ASSOCIATIONS OF AUTONOMIC NERVOUS SYSTEM WITH BLOOD PRESSURE AND HEART RATE RESPONSES DURING EXERCISE

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TIIVISTELMÄ

Haapakangas, S. 2020. Autonomisen hermoston toiminnan yhteys verenpaineeseen ja sykkeeseen liikunnan aikana. Liikuntatieteellinen tiedekunta, Jyväskylän yliopisto, liikuntalääketieteen pro gradu -tutkielma, 65 s., 2 liitettä.

Korkea verenpaine ja tyypin 2 diabetes ovat riskitekijöitä lukuisille sairauksille sekä kuolleisuudelle ja ovat yhteydessä autonomisen hermoston toiminnan häiriöihin. Liikunnanaikainen liiallinen verenpaineen kohoaminen, hidas palautuminen liikunnan jälkeen ja sykkeen epätavallinen käyttäytyminen ovat merkkejä autonomisen hermoston toiminnan häiriöistä. Sykevälivaihtelua (HRV) käytetään yleisesti kuvaamaan autonomisen hermoston tilaa. Tämän tutkimuksen tarkoitus oli tutkia yhteyksiä autonomisen hermoston toiminnan ja liikunnanaikaisen liiallisen verenpaineen nousun sekä sykekäyttäytymisen välillä.

Tämä tutkimus tehtiin osana Healthbeat-tutkimusta (”*Kunto, uni ja stressi diabetes- ja verenpainetautipotilailla*”), joka toteutettiin vuosina 2019-2020. Tutkimuspopulaatio koostui 18-64-vuotiaista, joilla oli todettu diabeteksen esiaste ja/tai diagnosoitu tyypin 2 diabetes viimeisen 5 vuoden sisällä ja/tai verenpainetauti. Tämän tutkimuksen otanta oli 28 henkilöä keski-iältään 54,4 vuotta. Korkea verenpaine oli diagnosoitu 23 (82,1 %), tyypin 2 diabetes 4 (14,3%) ja esidiabetes 10 henkilöllä (35,7%) osallistujista. Tutkimuksen data analysoitiin IBM SPSS Statistics (26.0) – ohjelmistolla. Analysointimenetelminä käytettiin t-testiä, ristiintaulukointia, Khiin neliö – testiä ja Mann Whitneyn U-testiä. Autonomisen hermoston toimintaa analysoitiin nousujohteisen maksimaalisen rasitustestin yhteydessä ryhmien välillä, jotka oli jaettu liikunnan aikaisen maksimaalisen systolisen verenpaineen (SBP) ja ikävakioidun maksimaalisen sykkeen mukaan. Lisäksi 72-tunnin HRV mittausta vertailtiin samoissa ryhmissä.

Parasympaattisen hermoston aktivaatio liikunnan jälkeen (5 min palautusjakson HF-arvo (ms^2)) oli ei-merkitsevästi nopeampaa ryhmässä, jossa liikunnan aikainen systolinen verenpaine oli matalampi (p=0,125). Korkeamman verenpaineen ryhmässä esiintyi enemmän diabeteksen esiasteita (p=0,036), liikunnan jälkeinen verenpaine oli korkeampi (1 min p=0,001, 3 min p=0,041) ja syke palautui liikunnasta ei-merkitsevästi hitaammin (1 min p=0,130, 3 min p=0,113). Korkeampi ikävakioitu syke liikunnan aikana oli yhteydessä alhaisempaan painoon (p=0,007), pienempään kehon painoindeksiin (BMI) (p=0,007), korkeampaan HDL-kolesterolipitoisuuteen (p=0,039) ja suurempaan sykereserviin (p=0,044).

Yhteenvetona alhaisemmat liikunnan aikaiset systolisen verenpaineen arvot voivat merkitä korkeampaa parasympaattisen hermoston toimintaa, kun indikaattoreina käytetään HRV:tä, liikunnan jälkeisiä SBP-arvoja ja sykevasteita. Korkeampi liikunnan aikainen ikävakioitu syke oli yhteydessä positiivisiin terveysmarkkereihin.

Asiasanat: Verenpaine, sydämen syke, autonominen hermosto, sykevälivaihtelu, rasituskoe

ABSTRACT

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Hypertension and type 2 diabetes are risk factors for morbidities and mortality and are associated with the autonomic nervous system (ANS) dysfunction. Dysfunction of the ANS represents itself as an exaggerated blood pressure response during and after exercise. Abnormal heart rate responses during and after exercise are a sign of imbalanced ANS as well. Heart rate variability (HRV) is commonly used to evaluate the state of the ANS. The aim of this study was to evaluate the associations of autonomic nervous system with blood pressure and heart rate responses during exercise.

This thesis was a part of the Healthbeat study ("*Kunto, uni ja stressi diabetes- ja verenpainetautipotilailla*") conducted in 2019-2020. Study population consisted of 18- to 64-year-old individuals with prediabetes and/or type 2 diabetes diagnosed during last five years and/or diagnosed hypertension. This study included 28 participants with a mean age of 54.4. High blood pressure was diagnosed with 23 (82.1%) of the participants, type 2 diabetes with 4 (14.3%) and prediabetes with 10 (35.7%) of the participants. The data were analyzed with the IBM SPSS Statistics (26.0) – software. T-test, crosstabs, Chi square ($\chi 2$) – test and Mann-Whitney-test were used as the analyzing methods. The participants were divided to groups according to the highest exercise-induced systolic blood pressure (SBP) response as well as the highest age-adjusted heart rate (HR) response during the maximal graded exercise test. The ANS function during the graded maximal exercise test was analyzed between groups. 72-hour recording of HRV values were compared within the groups.

Activation of the parasympathetic nervous system after exercise (5 min post exercise HF value (ms^2)) was non-significantly higher in the group with lower SBP values during exercise (p=0.125). Higher SBP group had a higher prevalence of prediabetes (p=0.036), higher post-exercise SBP (1 min p=0.001, 3 min p=0.041) and non-significantly slower heart rate recovery (1 min p=0.130, 3 min p=0.113) than lower SBP group. Higher age-adjusted HR was associated with lower body weight (p=0.007), lower body mass index (BMI) (p=0.007), higher serum HDL-cholesterol value (p=0.039) and higher HR reserve (p=0.044).

In summary, lower SBP values during maximal exercise may indicate higher parasympathetic domination when measured with HRV, post-exercise SBP and HR responses. Higher age-adjusted HR was associated with positive health indicators.

Key words: Blood pressure, heart rate, autonomic nervous system, heart rate variability, exercise test

ABBREVIATIONS

ANS	Autonomic nervous system
BP	Blood pressure
CAN	Cardiovascular autonomic neuropathy
CRF	Cardiorespiratory fitness
CVD	Cardiovascular diseases
DBP	Diastolic blood pressure
HR	Heart rate
HRV	Heart rate variability
PNS	Parasympathetic nervous system
SBP	Systolic blood pressure
SCD	Sudden cardiac death
SNS	Sympathetic nervous system

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1 INTRODUCTION

Hypertension is the main risk factor for the reduction of the healthy life years worldwide (Hypertension: Current Care Guidelines 2014). WHO (2019) has estimated that 1,13 billion people around the world have hypertension. Hypertension causes 9,4 million premature death every year (Hypertension: Current Care Guidelines 2014) and raises substantially the risk of heart, brain, kidney and other diseases (WHO 2019). Two million Finnish adults have hypertension and only fifth of them have hypertension under control (<140/90 mmHg) (Hypertension: Current Care Guidelines 2014). In addition to hypertension, type 2 diabetes affects hundreds of millions of people worldwide and is a risk factor for mortality and cardiovascular morbidity (Maser et al. 2003, Pop-Busui et al. 2010; Spallone et al. 2011; Cho et al. 2018). Hypertension, overweight and metabolic syndrome are common comorbidities with type 2 diabetes (Type 2 diabetes: Current Care Guidelines 2018). Risk for atherosclerosis is elevated already in prediabetic state (Syvänne 2017).

Autonomic nervous system (ANS), which consists of sympathetic and parasympathetic branches, regulates blood pressure together with local mediators (DeMers & Wachs 2019). Dysfunction of the ANS system is associated with the progression of the hypertension (Mancia & Grassi 2014). Imbalanced ANS manifest itself as an overly activated sympathetic nervous system and impaired parasympathetic activity (Mancia & Grassi 2014; Shaffer & Ginsberg 2017). The ANS has a crucial role of regulating the cardiovascular adjustment to exercise (Fadel 2015; Fisher et al. 2015). If the ANS is not in balance it can manifest itself as an exaggerated blood pressure response during exercise, which is also associated with heightened CVD risk as well as mortality (Schultz et al. 2013; Schultz et al. 2017; Dombrowski et al. 2018). Other mechanisms are related to the ANS dysfunction as well such as blunted heart rate during exercise or slow reduction of heart rate and blood pressure after exercise (Diller et al. 2006; Le et al. 2008; Brubaker & Kitzman 2011; Jae et al. 2016).

Heart rate variability (HRV), which means the change in the intervals between consecutive heartbeats, is used as an indicator of the ANS balance between the sympathetic and parasympathetic activity (Shaffer et al. 2014; Shaffer & Ginsberg 2017; Fatisson et al. 2016 Laukkanen et al. 2019). The behavior of HRV is associated with health and physical fitness (Stanley et al. 2013; Shaffer & Ginsberg 2017). Low HRV is associated with the increased risk for poor health outcomes such as hypertension, cardiovascular events, and sudden cardiac death (SCD), whereas higher HRV is related to health and higher fitness levels (Shaffer & Ginsberg 2017; Stanley et al. 2013). Investigating HRV responses during and after exercise might reveal the dysregulation of the ANS system (Fisher et al. 2015; Michael et al. 2017). Imbalanced ANS is associated to the risk population such as hypertensive and diabetic patients (Singh et al. 1998; Röhling et al. 2017a; Fatisson et al. 2016).

The aim of this thesis is to study the associations of the autonomic nervous system to blood pressure and heart rate responses during exercise. The associations of blood pressure, heart rate and HRV might reveal the state of the ANS and predict future health outcomes. Understanding the complex mechanism related to the autonomic regulation of the cardiovascular system might help to develop new future health promotion strategies since hypertension and type 2 diabetes can be affected via lifestyle modifications.

2 HYPERTENSION AND DIABETES

2.1 Hypertension

Blood pressure (BP) is reported as a systolic (SBP) and diastolic blood pressure (DBP) as well as mean arterial pressure (MAP). The cardiac cycle alternate between systole (ventricular contraction) and diastole (ventricular relaxation) (Shaffer et al. 2014). Systolic BP means this peak value while diastolic BP is measured when the BP is lowest as the left ventricle relaxes (Shaffer et al. 2014).

Cardiac output and systemic vascular resistance affect the magnitude of the BP (DeMers & Wachs 2019). The higher cardiac output and total peripheral resistance will create the rise in the BP (Fisher et al. 2015). Peripheral resistance or systemic vascular resistance is determined by the radius of the blood vessels (DeMers & Wachs 2019). Regulation of the resistance works through vasoconstriction and dilation of the blood vessels and is mediated by the local mediators and autonomic nervous system (ANS) (DeMers & Wachs 2019).

Hypertension generally does not result in observable symptoms but can cause a serious damage to heart among other complications (WHO 2019). It is one of the most common risk factors for cardiovascular morbidity and mortality (Le et al. 2008; Benjamin et al. 2017). Negative effects of hypertension are multifold if it is combined to other cardiovascular disease risk factors and even mildly elevated blood pressure can increase risk for cardiovascular diseases significantly (Hypertension: Current Care Guidelines 2014).

Hypertension is preventable with lifestyle modifications and physical activity (PA) has been proved to be effective way of reducing blood pressure among adults with normal BP, prehypertension and hypertension (Whelton et al. 2017; Pescatello et al. 2019). There are multiple risk factors for hypertension such as obesity, sedentary lifestyle, smoking, insulin resistance, dyslipidemia, high salt and alcohol intake, stress and increased age (Carretero & Oparil 2000; Whelton et al. 2017). Comorbidities of hypertension are cardiovascular diseases (CVD), diabetes mellitus, congestive heart failure and metabolic syndrome to name a few

(Whelton et al. 2017; Pescatello et al. 2019). Currently, blood pressure thresholds of hypertension are 140 mmHg for SBP and 90 mmHg for DBP (Hypertension: Current Care Guidelines 2014) but even lower values (130/80 mmHg) have been suggested in the Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines (Whelton et al. 2017).

Dysfunctional autonomic cardiovascular control is common hypothesis behind the progression of hypertension (Mancia & Grassi 2014). Especially overly activated sympathetic nervous system may have a role in hypertension as well as other cardiovascular diseases and it is likely accompanied by an impaired vagal activity (Mueller 2007; Mancia & Grassi 2014). Thus, altered autonomic nervous system may have a causative role in the development of hypertension (Mancia and Grassi 2014). Regular exercise may have a positive effect on resting sympathetic activation by reducing it (Grassi et al. 1994; Mueller 2007).

Sympathetic activity and arterial pressure during exercise may increase excessively, which can increase risk for adverse effects such as myocardial infarctions, myocardial ischemia, arrhythmia and stroke (Mittleman et al. 1993; Dombrowski et al. 2018). The mechanisms that cause exaggerated sympathetic response with hypertensive subjects is not clearly understood (Dombrowski et al. 2018). Therefore, it is important to understood mechanism and relationships behind the dysfunctional autonomic nervous system and its' effects on high blood pressure during exercise and everyday life.

2.2 Blood pressure during exercise

During exercise hypertensive subjects can experience very high arterial pressure to such levels that the heavy exercise is often not recommended (Fletcher et al. 2013; Dombrowski et al. 2018). Normal SBP response to a graded exercise testing is increase in a curvilinear fashion and a plateau at the peak or maximal effort (Schultz et al. 2017). This response is due to sympathetic nervous system activation, elevated heart rate and rising cardiac output (Le et al. 2008; Schultz et al. 2017). During exercise peripheral resistance drops but not enough to overcome the rise of SBP (Schultz et al. 2017). Normal diastolic response to exercise is a slight drop or remaining unchanged due to the physiological decline in vascular resistance (Fletcher et al. 2013).

Low or exaggerated BP response are abnormal during exercise (Schultz et al. 2017). Significant deviations from normal values can indicate a failure in body's response to exercise and be a marker of CVD (Le et al. 2008). Low BP or exercise-induced hypotension (EIH) is an established sign of existing and probably severe CVD such as coronary artery disease, severe left ventricular dysfunction and aortic outflow obstruction (Fletcher et al. 2013; Schultz et al. 2017; Le et al. 2008).

An exaggerated exercise BP is related to heightened CVD risk as well as mortality and it is associated with sub-clinical hypertension (Schultz et al. 2013; Schultz et al. 2017). Hypertensive patients often have high exercise BP regardless, if the resting BP levels are controlled (Fletcher et al. 2013). In addition, excessive elevation of SBP can be seen among individuals with normal office BP and with antihypertensive medication (Schultz et al. 2013; Schultz & Sharman 2014).

BP ≥210/110 mmHg for males and ≥190/110 mmHg for females have been used to define excessive response to exercise at maximal or peak intensities (Schultz et al. 2017). Values over these thresholds at moderate and high intensity exercise levels are linked to heightened risk for cardiovascular events as well as mortality (Schultz et al. 2013). Values over 250/115 mmHg are an indicator for exercise test termination (Fletcher et al. 2013).

