacceptable dispersion of the HRV parameters (figure 4-48 and 4-49).

Table 4-9: *Intergroup correlations coefficient (ICC) of trained and untrained men as well as of all men*

ICC	Untrained men (n=14)	Trained men (n=13)	All men (n=27)
meanNN	0.88 (0.96-0.71)	0.88 (0.94-0.76)	0.89 (0.94-0.83)
SDNN	0.71 (0.86-0.44)	0.72 (0.82-0.56)	0.74 (0.81-0.60)
RMSSD	0.82 (0.92-0.59)	0.78 (0.90-0.63)	0.81 (0.92-0.59)
pNN50	0.85 (0.95-0.75)	0.83 (0.91-0.78)	0.84 (0.95-0.75)
TP	0.57 (0.85-0.16)	0.59 (0.79-0.39)	0.58 (0.85-0.16)
VLF	0.31 (0.79-0.00)	0.52 (0.79-0.26)	0.41 (0.79-0.00)
LF power	0.53 (0.83-0.13)	0.40 (0.72-0.15)	0.47 (0.82-0.13)
HF power	0.71 (0.85-0.50)	0.63 (0.86-0.28)	0.68 (0.86-0.28)
LFnu	0.74 (0.86-0.66)	0.62 (0.71-0.33)	0.69 (0.86-0.33)
HFnu	0.74 (0.86-0.66)	0.62 (0.71-0.33)	0.69 (0.86-0.33)
LF/HF ratio	0.80 (0.91-0.64)	0.50 (0.77-0.23)	0.67 (0.91-0.23)

Table 4-10: *Coefficient of variation (CV) of trained and untrained as well as of all men*

CV [%]	Untrained men (n=14)	Trained men (n=13)	All men (n=27)
meanNN	8.1	7.8	8.0
SDNN	21.9	20.8	21.4
RMSSD	26.5	26.3	26.4
pNN50	45.4	31.0	38.5
TP	44.6	49.2	46.8
VLF	56.3	72.9	64.3
LF power	48.3	57.7	52.8
HF power	51.6	57.5	54.5
LFnu	22.0	24.1	23.0
HFnu	34.2	49.0	41.3
LF/HF ratio	49.8	76.3	62.6

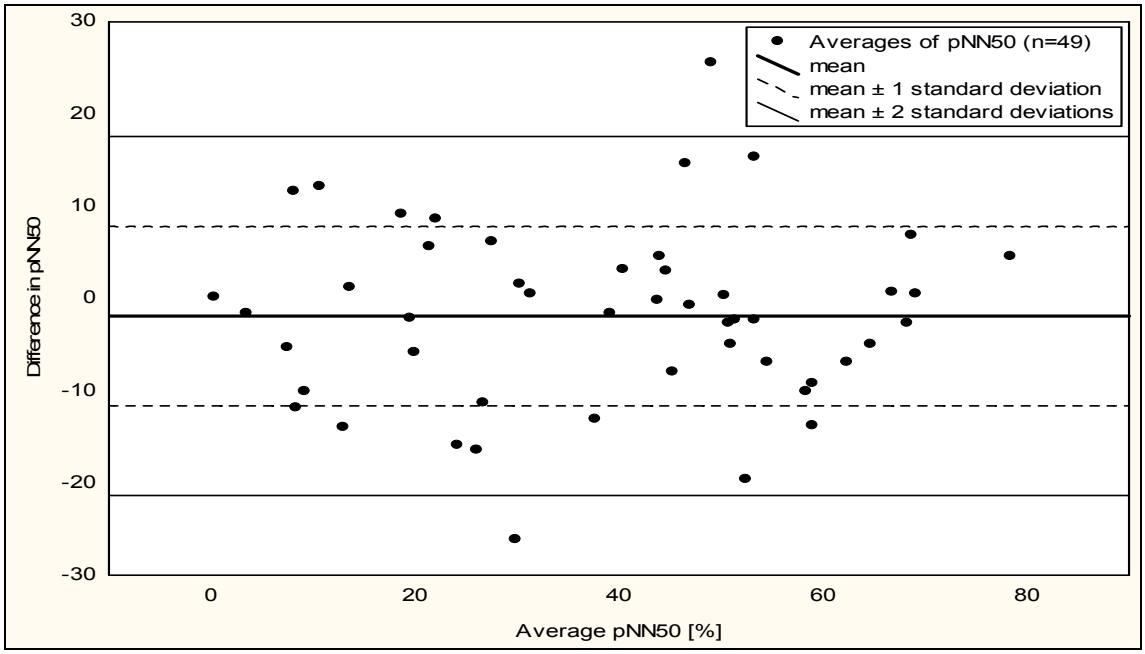


Figure 4-48: *Bland-Altman plot of pNN50 with an acceptable dispersion (96% of cases) within mean ± 2 standard deviations and acceptable limits of agreements (mean ± 2 standard deviations)*

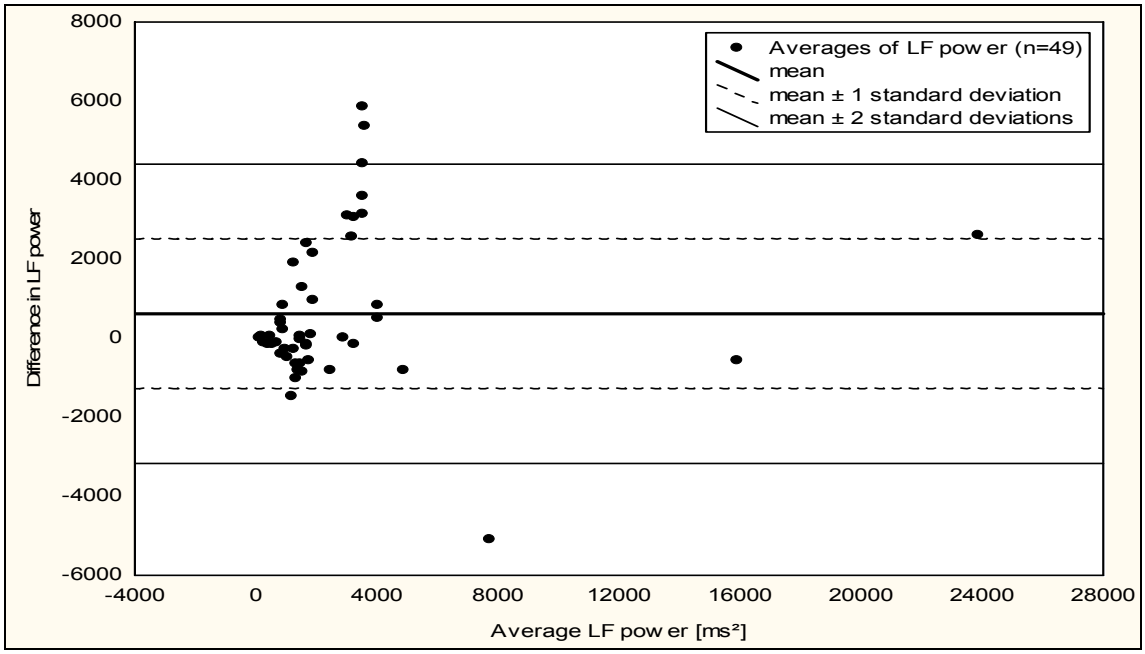


Figure 4-49: *Bland-Altman plot of LF power illustrates an unacceptable dispersion (92% of cases) because the limits of agreement (mean ± 2 standard deviations) are not related to the original scale of measurement; the mean is 616 ms² and the range of limits 7571 ms².*

5 Discussion

The analysis of the heart rate variability (HRV) has been applied to several examinations in different research areas as well as in clinical studies. The spectra of the power spectral analysis in particular give a non invasive insight in the vegetative control of the heart due to the classification of the sympathetic and parasympathetic activity with respective frequency bands. Therefore the sympathetic activity is classed with low (LF 0.15-0.04 Hz) and the vagal activity with high frequency (HF 0.4-0.15 Hz) fluctuations. The HF consists of pure vagal whereas the LF of a composition of sympathetic and vagal activity which may not be numerically quantized. Only the ratio between the sympathetic and parasympathetic activity may be expressed by the normalized LF and HF units as well as the LF/HF ratio which is often designated as a marker of the sympathovagal balance. Yet, the physiological significance of each HRV parameters and its correlations remains unknown so that provocation tests, e.g. the orthostatic test, are applied specifically to unbalance the autonomic nervous control of the heart which results in modulated HRV results.

Two main questions in relation to the HRV have been examined in the present study:

1. Can the analysis of the HRV be a valuable tool to compare the vegetative control of the heart in endurance trained subjects who were included in individual daily trainings with sedentary controls?
2. Do normal ovulatory athletic and sedentary females exhibit any effects in the autonomic nervous control of the heart investigated by the analysis of the HRV during the course of the menstrual cycle?

The results of the present study are presented in the following chapters.

5.1 Profile of mood state

In the present study, the daily mood state of trained and untrained women did not fluctuate during the menstrual cycle. The pre and the test month of all women were similar in the sub and the overall scores of the profile of mood states (POMS). No menstrual cycle influences which might have affected the POMS were noted in the pre and in the test month and the results of the POMS remained similar throughout the menstrual cycle. Therefore, our results are in line with the findings of Sato et al. [76] who also found no affected mood states in sedentary women in course of the menstrual cycle.

Additionally, the overall score of the pre and the test month in females was not significantly different compared with the overall score of men. Although differences in the sub scores of athletes compared with sedentary subjects were noted, the overall scores remained similar.

Several authors [15, 61, 67] investigated affected mood states by POMS during training periods in athletes which resulted in enhanced negative feelings. Nevertheless no difference in the overall score between trained and untrained subjects was noted and no inverted iceberg profiles (i.e. marker of enhanced training-induced negative feelings) found in the present study. Thereby, the individual training pattern in athletes did not affect the results of the daily POMS questionnaire. The overall scores of trained and untrained men and women remained in the normal range which implies unaffected mood states in course of the study. Thus, in accordance to Morgan et al. [59], no sign of staleness and/or overtraining was found in athletes during the study. Based on these findings, the requirements for the HRV analysis were met.

5.2 Blood borne parameters

5.2.1 Monitoring of the menstrual cycle

In our study, the menstrual cycle was divided into five different phases which depended on the characteristics of the hormonal fluctuations. The phases were individually determined according to the basal body temperature of the preceding month. All women had a normal ovulatory cycle including a typical course of the hormonal fluctuations despite of different individual hormonal levels. The concentrations of LH, FSH, E2 and P were at basal i.e. lowest level during the menstruation. In the follicular phase, LH,

FSH and E2 gradually increased with its peak at the ovulation phase (O). P only increased after O and remained enhanced till the pre menstruation phase whereas LH, FSH and E2 decline after O and approach to the basal level again in the pre menstruation phase. Therefore, cyclic changes of LH, FSH, E2 and P concentrations of women involving in this study could be shown. Moreover, no hormonal differences between trained and untrained women were noted. Based on these results, the requirements for the HRV analysis were met.