Prevalence of excessive BP response is higher among those with known CVD risk factors such as type 2 diabetes (Scott et al. 2008; Schultz et al. 2011; Schultz et al. 2017). Excessive exercise BP is associated with impaired vascular function including abnormal endothelial function as well as increased arterial stiffness (Thanassoulis et al. 2012). Impaired vascular function negatively affects the ability to compensate for the increased cardiac output, hence, resulting in rise of the BP (Thanassoulis et al. 2012). One of the proposed mechanisms behind the exaggerated exercise BP is excessive high sympathetic tone during exercise, which promotes vasoconstriction of the blood vessels **(**Schultz & Sharman 2014).

Higher post exercise SBP (≥163 mmHg after 5 minutes) is associated with higher prevalence of abnormal lipid profiles, hypertension, ischemic heart and cerebrovascular diseases (Yosefy et al. 2006). Laukkanen et al. (2014) found out that men with SBP over 195 mmHg at 2 minutes recovery was associated to 1.74-fold risk of a sudden cardiac death compared to men with SBP less than 170 mmHg. In addition, high percentage maximum SBP at 2 minutes after exercise (SBP at 2 minutes recovery divided by the maximum SBP) was associated with the risk of all strokes and ischemic strokes in study performed by Kurl et al. (2001). They proposed that impaired SBP decrease from peak value to rest might indicate increased vascular resistance.

Cardiorespiratory fitness is an important modifier of exercise BP response (Schultz et al. 2017). Both normotensive and hypertensive subjects experience lower SBP responses during exercise with higher fitness levels (Kokkinos et al. 2002; Kokkinos et al. 2007). However, fitness and SBP level during exercise may follow J-curve according to Prasad et al. (2015). The authors found that a poor fitness level produced the highest exercise SBP, but a high exercise SBP was also seen with highly fit men. This highlights the problems associated with the interpretation of maximal SBP as a health risk factor.

After exercise parasympathetic neural activity increases while sympathetic decreases which will drop heart rate and peripheral resistance (Fisher et al. 2010; Fisher et al. 2015). This will decrease SBP rapidly and it may remain below normal values for several hours after exercise (Le et al. 2008). A greater decrease of SBP after exercise can be a sign of good cardiorespiratory fitness and decreased vascular resistance after exercise (Laukkanen et al. 2014).

Patient with cardiac disease have frequently abnormal left ventricular function (Le et al. 2008) which can cause hypoperfusion to skeletal muscles (Kitaoka et al. 1995). Due to this the skeletal muscles can produce catecholamine-stimulating metabolites, which can induce sympathetic response resulting persistent vasoconstriction and diminished parasympathetic tone during recovery (Kitaoka et al. 1995; Le et al. 2008). This ANS dysfunction and increased vascular resistance may result to slower decrease in SBP after exercise (Le et al.

2008). In conclusion, both hypo- and hypertensive responses to exercise as well as abnormal recovery values have been linked to negative cardiovascular events, which reflects underlying ANS and endothelial dysfunction (Le et al. 2008).

2.3 Diabetes

Diabetes is one of the fastest growing diseases in Finland and worldwide (Type 2 diabetes: Current Care Guidelines 2018). There are over half a million diabetics in Finland (Type 2 diabetes: Current Care Guidelines 2018). Diabetes (type 1 and 2) is a risk factor for atherosclerosis and prognostic for coronary artery disease is more severe compared to other population (Syvänne 2017). Diabetes can be divided to type 1 and type 2 according to etiology, but there are other types as well (Type 2 diabetes: Current Care Guidelines 2018). Risk for atherosclerosis is elevated already in prediabetic states with impaired glucose tolerance and/or impaired fasting glucose (Syvänne 2017). Impaired fasting glucose is diagnosed if blood sugar levels are between 6,1-6,9 mmol/l after a minimum 8 hours of fasting (Ilanne-Parikka 2018). The diagnose of impaired glucose tolerance requires a glucose tolerance test (Ilanne-Parikka 2018). If blood sugar values after two hours are between 7,8-11,0 mmol/l, impaired glucose tolerance is diagnosed (Ilanne-Parikka 2018).

Diabetic neuropathies belong to the common complications of diabetes (Pop-Busui et al. 2017). Cardiovascular autonomic neuropathy (CAN) is a one form of diabetic neuropathies (Pop-Busui et al. 2017) and it increases with diabetes duration and age (Spallone et al. 2011). Prevalence is around 20%, but it may be present in up to 50-65% with long-standing type 2 diabetes (Spallone et al. 2011; Mustajoki 2019a). CAN is a risk factor for increased mortality and cardiovascular morbidity (Maser et al. 2003, Pop-Busui et al. 2010; Spallone et al. 2011). Association to mortality is stronger among diabetic patients with more severe autonomic dysfunction related to CAN (Maser et al. 2003). The symptoms of CAN include an impaired HRV and a higher resting heart rate (Pop-Busui et al. 2017). Higher resting heart rate is associated with increased risk of cardiovascular events and all-cause mortality (Lonn et al. 2014).

Diabetic patients with autonomic neuropathy show abnormalities in HRV values, which can be a sign of disturbed sympathovagal balance (Vinik & Ziegler 2007). Reduced HRV is seen already at the beginning of the cardiac autonomic dysfunction with type 1 and type 2 diabetes (Röhling et al. 2017b). Disturbed vagal and sympathetic modulation may lead to malfunction of cardiorespiratory system, which manifest itself in reduced cardiorespiratory fitness (CRF) (Vinik & Ziegler 2007). Exercise can improve cardiac autonomic function especially patients with type 2 diabetes (Röhling et al. 2017a).

If ANS activity is toward sympathetic predominance and vagal activity is diminished, it is linked to poor health outcomes such as metabolic syndrome and greater risk for cardiovascular events (Vinik & Ziegler 2007; Stuckey et al. 2014; Shaffer & Ginsberg 2017). Insulin resistance may be one of the reasons linking metabolic syndrome and autonomic dysfunction since risk factors of metabolic syndrome are linked to insulin resistance (Stuckey et al. 2014). Röhling et al. (2017) found that recently diagnosed patients with type 1 and type 2 diabetes show signs of cardiac autonomic dysfunction measured with HRV values. They suggest that HRV is impaired at the recent-onset diabetes due to early glucometabolic disturbances.

3 CARDIOVASCULAR ADJUSTMENTS TO EXERCISE

Cardiovascular and hemodynamic adjustments are necessary during exercise to ensure both the oxygen supply to the muscles and adequate perfusion pressure to vital organs such as the brain (Fadel 2015; Joyner & Casey 2015). During exercise the cardiac output can rise to 25-30 l/min compared to resting 5 l/min (Kiilavuori 2014). Normally functioning ANS is important for correct cardiac response during exercise (Fisher et al. 2015*)*. ANS includes the sympathetic and parasympathetic branches, which are responsible for many cardiovascular responses during exercise in an intensity-dependent manner including increase in cardiac output, skeletal muscle blood flow and arterial blood pressure (Fadel 2015).

The sympathetic nervous system (SNS) dominates during exercise and prepares body for a strenuous physical activity as well as increases the blood flow to the working muscles (McCorry 2007). The parasympathetic nervous systems (PNS) dominates during rest and digest conditions (McCorry 2007). The most important part of the PNS is vagus nerve (McCorry 2007). 75% of the parasympathetic fibers are in the vagus nerve (McCorry 2007). Therefore, the PNS is often referred as a vagal tone or vagal activation.

3.1 Heart rate

Heart rate (HR) rises rapidly at the beginning of the exercise, which has been suggested to occur due to combined actions of central command and the muscle mechanoreflex resulting in the withdrawal of parasympathetic activation (Fisher et al. 2015). The sympathetic contribution to HR manifests with the longer latency but increases with higher workloads as HR exceeds around 100 beats/min (Fisher et al. 2015).

HR recovery after exercise is faster with trained individuals, whose parasympathetic activation is elevated (Imai et al. 1994; Du et al. 2005). The reduction of HR is a result of the restoration of parasympathetic activation **(**Imai et al 1994). Rapid recovery of HR can be an important mechanism for avoiding excessive cardiac loading after exercise (Imai et al. 1994). If the parasympathetic activation is delayed and reduction of sympathetic activation is slower after exercise, cardiac output remains higher and preserves perfusion pressure (Fisher et al. 2015). Slower HR recovery (e.g. fall from peak exercise HR is ≤ 12 beats/min in recovery) is a predictor of increase risk of all-cause mortality (Cole et al. 1999). The rate of the HR recovery after exercise seems to be directly associated with the magnitude of the parasympathetic activity (Brubaker & Kitzman 2011).

In addition, chronotropic incompetence (CI), which means heart´s inability to increase its rate during increased physical activity or demand is an independent predictor of cardiovascular events or overall mortality (Brubaker & Kitzman 2011). Chronotropic incompetence is a common state in patients with cardiovascular disease (Brubaker & Kitzman 2011). Failure to achieve maximal HR during exercise is a sign of impaired chronotropic response (Brubaker & Kitzman 2011). It has been well established that there is an age-related decrease with maximum HR response to exercise (Ozemek et al. 2015). Traditional equation to predict one's maximal HR is 220 bpm – age, but other equations are available as well (Tanaka et al. 2001). However, Ozemek et al. (2015) found out an inverse association between cardiovascular fitness and rate of decline with HR peak during exercise. Individuals with higher fitness level had a slower rate of decline with HR peak values, but this finding contrasts with many previous reports (Ozemek et al. 2015).

CI can also be determined with heart rate reserve (HRR) (Brubaker & Kitzman 2011). HRR means the difference between maximal HR and resting HR during the exercise test (Brubaker & Kitzman 2011). If the HR during exercise failures to attain 80% of the age predicted maximum HR or HRR, it is a criterion for the CI (Brubaker & Kitzman 2011). Exercise training can give favorable results to chronotropic function such as decreased resting HR and more rapidly recovering post-exercise HR (Brubaker & Kitzman 2011). These changes can indicate alteration in balance of sympathetic and parasympathetic nervous system (Brubaker & Kitzman 2011).

Abnormal HR response to exercise is related to autonomic dysfunction (Diller et al. 2006). Blunted HR response to exercise was predictor for higher mortality among patients with adult congenital heart disease (Diller et al. 2006). In addition, attenuated heart rate recovery after exercise was an important prognostic marker for mortality in same study population (Diller et al. 2006). Jae et al. (2016) found out that exercise heart rate reserve as well as recovery predicted incidence of type 2 diabetes among healthy men indicating that autonomic dysfunction may be associated with development of type 2 diabetes.

3.2 Autonomic nervous system

The brainstem in the brain collects sensory information (Shaffer et al. 2014). Through this regulation center happens adjustment of heart rate and blood pressure via sympathetic and parasympathetic efferent pathways (Shaffer et al. 2014). Both branches are tonically active and can work together simultaneously (McCorry 2007; Shaffer & Ginsberg 2017). The sympathetic and parasympathetic outflow can accelerate or slower the heart rate, respectively, and the HR estimated at any given time represents the net effect of neural outflow (Shaffer et al. 2014).

In healthy subjects the reflexes that mediate the cardiovascular responses to exercise are the following:

- central command (originated form the cerebral cortex and/or subcortical nuclei),
- arterial baroreflex (a negative feedback mechanism from the carotid sinus and aortic arch),
- cardiopulmonary baroreflex (a negative feedback mechanism originated form the heart, great veins and blood vessel of the lungs),
- exercise pressor reflex (stimulation of skeletal muscle mechano-sensitive and metabo-sensitive afferents),
- carotid chemoreflex respiratory metaboreflex (Figure 1) (Dombrowski et al. 2018; Fadel 2015).

All the neural mechanisms are capable of modulating the autonomic adjustments to exercise and they operate in an intensity-dependent manner (Fisher et al. 2015).

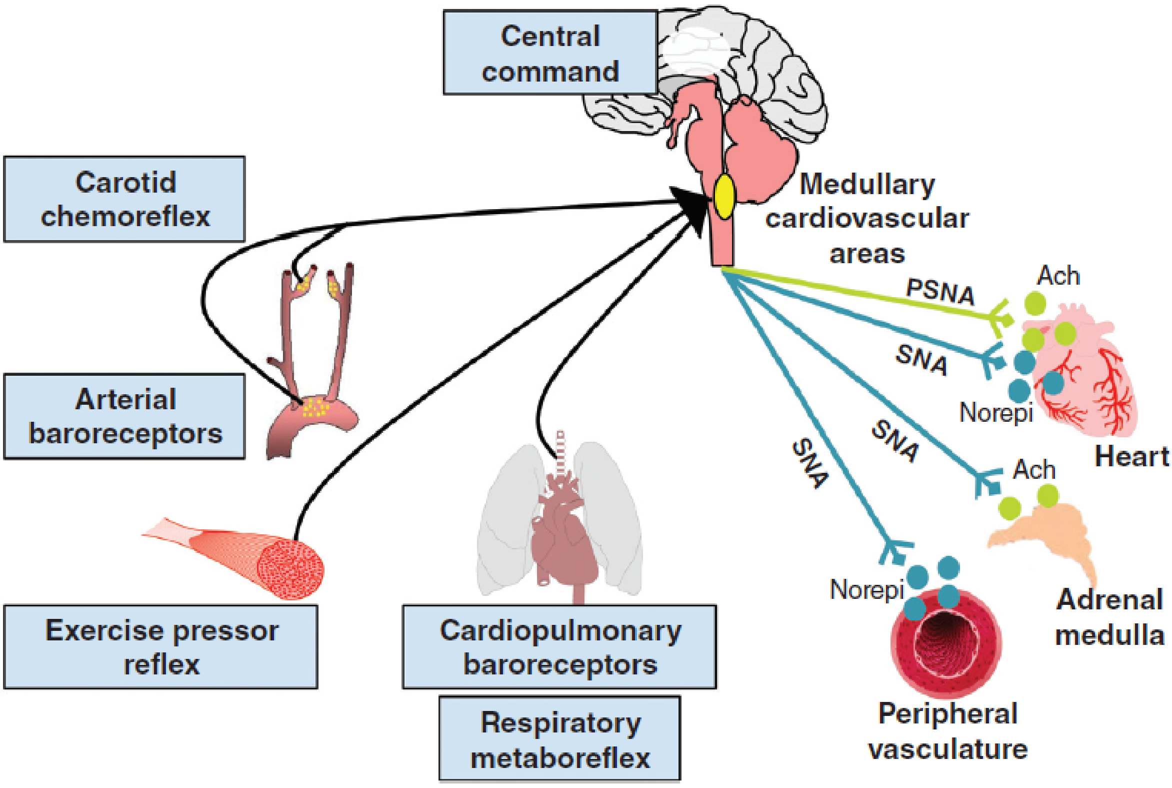


FIGURE 1. Summarization of the mechanism involved mediating the autonomic nervous system adjustments during exercise (Fisher et al. 2015). (SNA=Sympathetic nervous activity, PSNA=Parasympathetic nervous activity, Ach=Acetylincholine, Norepi=norepinephrine)

As depicted in Figure 1, brain cardiovascular control area commands the sympathetic and the parasympathetic branches according to signals from central command, arterial baroreceptors, carotid chemoreflex, exercise pressor reflex, cardiopulmonary baroreflex and respiratory metaboreflex. These ANS functions evoke changes in the cardiac and vascular functioning and release catecholamines from the adrenal medulla.

3.3 Autonomic nervous system during exercise

Exercise causes immediate changes to the function of autonomic nervous system termed by central command (Dombrowski et al. 2018). Figure 2 illustrates the autonomic nervous system activation and its influences on HR during exercise. In the beginning the parasympathetic activity is reduced by the central command and feedback from muscle mechanoreceptors allowing higher heart rate and cardiac output (Dombrowski et al. 2018; Fisher et al. 2015). As exercise continues the sympathetic nerve system (SNS) activity is increased by the central command and feedback from the muscle metaboreceptors (Fadel 2015; Fisher et al. 2015). This will contribute to the rise of the HR (Fisher et al. 2015). After the exercise the vagal activation is immediate, and it will rapidly lower the heartrate. During the recovery phase vagal activation continues to increase while the SNS decreases.