5.2.2 Monitoring of hormonal fluctuation in men

The hormonal fluctuations in men were analysed to determine whether there were training induced hormonal fluctuations in athletes compared with sedentary men and in course of one month. SHBG and the total testosterone (tT) levels were analysed in trained and untrained men during the study whereas the free androgen index (fAi) and the percentage of free testosterone (fT) were calculated. In spite of individually different hormonal levels, SHBG, tT, fAi and fT remained similar in athletes and sedentary males during the study month. These findings suggest that the hormonal levels were not affected by the individual training pattern in athletes and that there were no hormonal fluctuations in course of one month in males.

5.2.3 Glucose and insulin concentration

The blood glucose (BG) and the insulin (INS) concentration were supposed to have an influence on the vegetative control of the heart i.e. the HRV. Therefore, subjects were requested to refrain from eating and drinking for at least 8 hours before the ECG recordings. The determination of the BG and the INS served to control the fasting value of subjects´ BG and INS level. The results of the present study showed that the BG levels remained low during the study and were similar in athletes and sedentary subjects without any difference between males and females. Based on these findings it could be assumed that the subjects were fasting 8h prior to the study days. The insulin (INS) levels were also at fasting values and stable throughout the study month. Still lower INS concentrations were found in male and female athletes compared to the sedentary subjects. Nevertheless, no relation between the BG and INS levels as a marker of the metabolic supply was found in athletes or sedentary subjects. Furthermore, diabetes mellitus could be excluded and fasting metabolic supply controlled during the study.

5.2.4 Electrolytes and blood count

The electrolytes sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), chloride (Cl^-) were determined in the blood serum to control its homeostasis during the study. Affected Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^- concentrations may lead to modulation of the HRV because of its effect on the conduction system of the heart and therefore the control of electrolytes. During the five study days, the electrolytes remained stable and in the normal range in athletes and sedentary subjects. No intergroup difference was noted.

The haemoglobin (Hb) and the hematocrit (Hc) concentration also remained stable and in the normal range in trained and untrained subjects. Differences between men and women were only noted in the Hc level which was lower in females than in males. Still, the Hc was similar throughout the study month in all subjects. Based on these findings, any kind of anaemia as well as affected balance of the fluid concentration could be excluded in athletes and sedentary volunteers and the requirements for the HRV analysis were thereby met.

5.3 Heart rate variability at rest

In this study, the heart rate variability (HRV) of endurance trained athletes, who were supposed to show an improvement of the vegetative control expressed by enhanced vagal and/or reduced sympathetic activity due to the lower resting heart rate were compared with sedentary i.e. moderately active people. The HRV of male and female subjects were investigated by short time ECG recordings at rest in the time and the frequency domain.

The present results show that male and female athletes had a significantly lower resting heart rate as well as increased HRV parameters in the time domain compared with sedentary subjects. The enhanced variability was expressed by an increased SDNN, by higher RMSSD and pNN50 values in athletes which reflect augmented vagal activity. In the frequency domain, the total power and the LF power were significantly enhanced in athletes whereas the HF power was similar in trained and untrained subjects.

Although the results of the time domain indicated an increased vagal activity in trained subjects, the power spectral analysis did not because the HF power as a measure of short term variability and vagal activity was not increased. On the basis of these findings, the spontaneous breathing frequency and the individual training pattern were

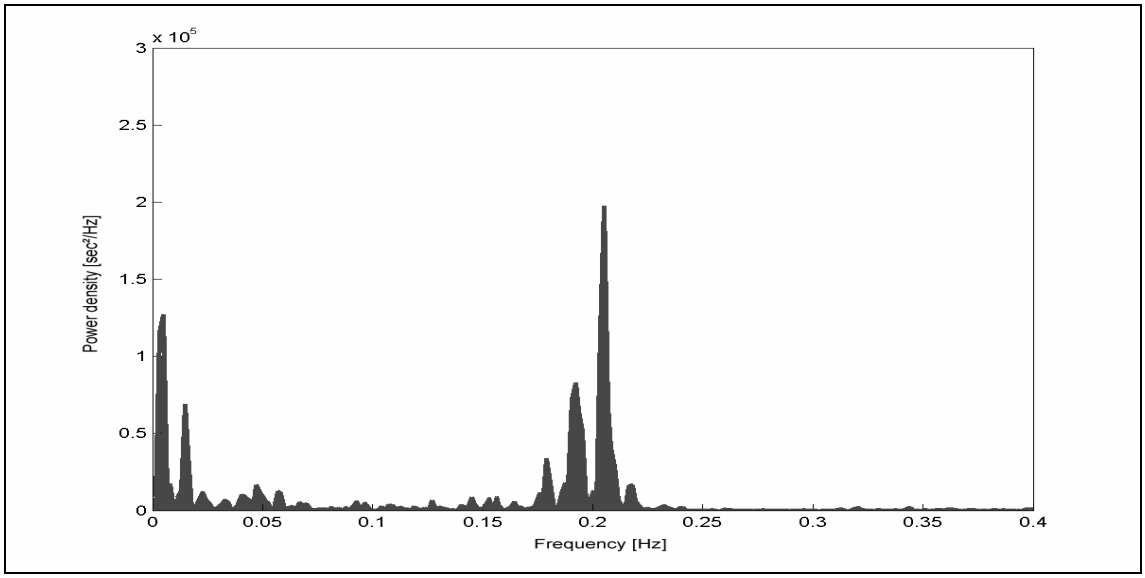
supposed to affect the HRV results in the frequency domain whereas any kind of overtraining e.g. enhanced negative feeling and fatigue could primary be excluded because of the POMS evaluation.

5.3.1 Heart rate variability affected by the breathing frequency

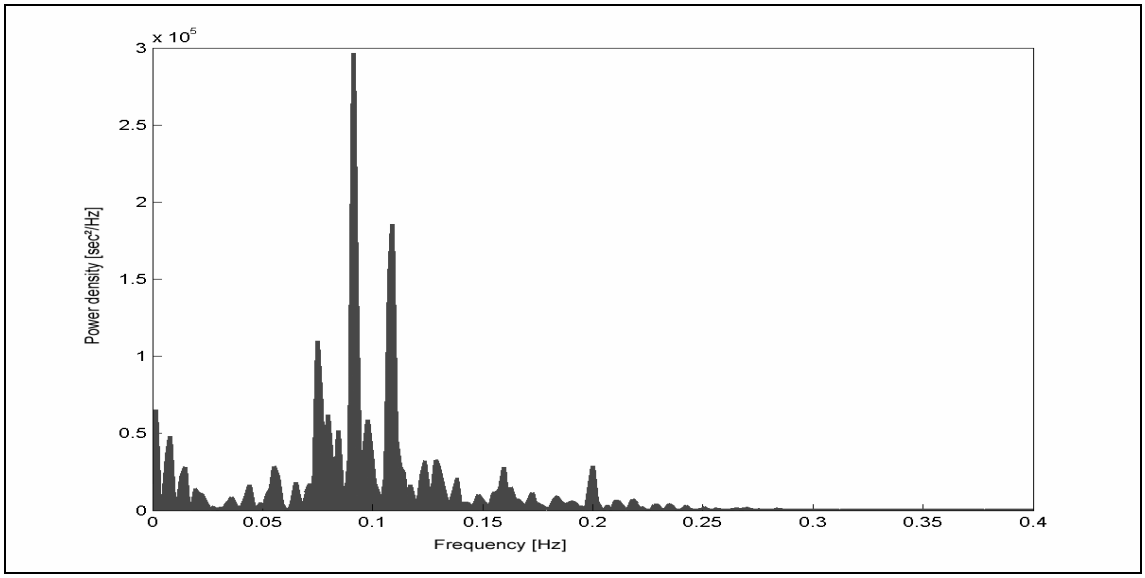
The breathing frequency (BF), which modulates the HF power by its strong relation with the respiratory sinus arrhythmia (RSA), was significantly lower in trained than in untrained men whereas the opposite was noted in the female groups. The main BF of male athletes was found inside the LF instead of the HF power band whereas the other groups did not show any main respiratory frequencies in the LF area.

The slow BF in male athletes was supposed to induce a roll-off of the respiratory-linked oscillations into the area below 0.15 Hz. That means that the HF power peak shifted into the LF power band, which resulted in an augmented LF and a reduced HF power. For the first time, Melanson et al. [57] observed such a shift in a pilot study with endurance trained athletes who had an extremely low spontaneous BF. They supposed an overlap of the HF in the LF power band, which had affected the results in the power spectral analysis.

In the present study, male athletes showed such a shift of the respiratory-linked oscillations from the HF into the LF power band which can be illustrated best by two different power spectra of the same male athlete. Primary spectrum 5-1 presents a HF power peak inside the HF bands and secondary spectrum 5-2 illustrates a shift of the HF peak inside the LH power band due to slower BF.



Spectrum 5-1: *Power spectrum with a BF of 11.2 breaths/min and its main frequency around 0.19 Hz; the HF power peak can be noted between 0.15-0.25 Hz inside the HF band*



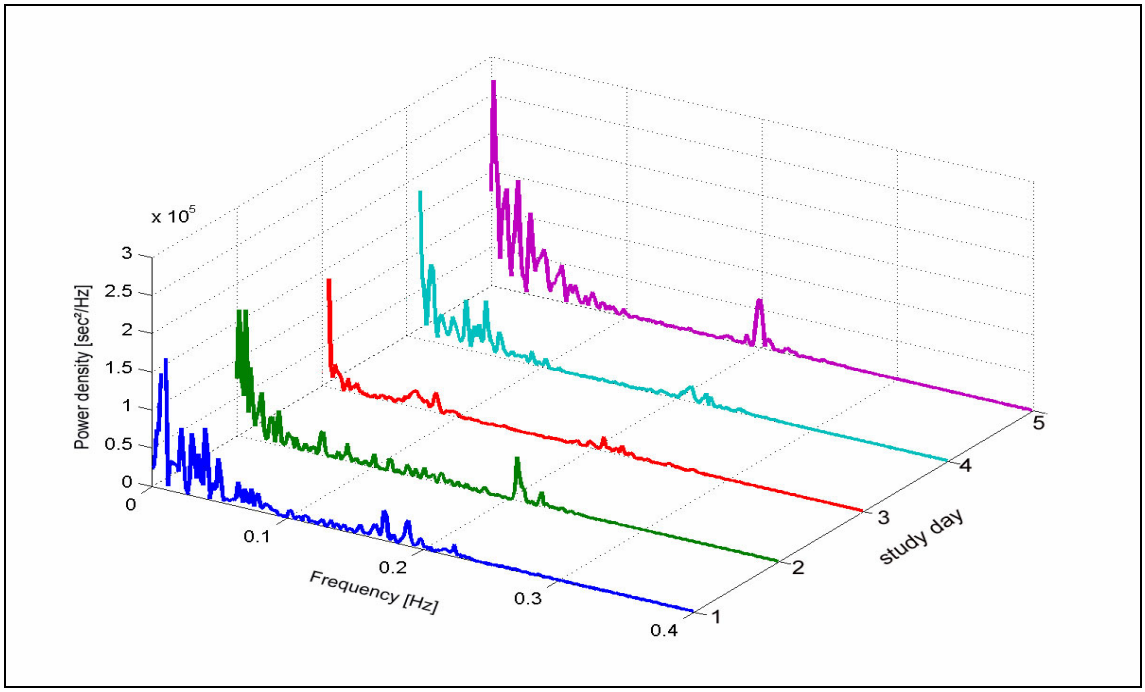
Spectrum 5-2: *Power spectrum with a BF of 5.2 breaths/min and its main frequency around 0.09 Hz; the HF power peak shifted inside the LF band between 0.15-0.04 Hz where several peaks can be noted*

In summary, male athletes did not show significantly increased HF power due to an overlap of the HF into the LF power area which affected the HRV results in the frequency domain. However, we found significantly enhanced LF power which resulted from the shift of the HF inside the LF power in trained compared with untrained subjects. This indicates that absent HF power not necessary correlates with reduced vagal activity in male athletes. Lower BF which is often accompanied with a higher

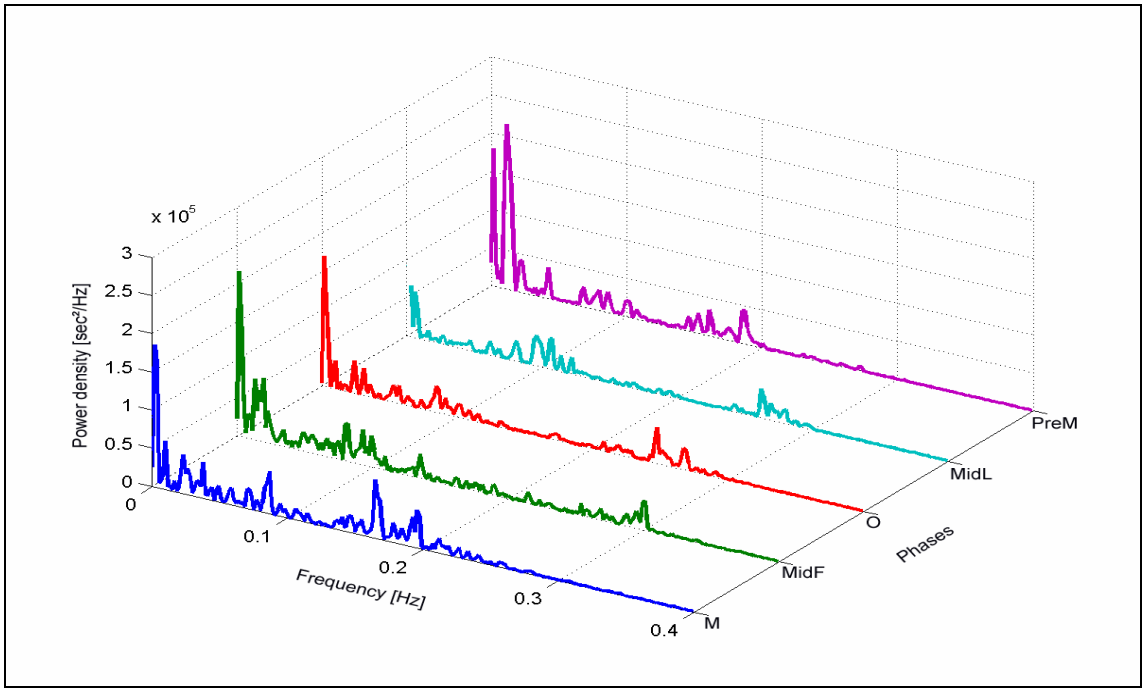
level of endurance capacity could therefore prevent from the application of power spectral analysis to determine the autonomic nervous activity to the heart.

Although female athletes did not cause a power shift induced by slow BF, a permanent increase of the BF was noted in course of the menstrual cycle. Trained women showed significantly enhanced BF in the middle of the luteal (MidL) and the pre menstruation (PreM) compared with the menstruation (M) phase whereas the untrained females failed to demonstrate this. The main respiratory frequency remained in the HF power band i.e. beyond 0.15 Hz in athletic women. Nevertheless, a shift of the HF power could be found in female athletes due to the permanently enhanced BF during the five phases. Thereby the main respiratory frequency changed from phase to phase. Some authors [6, 9, 43, 44, 86] investigated the HRV at different BF and noted a decreased HRV, i.e. HF power, in an inverse relationship to the BF. Still we failed to demonstrate a diminished HF power at increased BF in female athletes. On the one hand, simultaneously increased tidal volume which results in higher HRV, i.e. HF power might have neutralized the effect of the enhanced BF. On the other hand, the individually different training pattern of the female athletes might have affected the HF power and thus the HRV results in the frequency domain. And finally, other mechanisms as a modulated sensitivity of the respiratory as well as cardio circulatory control, which includes the neurons to P_{CO_2} and to the pH during the menstrual cycle, have to be assumed.

Yet, the HF power in female athletes remained nearly unaffected in the present study. Nevertheless the enhanced BF caused a shift within the HF band which can be illustrated best by the two different spectra. Spectrum 5-3 presents the power spectrum of an untrained man with similar main respiratory frequencies which resulted in stable HF power peaks around 0.2 Hz. In comparison, spectrum 5-4 presents the power spectrum of a woman who showed permanently enhanced BF which caused a change of the main respiratory frequencies in course of the menstrual cycle. This change resulted in a shift of the HF power peak from phase to phase however remaining within the HF band.



Spectrum 5-3: *Five power spectra of an untrained man with HF peaks around 0.2 Hz; unaffected HF power due to the BF*



Spectrum 5-4: *Power spectra of a woman with HF peaks shifting between 0.15-0.3 Hz in course of the menstrual cycle; still the HF power peaks remained in the HF area*

5.3.2 Heart rate variability and individual training pattern

In the present study, LF power was significantly increased, but HF power was not changed in trained subjects. Significantly lower resting heart rate (HR) as well as increased HRV was noted in the time domain. The reason for the discrepancy between low HR and missing increased HF power, which is an indicator for the vagal activity, is due to the low breathing frequency in athletes as pointed out in the previous chapter.

The present findings can be compared to other authors. Primarily the study of Furlan et al. [28] noted a depressed HF power for 48h in untrained subjects after maximal exercise training. They found greater HF power in trained swimmers during a break in the yearly training compared with those swimmers who were involved in the training. Moreover, the HF power was noted to be reduced when the swim training resumed. Based on the findings of Furlan et al. [28], the HVR was supposed to be affected by maximal exercise in untrained as well as by yearly training periods in athletes. This affection was found to result in a depressed HF power up to 48h. Additionally, Melanson et al. [57] investigated the HRV in three different kinds of training groups (high, moderate, low) which were separated in relation to their self reported physical activity level. They found a significantly increased HRV in the time and the frequency domain in the low compared with the moderately and highly active subjects. But they missed to demonstrate any differences in the HRV i.e. the HF power between the moderately and highly active groups. Due to this, Melanson et al. [57] supposed that a depressed HF power was noted in highly active subjects because their training happened more often and with higher intensity than the training of the moderate active group. Furthermore, Janssen et al. [35] compared cyclists and sedentary subjects and finally found a persistent sympathetic activation in athletes up to 24h after exercise in cyclists. Based on these three studies [28, 35, 57] it may be argued that exercise could be supposed to diminish the vagal activity shown by depressed HF power up to 48h and to increase the sympathetic activation up to 24h in athletes [35].

The athletes included in this study were all long term endurance trained with at least 2 years of training experience. Male and female athletes did their individual training but none were involved in competitions at the time of the study. Still, the aerobic capacity of athletes was examined by a maximal ergometric test. The median of the maximal oxygen uptake in relation to the body weight (VO_{2rel}) was 62.8 ml/min/kg for men and 50.9 ml/min/kg for women. During the course of the study, athletes were allowed to maintain their habitual training pattern which was recorded daily in a training diary

including the training duration and its intensity. Based on these self-reported data, men and women showed an average of 4 training sessions per week consisting of 8 hours/week in male and 6.5 hours/week in female athletes at comparable intensity. The different individual range of the sessions lasted up from 3-7 sessions/week. This would mean that athletes were not able to rest 24-48h prior to the HRV measurements. In consequence, the vegetative control of the heart may have been affected by the training intensity in athletes. Finally, this affection contributes to enhanced LH and diminished HF power during the present study which would be consistent with the above mentioned findings [28, 35, 57].

5.3.3 Summary

The results of the present study showed a significantly enhanced HRV in endurance trained athletes in the time domain whereas in the frequency domain the LF rather than the HF power was increased in athletes compared with sedentary controls.

These findings can be explained primarily by the low breathing frequencies noted only in male athletes which caused a shift of the HF into the LF power band. Due to the overlapping of both frequencies in one, an enhanced LF power was found in athletes as well as similar HF power in trained and untrained groups.

The BF nearly unaffected the HF power of female athletes, which implied to examine other mechanisms affecting the spectral power analysis. Therefore, the different individual training in course of the study was additionally supposed to affect the HRV results. Several studies [28, 35, 57] noted an increased sympathetic activity up to 24h and a depressed vagal activity up to 48h after the training intervention. Considering the training patterns during the study which were led by a protocol, the above mentioned rest between 24-48h after the training was not given prior to the HRV measurements. In consequence, the results of the power spectral analysis were supposed to be affected in trained due to the regular training.

In summary, the HRV was modulated primarily by the training influence in athletes and additionally by the low breathing frequency in trained men.

5.4 Heart rate variability during the menstrual cycle

An additional aim of this study was to determine whether the vegetative control of the heart was modulated by the hormonal fluctuations in normal ovulatory women during

one menstrual cycle. Thus, endurance trained and sedentary females were investigated in five different phases which had been individually determined based on the basal body temperature of one month prior to the study.