The contribution of sympathetic and parasympathetic activity, influence of central command, feedback from muscle metaboreceptors and mechanoreseptors (muscle tetanoreceptors) is shown in Figure 2.

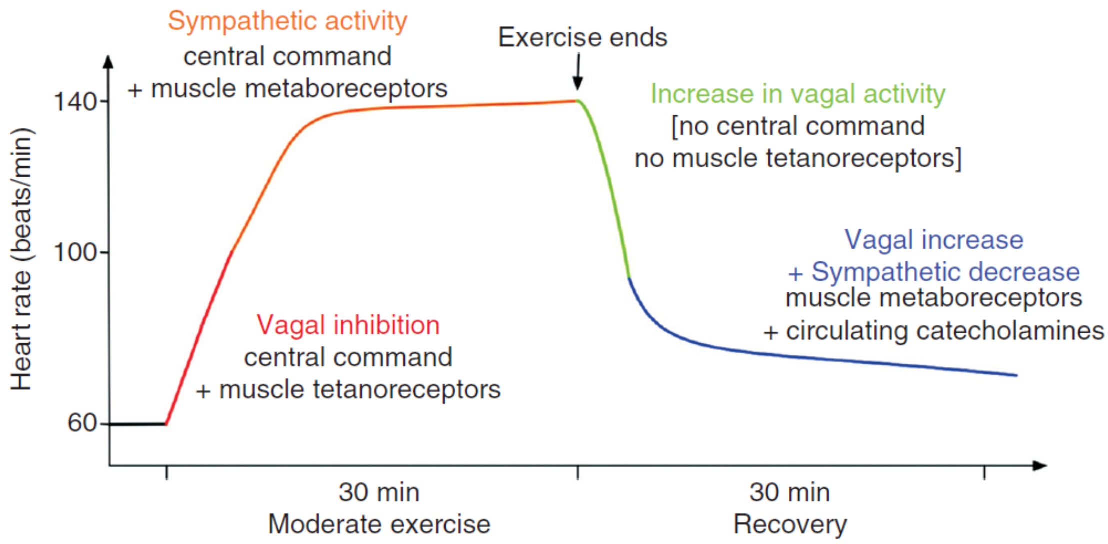


FIGURE 2. Summary of the neural control mechanism that affect the HR response during different phases of exercise (Coote 2010).

In addition to HR variations, exercise has a large effect on BP through ANS functioning. During exercise the systolic blood pressure and mean arterial pressure increase due to the vasoconstriction of the blood vessels via SNS and rising of the cardiac output (Kiilavuori 2014). The SNS causes vasoconstriction in non-exercise muscle and visceral organs (Fadel 2015). The cardiac output is directed to the exercising skeletal muscles by the metabolic modulation of sympathetic vasoconstrictor activity in the active muscles as well as due to higher blood pressure (Fisher et al. 2015; Kiilavuori 2014). The systemic blood pressure rises during exercise despite of the vasodilation in the active muscles (Joyner & Casey 2015). Abnormal autonomic adjustments during exercise are a sign of dysregulation of the mentioned neural mechanism and this is involved with many cardiovascular disease states such as hypertension (Fisher et al. 2015).

3.4 Exercise pressor reflex and baroreflexes

The mechanoreflex is activated by mechanical deformation such as changes in pressure or stretch at the immediate onset of muscle contraction (Fadel 2015; Fisher et al. 2015). The metaboreflex comprises afferent neurons that include chemically sensitive receptors (Fadel 2015). Both mechano- and metaboreflex are important part of the normal blood pressure (BP) response during exercise (Fadel 2015; Raven et al. 2019). The activation of mechanically sensitive muscle afferents can increase HR via inhibition of cardiac parasympathetic activity (Fisher et al. 2015).

Arterial baroreceptors (ABR) are mechanically sensitive receptors in aortic arch and carotid sinuses, and function as the afferent sensors in a negative feedback loop that is based on beat-to-beat changes in BP (Kougias et al. 2010; Fadel 2015; Dombrowski et al. 2018). These receptors affect to cardiac output and total vascular conductance via alteration of autonomic neural outflow and they are the main regulators of BP (Fadel 2015, Dombrowski et al. 2018, Raven et al. 2019).

Alterations in BP causes conformation in the baroreceptors which leads to changes in afferent neuronal firing (Fisher et al. 2015; Raven et al. 2019). Inhaling and exhaling changes HR and through this creates short-term changes to baroreceptors activity according acceleration and deceleration of HR (Shaffer et al. 2014; Shaffer & Ginsberg 2017). Rising BP will stretch the receptors and cause an increase in afferent neuronal feedback (Fisher et al. 2015; Raven et al. 2019). This will increase parasympathetic activity and decrease of the sympathetic outflow to the peripheral vessels and the heart to lower the BP (Kougias et al. 2010; Shaffer et al. 2014; Raven et al. 2019). The result is reduction of the HR, cardiac output and total peripheral resistance, which will reduce BP back to setpoint value (Raven et al. 2019). This reflex-mediated regulation mechanism works both ways reducing and increasing BP according to afferent signals (Fisher et al. 2015).

The arterial baroreceptors have an ability to reset themselves to work around exercise-induced elevation in BP with maintained sensitivity (Fisher et al. 2015; Raven et al. 2019). If the sensitivity of the arterial baroreflex is impaired it will lead to altered neural cardiovascular responses during exercise (Fisher et al. 2015). Baroreflex function in hypertension may be attenuated which affects its ability to restrain pressor responses from the muscle metaboreflex and in turns allows exaggerated increase in blood pressure during exercise (Dombrowski et al. 2018). Impaired baroreflex control is commonly associated with high blood pressure (Mancia & Grassi 2014). In addition, a cardiopulmonary baroreflex has a role in alteration of the SNS, the BP and ABR resetting during exercise (Fadel 2015; Raven et al. 2019). The loading of these receptors will exert an inhibition of the SNS during dynamic exercise (Raven et al. 2019).

4 HEART RATE VARIABILITY

Heart rate variability (HRV) is commonly used as an index of health and it corresponds to the adaptation of the heart during any stimulus (Fatisson et al. 2016). HRV represent heart's ability to be responsive and resilient (Shaffer et al. 2014). Reduction of HRV correlates with disease and mortality, which may be due reduced regulatory capacity meaning ability to adapt stressors like exercise and stress (Shaffer et al. 2014). Low HRV is seen in patients with reduced cardiac regulatory capacity and increased likelihood of prior myocardial infraction (Shaffer et al. 2014) when an optimal level of HRV is linked to health and resilience (Shaffer & Ginsberg 2017).

Low HRV is associated with increased risk for hypertension, cardiovascular events, and sudden cardiac death (SCD) (Singh et al. 1998; La Rovere et al. 2003; Hillebrand et al. 2013). Pathophysiology of SCD involves autonomic nervous system imbalance with reduced vagal tone or possibly higher sympathetic tone (Maheshwari et al. 2016). ANS dysfunction can be considered as a critical process that manifests itself with wide range of symptoms of poor health (Shaffer & Ginsberg 2017).

HRV values can be used as an indicator of the autonomic nervous system balance between parasympathetic and sympathetic nervous system (Fatisson et al. 2016; Laukkanen et al. 2019). Time and frequency domain of the HRV is commonly used as an indirect and noninvasive method to assess sympathetic and parasympathetic activity (Michael et al. 2017).

4.1 HRV as a marker for ANS function

HRV means the change in the intervals between consecutive heartbeats and all HRV values are calculated from interbeat intervals (IBI) (Shaffer et al. 2014, Shaffer & Ginsberg 2017). The heart operates according to sympathetic and parasympathetic outflow and heart-brain interactions all of which contribute to beat-to-beat changes (Shaffer et al. 2014). HRV is affected by multiple physiological and pathological factors as well as environmental and lifestyle factors (Fatisson et al. 2016). These factors include age, sex, HR, health status, physical activity, stress, emotions and alcohol consumption to name a few (Fatisson et al. 2016, Shaffer & Ginsberg 2017). Higher HR lowers HRV and vice versa (Shaffer & Ginsberg 2017). Patients with hypertension have reduced HRV when compared to normotensive individuals and autonomic dysregulation can be present already in the early stages of hypertension (Singh et al. 1998). Decreased HRV is as well associated with high levels of blood glucose (Fatisson et al. 2016) which is seen with diabetic patients.

The short-term measurements of HRV are affected by the relationship between the sympathetic and parasympathetic branches (Shaffer & Ginsberg 2017). Heart rate changes according to breathing by accelerating during inspiration and slowing during exhalation (Shaffer et al. 2014). This is due inhibition and restoration of vagal outflow (Shaffer et al. 2014). This HR variation is called respiratory sinus arrhythmia (RSA) and it affects HRV variations, which represent vagal activity (Shaffer et al. 2014). In addition, short-term variations of HRV is affected by baroreflex, which links together changes in HR, BP and vascular tone (Shaffer & Ginsberg 2017). Change in the baroreceptor activity due to BP shift affects the mechanism that changes HR and vascular tone (Shaffer & Ginsberg 2017). HR and vascular tone decrease if BP rises and in increase if BP falls (Shaffer & Ginsberg 2017). The longer 24-h HRV measurements are influenced by circadian rhythms, core body temperature, metabolism and hormones as well as environmental, lifestyle and neuropsychological factors (Shaffer et al. 2014; Fatisson et al. 2016).

4.2 HRV during and after exercise

Investigating HRV responses during and after stress (e.g. exercise) might provide useful information of the autonomic stress reactivity (Michael et al. 2017). The reactivity hypothesis suggests that cardiovascular responses to a stressor might predict development of cardiovascular disease (Treiber et al. 2003). Autonomic adjustments to exercise might reveal dysregulation on the neural mechanism that accompany many cardiovascular disease states such as hypertension (Fisher et al. 2015).

All exercise intensities will result a very large reduction in cardiac parasympathetic activity in the first 10 minutes of exercise (Stanley et al. 2013). HRV returns rapidly to baseline values after manipulations such as mild exercise. A more powerful stimulus (i.e. maximum exercise) may result more longer effect to HRV before it can return to baseline values (Task Force Report 1996; Stanley et al. 2013). Recovery from exercise involves cardiac parasympathetic reactivation and this mechanism occurs more rapidly in individuals with greater aerobic fitness level (Stanley et al. 2013). Investigation of parasympathetic activation is possible with post-exercise HRV values (Buchheit et al. 2007).

4.3 Methods of analysing HRV

Two most common HRV analyzing methods are frequency domain or power spectral density and time domain analysis (Shaffer et al. 2014; Fatisson et al. 2016). There are other methods as well (Shaffer & Ginsberg 2017) but those are not discussed in this study. Each method starts with defining time intervals between each successive normal QRS complex from the electrocardiographic (ECG) record and excluding abnormal beats (Shaffer et al. 2014).

4.3.1 Frequency bands

HRV can be separate to component rhythms that operate in different frequency ranges (Shaffer et al. 2014). The HRV waveform of heart rhythm oscillations can be divided with filtering techniques to high-frequency (HF), low-frequency (LF), very-low-frequency (VLF) and ultra-low-frequency (ULF) bands (Task Force Report 1996; Shaffer et al. 2014; Shaffer & Ginsberg 2017). Following recording periods are often used and recommended: ULF (24 h), VLF (5 min, 24 h), LF (2 min) and HF (1 min) (Shaffer et al. 2014; Shaffer & Ginsberg 2017).

The HF band operates within frequency of 0.15 to 0.4 Hz and it reflects parasympathetic activity (Task Force Report 1996; Vinik & Ziegler 2007; Shaffer et al. 2014). Patient under stress or anxiety and with pathological cardiac conditions have been found to have reduced parasympathetic activity and HF band (Shaffer et al. 2014). The HF band is often called the respiratory band because it reflects the HRV values related to HR variations of the respiratory cycle (Shaffer et al. 2014; Shaffer & Ginsberg 2017).

The range of the LF band is from 0.04 to 0.15 Hz or rhythms with periods between 7-25 s (Shaffer & Ginsberg 2017). The power of LF band can be influenced with parasympathetic or sympathetic mechanisms and with baroreflex activity depending on the situation (resting vs. ambulatory) (Shaffer et al. 2014). Previously the LF band has been suggested to describe the sympathetic activity but at present it is thought to reflect mix of sympathetic and parasympathetic activity with other unidentified factors (Vinik & Ziegler 2007; Billman 2013; Michael et al. 2017)

The LF/HF ratio may estimate the ratio between SNS and PNS activity under controlled conditions (Shaffer & Ginsberg 2017). In 24 h recordings both PNS and SNS activity contribute to LF band and PNS to HF band (Shaffer & Ginsberg 2017). It is stated that low LF/HF ratio is a sign of a greater parasympathetic activity (Shaffer & Ginsberg 2017). A high LF/HF ratio may indicate higher sympathetic activity which is seen when people engage in fight-or-flight behaviors or parasympathetic withdrawal (Shaffer et al. 2014; Shaffer & Ginsberg 2017). However, interpretation of both LF and LF/HF should be done with caution due to complex interactions of SNS and PNS to LF band (Billman 2013; Michael et al. 2013).

The very-low-frequency (VLF) band operates between 0.0033 and 0.04 Hz or rhythms between 25-300 s (Shaffer et al. 2014; Shaffer & Ginsberg 2017). All low values of 24 h clinical HRV measurements are associated with greater risk of adverse outcomes (Shaffer et al. 2014), but the VLF band may have stronger associations with all-cause mortality than LF or HF band (Schmidt et al. 2005). VLF frequency is thought to be modulated by the sympathetic activity (Vinik & Ziegler 2007; Shaffer et al. 2014).

4.3.2 Time domain measurements

The three most common measurements for the 24-hour recordings are the SDNN, the SDNN index and the RMSSD (Shaffer et al. 2014). The SDNN means standard deviation of the normal-to-normal (NN) heartbeats in milliseconds, i.e. the square root of variance (Task Force Report 1996; Shaffer et al. 2014). With short-term recordings in resting conditions the SDNN is mainly affected by the parasympathetically-mediated RSA (Shaffer et al. 2014). Low values of SDNN with age-adjustment are associated with morbidity and mortality when higher SDNN values relate to higher survival (Shaffer et al. 2014).

The SDNN index is the mean value of the standard deviations of all the NN intervals from 24-h recording divided to 5-min segments (Task Force Report 1996; Shaffer et al. 2014). It represents an average of all the 5-min recordings values from one 24-h recording (Task Force Report 1996; Shaffer et al. 2014). The SDNN index is believed to represent the autonomic influence on HRV and it correlates with VLF band in 24-h recordings (Shaffer et al. 2014). The SDNN methods can be used with both short- and long-term recordings (Task Force Report 1996).

The RMSSD value means root mean square of successive differences between normal heartbeats in milliseconds (Task Force Report 1996; Shaffer et al. 2014; Shaffer & Ginsberg 2017). It represents the beat-to-beat variance of heart rate and it is most commonly used to measure the parasympathetically-mediated changes in HRV (Shaffer et al. 2014). The RMSSD correlates highly with HF power (Task Force Report 1996). The RMSSD estimate short-term components of HRV (Task Force Report 1996) and conventional minimum recording is 5-min (Shaffer & Ginsberg 2017).