Several studies [30, 50, 75, 76, 97] already compared the HRV in different menstrual cycle phases in sedentary females but with methodological differences. Most investigators [30, 75, 76, 97] suggested a modulated vegetative control based on some selected HRV results whereas one author [50] did not find any HRV modulations in the time and the frequency domain in course of the menstrual cycle. Due to these disagreements and the differences between the studies, the HRV was investigated by short time ECG recording at rest in the present study. The parameters of the time and the frequency domain were evaluated in two female groups; trained and untrained women. The monitoring of the menstrual cycle was done by hormonal analysis which represented hormonal fluctuations of LH, FSH, E2 and P of normal ovulatory females. In addition to this, no affected mood state were noted in women based on the evaluation and the comparison between pre and test month of the daily POMS questionnaire. In summary, menstrual disturbances and/or dysfunctions as well as any affected mood states could be definitely excluded as having a modulating effect on the HRV in athletic and sedentary females in the present study.

Nevertheless, no significant difference was noted in the HRV parameters between five different phases i.e. menstruation, middle of follicular, ovulation, middle of luteal and pre menstruation phase. The results remained similar in the time and the frequency domain in course of one menstrual cycle. No significance was noted between the athletic and the sedentary females; both groups reacted comparably throughout the five study days. That means that the individual training pattern of the endurance trained women did not affect the vegetative control of the heart in the course of the menstrual cycle.

The present findings are in line with Leicht et al. [50], who also did not find any affected HRV during the menstrual cycle. Due to this consistency and based on our entire HRV investigations we conclude that the autonomic nervous control of the heart is not directly modulated by the hormonal fluctuations in normal ovulatory women. Additionally, no difference was noted between athletes and sedentary women; this implies that the physical activity level did not affect the course of the HRV throughout five menstruation phases. Thus, athletes and sedentary females showed an unaffected HRV course throughout one menstrual cycle.

5.5 HRV during the orthostatic test

The orthostatic test is the best known test to provoke an increase of the sympathetic and/or a decrease of the vagal activity by an active or passive body position change from supine to standing. The vagal predominance i.e. the enhanced HF power in supine position and the shift towards the increased sympathetic and/or the decreased vagal activity i.e. the enhanced LF power while standing are well described [44, 84, 90]. However, the different reaction of trained and untrained subjects during the orthostatic test was rarely investigated. Janssen et al. [35] compared cyclists and sedentary controls during supine rest and while standing. They found significantly decreased meanNN while standing and suggested a reduced parasympathetic activity in cyclists in this position. Nevertheless while lying supine, a vagal predominance was noted in cyclists.

In the present study the orthostatic test was done primarily to compare the provocation in athletes and sedentary subjects. The active standing was considered as a physical work-load and therefore differences between endurance trained and moderately active subjects were supposed while standing as well as in the second supine position. Furthermore, the orthostatic test was investigated in women to examine the provocation in five different menstrual cycle phases. The orthostatic tests were assumed to be affected by the hormonal fluctuations in females as shown by Saeki et al. [75], who noted differences between the orthostatic tests in five menstrual cycle phases and suggested a modulated reflex control of the autonomic functions during the menstrual cycle in sedentary women.

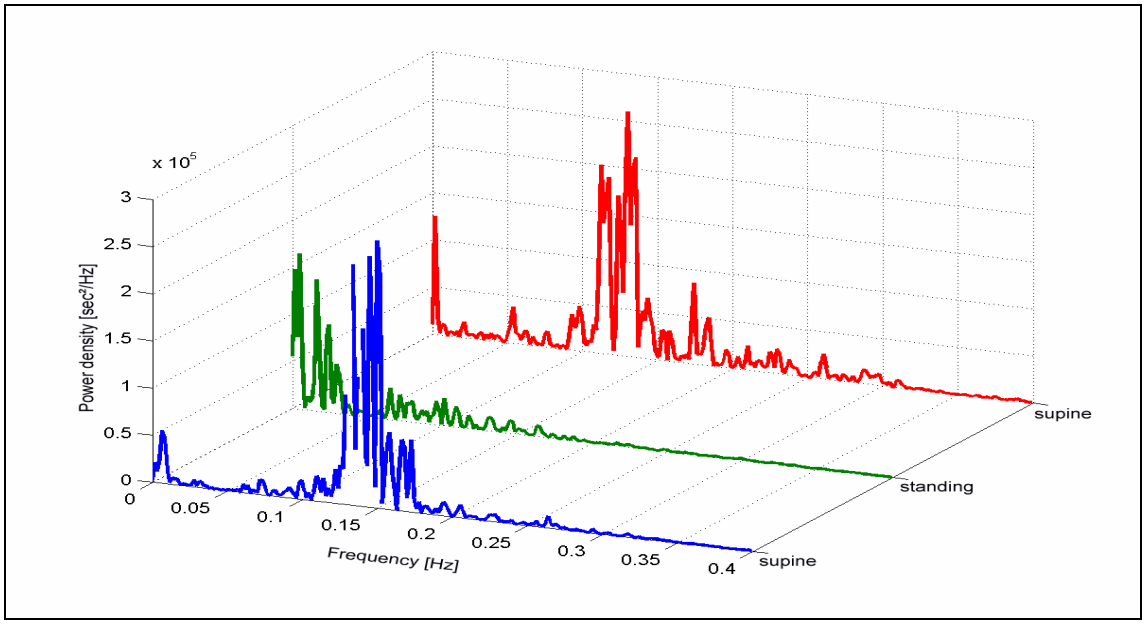
5.5.1 Orthostatic test in athletes and sedentary subjects

In the time domain, the orthostatic reaction was similar in males and females and without any differences between athletes and sedentary subjects. While standing the HRV parameters of the time domain were reduced compared with the supine position. Based on these findings, we could prove the assumed vagal predominance at rest as well as the increased sympathetic and/or decreased parasympathetic activity while standing. In the frequency domain the reaction of the total power (TP) was comparable with the results of the time domain due to the reduced TP while standing and its enhancement at rest. Also an increased LF power was only noted in trained men whereas the LH power remained similarly or slightly decreased in the three other groups while standing. Nevertheless, the HF power was similarly reduced while standing and thus a comparable reaction of the HF power was noted in all subjects.

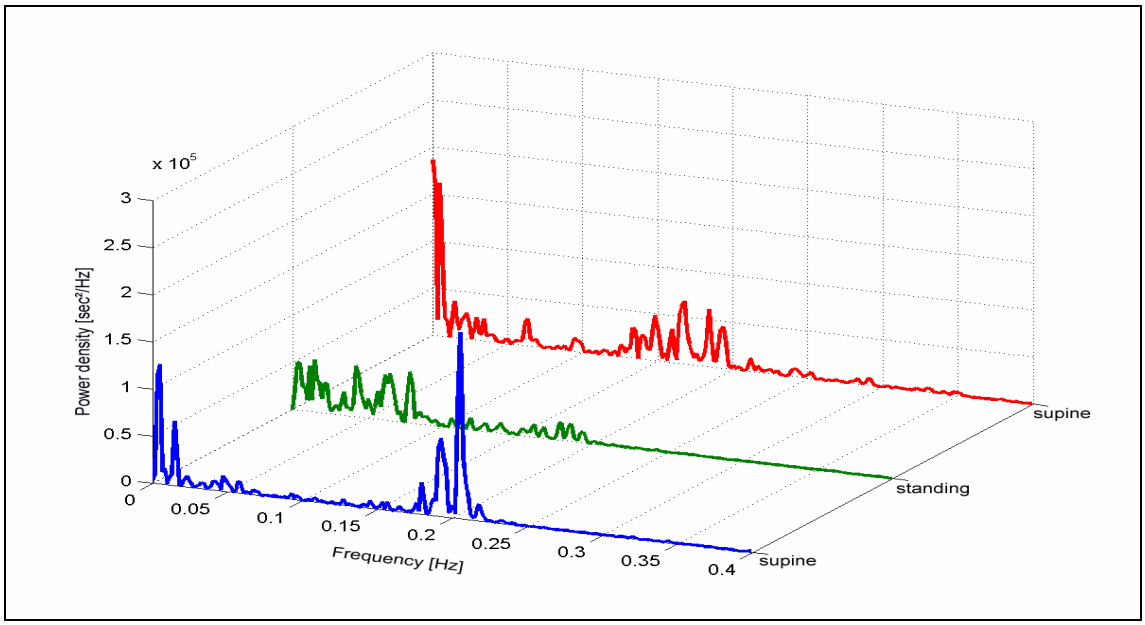
The interplay of LF and HF components during the orthostatic provocation can be also demonstrated by the normalized units (LFnu and HFnu) as well as by the LF/HF ratio. But it has to be taken into consideration that the normalized units only represent the relative value of each power component in proportion to the total power minus the VLF component. This representation only emphasizes the controlled and balanced behaviour of the sympathetic and parasympathetic nervous branches which tends to minimize the effect of the TP changes on the LF and HF values. Therefore, the normalized units have to be quoted by the absolute power values which were described before.

Athletes and sedentary subjects showed a reduced HFnu and an increased LFnu in the standing position whereas the opposite reaction was noted at rest which resulted in an increased LF/HF ratio while standing and a decreased one in supine position. No difference was noted in the orthostatic reaction between men and women and between trained and untrained subjects. Based on these results we supposed that the orthostatic test was not affected by enhanced physical activity e.g. endurance training in male and female athletes. The present findings are still not consistent with the study of Janssen et al. [35] who noted reduced vagal activity while standing in cyclists compared to sedentary controls during the orthostatic test.

In summary, the orthostatic provocation led to a comparable shift of the sympathovagal balance in trained and untrained subjects. The only exception of the provocation was found in the LF power which reacted different in male athletes compared with the other groups. On one hand, the different course of the LF power during the orthostatic test was assumed to be due to the similar affection of the slow breathing frequency in male athletes as seen in chapter 5.5.3. On the other hand, the diminished TP while standing was also supposed to affect the LF and the HF power composition in the spectrum. These possible explanations were additionally confirmed by the similar orthostatic reaction in normalized units as well as by the LF/HF ratio seen in all groups. The orthostatic provocation can be illustrated best with the help of a power spectrum. Spectrum 5-5 presents the orthostatic provocation of a trained woman whereas spectrum 5-6 presents the similar reaction of a sedentary male. Although the female athlete shows higher power density peaks than the untrained men, the orthostatic reaction remained comparable.



Spectrum 5-5: *The orthostatic test presented in three different parts (i.e. supine, standing and supine) of a trained woman*



Spectrum 5-6: *The orthostatic test presented in three different parts (i.e. supine, standing and supine) of a trained man*

5.5.2 Orthostatic test during the menstrual cycle

In normal ovulatory women, the orthostatic test was investigated in five different phases i.e. menstruation, middle of follicular, ovulation, middle of luteal and pre menstruation phase. Despite different hormonal fluctuations which have been demonstrated in chapter 5.2.1, similar orthostatic reactions were noted in athletic and sedentary women. No

significant difference was found in the HRV parameters of the time and the frequency domain during the course of the menstrual cycle. Thus, the orthostatic provocation remained comparable in trained and untrained women over the five phases. This implies that the provocation of the orthostatic test was not directly affected by the menstrual cycle and its hormonal fluctuations in females. These findings are not in line with the results of Saeki et al. [75] who found modulated orthostatic reactions in the luteal and pre menstruation compared with the menstruation phase in sedentary women.

5.5.3 Summary

In the present study the orthostatic test has been proven in all groups. Due to the reduced TP while standing, the vagal predominance at rest as well as the enhanced and/or diminished sympathetic activity could be shown by results of the normalized units. Nevertheless we failed to demonstrate a different orthostatic reaction between trained and untrained subjects. Moreover, the orthostatic reaction was also not affected by the hormonal fluctuations of normal ovulatory females. No relation between the orthostatic reaction and the menstrual cycle in athletic and sedentary females could be found in this study.

5.6 Reliability

In the present study the reliability of the repeated measurements was controlled by the intragroup correlations coefficient (ICC), the coefficient of variation (CV) and the Bland-Altman plot which tested the intra- as well as the intergroup reliability. Although the data of this study were not normally distributed, the ICC, the CV and the Bland-Altman plots were analysed because equally good tests for non parametric data do not exist. In addition to this, universal standards for the ICC and the CV are missing. Thereby the ICC was determined in accordance to Lee et al. [49] whereas the CV was subjectively discussed considering the present data.

The reliability was determined from the ICC, the CV and the Bland-Altman plots ranged from acceptable to good in the time domain. However the reliability was poor in the frequency domain. Due to these findings, we suggest individual biological variance and their effects leading to the debased reliability in the frequency domain. Thus, further investigations are needed to examine these biological variances and their effects on the heart rate variability.

The HRV reproducibility has been extensively assessed by other authors and found to be acceptable for measurement durations as short as 2.5 min in resting position in the time as well as the frequency domain [49]. Nevertheless, the reproducibility has not been investigated in the present study because the comparison of the algorithms used in this study with algorithms of a so-called golden standard is missing in the HRV investigation. An additional problem is that the hard- and software as well as the combination of algorithms, used to detect and evaluate the HRV, are mostly proprietary, not well described and therefore not always duplicated.

In accordance to the Task Force [84] comparable accuracy of the HRV measurements, reported by different commercial equipment, may only be achieved by devices, which have been tested independently of the manufacturer. Several short and long time inventions with precisely known HRV parameters and with different morphological characteristics of the ECG signal should be involved. In particular, both the recording and the analytical part of the device should be tested. Alongside, an appropriate technology should be used to record fully reproducible signal with precisely known HRV parameters, which have to be done by generated computer software and/or hardware. In summary, technical reports of the testing should be prepared and established for further HRV measurements [84].

5.7 Critics of the method

A possible limitation of this study was the density of the different menstrual cycle phases in women. Alternatively it might be possible, that the examination of more than five phases could demonstrate other HRV results because of a menstrual cycle monitoring with close-meshed control. At least, the influence of the menstrual cycle and its hormonal fluctuation of the vegetative control of the heart in women might only be completely excluded by studies which investigate daily HRV measurements in course of the menstrual cycle. Beside this, the duration of the study which consisted of a pre and a test month in women might have been too short to examine the influence of the menstrual cycle on the HRV in women.

It was of interest in this study to determine whether a short time recording of the HRV might be a practicable tool to investigate physiological mechanisms during individual training interventions. Because of this, a short time instead of a 24h ECG recording was used. On point of critic could be that the recording period of 10 min used in the present study might have limited the examination of the vegetative control of the heart in daily active athletes. Still it is not proven whether a 24h ECG recording really contains more detailed information of the HRV. In addition to this, the intraindividual differences of the subjects´ pre analysis between the single study days which were found beside standardized study conditions can also be mentioned, here. But even under more controlled conditions, the differences between biological systems as humans would remain consistent. And thus, the total standardisation of the pre analytics in human beings keeps impossible. The individual differences between subjects will always remain.

Finally, the HRV in the frequency domain was analysed by the Fast Fourier transform (FFT) which requires stationary ECG signals. Although biological systems may not reach conditions producing stationary signals, the FFT is used to investigate the power spectral analysis of the HRV because of poor available alternatives in mathematical calculations. On this account, other mathematical possibilities which might be applicable in further studies have to be assessed for non stationary ECG signals.

5.8 Applicable consequences in sports medicine

Based on the reliability of the present data, the applicability of the HRV analysis is given in the time but not in the frequency domain. Repeated measurements as well as short time recordings are applicable in relation to training interventions of male and female athletes. Unfortunately, data of comparable work-loads and/or training interventions in sports medicine are still missing. Additionally, the diagnostic significance in relation to sensitivity and specification of the HRV analysis remains unclear. Thus, the HRV analysis is not yet a practicable tool to supervise and/or control the training process in athletes which might prevent a developing overstrain and overtraining. Further investigations are needed.

However, we found that the vegetative control of the heart in normal ovulatory females is not affected during the menstrual cycle; trained and untrained women reacted similar. Thus, female athletes do not have any affected autonomic nervous control of the heart by endogenous hormonal fluctuations. Based on these findings, we suggest that the HRV can be analysed in normal ovulatory females irrespective of the menstrual cycle.

5.9 Future prospective

Future prospective should involve the standardisation of the HRV procedures and its methods which include the testing of the measurement equipment as well as the hard- and software used to detect and calculate the HRV. Besides, new mathematical calculations (i.e. algorithms) have to be developed to analyse primary non stationary ECG signals and secondary ECG signal affected by slow breathing frequencies found mostly in athletes. Such mathematical possibilities would finally allow improving the investigation of the HRV i.e. the vegetative control of the heart in athletes. And thus, mechanisms and parameters which control the physical work load might be better examined in athletes. Unfortunately, the examination of the HRV in athletes is limited till today due to the missing mathematical alternatives in sports medicine. At least the available concepts used to examine the sympathovagal control of the heart should also have been taken under consideration. What, if these would be the limitations to improve the determination of the vegetative control of the heart in biological systems?

6 Summary

The HRV analysis, which gives a good insight into the autonomic control of the heart, has been rarely investigated in athletes; data particularly of female athletes are still missing. Due to the unclear physiological significance in athletes, the HRV analysis is not yet practicable in relation to different training interventions, which may cause short and long time disturbances beside acute or chronically adaptations of the autonomic nervous system. Nevertheless, short time recording of the HRV as well as the orthostatic reflex control are assumed to be a valuable tool to supervise and/or control the training process in male and female athletes to prevent overstrain and overtraining syndromes.

Therefore, these two main questions in relation to the HRV have been investigated in the present study:

1. Can the analysis of the HRV be a valuable tool to compare the vegetative control of the heart in endurance trained subjects who were included in individual daily trainings with sedentary controls?
2. Do normal ovulatory athletic and sedentary females exhibit any effects in the autonomic nervous control of the heart investigated by the analysis of the HRV during the course of the menstrual cycle?

HRV measurements repeated five times during one month, i.e. one menstrual cycle, were examined by short time ECG recordings of 20 min at rest as well as during an orthostatic test consisting of three parts: 20 min supine, 10 min standing and 20 min

supine. On one hand the HRV was compared between 24 long term endurance trained (≥2 years of training) athletes who were involved in marathon running, cycling and/or triathlon and 27 sedentary controls. And on the other hand, the HRV was investigated in trained (n=11) and untrained (n=11) normal ovulatory women in course of five different menstrual cycle phases which were individually determined based on the basal body temperature of the preceding month.

The measurement conditions of the ECG recording were standardized for each study day because of the known effects on the HRV of internal and external stimuli. The ECG recordings were done by commercial equipment designed to analyse short time HRV. Prior to the evaluation, the ECG signals at rest were shortened to 10 min sequences. First the correction of the ECG signals was done by hands followed by the application of an automatic detection algorithm implemented by RASCHlab; no interpolation was used. Finally the HRV was calculated in the frequency domain by a Fast Fourier Transform after a band pass filtering by a Hamming window and in the time domain by descriptive mathematical methods. Due to the missing golden standard in the analysis of the HRV, the guidelines of the Task Force [84] were followed in the present study.

In the present study, male and female athletes showed significantly higher HRV which explained enhanced vagal activity in the time domain whereas an increased HF power in the frequency domain was missing. However, enhanced LF power was noted in male and female athletes. Moreover, male athletes showed a BF which had its main frequency inside the LF power band. These resulted in an overlapping of the HF inside the LF power band which leaded to an increased LF power in trained subjects. Based on these findings, we conclude that we did not fail to demonstrate an increased vagal activity in trained males due to the missing enhanced HF power in athletes compared with controls. However, trained females did not show an affected HF power by slow BF. The missing HF as well as the augmented LF power was suggested to be induced by other mechanisms. Therefore, the influence of the individual daily training program during the study was taken into consideration. Several authors [28, 35, 57] described depressed vagal and enhanced sympathetic activity which lasted up between 24-48h after trainings of higher work load. The individually different training pattern of each athlete was led by daily protocol during the study. On the basis of these data, athletes trained in average 4 times per week with an individual variance between 3-7 times per

week. Based on the above mentioned studies [28, 35, 57] this would imply, that male and female athletes would have a training induced modulation of the vegetative control of the heart due to lacking rest prior to the HRV measurements. Nevertheless the orthostatic provocation was similar in trained and untrained subjects with a vagal predominance at rest and an augmented sympathetic and/or reduced parasympathetic activity while standing induced by an active body position change.

In course of the menstrual cycle, hormonal fluctuations of LH, FSH, E2 and P could be demonstrated without any differences in athletes and sedentary women. Still the parameter of the time as well as the ones of the frequency domain remained similar in the five phases. No differences were noted between the HRV of trained and untrained women in course of one menstrual cycle. Even the orthostatic provocation reacted not significantly different between the menstruation, the middle of the follicular, the ovulation, the middle of the luteal and the pre menstruation phase. These findings which are consistent with Leicht et al. [50] induce, that the hormonal fluctuation in normal ovulatory active and sedentary women did not directly influence the vegetative control of the heart. Although an enhanced BF throughout the menstrual cycle was noted, no affected HRV was found because the BF remained inside the HF power band in women. Therefore, the hormonal induced modulation of the BF did not affect the HRV results in the present study.

In summary, male and female athletes showed enhanced HRV parameters in the time domain compared with sedentary subjects. Nevertheless, we failed to demonstrate enhanced HF power in athletes at rest primarily because of respiration and secondarily because of training induced modulations of the autonomic nervous control of the heart. Still, the orthostatic provocation was similar in both groups. Furthermore, no menstrual cycle related HRV changes could be found at rest or during the orthostatic provocation in normal ovulatory females. Based on these findings, a direct influence of the endogenous hormones on the autonomic nervous control of the heart can be excluded.