5 AIM OF THE RESEARCH AND RESEARCH QUESTIONS

Aim of this thesis is to demonstrate associations of dysfunctional autonomic nervous system to the exercise-induced exaggerated blood pressure and blunted heart rate responses among hypertensive individuals and individuals with impaired glucose metabolism. Exaggerated blood pressure responses during exercise and slow reduction to baseline values are a risk factor for future cardiovascular events and diseases (Yosefy et al. 2006; Schultz et al. 2013, Laukkanen et al. 2014; Dombrowski et al. 2018; Schultz et al. 2017). Blunted HR response and slow post-exercise HR recovery are as well associated with adverse cardiovascular events and mortality (Diller et al. 2006; Brubaker & Kitzman 2011; Jae et al. 2016). ANS system is a regulator of the cardiovascular responses to exercise. Therefore, dysfunction of the ANS system during exercise and in daily activities may contribute to negative health outcomes such as hypertension and type 2 diabetes as well increase risk for mortality and morbidities.

Dysfunctional autonomic nervous system means imbalance between two branches of the ANS system also known as a reduced parasympathetic and an exaggerated sympathetic activation. In this study it is measured with heart rate variability (HRV) values during maximal exercise test and three continuous 24-h recordings.

Research question 1. Is there an association between the autonomic nervous system functioning and the exaggerated blood pressure and/or attenuated heart rate response during the maximal exercise test?

Hypothesis 1. The blood pressure responses to the exercise are lower with those who have higher parasympathetic and/or smaller sympathetic activity before, during and/or after the exercise test. The heart rate response to the exercise is higher with those who have higher parasympathetic activity and/or smaller sympathetic activity before and after the exercise test.

Research question 2. Is there an association between the three continuous 24-h recordings of heart rate variability and the exaggerated blood pressure and/or attenuated heart rate response during the exercise test?

Hypothesis 2. Balanced ANS function is associated with lower blood pressure and higher heart rate responses during the exercise test.

6 METHODS

This chapter describes the study protocol of this thesis, the research methods and data, as well as statistical methods. The statistical methods are chosen to suit the data and the research questions.

6.1 Research data and data collection

This thesis is done as a part of the Healthbeat – study (“*Kunto, uni ja stressi diabetes- ja verenpainetautipotilailla”)* performed by the Central Finland Health Care District, University of Jyväskylä (the Faculty of Sport and Health Sciences, the Department of Psychology), and Firstbeat Technologies Oy in 2019-2020. Firstbeat Technologies Oy is specialized to measurement of heart rate variability. Aim of the Healthbeat – study was to investigate association with physical fitness, sleep and stress among hypertensive and/or diabetic patients and/or patients with impaired glucose metabolism. The research frame was cross-sectional study. This thesis includes only part of the study population (28 participants) because the thesis was done before the main study was finished. Aim of the Healthbeat study was to include 100 participants.

Study protocol consisted of six different tests that participants went through approximately in four weeks: 1) medical examination with the resting ECG-test, 2) blood test after overnight fast, 3) a cardiopulmonary exercise test (i.e. maximal graded exercise test), 4) 30-minute self-paced walking test, 5) monitoring of sleep with polysomnography during one night and 6) a psychosocial stress test. In addition, study included three 72-hour HRV measurements, which were done with the Firstbeat Bodyguard 2 - device. This thesis concentrates on the maximal graded exercise test and blood pressure, heart rate and HRV values interrelate to the exercise test and one 72-hour HRV-measurement. The data of this thesis was collected during the medical examination and the maximal graded exercise test. The data from the blood samples was also used in this thesis.

6.1.1 Exercise test

The cardiopulmonary exercise test was a graded maximal exercise test what is commonly used to measure aerobic capacity and to define person's VO_{2max} (Beltz et al. 2016). The exercise test was done by walking with treadmill (JUOKSUMATTO OJK-1, Telineyhtymä, Kotka, Finland). Incline of the treadmill was lifted with every 3-minute period to make the exercise harder due to uphill walking. The test participants walked until exhaustion was reached. The test was terminated if the participant experienced symptoms that demanded ending of the test or if the supervising research doctor observed reason to test termination.

Pre-exercise test arrangements. The conditions of the test subjects were standardized. The participants were instructed to avoid food, smoking, coffee, tea or other stimulating substances 2 hours before the test. Heavy exercise as well as alcohol was avoided 1,5 day before the test. The 12-lead ECG (CardioSoft V5.02, GE Medical Systems Information Technologies GmbH, Freiburg, Germany), resting BP and blood glucose level (only with diabetic patients) was measured before the test. Resting ECG and resting BP (SunTech Tango M2, SunTech Medical, Inc., Morrisville, USA) from the left hand were measured in supine position after 5-min rest. Weight, body composition, waist cicumference and height were measured before the exercise test. The bioimpedance device (InBody770, InBody Co., Ltd., Seoul, South Korea) was used to measure body composition and weight.

Exercise test. During the exercise test 12-lead ECG (CardioSoft V5.02, GE Medical Systems Information Technologies GmbH, Freiburg, Germany), BP, HR and HRV of the test participants were monitored and measured. Blood pressure was measured from the left hand with automated blood pressure meter (SunTech Tango M2, SunTech Medical, Inc., Morrisville, USA), which have been validated for the maximal treadmill test as well as rest BP measurements (Cameron et al. 2004). The BP measurements were done at the end of the 5-min rest phase before the exercise test, at the end of the every 3-min exercise phase, immediately after or prior to the exercise test cessation, after 1-minute recovery phase (standing) as well as after 3- and 5-minute recovery phase in supine position. HRV was measured with the Firstbeat Bodyguard 2 – device (Figure 3). The ventilatory gas analysis was performed during the test and the subjects wore oro-nasal mask for this purpose. Inspired and expired gases were collected breath-by-breath and O_2 as well as CO_2 concentrations analyzed (Oxycon Pro® Version 5.0, VIASYS Healthcare GmbH, Hoechberg, Germany). Volumes and flows of inspiratory and expiratory gases were measured (Triple V®, Erich Jaeger, Friedberg, Germany). Perceived exertion was asked with Borg scale (6-20) at the end of every 3-minute period during the test. In addition, perceived dyspnea with Borg scale 0-10 was asked at the same time.

The exercise test protocol consisted following stages (Wolthuis et al. 1977):

- 5 min rest phase (the subject stands on the treadmill)
- 3 min light walking (3,2 km/h with 0% incline of the treadmill)
- 3 min walking (5,3 km/h with 0% incline of the treadmill)
- 3 min walking periods until maximal exhaustion (5,3 km/h with +5% incline with every 3-minute period)
- 1 min rest phase in standing position on the treadmill
- 4 min rest phase in supine position

All the mentioned measuring devices where in place during the whole test protocol except the breathing mask, which was removed when the subject was moved to the supine position.

6.1.2 HRV measurements

In addition to the exercise, three continuous 72-hour measurement was recorded during the whole Healthbeat-study per one study participant. This thesis concentrates on one 3-day measurement following the exercise test. 3-day HRV recording was done with Bodyguard 2-device (Figure 3). Study participants were instructed to use the Bodyguard device and to fill out the 3-day diary. Participants could mark exercise, medications, alcohol use and sleep time (mandatory) to diary. There were no limitations to lifestyle (e.g. alcohol use or smoking) during this measurement. The 3-day recording period consisted two working days and one weekend day. After the whole test protocol, the participants received lifestyle assessment report based on their 72-hour HRV measurements.

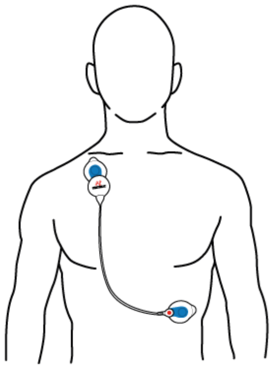


FIGURE 3. Firstbeat Bodyguard 2-device measures HRV with two electrodes attached according to figure (Firstbeat 2019).

6.1.3 Study data

The data of this thesis consisted the record of the maximal exercise test, data collected during the first medical exam (e.g. medication use) and 72-hour Bodyguard 2 – recording after the exercise test. Study populations consisted of 18- to 64-year-old individuals with prediabetes (i.e. elevated fasting glucose and/or impaired glucose tolerance), diagnosed type 2 diabetes during last five years and/or diagnosed hypertension. In Finland, hypertension is diagnosed with values 140/90 mmHg or higher (Mustajoki 2020). BMI of the research participants had to be under 40 kg/m^2. In addition, exclusion criteria included specific medications (i.e. beta blockers and insulin medication), specific diseases (i.e. cardiovascular and pulmonary diseases, cancer), pregnancy and nursing as well as misuse of substances. More specific inclusion and exclusion criteria can be found from the attachment 1. Subject were recruited by online and local noticeboard advertisements and through local health care providers. Target area was Central Finland Health Care District.

There were 28 participants with a mean age 54,4. High blood pressure was diagnosed with 23 (82,1%) of the participants, type 2 diabetes with 4 (14,3%), impaired fasting glucose metabolism (IFG) or/and impaired glucose tolerance (IGT) with 10 (35,7%) of the participants. Descriptive data of the study participants is represented in Table 1.

TABLE 1. Descriptive data of the study participants.

Variable	**N=28**	**Value** [a] (mean ±SD)
General		
Age (years)	28	54,4 ±7,2
Sex (Women, %)	19 (67,9%)	
Body weight (kg)	28	82,6±15,7
Body mass index (kg/m^2)	28	28,5±4,5
Hypertension (yes, %)	23 (82,1%)	
Diabetes (yes, %)	4 (14,3%)	
Type 2 diabetes (yes, %)	4 (14,3%)	
IFG or/and IGT (yes, %) [b]	10 (35,7%)	
Systolic blood pressure, supine	28	136,8±13,8
Diastolic blood pressure, supine	28	83±6,8
Minimum HR (bpm) [c]	28	48,0±6,9
Smoking, currently (yes, %)	0 (0,0%)	
Hypertension medication (yes, %)	21 (75,0%)	
Total cholesterol (mmol/l)	28	4,9±0,9
Serum HDL cholesterol [d] (mmol/l)	28	1,5±0,4
Serum LDL cholesterol [f] (mmol/l)	28	3,2±0,8
Triglycerides (mmol/l)	28	1,3±0,7
Lipid metabolism medication (yes, %)	4 (14,3%)	

Table 1 explanations. [a] Continuous value are reported as mean ± standard deviation; categorial values are shown as number of the participants (N) and percentage (%). [b] IFG=impaired fasting glucose, IGT=impaired glucose tolerance. [c] Minimum heart rate value measured with Bodyguard 2 device during 72 h recording. [d] High density lipoprotein. [f] Low density lipoprotein.

6.2 Statistical methods

Main variables of this thesis are the maximal systolic blood pressure and maximal heart rate during the exercise test. Autonomic nervous system functioning was measured with the heart rate variability variables including HF, LF, VLF, LF/HF ratio, RMSSD, SD and SDNN Index. These HRV variables are commonly used when HRV is measured (Task Force Report 1996; Shaffer et al. 2014; Shaffer & Ginsberg 2017).

The study data were analyzed with IBM SPSS Statistic 26.0 – software. Normal distribution of the data were tested with Shapiro-Wilk test and by analyzing the skewness and kurtosis of the distribution. Group comparisons were done with t-test, crosstabs, Chi square ($\chi 2$) – test and Mann-Whitney-test depending of the normality of the variable. Statistical significance value was $p<0,05$. Used statistical methods are reported with the results.

T-test is a method for testing differences between mean values (Metsämuuronen 2006, 374). The T-test can be used when the variables are normally distributed and when the scale of the variable is at least interval (Metsämuuronen 2006, 374). In this thesis, t-test was used to compare means between normally distributed variables such as weight, blood pressure, heart rate and age.

Crosstabs analyze detects associations between two variables and independence of the variables is analyzed with Chi square ($\chi 2$) – test (Metsämuuronen 2006, 347). Categorial variables can be analyzed with crosstabs and Chi square ($\chi 2$) – test. In this thesis these tests were used to compare differences of the categorial variables between different groups such as gender, disease state and medication.

HRV data were compared between the groups with the Mann Whitney U - test since HRV data were not normally distributed and the sample size was small ($N<30$) (Metsämuuronen 2006, 369). Mann Whitney U – test arranges variables in sequence by the magnitude of the variable from the smallest to the largest (Metsämuuronen 2006, 370). There are differences between groups, if the means of the U-test significantly diverge from one another

(Metsämuuronen 2006, 371). With small sample sizes non-parametrical test like Mann Whitney can be more reliable than the parametric tests (Metsämuuronen 2006, 370).

The HRV data are represented in median values due to its very wide and non-normal distribution. Median value represents the middle value of all the values, and it is not affected by extremely small or large numbers that deviate from other values (Mattila 2003). Significantly deviating values affect greatly to mean values when the sample size is small (Mattila 2003). Therefore, median value can give better picture of the middle value than the mean value in situations with small sample size and deviating values (Mattila 2003).

7 RESULTS

This thesis researches the associations of the HRV values to the blood pressure and heart rate during maximal exercise test within participants with hypertension, diabetes or prediabetic state. The results in this chapter demonstrate differences of HRV values during exercise test and separate 72-hour recording between low and high blood pressure as well as age-adjusted HR groups. Group differences between related variables was analyzed to find possible confounding variables.

7.1 Descriptive data of the subjects

The study population was divided into two groups according to median values of the maximum SBP and maximum age-adjusted HR value during exercise test. Median value was used due to fact that study population was small and no absolute value for high SBP during exercise is represented (Currie et al. 2018). Median value divides population in half (Mattila 2003), which makes the group comparison meaningful with small study populations.

Table 2 represents the descriptive data of the study groups. There were 14 participants per group in both SBP and HR comparisons. SBP values for low and high SBP group were under (<) 218 mmHg and over (≥) 218 mmHg, respectively. Age-adjusted maximum HR values (%) for low and high HR group were under (<) 104% and over (≥) 104%, respectively. The age-adjusted HR value was calculated dividing maximum HR during exercise test with age-predicted HR (220-age). Maximum HR values decline with age (Ozemek et al. 2015). Therefore, using of age-adjusted percent value standardizes the effect of the age to the maximum HR.

Only statistically significant difference between low and high SBP group in general variables (Table 2) was within IFG or/and IGT variable (low SBP N=2 vs. high SBP N=8, p=0.036). There were no statistically significant differences between low and high SBP group within cholesterol related values (Table 3). There was statistically significant difference in maximum

SBP ($p<0.001$) and MAP ($p=0.007$) values within SBP groups (Table 4) due to fact that group were divided according to median value of the SBP. In addition, there was a nearly statistically significant difference in rest SBP (supine) values ($p=0.073$). Maximum HR value was non-significantly higher in lower SBP group (177 vs. 171 bpm, $p=0.141$). Mean aerobic fitness (VO_2max values) or exercise time did not vary between BP groups (Table 4).

In recovery phase significant differences were found in related to recovery SBP measured 1- and 3-minute post-exercise (Table 4). 1-minute post-exercise value was lower among participants with lower maximal SBP during the exercise test (196±17 vs. 230±27 mmHg, $p=0.001$) as well as 3-minute post-exercise values (179±15 vs. 193±19 mmHg, $p=0.041$). HR recovery was larger in lower SBP group post-exercise (1- and 3-minute post-exercise values), but the difference was not statistically significant (Table 4).

Statistical difference in body weight (kg) and body mass index (BMI) (kg/m^2) between low and high HR group ($p=0.007$) was found. Mean value of the body weight in low HR group was 90,3 kg (SD ±17,2) and in high HR group 75,0 kg (SD±9,4). Mean values for BMI in low and high HR group were 30,7 kg/m^2 (SD±4,8) and 26,4 kg/m^2 (SD±2,8), respectively. In addition, HDL-cholesterol values were larger in high HR group (1,7±0,3 vs. 1,4±0,4 mmol/l, $p=0.039$). There were no statistically significant differences between HR groups related to exercise values except in maximum HR (bpm) and maximum age-adjusted HR (%). This is a result of the groups being divided based on maximum HR. Mean aerobic fitness (Vo_2max values) did not vary within different HR groups (Table 4). In higher HR group exercise test time was non-significantly higher (15,4 vs. 16,5 min, $p=0.367$). Higher HR group had a higher HR reserve (84±13 vs. 96±17 bmp, $p=0.044$), which means difference between the minimal HR during pre-rest phase and maximal HR during exercise phase (Table 4).

TABLE 2. Descriptive data of the study groups. Percent values (%) within groups.

Variable	Systolic blood pressure Low	Systolic blood pressure High	p-value	Max. age-adjusted HR (%) Low	Max. age-adjusted HR (%) High	p-value
General	N (%) / Mean±SD	N (%) / Mean±SD		N (%) / Mean±SD	N (%) / Mean±SD	
N	14 (50,0%)	14 (50,0%)	1.000 [b]	14 (50,0%)	14 (50,0%)	1.000 [b]
Age (years)	53,4±5,6	55,4±8,7	0.104 [a]	52,5±8,3	56,3±5,7	0.194 [a]
Sex (Women, %)	10 (52,6%)	9 (47,4%)	1.000 [b]	9 (47,4%)	10 (52,6%)	1.000 [b]
Sex (Men, %)	4 (44,4%)	5 (55,6%)	1.000 [b]	5 (55,6%)	4 (44,4%)	1.000 [b]
Body weight (kg)	85,1±16,5	80,2±15,0	0.414 [c]	90,3±17,2	75,0±9,4	**0.007*** [c]
Body mass index (kg/m2)	29,4±4,5	27,7±4,4	0.328 [c]	30,7±4,8	26,4±2,8	**0.007*** [c]
Hypertension (yes, %)	13 (56,5%)	10 (43,5%)	0.326 [b]	12 (52,2%)	11 (47,8%)	1.000 [b]
Type 2 diabetes (yes, %)	2 (50,0%)	2 (50,0%)	1.000 [b]	3 (75,0%)	1 (25,0%)	0.596 [b]
IFG or/and IGT (yes, %)	2 (20,0%)	8 (80,0%)	**0.036*** [b]	4 (40,0%)	6 (60,0%)	0.697 [b]
Systolic blood pressure, supine	132±10	141±16	0.073 [c]	136±16	137±11	0.894 [c]
Diastolic blood pressure, supine	82±8	84±6	0.353 [c]	82±7	84±7	0.625 [c]
Minimum HR (bpm) [d]	48±8	48±7	0.909 [c]	49±7	47±7	0.514 [c]
Hypertension medication (yes, %)	11 (52,4%)	10 (47,6%)	1.000 [b]	12 (57,1%)	9 (42,9%)	0.385 [b]

[a] Mann-Whitney test (U=134,0 SBP class, U=127 Max HR class), exact p-value. [b] Chi-Square test, exact p-value. [c] Independent Sample t-test. * p<0.05. [d] Minimum heart rate value measured with Bodyguard 2 device during 72 h recording.

TABLE 3. Cholesterol values and medication between the groups.

Variable	Systolic blood pressure Low	High	p-value	Max. age-adjusted HR (%) Low	High	p-value
Cholesterol values	N (%) / Mean±SD	N (%) / Mean±SD		N (%) / Mean±SD	N (%) / Mean±SD	
Total cholesterol (mmol/l)	4,9±0,8	4,9±1,0	0.984 [b]	4,6±0,9	5,3±0,8	0.070 [b]
Serum HDL cholesterol (mmol/l)	1,5±0,3	1,6±0,5	0.507 [b]	1,4±0,4	1,7±0,3	**0.039*** [b]
Serum LDL cholesterol (mmol/l)	3,2±0,8	3,1±0,9	0.843 [b]	2,9±0,8	3,4±0,8	0.119 [b]
Triglycerides (mmol/l)	1,5±0,6	1,2±0,8	0.384 [b]	1,5±0,8	1,2±0,6	0.232 [b]
Lipid metabolism medication (yes, %)	2 (50%)	2 (50%)	1.000 [a]	4 (100,0%)	0 (0,0%)	0.098 [a]

[a] Chi-Square test, exact p-value. [b] Independent Sample t-test. * p<0.05

TABLE 4. Exercise test parameters between the groups.

Variable	Systolic blood pressure Low	Systolic blood pressure High	p-value [a]	Max. age-adjusted HR (%) Low	Max. age-adjusted HR (%) High	p-value [a]
Exercise test	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
Max HR[b] (bpm)	177±12	171±10	0.141	167±10	181±8	**>0.001***
Max HR predicted[c] (%)	107±8	104±6	0.353	100±3	111±5	**>0.001***
Max VO_2 [d] (l/min)	2,7±0,7	2,6±0,7	0.633	2,8±0,7	2,5±0,6	0.283
Max VO_2 BM [e] (ml/min/kg)	31,7±4,8	32,8±8,5	0.691	31,1±8,0	33,3±5,3	0.401
Max VO_2 FFM [f] (ml/min/kg)	47,3±4,0	46,0±7,4	0.582	46,3±7,7	47,0±3,4	0.780
Max exercise SBP (mmHg)	207±10	237±15	**>0.001***	222±19	221±21	0.844
Max exercise DBP (mmHg)	99±15	105±8	0.157	100±16	104±7	0.471
Max exercise MAP (mmHg)	132±9	143±10	**0.007***	136±14	138±8	0.616
Exercise time (min)	16,0±1,8	16,0±3,9	0.998	15,4±3,4	16,5±2,5	0.367
Recovery phase						
Heart rate reserve (bpm)	94±15	86±16	0.203	84±13	96±16	**0.044***
Heart rate recovery after 1 min (bpm)	27±7	23±8	0.130	25±7	25±9	0.981
Heart rate recovery after 3 min (bpm)	72±14	64±11	0.113	66±11	70±15	0.353
Systolic BP 1 min recovery	196±17	230±27	**0.001***	213±30	213±28	0.979
Systolic BP 3 min recovery	179±15	193±19	**0.041***	183±20	189±17	0.411
Systolic BP 5 min recovery	153±16	165±15	0.059	158±21	160±11	0.726

[a] Independent Sample t-test. * $p<0.05$, [b] Maximum HR during exercise test, [c] Maximum HR of age predicted (age-220) HR (%), [d] Maximum VO_2 , [e] Maximum VO_2 in relation to body weight, [f] Maximum VO_2 in relation to fat free mass

7.2 HRV values in exercise test

Comparison of the HRV values during the exercise test within the SBP and HR groups is represented in Table 5. The HRV values recorded in 5-minute rest phase before the exercise test did not separate between groups. The HRV values related to the exercise test were compared within the SBP and the HR group in different 3-minute walking phases and last 30s of the exercise test. Only first three 3-minute walking phases and last 30 second of the exercise test were analyzed due to fact that all the participants executed these phases. Beyond these phases sample size would have dropped. There were few statistically significant differences in HR group comparison but not within the SBP groups. The attachment 2 contains the comparison data with Mann Whitney U-test values. Table 5 represents HRV values in rest phase, peak exercise phase, 1- and 5-minute recovery phase.

In peak exercise phase HF values were higher in low HR group compared to high (median 2,2 vs. 0,7 ms^2, p=0.011). LF values were as well higher in low HR group (median 1,0 vs. 0,3 ms^2, p=0.024) in peak phase. There were no significant differences in recovery phase (0-5 minute) between lower and higher HR group. In recovery phase (0-1 minute) there were no differences between low and high SBP group. However, HF values were non-significantly higher in low SBP group during recovery (median 30,0 vs. 13,8 ms^2, p=0.125) as well as LF values (median 52,2 vs. 26,4 ms^2, p=0.164).

TABLE 5. HRV median values between different SBP and HR groups.

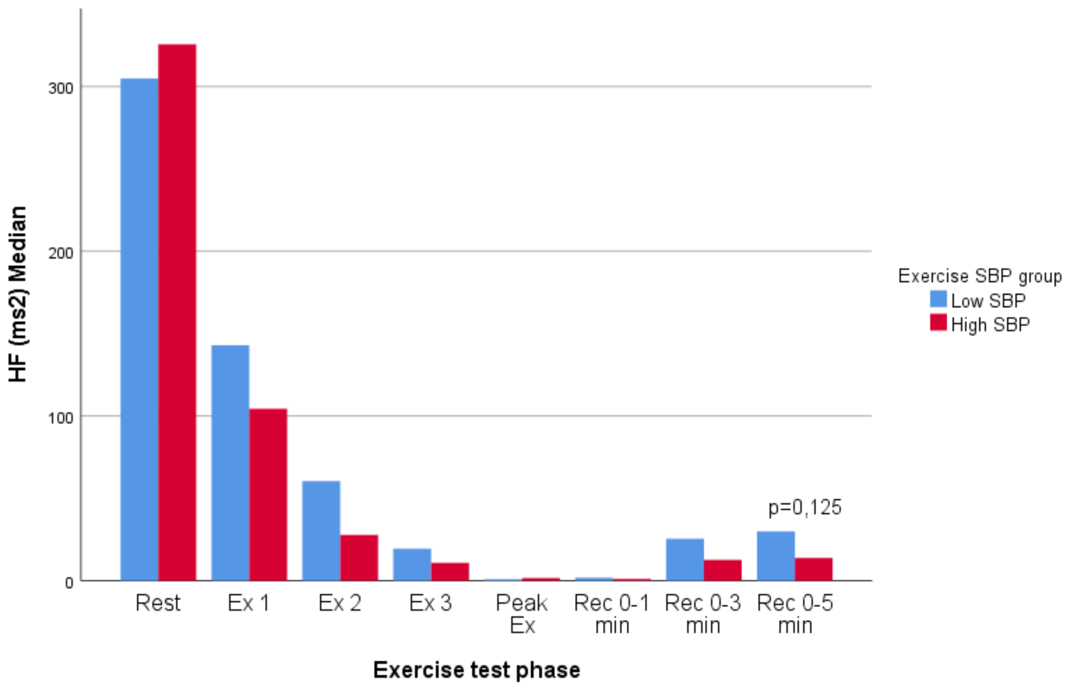
	Systolic blood pressure			Max. age-adjusted HR (%)		
HRV values	Low	High	p-value [a]	Low	High	p-value [a]
Rest 5 min	Median	Median		Median	Median	
HF (ms^2)	304,9	325,6	0.701	325,6	304,9	0.804
LF (ms^2)	663,2	640,0	0.401	565,8	663,2	0.603
LF/HF Ratio	2,3	1,8	0.635	1,9	2,1	0.701
VLF (ms^2)	70,8	68,5	0.376	51,2	75,3	0.329
RMSSD (ms)	14,3	13,6	0.635	12,9	14,7	0.701
SD (ms)	35,8	30,1	0.701	30,1	36,8	0.874
Peak exercise [b]						
HF (ms^2)	0,9	1,6	0.667	2,2	0,7	**0.011***
LF (ms^2)	0,5	0,6	0.804	1,0	0,3	**0.024***
LF/HF Ratio	0,6	0,5	0.227	0,5	0,5	0.946
RMSSD (ms)	4,5	4,4	0.804	4,8	4,1	0.104
SD (ms)	11,4	9,8	0.265	13,2	9,5	0.094
Recovery 0-1 min						
HF (ms^2)	1,9	1,1	0.329	2,2	1,1	0.077
LF (ms^2)	2,2	1,1	0.329	2,5	1,4	0.352
LF/HF Ratio	1,1	1,2	0.874	1,0	1,3	0.427
RMSSD (ms)	4,2	4,3	0.769	4,8	3,2	0.077
SD (ms)	18,9	17,2	0.635	17,9	16,6	0.210
Recovery 0-5 min						
HF (ms^2)	30,0	13,8	0.125	23,8	19,2	0.306
LF (ms^2)	52,2	26,4	0.164	39,7	29,5	0.352
LF/HF Ratio	1,5	2,2	0.056	1,7	2,1	0.306
VLF (ms^2)	5,6	3,9	0.454	6,3	4,3	0.246
RMSSD (ms)	6,1	5,6	0.454	6,4	4,9	0.210
SD (ms)	85,7	94,9	0.874	98,5	86,0	0.265

[a] Mann-Whitney test (exact p-value), [b] Last 30s of the maximal exercise, * $p<0.05$

Figures 4-9 represent HF, LF and RMSSD median values during exercise test within the SBP and the HR groups. Statistically significant and near-significant values are represented in the pictures. The explanations for the abbreviations used in the figures are following: rest=5 min rest before the exercise test; Ex1=first 3-minute walking phase; Ex2=second 3-minute walking phase; Ex3=third 3-minute walking phase, Peak Ex=last 30 s of the exercise test; Rec 0-1 min= recovery time between 0-1 minute; Rec 0-3 min=recovery time between 0-3 minute; Rec 0-5 min=recovery time between 0-5 minute.

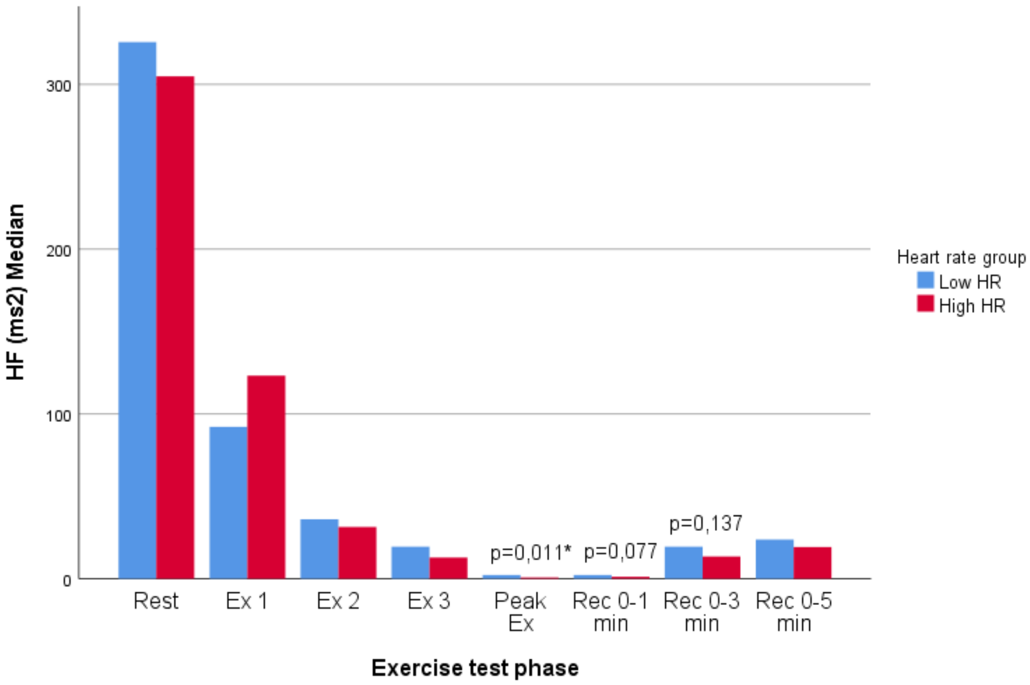
Figure 4 represents HF (ms^2) values in low and high SBP groups during the maximal graded exercise test including the rest phase before the actual exercise and the 5-min recovery phase post-exercise. In the figure, results of the first three 3-min walking stages and last 30 s of the exercise (peak exercise phase) are represented. There were no statistically significant differences in HF values between low and high SBP groups. Highest non-significant difference was found in the recovery phase, in which higher HF values were found in the lower SBP group (30,0 vs. 13,8 ms^2, $p=0.125$).