Literature

1. Akselrod, S, Gordon, D, Ubel, FA, Shannon, DC, Barger, AC, Cohen, RJ, Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat to beat cardiovascular control, Science, 213:220-222, 1981.
2. Aubert, A. E, Seps, B, Beckers, F, Heart rate variability in athletes, Laboratory of Experimental Cardiology, School of Medicine, K.U. Leuven, Belgium, 2003.
3. Beidleman, BA, Rock, PB, Muza, SR et al, Exercise VE and physical performance at altitude are not affected by menstrual cycle phase, J Appl Physiol, 86(*5*):1519-26, 1999.
4. Beitins, I. Z, et al, Exercise induces two types of human luteal dysfunction: confirmation by urinary free progesterone, JCE&M, Volume 72, 1991.
5. Bland, J. M, Altman, D. G, Statistical methods for assessing agreement between two methods of clinical measurement, The Lancet, Feb(*8*):307-311, 1986.
6. Bloomfiled, D.M, et al, Comparison of spontaneous vs. metronome-guided breathing on assessment of vagal modulation using RR variability, Am J Physiol Heart Circ Physiol, Vol.280 (*3*):H1145-H1150, 2001.
7. Bonekat, HW, Dombovy, ML, Staats, BA, Progesterone-induced changes in exercise performance and ventilatory response, Med Sci Sports Exerc, Apr;19(*2*):118-23, 1987.
8. Bonen, A, Keizer, H.A, Pituitary, ovarian and adrenal hormone responses to marathon running, Int J Sports Med, 8:161-167, 1987.
9. Brown, T.E, Beightol, L.A, Koh, J, Eckberg, D.L, Important influence of respiration on human RR interval power spectra is largely ignored, J Appl Physiol, Nov; 75(*5*):2310-1, 1993.
10. Bunt, JC, Metabolic actions of estradiol: significance for acute and chronic exercise responses, Sports Med, Apr;29(*4*):221-7, 2000.
11. Byrne, A, Byrne, D. TG, The Effect of exercise on depression, anxiety and other mood states, a review, J. Psychosom. Res., 37(*6*): 565-574, 1993.
12. Cagnacci, A, Arangino, S, Tuveri, F, Paoletti, AM, Regulation of the 24h body temperature rhythm of women in luteal phase: role of gonadal steroids and prostaglandins, Chronobiol Int, Jul;19(*4*):721-30, 2002.
13. Catai, A. M, et al, Effects of aerobic exercise training on heart rate variability during wakefulness and sleep and cardiorespiratory responses of young and middle-aged healthy men, Braz J Med Biol Res, 35(*6*):741-752, 2002.

14. Cockerill, IM, Nevill, AM, Lyons, N, Modelling mood states in athletic performance, J Sports Sci, Summer;9(*2*):205-12, 1991.
15. Coker, CA, Mickle, A, Stability of the Iceberg profiles as a function of perceived difficulty in defeating an opponent, Percept Mot Skills, Jun;90(*3Pt2*):1135-8, 2000.
16. Conny, M.A, et al, Heart rate variability, review, Annuals of Internal Medicine, 118:436-447, 1993.
17. Consitt, L.A, Copeland, J.L, Tremblay, M.S, Endogenous anabolic hormone responses to endurance versus resistance exercise and training in women, Sports Med, 32(*1*):1-22, 2002.
18. Cullen, JH, Brum, VC, Reidt, WV, The respiratory effects of progesterone in severe pulmonary emphysema, American Journal of Medicine, Oct:34-41, 1960.
19. Damas-Mora, J, Davies, L, Taylor, W, Jenner, FA, Menstrual respiratory changes and symptoms, Br. J. Psychiatry, 136:492-497, 1980.
20. Davidson, NS, Goldner, S, McCloskey, DI, Respiratory modulation of baroreceptor and chemoreceptor reflexes affecting heart rate and cardiac vagal efferent nerve activity, J Physiol (London), 259:523-530, 1976.
21. De Meersman, RE, Reisman, SS, Daum, M, Zorowitz, R, Leifer, M, Findley, T, Influence of respiration on metabolic, hemodynamic, psychometric and RR interval power spectral parameters, Am J Physiol, Oct;269(*4Pt2*):H1437-40, 1995.
22. Dutton, K, Blanksby, B.A, Morton, A.R, CO2 sensitivity changes during the menstrual cycle, J Appl Physiol, Aug;67(*2*):517-22, 1989.
23. Ewing, DJ, Martin, CN, Young, RJ, Clarke, BF, the value of cardiovascular autonomic function tests: 10 years´ experience in diabetes, Diabetes Care, 8:491-498, 1985.
24. Faber, P, Lammert, O, Johansen, O, Garby, L, A fast responding combined direct and indirect calorimeter for human subjects, Medical Engineering & Physics, 20:291-301, 1998.
25. Filaire, E, Bernain, X, Sagnol, M, Lac, G, Preliminary results on mood state, salivary testosterone: Cortisol ratio and team performance in a professional soccer team, Eur J Appl Physiol, Dec;86(*2*):179-84, 2001.
26. Friesen, GM, Jannett, TC, Jadallah, MA, Yates, SL, Quint, SR, Nagle, HT, A comparison of the noise sensitivity of nine QRS algorithms, IEEE Trans Biomed Eng, Jan;37(*1*):85-98, 1990.
27. Fuenmayor, A.J, Ramirez, L, Fuenmayor, A.M, Left ventricular function and autonomic nervous system balance during two different stages of the menstrual cycle, Int J Cardiolo, 72:243-246, 2000.
28. Furlan, R, Piazza, S, Dell`Orto, S, Gentile, E, Cerutti, S, Pagani, M, Malliani, A, Early and late effects of exercise and athletic training on neural mechanisms controlling heart rate, Chest, May;101(*5*):226S-230S, 1992.
29. Goldsmith, RL, Bigger, Jr JT, Steinmann, RC, Fleiss JL, Comparison of 24hour parasympathetic activity in endurance-trained and untrained young men, J Am Coll Cardiol, 20:552-558, 1992.
30. Guasti, L, et al, Autonomic function and baroreflex sensitivity during a normal ovulatory cycle in humans, Acta Cardiol, 54(*4*):209-213, 1999.
31. Hartung, J, Statistik, Lehrbuch-Handbuch der angewandten Statistik, 13. Auflage, R. Oldenburger Verlag, München.
32. Hon, EH, Lee, ST, Electronic evaluations of fetal heart rate patterns preceding fetal death: further observations, Am J Obstet Gynecol, 87:814-826, 1965.
33. Huang, CT, Lyons, HA, Ventilatory effect of progesterone in acute metabolic acidosis and alkalosis with relevance to the changes in C. S. F, Physiologist, 9:207, 1966.
34. Janse de Jonge, X.A, Effects of menstrual cycle on exercise performance, Sports Med,

33(*11*):833-51, 2003.

35. Janssen, MJ, de Bie J, Swenne, CA, Oudhof, J, Supine and standing sympathovagal balance in athletes and controls, Curr Opin Cardiol, Jan;13(*1*):36-44, 1998.
36. Jurkowski, JE, Jones, NL, Walker, C, Younglai, EV, Sutton, JR, Ovarian responses to exercise, Med Sci Sports Exerc, Jun;22(*3*):286-90, 1990.
37. Keizer, HA, et al, Effect of 3-month endurance training program on metabolic and multiple hormonal responses to exercise, Int J Sports Med, 8(*3*):154-160, 1987.
38. Keizer, HA, General discussion, Int J Sports Med, 8: 168-174, 1987.
39. Keizer, HA, Kuipers, H, de Haan, J, Beckers, E, Habets, L, Multiple hormonal response to physical exercise in eumenorrheic trained and untrained women, J Appl Physiol, May;48(*5*):765-9, 1980.
40. Keizer, HA, Poortman, J, Bunnik, GS, Influence of physical exercise on sex-hormone metabolism, J Appl Physiol, Jan;44(*1*):109-14, 1978.
41. Keizer, HA, Rogol, AD, Physical exercise and menstrual cycle alterations. What are the mechanisms? Sports Med, 10(*4*):218-35, 1990.
42. Kobayashi, H, Ishibashi, K, Noguchi, H, Heart rate variability; an index of monitoring and analysing human autonomic activities, Appl Human Sci, 18(*2*):53-59, 1999.
43. Kobayashi, H, Normalization of respiratory sinus arrhythmia by factoring in tidal volume, Appl Human Sci, 17(*5*):207-13, 1998.
44. Kobayashi, H, Postural Effect on Respiratory sinus arrhythmia with various frequencies, Appl Human Sci, 15(*2*):87-91, 1996.
45. Koh, J, Brown, T.E, Beightol, L.A, Eckberg, D.L, Contributions of tidal lung inflation to human RR interval and arterial pressure fluctuations, J Autonom Nerv System, 68:89-95, 1998.
46. Kouidi, E, Haritonidis, K, Koutlianos, N, Deligiannis, A, Effects of athletic training on heart rate variability triangular index, Clin Physiol & Func Im, 22:279-284, 2002.
47. Kruijver, FP, Swaab, DF, Sex hormone receptors are present in the human suprachiasmatic nucleus, Neuroendocrinology, May;75(*5*):296-305, 2002.
48. Lebrun, CM, McKenzie, DC, Prior, JC, Taunton, JE, Effects of menstrual cycle phase on athletic performance, Med Sci Sports Exerc, 27(*3*):437-444, 1995.
49. Lee, K, Buchanan, D.B, Flatau, A.B, Franke, W.D, Reproducibility of heart rate variability responses to graded lower body negative pressure, Eur J Appl Physiol, 92:106-113, 2004.
50. Leicht, A.S, Hirning, D.A, Allen, D.A, Heart rate variability and endogenous sex hormones during the menstrual cycle in young women, Experimental Physiology, 88(*3*):441-446, 2003.
51. Loimaala, A, Huikuri, H, Oja, P, Pasanen, M, Vuori, I, Controlled 5-mo aerobic training improves heart rate but not heart rate variability or baroreflex sensitivity, J Appl Physiol, 89:1825-1829, 2000.
52. Lyons, HA, Antonio, R, The Sensitivity of the respiratory centre in pregnancy and after the administration of progesterone, Trans Assoc Am Phys, 72:173-181, 1959.
53. Malliani, A, The pattern of sympathovagal balance explored in the frequency domain, News Physiol Sci, 14:111-117, 1999.
54. Martin, BJ, Sparks, KE, Zwillich, CW, Weil, JV, Low exercise ventilation in endurance athletes, Med Sci Sports, Summer;11(*2*):181-5, 1979.
55. McGurk, S. P, Blanksby, B. A, Anderson, M. J, The relationship of hypercapnic ventilatory responses to age, gender and athleticism, review article, Sports Medicine, 3:173-183, 1995.
56. McNair, D. M, Lorr, M, Dropplemann L. F, Manual of profile of mood state, Educational and

Industrial Testing Service, San Diego CA, 1971.