FIGURE 4. Median HF (ms^2) values during the exercise test between low and high SBP group.



In Figure 5 HF (ms^2) values during the exercise test including the rest and 5-min recovery phase in lower and higher age-adjusted HR group are depicted. Significantly higher HF value was found in lower HR group during the peak exercise phase (2,2 vs. 0,7 ms^2, p=0.011). During the recovery phase (0-1 min and 0-3 min) higher, but non-significant, HF values were seen in the lower HR group.

FIGURE 5. Median HF (ms^2) values between low and high HR group during the exercise test.



LF (ms^2) values during the exercise test including pre-rest and recovery phase are represented in Figures 6 and 7. There were no statistically significant differences in LF values between lower and higher SBP groups (Figure 6). Highest non-significant difference was after 5-min recovery when higher LF values were found in the lower SBP group (52,2 vs. 26,4 ms^2, p=0.164). Between HR groups (Figure 7), the only statistically significant difference was found in peak exercise phase where higher LF values were found in lower age-adjusted HR group (1,0 vs. 0,3 ms^2, p=0.024).

FIGURE 6. LF (ms^2) values during the exercise test in different SBP groups.

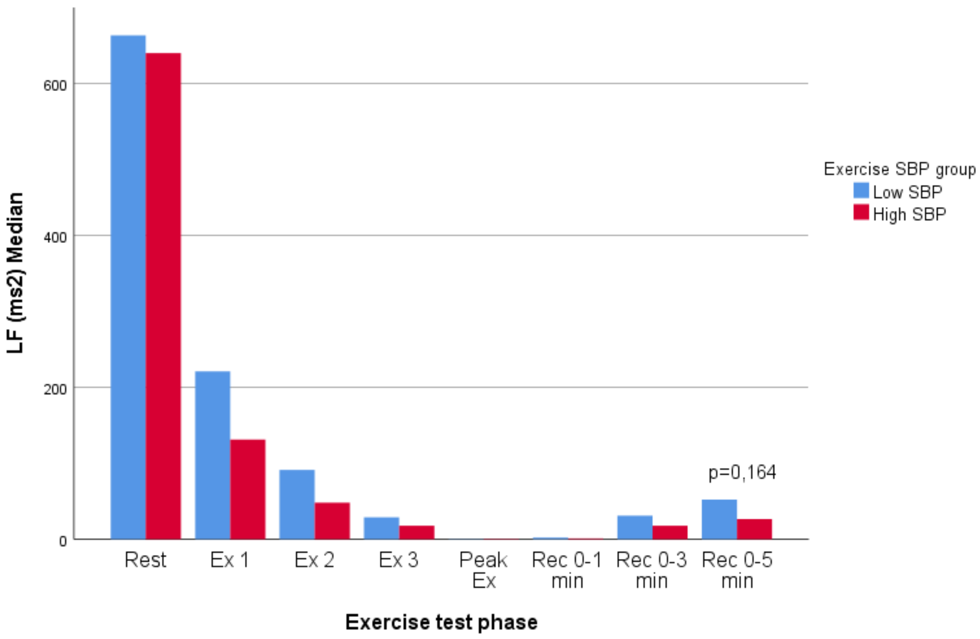
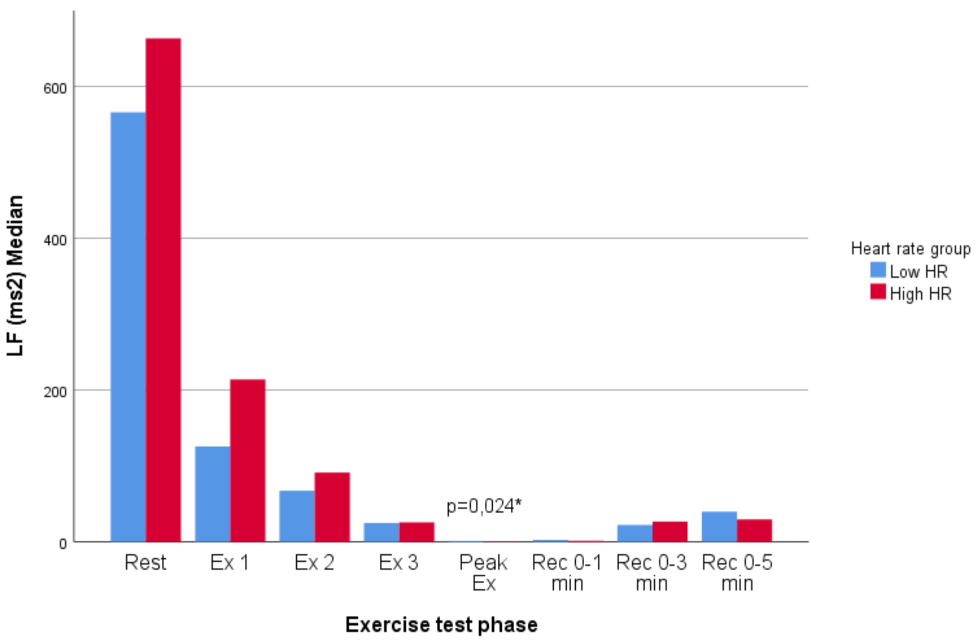
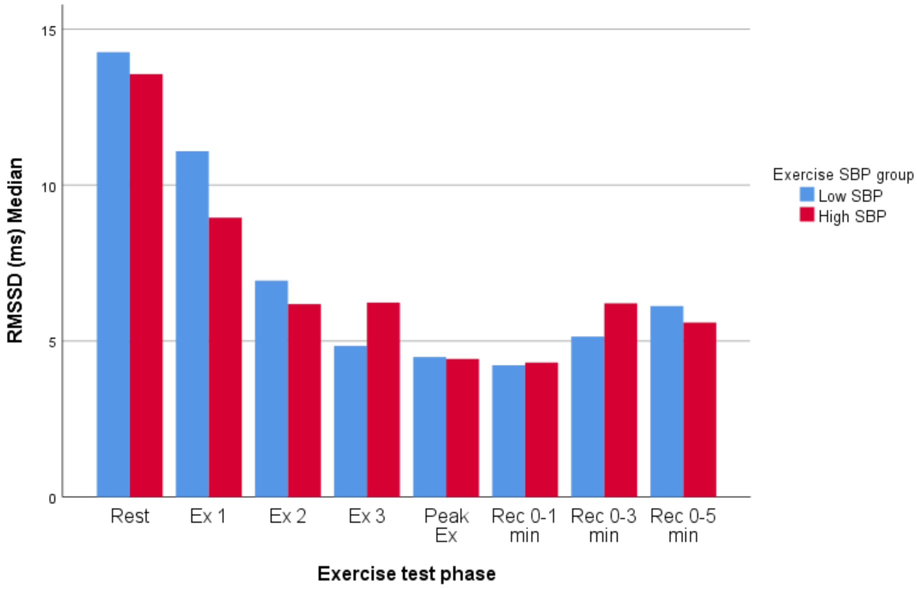


FIGURE 7. LF (ms^2) values during the exercise test in low and high HR group.



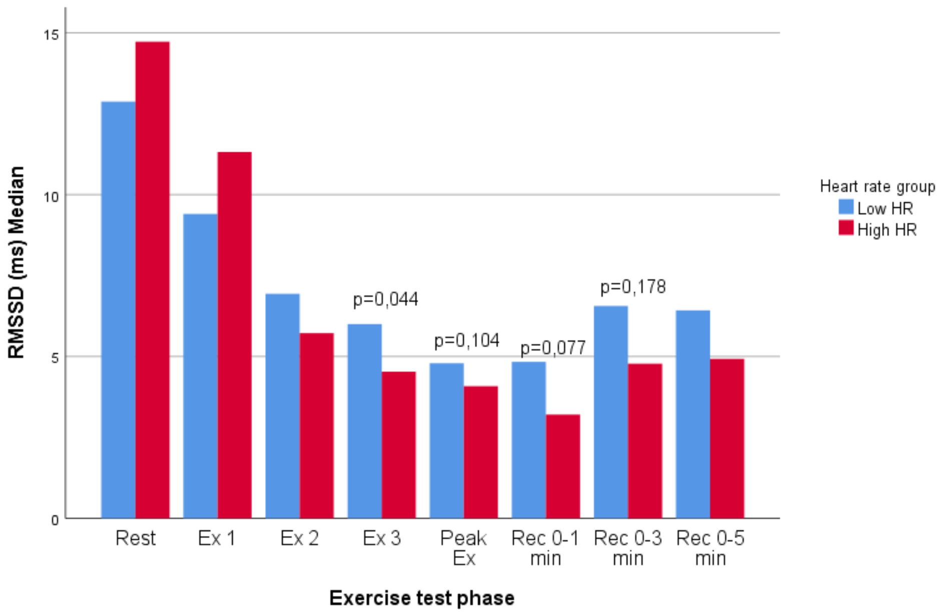
RMSSD (ms) values during the exercise test as well as pre-rest and post-recovery phases are represented in Figures 8 and 9. No statistically significant differences in RMSSD values were found between lower and higher SBP groups (Figure 8) during the 5-min pre-rest, the exercise phase or during the 5-min recovery phase.

FIGURE 8. RMSSD (ms) values during exercise test in different SBP groups.



Within HR group comparison of the RMSSD values (Figure 9) the only statistically significant difference was during third 3-min exercise phase (p=0.044) where higher RMSSD values were seen in the lower HR group. Non-significantly higher RMSSD values were seen in the pre-rest phase and during the first 3-min exercise phase in the higher HR group. After the first exercise phase as well as during the recovery phase non-significantly higher RMSSD values were recorded in lower HR group.

FIGURE 9. RMSSD (ms) values during exercise test in different HR groups.



7.3 HRV values in 72-hour recordings

Table 6 represents the median HRV values in 72-hour recordings between groups. 72-hour continuous recording was analyzed in three different segments: the whole 72-hour recording and only daytime or nighttime of the same 72-hour recording. Group comparisons of the HRV values was done with Mann-Whitney U-test.

Statistically significant differences were found only between LF/HF ratio in SBP group comparison. 72-hour continuous recording (day and night values) of LF/HF ratio was 3,5 on low SBP group and 2,7 in high SBP group (p=0.044). LF/HF ratio what consisted only daytime values of 72-hour recording was higher in low SBP group (3,9 vs. 3,0, p=0.035). In addition, the nighttime values of LF/HF ratio were non-significantly higher in lower SBP group (2,2 vs. 1,7, p=0.085).

There were no statistically significant differences between low and high HR group. LF (ms^2) values were non-significantly higher in high HR group, 694,2 ms^2 vs. 1243,4 ms^2 (p=0.114) as well as VLF (ms^2) values (110,6 ms^2 vs. 159,9 ms^2, p=0.150) in recording that considered both day and night. SDNN Index was non-significantly higher in the high HR group (45,5 vs. 62,0, p=0.125) in the same recording. Analyzing daytime and nighttime separately did not resulted statistically significant results in HR group comparison.

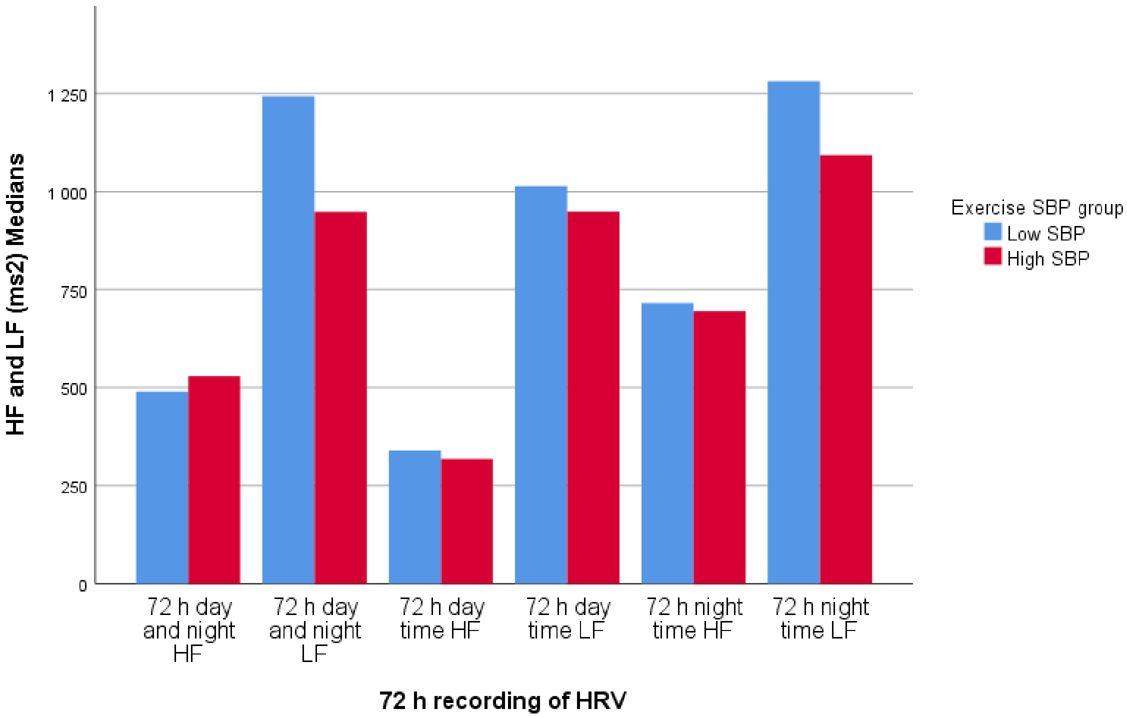
TABLE 6. 72-hour HRV recordings between groups. Mann-Whitney U-test.

	Systolic blood pressure			Max. age-adjusted HR (%)		
HRV values	Low	High	p-value [a]	Low	High	p-value [a]
72-h (day and night)	Median	Median		Median	Median	
HF (ms^2)	489,7	529,3	0.603	478,3	534,2	0.401
LF (ms^2)	1243,4	948,4	0.635	694,2	1243,4	0.114
LF/HF Ratio	3,5	2,7	**0.044***	2,9	3,2	0.603
VLF (ms^2)	147,3	132,6	0.603	110,6	159,9	0.150
RMSSD (ms)	24,6	24,1	0.946	23,8	26,6	0.454
SD (ms)	140,8	168,7	0.352	146,4	166,5	0.265
SDNN Index (ms)	53,9	52,1	0.804	45,5	62,0	0.125
72-h (daytime)						
HF (ms^2)	339,0	318,0	0.734	302,7	384,2	0.265
LF (ms^2)	1013,9	949,0	0.804	557,5	1064,7	0.194
LF/HF Ratio	3,9	3,0	**0.035***	3,5	3,4	1.000
VLF (ms^2)	119,1	115,4	0.769	79,4	133,4	0.178
RMSSD (ms)	20,2	21,5	0.839	20,2	22,9	0.376
SD (ms)	111,2	134,3	0.285	108,2	138,6	0.137
SDNN Index (ms)	49,7	53,7	0.571	43,0	60,6	0.085
72-h (nighttime)						
HF (ms^2)	715,7	694,1	0.901	614,6	813,4	0.603
LF (ms^2)	1280,7	1092,7	0.571	865,9	1280,7	0.137
LF/HF Ratio	2,2	1,7	0.085	1,4	2,3	0.194
VLF (ms^2)	175,6	183,5	0.667	174,4	184,4	0.246
RMSSD (ms)	35,0	31,5	1.000	30,2	35,5	0.454
SD (ms)	92,8	109,2	0.734	87,2	111,0	0.125
SDNN Index (ms)	58,5	54,8	0.839	50,0	61,6	0.178

[a] Mann-Whitney U-test. * p<0.05

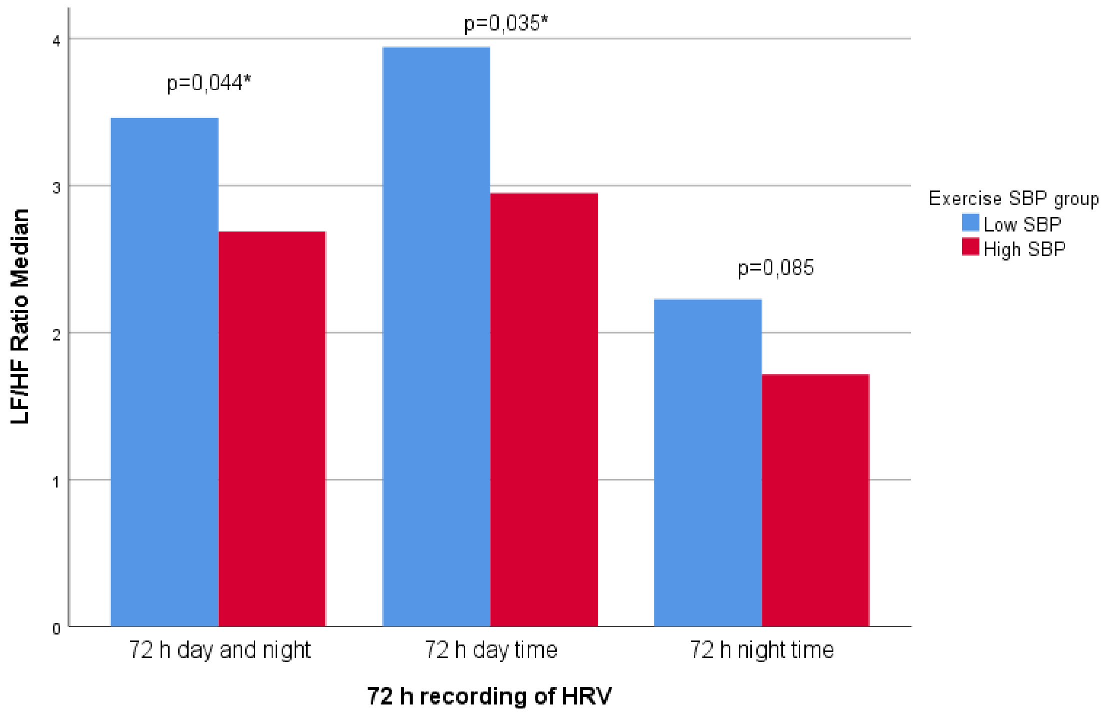
72-hour HF and LF values in lower and higher SBP group are presented in Figure 10, however there were no statistically significant differences. Both HF and LF values are represented in continuous 72-hour recording as well as only daytime and nighttime values of the same recording.

FIGURE 10. HF and LF median values (ms^2) in 72-hour recording between SBP groups.



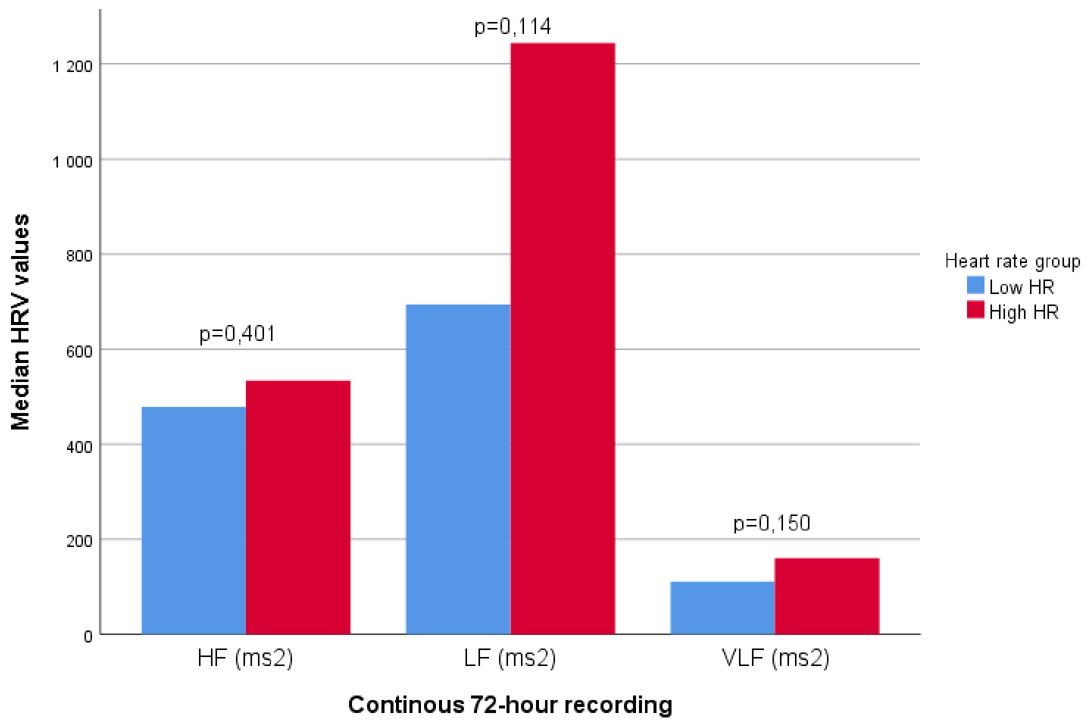
For the LF/HF ratio 72-hour continuous, daytime and nighttime values between SBP groups are represented in Figure 11. Continuous 72-hour recording of the LF/HF ratio was significantly higher in the group with lower SBP values during the exercise test (3,5 vs. 2,7, p=0.044). The daytime values of LF/HF ratio was significantly higher and the nighttime values was non-significantly higher in lower SBP group.

FIGURE 11. LF/HF Ratio on 72-hour recording between SBP groups.



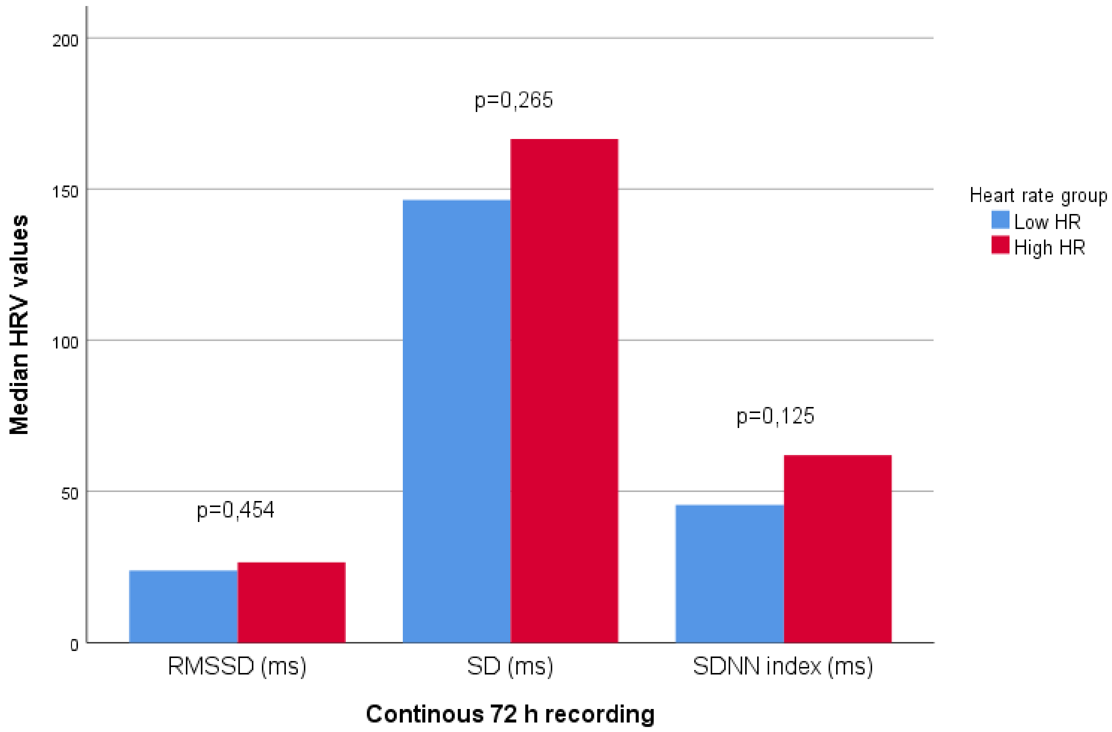
In Figure 12 HF, LF and VLF values in 72-hour continuous recording between lower and higher age-adjusted HR group are depicted. There were no statistically significant associations between HF, LF or VLF 72-hour values depending on the HR during the exercise test. However, all of the presented frequency domain HRV values were non-significantly higher in higher HR group.

FIGURE 12. Frequency HRV values (ms^2) and continuous 72-hour recording within HR groups.



Comparison of the 72-hour continuous recording of the time domain HRV values (RMSSD, SD and SDNN Index) between lower and higher HR group are depicted in Figure 13. No statistically significant differences were found depending on the HR during exercise.

FIGURE 13. Time domain HRV values and continuous 72-hour recording between HR groups.



8 DISCUSSION

The aim of this thesis was to study associations between an imbalanced autonomic nervous system and the exercise-induced exaggerated blood pressure as well as attenuated HR response. SBP and HR responses during as well as after exercise and their associations to the autonomic nervous system were analyzed. The study population consisted of risk population with hypertension, type 2 diabetes and/or impaired glucose metabolism.

The ANS function was evaluated during the exercise test between low and high exercise-induced SBP group as well as low and high age-adjusted HR group. HRV values were used to describe the function of the ANS. In addition, ANS function during 72-hour recording was compared within the SBP and the HR groups. Systolic blood pressure (SBP) value was used to measure blood pressure response since variation of the diastolic blood pressure (DBP) response is small during exercise (Fletcher et al. 2013).

In general, HRV values reacted to the exercise test according to theory represented in this thesis. When the exercise intensity grew it resulted large reduction to all of the HRV values. During the peak exercise phase, HF and LF values were near zero, but started to rise during recovery. Greater reduction during exercise and slower recovery of HRV values was seen with more intense exercise. More intense exercise has a longer HRV lowering effect (Task Force Report 1996; Stanley et al. 2013).

8.1 Associations of HRV and blood pressure

Hypothesis was that the parasympathetic activity would be higher within low SBP group since higher parasympathetic activity lowers BP responses (Kougias et al. 2010; Shaffer et al. 2014; Raven et al. 2019). Higher HRV is the sign of healthy heart, which have more flexibility to react in stress situations (Fatisson et al. 2016) such as exercise. Activation of the parasympathetic nervous system (HF) was non-significantly higher in the low SBP group after the exercise when the mean value of 5-minute recovery was analyzed. If the population would have been higher, the difference might have been significant due to fact that the p-value is affected by the population size (Thiese et al. 2016). This can indicate that the parasympathetic activation might be higher or faster with lower exercise induced SBP values, and dysregulation of the ANS might be associated with higher SBP values during exercise. Dysregulation of the ANS accompanies many CVD's, such as hypertension (Fisher et al. 2015). Dynamic autonomic regulation of the vagal outflow is important to cardiovascular health (Shaffer et al. 2014). More rapid parasympathetic activation is considered to represent more resilient and healthier ANS function.

When the LF values recorded during the exercise test were compared between SBP groups, largest non-significant difference was a mean value of 5-minute recovery with higher LF values in the lower SBP group. The LF can represent sympathetic nervous system activity, but it can be influenced with parasympathetic mechanisms as well (Vinik & Ziegler 2007; Shaffer et al. 2014). Exercise increases sympathetic activation (Fadel 2015; Fisher et al. 2015), but during the exercise test LF values acted in a similar way compared to HF values decreasing near zero in the peak exercise phase and starting again rise during the recovery phase. In addition, the LF values were higher, but not with statistical significance, in the lower SBP group in recovery phase when the activation of parasympathetic nervous systems occurs. According to these results, LF values can represent parasympathetic activation as well. Parasympathetic activation may have even stronger influence on the LF band compared to sympathetic activation (Billman 2013).

With RMSSD values no clear differences were found between SBP groups. RMSSD values represent parasympathetic activation (Shaffer et al. 2014). During exercise RMSSD values dropped when the intensity increased and raised again during recovery. The RMSSD values should correlate to HF values (Task Force Report 1996). However, there were no notable differences in the RMSSD values between SBP groups after the 5-minute recovery time, although higher, but not statistically significant, HF values were found in the lower SBP group.

The LF/HF ratio was non-significantly lower in low SBP group (1,47 vs. 2,20, $p=0.056$) after 5-minute recovery time. The ratio can estimate ratio between SNS and PNS activity (Shaffer

& Ginsberg 2017). As stated, both SNS and PNS contribute to LF, but HF reflects PNS activity. For this reason, LF/HF ratio can indicate balance of the ANS function. Lower values in low SBP group can indicate higher PNS activation and whereas higher ratio values in high SBP group can represent larger SNS activation.

When the 72-hour recording of the LF/HF ratio was compared within SBP groups the significantly higher LH/HF ratio values were found in the group with lower SBP values during the exercise test. During exercise test, LF/HF ratio indicated higher PNS activation association to lower SBP value, longer recordings during everyday life did not indicate similar results. During the 72-hour HRV recording the circumstances of the study participants (e.g. exercise or alcohol consumption) were not controlled, which can greatly affect the HRV. Therefore, interpretation of the 72-hour recordings should be done with caution.

Although HRV has been considered useful method to evaluate autonomic activity, especially parasympathetic activity, there are controversies considering interpretation of HRV as a marker of cardiac sympathetic activity or “sympatho-vagal balance” (Michael et al. 2017). Due to the complex nature of LF band and its’ contributions, the LF/HF ratio should be interpreted with caution especially with short-term recordings (Shaffer et al. 2014). Billman (2013) suggests that LF is not an index of sympathetic activity, but rather reflects a complex mix of SNS and PNS activity with unidentified factors and LH/HF ratio does not accurately measure “sympatho-vagal balance”. As a consequence, the LH/HF ratio is difficult to interpret. The HRV measures that reflect parasympathetic activity (e.g. RMSSD and HF) are widely accepted and supported by multiple studies (Michael et al. 2017). Therefore, HF and RMSSD might provide more reliable insight to ANS status, especially to the activity of the PNS.

When secondary variables (Tables 2-4) were compared between lower and higher SBP group differences were found with important prognostic markers. IFG and IGT indicate early sign of type 2 diabetes and are a risk factor for CVD (Syvänne 2017; Ilanne-Parikka 2018). In addition, autonomic dysfunction is accompanied with type 2 diabetes (Röhling et al. 2017).

Higher IFG and IGT prevalence in higher SBP group might indicate higher probability to autonomic dysfunction, which is associated with higher exercise induced SBP values.

Higher post-exercise SBP values are associated with adverse health outcomes and SCD (Yosefy et al. 2006; Laukkanen et al. 2014). Significantly higher 1- and 3-minute post-exercise SBP values were found in the group with the higher SBP during exercise indicating slower parasympathetic activation. Cessation of the exercise should normally result to increased parasympathetic activity and rapidly lower the peripheral resistance as well as HR (Fisher et al. 2010; Fisher et al. 2015). Dysfunction of the ANS may result to increased vascular resistance and slower recovery of the SBP (Le et al. 2008).

In addition, the higher SBP group had higher, but not statistically significant, supine SBP and post-exercise SBP after 5 minutes. Non-significantly lower maximal HR, a non-significantly smaller heart rate reserve and a non-significantly slower heart rate recovery post-exercise were found in the higher SBP group. Smaller HR reserve and slower reduction of the HR can indicate the dysfunctional ANS system as well (Diller et al. 2006; Brubaker & Kitzman 2011).