57. Melanson, E.L, Resting heart rate variability in men varying in habitual physical activity, Med Sci Sports Exerc, 32(*11*):1894-1901, 2000.
58. Montagnani, C, Arena, B, Maffuli, N, Estradiol and progesterone during exercise in healthy untrained women, Med Sci Sports Exerc, 24(*7*):746-8, 1992.
59. Morgan, WP, Brown, DR, Raglin, JS, O'Connor, PJ, Ellickson, KA, Psychological monitoring of overtraining and staleness, Br J Sports Med, Sep;21(*3*):107-14, 1987.
60. Nakayama, T, Suzuki, M, Ishizuka, N, Action of progesterone on preoptic thermosensitive neurons, Nature, 258(*5530*):80, 1975.
61. O'Connor, PJ, Morgan, WP, Raglin, JS, Barksdale, CM, Kalin, NH, Mood state and salivary Cortisol levels following overtraining in female swimmers, Psychoneuroendocrinology, 14(*4*):303-10, 1989.
62. Patzak, A. et al, Herzfrequenzvariabilität-Methoden, Physiologie und Applikation im pädiatrischen Schlaflabor, Wien Klin Wochenschr, 112/5:234-250, 2000.
63. Perini, R, Veicsteinas, A, Heart rate variability and autonomic activity at rest and during exercise in various physiological conditions, Eur J Appl Physiol, 90:317-325, 2003.
64. Pigozzi, F, et al, Effects of aerobic exercise training on 24 hr profile of heart rate variability in female athletes, J Sports Med Phys Fitness, 41:101-107, 2001.
65. Pomeranz, B, et al, Assessment of autonomic function in humans by heart rate spectral analysis, Am J Physiol, 248:151-153, 1985.
66. Pöyhönen, M, Syväoja, S, Hartikainen, J, Ruokonen, E, Takala, J, The effect of carbon dioxide, respiratory rate and tidal volume on human heart rate variability, Acta Anaesthesiol Scand, Jan;48(*1*):93-101, 2004.
67. Pronk, N. P, Crouse, S. F, Rohack, J. J, Maximal exercise and acute mood response in women, Physiology and Behaviour, Vol. 57, *No. 1*, pp.1-4, 1994.
68. Raglin, JS, Morgan, WP, Luchsinger, AE, Mood and self-motivation in successful and unsuccessful female rowers, Med Sci Sports Exerc, Dec;22(*6*):849-53, 1990.
69. Rebuck, A.S, Measurement of ventilatory response to CO2 by rebreathing, Chest, 70:118-121, 1976.
70. Rennie, K.L, et al., Effects of moderate and vigorous physical activity on heart rate variability in a British study of civil servants, Am J Epidemiol, 158:135-143, 2003.
71. Robinson, JE, Birch, RA, Grindrod, JA, Taylor, JA, Unsworth, WP, Sexually differentiated regulation of GnRh release by gonadal steroid hormones in sheep, Reprod Suppl, 61:299-310, 2003.
72. Rost, R, Hollman, W, Athlete's heart-a review of its historical assessment and new aspects, Int Sports Med, 4:147, 1983.
73. Ryan, A.D, Larsen, P.D, Galletly, D.C, Comparison of heart rate variability in supine, and left and right lateral positions, Anaesthesia, 58:432-436, 2003.
74. Sacknoff, D. M, Gleim, G. W, Stachenfeld, N, Coplan, N. L, Effect of athletic training on heart rate variability, American Heart Journal, 127:1275-1278, 1994.
75. Saeki, Y, Atogami, F, Takahashi, K, Yoshizawa, T, Reflex control of autonomic function induced by posture change during the menstrual cycle, J Auton Nerv Syst, 66:69-74, 1997.
76. Sato, N, Miyake, S, Akatsu, J, Kumashiro, M, Power spectral analysis of heart rate variability in healthy young women during the normal menstrual cycle, Psychosomatic Medicine, 57:331-335, 1995.
77. Schmidt, R. F, Thews, G, Physiologie des Menschen, 25. Auflage, Springer Verlag, Berlin,

1993.

78. Schoene, RB, Robertson, HT, Pierson, DJ, et al, Respiratory drives and exercise in menstrual cycle of athletic and nonathletic women, J Appl Physiol, 50(*6*):1300-05, 1981.
79. Silva, NL, Boulant, JA, Effects of testosterone, estradiol and temperature on neurons in preoptic tissue slices, Am J Physiol 250:R625-32, 1986.
80. Skatrud, JB, Dempsey, JA, Kaiser, DG, Ventilatory response to medroxyprogesterone acetat in normal subjects, time course and mechanism, J Appl Physiol, 44(*6*):939-944, 1978.
81. Snow, RC, Barbieri, RL, Frisch, RE, Estrogen 2-hydroxylase oxidation and menstrual function among elite oarswomen, J Clin Endocrinol Metab, Aug;69(*2*):369-76, 1989.
82. Steptoe, A, Cox, S, Acute Mood responses to maximal and sub maximal exercise in active and inactive men, Health Psychol, 7(*4*):329-340, 1993.
83. Strano, S, et al, Respiratory sinus arrhythmia and cardiovascular neural regulation in athletes, Med Sci Sports Exerc, 30(*2*):215-9, 1998.
84. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, Heart rate variability: standards of measurements, physiological interpretation and clinical use, Circulation, 93:1043-1065, 1996.
85. Uusitalo, Al, Uusitalo, AJ, Rusko, HK, Endurance training, overtraining and baroreflex sensitivity in female athletes, Clinical Physiology, 18(*6*):510, 1998.
86. Van de Borne, et al, Importance of ventilation in modulating interaction between sympathetic drive and cardiovascular variability, Am J Physiol Heart Circ Physiol, 280:H722-H729, 2001.
87. Warren, MP, Perlroth, NE, The effects of intense exercise on the female reproductive system, J Endocrinol, 170(*1*):3-11, 2001.
88. Warren, MP, Shanta, S, The female athlete, Baillieres Best Pract Res Clin Endocrinol Metab, 14(*1*):37-53, 2000.
89. Wayne, C.L, et al., Effect of endurance exercise training on heart rate variability at rest in healthy young and older men, Am J Cardiol, 82:1236-41, 1998.
90. Weise, F, Heydenreich F, Effects of modified respiratory rhythm on heart rate variability during active orthostatic load, Biomed Biochem Acta, 48(*8*):549-556, 1989.
91. White, DP, Douglas, NJ, Pickett, CK, Weil, JV, Zwillich, CW, Sexual influence on the control of breathing, J Appl Physiol, Apr;54(*4*):874-9, 1983.
92. White, MD, Cabanac, M, Exercise hyperpnoea and hypothermia in humans, J Appl Physiol, 81(*3*):1249-54, 1996.
93. Williams, CA, Lopes, P, The influence of ventilatory control on heart rate variability in children, J Sports Sci, May;20(*5*):407-15, 2002.
94. Williams, TJ, Krahenbuhl, GS, Morgan, DW, Mood state and running economy in moderately trained male runners, Med Sci Sports Exerc, Jun;23(*6*):727-31, 1991.
95. Winters, KM, Adams, WC, Meredith, CN, et al, Bone density and cyclic ovarian function in trained runners and active controls, Med Sci Sports Exerc, 28(*7*):776-85, 1996.
96. Wolf, MM, Varigos, GA, Hunt, D, Sloman, JG, Sinus arrhythmia in acute myocardial infarction, Med J Aust, 2:52-52, 1978.
97. Yildirir, A, et al, Effects of menstrual cycle on cardiac autonomic innervation as assessed by heart rate variability, A. N. E, 7(*1*):60-63, 2002.
98. Zwillich, CW, Natolino, MR, Sutton, FD, et al., The effects of progesterone on chemosensitivity in normal men, J. Lab. Clin. Med, 92(*2*):262-269, 1978.

A Abbreviations

ANS	autonomic nervous system
BBT	basal body temperature
BMI	body mass index
BP	blood pressure
BR/BF	breathing rate or breathing frequency
Ca^{2+}	calcium
Cl^{-}	chloride
CNS	central nervous system
E2	β17-estradiol
ECG	electrocardiogram
fAi	free androgen index
FFT	Fast Fourier Transform
FSH	follicle-stimulating hormone
fT	free Testosterone
GnRH	gonadotrophic releasing hormone

HF	power in high frequency bands (0.15-0.4 Hz)
HFnu	high frequency in normalized units HFnu=HF(HF+LF)
HR	heart rate
HRV	heart rate variability
INS	insulin
K^+	potassium
LF	power in low frequency bands (0.04-0.15 Hz)
LFnu	low frequency in normalized units; LFnu=LF(LF+HF)
LH	luteinizing hormone
LHRH	hypothalamic releasing hormone
M	menstruation phase
meanNN	mean of all NN or RR intervals
Mg^{2+}	magnesium
MidF	middle of the follicular phase
MidL	middle of the luteal phase
MT	trained men
MUT	untrained men
Na^+	sodium
NN/RR intervals	normal to normal intervals
O	ovulation phase
P	progesterone
P_{CO2}	partial pressure of CO_2

pNN50	relation of NN differences <50ms to total NN interval number
POMS	profile of mood state questionnaire
PreM	pre menstruation phase
RMSSD	square root of means of squared differences of consecutive NN intervals
RSA	respiratory sinus arrhythmia
SDNN	standard deviation of all NN intervals
SHBG	sexual hormone binding globulin
T_{Age}	trainings age of certain sport
TP	total power; sum of ULF, VLF, LF and HF power
TS_{pweek}	training sessions per week
tT	total testosterone
TU_{pweek}	training units per week
ULF	power in ultra low frequency band (0-0.0033 Hz)
V_E	ventilation volume
VLF	power in very low frequency band (0.0033-0.04 Hz)
VO_{2max}	maximal oxygen uptake
VO_{2rel}	oxygen uptake in relation to the body weight
V_t	tidal volume
WT	trained women
WUT	untrained women

B Appendix

Intragroup correlation coefficient in the time domain

Intragroup correlation coefficient of untrained men (MUT) presented in five different study days

MUT	Study day 1	Study day 2	Study day 3	Study day 4	Study day 5
meanNN 1	**1.00**	**0.96**	**0.91**	**0.71**	**0.92**
meanNN 2	**0.96**	**1.00**	**0.93**	**0.79**	**0.95**
meanNN 3	**0.91**	**0.93**	**1.00**	**0.81**	**0.94**
meanNN 4	**0.71**	**0.79**	**0.81**	**1.00**	**0.85**
meanNN 5	**0.92**	**0.95**	**0.94**	**0.85**	**1.00**
SDNN 1	**1.00**	**0.86**	**0.82**	**0.51**	**0.81**
SDNN 2	**0.86**	**1.00**	**0.85**	**0.44**	**0.78**
SDNN 3	**0.82**	**0.85**	**1.00**	**0.61**	**0.79**
SDNN 4	**0.51**	**0.44**	**0.61**	**1.00**	**0.62**
SDNN 5	**0.81**	**0.78**	**0.79**	**0.62**	**1.00**
RMSSD 1	**1.00**	**0.85**	**0.91**	**0.79**	**0.92**
RMSSD 2	**0.85**	**1.00**	**0.92**	**0.59**	**0.81**
RMSSD 3	**0.91**	**0.92**	**1.00**	**0.78**	**0.87**
RMSSD 4	**0.79**	**0.59**	**0.78**	**1.00**	**0.79**
RMSSD 5	**0.92**	**0.81**	**0.87**	**0.79**	**1.00**
pNN50 1	**1.00**	**0.95**	**0.88**	**0.78**	**0.89**
pNN50 2	**0.95**	**1.00**	**0.92**	**0.75**	**0.84**
pNN50 3	**0.88**	**0.92**	**1.00**	**0.80**	**0.86**
pNN50 4	**0.78**	**0.75**	**0.80**	**1.00**	**0.86**
pNN50 5	**0.89**	**0.84**	**0.86**	**0.86**	**1.00**

Intragroup correlation coefficient of trained men (MT) presented in five different study days

MT	Study day 1	Study day 2	Study day 3	Study day 4	Study day 5
meanNN 1	**1.00**	**0.93**	**0.90**	**0.91**	**0.94**
meanNN 2	**0.93**	**1.00**	**0.76**	**0.92**	**0.9**
meanNN 3	**0.9**	**0.76**	**1.00**	**0.82**	**0.87**
meanNN 4	**0.91**	**0.92**	**0.82**	**1.00**	**0.89**
meanNN 5	**0.94**	**0.9**	**0.87**	**0.89**	**1.00**
SDNN 1	**1.00**	**0.76**	**0.81**	**0.73**	**0.82**
SDNN 2	**0.76**	**1.00**	**0.64**	**0.74**	**0.79**
SDNN 3	**0.81**	**0.64**	**1.00**	**0.56**	**0.74**
SDNN 4	**0.73**	**0.74**	**0.56**	**1.00**	**0.62**
SDNN 5	**0.82**	**0.79**	**0.74**	**0.62**	**1.00**
RMSSD 1	**1.00**	**0.90**	**0.86**	**0.75**	**0.82**
RMSSD 2	**0.9**	**1.00**	**0.82**	**0.80**	**0.86**
RMSSD 3	**0.86**	**0.82**	**1.00**	**0.70**	**0.81**
RMSSD 4	**0.75**	**0.80**	**0.70**	**1.00**	**0.63**
RMSSD 5	**0.82**	**0.86**	**0.81**	**0.63**	**1.00**
pNN50 1	**1.00**	**0.91**	**0.82**	**0.83**	**0.81**
pNN50 2	**0.91**	**1.00**	**0.79**	**0.84**	**0.85**
pNN50 3	**0.82**	**0.79**	**1.00**	**0.81**	**0.88**
pNN50 4	**0.83**	**0.84**	**0.81**	**1.00**	**0.78**
pNN50 5	**0.81**	**0.85**	**0.88**	**0.78**	**1.00**

Intragroup correlation coefficient in the frequency domain

Intragroup correlation coefficient of untrained men (MUT) presented in five different study days

MUT	Study day 1	Study day 2	Study day 3	Study day 4	Study day 5
TP 1	**1.00**	**0.85**	**0.65**	**0.16**	**0.68**
TP 2	**0.85**	**1.00**	**0.65**	**0.24**	**0.73**
TP 3	**0.65**	**0.65**	**1.00**	**0.46**	**0.66**
TP 4	**0.16**	**0.24**	**0.46**	**1.00**	**0.57**
TP 5	**0.68**	**0.73**	**0.66**	**0.57**	**1.00**
VLF 1	**1.00**	**0.40**	**0.79**	**0.00**	**0.49**
VLF 2	**0.40**	**1.00**	**0.46**	**0.18**	**0.31**
VLF 3	**0.79**	**0.46**	**1.00**	**0.12**	**0.30**
VLF 4	**0.00**	**0.18**	**0.12**	**1.00**	**0.10**
VLF 5	**0.49**	**0.31**	**0.30**	**0.10**	**1.00**
LF power 1	**1.00**	**0.82**	**0.57**	**0.13**	**0.66**
LF power 2	**0.82**	**1.00**	**0.61**	**0.19**	**0.72**
LF power 3	**0.57**	**0.61**	**1.00**	**0.33**	**0.69**
LF power 4	**0.13**	**0.19**	**0.33**	**1.00**	**0.53**
LF power 5	**0.66**	**0.72**	**0.69**	**0.53**	**1.00**
HF power 1	**1.00**	**0.68**	**0.85**	**0.77**	**0.84**
HF power 2	**0.68**	**1.00**	**0.72**	**0.50**	**0.53**
HF power 3	**0.85**	**0.72**	**1.00**	**0.76**	**0.71**
HF power 4	**0.77**	**0.50**	**0.76**	**1.00**	**0.75**
HF power 5	**0.84**	**0.53**	**0.71**	**0.75**	**1.00**
LFnu 1	**1.00**	**0.85**	**0.72**	**0.67**	**0.71**
LFnu 2	**0.85**	**1.00**	**0.68**	**0.75**	**0.78**
LFnu 3	**0.72**	**0.86**	**1.00**	**0.66**	**0.79**
LFnu 4	**0.67**	**0.75**	**0.66**	**1.00**	**0.66**
LFnu 5	**0.71**	**0.78**	**0.79**	**0.66**	**1.00**

MUT	Study day 1	Study day 2	Study day 3	Study day 4	Study day 5
HFnu 1	**1.00**	**0.85**	**0.72**	**0.67**	**0.71**
HFnu 2	**0.85**	**1.00**	**0.86**	**0.75**	**0.78**
HFnu 3	**0.72**	**0.86**	**1.00**	**0.66**	**0.79**
HFnu 4	**0.67**	**0.75**	**0.66**	**1.00**	**0.66**
HFnu 5	**0.71**	**0.78**	**0.79**	**0.66**	**1.00**
LF/HF ratio 1	**1.00**	**0.86**	**0.75**	**0.79**	**0.91**
LF/HF ratio 2	**0.86**	**1.00**	**0.64**	**0.76**	**0.74**
LF/HF ratio 3	**0.75**	**0.64**	**1.00**	**0.90**	**0.82**
LF/HF ratio 4	**0.79**	**0.76**	**0.90**	**1.00**	**0.82**
LF/HF ratio 5	**0.91**	**0.74**	**0.82**	**0.82**	**1.00**

Intragroup correlation coefficient of trained men (MT) presented in five different study days

MT	Study day 1	Study day 2	Study day 3	Study day 4	Study day 5
TP 1	**1.00**	**0.66**	**0.79**	**0.48**	**0.72**
TP 2	**0.66**	**1.00**	**0.59**	**0.59**	**0.62**
TP 3	**0.79**	**0.59**	**1.00**	**0.39**	**0.65**
TP 4	**0.48**	**0.59**	**0.39**	**1.00**	**0.56**
TP 5	**0.72**	**0.62**	**0.65**	**0.56**	**1.00**
VLF 1	**1.00**	**0.53**	**0.59**	**0.62**	**0.62**
VLF 2	**0.53**	**1.00**	**0.37**	**0.49**	**0.71**
VLF 3	**0.59**	**0.37**	**1.00**	**0.26**	**0.36**
VLF 4	**0.62**	**0.49**	**0.26**	**1.00**	**0.79**
VLF 5	**0.62**	**0.71**	**0.36**	**0.79**	**1.00**
LF power 1	**1.00**	**0.56**	**0.26**	**0.67**	**0.72**
LF power 2	**0.56**	**1.00**	**0.52**	**0.45**	**0.33**
LF power 3	**0.26**	**0.52**	**1.00**	**0.15**	**0.25**
LF power 4	**0.67**	**0.45**	**0.15**	**1.00**	**0.46**
LF power 5	**0.72**	**0.33**	**0.25**	**0.46**	**1.00**
HF power 1	**1.00**	**0.86**	**0.82**	**0.41**	**0.70**
HF power 2	**0.86**	**1.00**	**0.79**	**0.61**	**0.79**
HF power 3	**0.82**	**0.79**	**1.00**	**0.60**	**0.62**
HF power 4	**0.41**	**0.61**	**0.60**	**1.00**	**0.28**
HF power 5	**0.70**	**0.79**	**0.62**	**0.28**	**1.00**
LFnu 1	**1.00**	**0.55**	**0.49**	**0.33**	**0.49**
LFnu 2	**0.55**	**1.00**	**0.63**	**0.63**	**0.68**
LFnu 3	**0.49**	**0.63**	**1.00**	**0.65**	**0.71**
LFnu 4	**0.33**	**0.63**	**0.65**	**1.00**	**0.69**
LFnu 5	**0.49**	**0.68**	**0.71**	**0.69**	**1.00**

MT	Study day 1	Study day 2	Study day 3	Study day 4	Study day 5
HFnu 1	**1.00**	**0.55**	**0.49**	**0.33**	**0.49**
HFnu 2	**0.55**	**1.00**	**0.63**	**0.63**	**0.68**
HFnu 3	**0.49**	**0.63**	**1.00**	**0.65**	**0.71**
HFnu 4	**0.33**	**0.63**	**0.65**	**1.00**	**0.69**
HFnu 5	**0.49**	**0.68**	**0.71**	**0.69**	**1.00**
LF/HF ratio 1	**1.00**	**0.58**	**0.5**	**0.50**	**0.72**
LF/HF ratio 2	**0.58**	**1.00**	**0.23**	**0.40**	**0.32**
LF/HF ratio 3	**0.50**	**0.23**	**1.00**	**0.58**	**0.77**
LF/HF ratio 4	**0.50**	**0.40**	**0.58**	**1.00**	**0.59**
LF/HF ratio 5	**0.72**	**0.32**	**0.77**	**0.59**	**1.00**

SPECIAL THANKS TO

ANDREAS-ANDREAS M-CIARON-ELLEN-MARC-
MICHAEL-MY FAMILY-MY FRIENDS