8.2 Associations of HRV and heart rate

Associations between HRV and HR were analyzed between lower and higher age-adjusted HR group. In the higher HR group subjects exceeded age predicted HR more than in the lower group. HRpeak (peak value of HR during an exercise test) declines with age (Ozemek 2015). Therefore, it is an important fact to take into consideration when comparing peak HR and HRV values. Failure to reach maximal HR value during an exercise test is a sign of chronotropic incompetence, which is a predictor of cardiovascular events and mortality (Brubaker & Kitzman 2011). In addition, there might be inverse association between CRF levels and the rate of decline in HRpeak value (Ozemek et al 2015).

When HR values were compared to HRV values during rest phase before the exercise test, there were no significant differences between groups. During peak exercise phase HF and LF values were significantly lower in higher HR group. HRV values drop more when the exercise intensity increases (Task Force Report 1996; Stanley et al. 2013) and with higher HR group exercise might have been more intense due to higher HR. HF and RMSSD values were non-significantly lower in the higher HR group after 1-min recovery. Significantly lower HF and LF values during exercise as well as non-significantly lower HF and RMSSD recovery values are probably a result of absolute higher HR values in the higher HR group. The exercise time was non-significantly shorter, and subjects were significantly more overweight in lower HR group. These facts might affect the maximum HR during exercise resulting in lower absolute HR values and therefore higher HRV during exercise.

No significant differences were found when 72-hour HRV recordings were compared between lower and higher HR group. However, all the HRV values were non-significantly higher in high HR group during the whole 72-hour recording. This can indicate higher overall HRV values during everyday life with subjects who exceed age-predicted HR more.

The cardiac parasympathetic reactivation after exercise occurs more rapidly in individuals with greater aerobic fitness level (Stanley et al. 2013). If the subject exceeds the age-predicted HR it might represent better fitness level (Ozemek et al. 2015). However, there were no differences in VO_2max values between the HR groups (Table 4). HR reserve was significantly larger with the higher HR group. Failure to reach 80% of the age-predicted HR reserve can be used as a criterion for CI, which is a predictor for cardiovascular event and mortality (Brubaker & Kitzman 2011). Therefore, higher HR reserve might indicate more resilient heart and balanced ANS system as well as be a positive marker for one’s health. In this thesis the mean value of maximal age-adjusted HR (%) in the lower HR group was nearly 100% which indicates that almost the whole study population reached their age-predicted HR.

When the background variables between the higher and lower HR group were compared largest differences were found in body weight, BMI and HDL-cholesterol values (Tables 2-3). Body weight (kg) and BMI (kg/m^2) were significantly lower among subjects in the higher HR group. Overweight is a known risk factor for morbidities such as type 2 diabetes (Mustajoki 2019b). In addition, HDL-cholesterol was significantly higher among subjects belonging to higher the HR group. Low HDL-cholesterol values increase the risk for CVD (Mustajoki 2019c). However, significantly lower HDL cholesterol values in lower HR group were in the recommend range of the HDL cholesterol values (Eskelinen 2016) and not pathological. Differences in background variables can indicate association of a higher age-adjusted maximal HR to more positive health markers.

8.3 Reliability and ethics

Reliability of the study. Reliability of this thesis could be affected by multiple factors. The sample size is an important factor when the reliability of the study is considered. Larger sample size gives more reliable results that can be generalized to larger population. The sample of this study was small (N=28). Therefore, the results of this study might not represent the results that can be achieved with larger sample size. With small sample size one or few deviating values can affect greatly to mean values (Mattila 2003) that are used as a base of statistical analyzes. Majority of the group comparisons with normally distributed values was analyzed based on the mean values.

HRV varies greatly depending of the persons due to fact that multiple factors affect HRV (Fatisson et al. 2016). Environmental factors such as fatigue can affect HRV (Fatisson et al. 2016). Stress and negative emotions can also lower HRV (Fatisson et al. 2016). Test circumstances during the exercise test can create emotional stress as well as negative emotions and have lowering effect to HRV. In addition, stress from the exercise test can affect previous night sleep resulting fatigue in the test day. Due to individual variation of the HRV, the mean HRV values were not used in this study and the analyzing was done with Mann Whitney U – test, which is not affected by the variation of the data (Metsämuuronen 2006, 439). Similar analyzing methods with non-normally distributed HRV data have been used by others as well. Bobkowski et al. (2017) used Mann-Whitney U-test to compare sex differences in non-Gaussian HRV values among healthy children. Voss et al. (2015) compared HRV differences between different age and gender groups using Mann Whitney U-test due to fact that HRV values were not normally distributed.

However, other statistical methods are commonly used with HRV values such as normalization of the HRV data with natural logarithm (Shaffer & Ginsberg 2017). Normalization of the HRV values might have given different results due to fact that other and possibly wider statistical methods could have been used. However, this was not done due to fact that even after normalization there might have been too much variation within the HRV values. In addition, non-parametric Spearman correlation could have been used to study the associations between SBP, HR and HRV. Bobkowski et al. (2017) used both Mann-Whitney and non-parametric Spearman correlation to study associations between HRV, age and sex. Using both methods would have made the results to more reliable. However, this was not conducted in order to limit the already wide result section.

The group comparisons were done inside the study groups due to fact that this study did not include control group. The study population consisted population with confounding factors such as diseases, medication and overweight, which can affect to HRV values. Standardizations of the cofactors were not done due to fact that statistical test used in this thesis did not allow adding confounding factors to the model. If the control group consisting of healthy population would have been included in this study, the comparisons of the groups would have most likely given different results.

Exercise test was maximal exercise test, but several participants did not reach VO_2max value rather than VO_2peak value. VO_2peak means highest value reached during the exercise test while VO_2max represents highest physiologically reachable value for individual (Beltz et al. 2016). Thus, VO_2peak is not always same as the true VO_2max. This can alter the study results due to the fact that maximal HR and SBP were the base of the division of the comparison groups. If the subject is not actually achieving his or her maximal capacity, the maximal HR and SBP values are not truly maximal values. If a true maximal intensity would have been reached with all of the participants, the group divisions might have been different as well the results.

During the 72-hour continuous HRV measurement, the lifestyle of the participants was not controlled such as consumption of alcohol or exercise habits. Therefore, multiple everyday factors affected HRV values during this longer measurement. Participants were instructed to keep diary during this 72-hour measurement, but the analyzation of the diaries was not included to this thesis.

Ethics of the study. The privacy of the study participants was respected during the whole study. The data was managed and stored carefully according to privacy protection law and EU regulation so that the identification of the study participants was secured. The data of this thesis was anonymized so that identification of the participants is impossible. During the test procedures, the study participants were treated with respect. Study personnel were educated regarding to the data protection as well as the test procedure and qualified to execute the study protocols. The participants were encouraged to speak out any concerns or questions regarding to the study. If the participants wished to stop the exercise test, test was stopped immediately. The whole test protocol was based on voluntary participation. If the study participant wished to withdraw from the study at any given point, he or she had a right to do so without any consequences. Participants had to give written consent before participation in the study. The result of this thesis was analyzed and reported precisely. The data was inspected in multiple points during the analyzing phase to exclude possible errors. The literature review of this thesis was done based on reliable sources and the references were done according to university instructions with respect to original writers.

8.4 Future research proposal and conclusion

Exaggerated systolic blood pressure during exercise is a risk factor for future hypertension and negative health outcomes. Reasons for the exaggerated systolic blood pressure during exercise originated from multiple physiological mechanisms that autonomic nervous system regulates. The dysregulation of the ANS impacts to the physiological regulatory factors of blood pressure and heart rate which manifest itself as abnormal physiological responses during and immediately after exercise.

In this study population, HF values, which describe parasympathetic activation, were non-significantly higher with those who had lower SBP values during maximal exercise test.

These results might indicate that lower SBP values during exercise are associated with more balanced autonomic nervous system in study population with hypertension, type 2 diabetes and/or prediabetic state.

LF/HF ratio that have been used to describe balance between sympathetic and parasympathetic nervous system gave conflicting results in this study. 72-hour LF/HF ratio was higher among those with lower SBP during exercise. Lower LF/HF ratio is associated with better health outcomes, but the results are conflicting with this value (Billman 2013; Michael et al. 2017). Therefore, no clear conclusions can be drawn from these results in this thesis.

There were more positive health markers with those who had lower SBP values during the maximal exercise test. Statistically significant differences were lower prevalence of prediabetic state and lower SBP during recovery (measured in 1- and 3-minutes post exercise). In addition, post-exercise heart rate recovery (1- and 3-minute value) was non-significantly higher among those who had lower maximal SBP value during the exercise test. Lower SBP and HR values after exercise indicate rapid restoration of parasympathetic nervous system, which is a sign of more resilient ANS function. Glucometabolic disturbances, which are seen in prediabetic state, affect negatively to HRV values and ANS function. Higher cardiac parasympathetic activation might have protective effects after exercise as well as in everyday life.

Subjects in the higher HR group had significantly higher HR reserve. Higher HR reserve might predict more resilient heart function and more balanced ANS system. HRV in everyday life was non-significantly higher with subjects in higher HR group. In addition, subjects had significantly lower body weight and BMI in higher HR group. Overweight is significant risk factor for adverse health outcomes (Mustajoki 2019b). Overall, subjects in the higher HR group had more positive health indicators.

In future these analyzing methods could be repeated with larger sample size to found out if the differences would be statistically significant. Especially, exaggerate SBP values during exercise and their associations to HRV values that indicate PNS activation should be analyzed. In addition, the concentration to the post-exercise values might give new results. Investigation of the post-exercise SBP values and associations to HRV might reveal association with higher post-exercise SBP values to lower HRV after exercise or everyday life. This could indicate associations with dysfunctional ANS system and lower HRV. With larger sample size effects of different medications could be interpreted. This was not in this thesis due to too small sample size. Healthy control group would give more information about the differences between risk and healthy population. This could reveal the associations of the ANS dysfunction to hypertension, type 2 diabetes and prediabetic state. Strong association can highlight the importance of HRV as a marker of overall health.

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ATTACHMENT 1

Inclusions and exclusions criteria of the study population

Inclusion criteria:

- age 18-64 years
- diagnosed prediabetic state (i.e. elevated IFG/IGT) and/or type 2 diabetes and/or hypertension during the last 5 years
- BMI under 40 kg/m^2
- no disease or symptom that would affect fundamentally to the measurements of the study

Exclusion criteria:

- Medication: β-blockers, insulin, regular SNRI-, regular tricyclic medication
- Cardiovascular diseases: secondary hypertension, coronary artery disease, clinically significant hypertension-mediated organ damage, heart failure, cerebrovascular disease, chronic atrial fibrillation, significant or nonspecified valvular disease, cerebrovascular disease, left bundle branch block
- Pulmonary diseases: symptomatic or unstable asthma, chronic obstructive pulmonary disease
- Other diseases: cancer, anemia, diabetic neuropathy/nephropathy/retinopathy, symptomatic or unstable disorder of thyroid gland, obstructive sleep apnea with CPAP-treatment, psychotic disorder or other unstable psychiatric disorder, disease that limits overall physical function, or other disease that is contraindication for clinical exercise test
- Other factors: pregnancy, nursing, substance abuse

TABLE 7. HRV comparison between SBP and HR groups. Mann-Whitney U-test (* p<0,05).

	Systolic blood pressure				Max. age-adjusted HR			
HRV values		Low	High	p-value		Low	High	p-value
Rest 5 min	U-value	Median	Median		U-value	Median	Median	
HF (ms^2)	89,00	304,89	325,64	0,701	104,00	325,64	304,89	0,804
LF (ms^2)	79,00	663,16	639,97	0,401	110,00	565,76	663,16	0,603
LF/HF Ratio	87,00	2,31	1,80	0,635	107,00	1,85	2,06	0,701
VLF (ms^2)	78,00	70,83	68,45	0,376	120,00	51,22	75,34	0,329
RMSSD (ms)	87,00	14,26	13,56	0,635	107,00	12,88	14,73	0,701
SD (ms)	89,00	35,79	30,10	0,701	102,00	30,10	36,82	0,874
Exercise [a]								
HF (ms^2)	108,00	0,89	1,59	0,667	43,00	2,24	0,72	**0,011***
LF (ms^2)	103,50	0,47	0,58	0,804	49,00	0,97	0,27	**0,024***
LF/HF Ratio	71,50	0,62	0,46	0,227	100,00	0,52	0,48	0,946
RMSSD (ms)	92,50	4,49	4,43	0,804	62,50	4,79	4,08	0,104
SD (ms)	73,00	11,36	9,75	0,265	61,00	13,20	9,46	0,094

Recovery 0-1 min								
HF (ms ^{2})	76,50	1,86	1,09	0,329	59,00	2,19	1,13	0,077
LF (ms ^{2})	76,00	2,15	1,09	0,329	77,00	2,49	1,36	0,352
LF/HF Ratio	102,00	1,11	1,17	0,874	116,00	0,97	1,31	0,427
RMSSD (ms)	105,00	4,22	4,31	0,769	59,00	4,84	3,21	0,077
SD (ms)	87,00	18,85	17,17	0,635	70,00	17,93	16,55	0,210
Recovery 0-5 min								
HF (ms ^{2})	64,00	29,95	13,84	0,125	75,00	23,76	19,15	0,306
LF (ms ^{2})	67,00	52,19	26,43	0,164	81,00	39,68	29,46	0,352
LF/HF Ratio	140,00	1,47	2,20	0,056	121,00	1,71	2,07	0,306
VLF (ms ^{2})	81,00	5,59	3,89	0,454	72,50	6,30	4,28	0,246
RMSSD (ms)	81,50	6,12	5,59	0,454	70,50	6,43	4,93	0,210
SD (ms)	102,00	85,68	94,80	0,874	73,00	98,49	86,01	0,265
72 h (continuous)								
HF (ms ^{2})	110,00	489,66	529,31	0,603	117,00	478,32	534,18	0,401
LF (ms ^{2})	87,00	1243,41	948,42	0,635	133,00	694,15	1243,41	0,114
LF/HF Ratio	54,50	3,46	2,69	**0,044***	109,50	2,88	3,21	0,603
VLF (ms ^{2})	86,00	147,31	132,60	0,603	130,00	110,62	159,89	0,150
RMSSD (ms)	100,00	24,60	24,12	0,946	115,00	23,83	26,55	0,454
SD (ms)	119,00	140,81	168,73	0,352	123,00	146,41	166,49	0,265
SDNN Index (ms)	104,00	53,86	52,06	0,804	132,00	45,46	62,03	0,125

72 h (daytime)								
HF (ms^2)	106,00	338,97	318,03	0,734	123,00	302,7	384,2	0,265
LF (ms^2)	92,00	1013,86	948,96	0,804	127,00	557,50	1064,72	0,194
LF/HF Ratio	52,00	3,94	2,95	**0,035***	99,00	3,54	3,44	1,000
VLF (ms^2)	91,00	119,12	115,42	0,769	128,00	79,40	133,37	0,178
RMSSD (ms)	103,00	20,22	21,47	0,839	118,00	20,16	22,90	0,376
SD (ms)	122,00	111,22	134,29	0,285	131,00	108,15	138,59	0,137
SDNN Index (ms)	111,00	49,74	53,68	0,571	136,00	43,00	60,56	0,085
72 h (nighttime)								
HF (ms^2)	101,00	715,72	694,10	0,901	110,00	614,6	813,4	0,603
LF (ms^2)	85,00	1280,73	1092,69	0,571	131,00	865,89	1280,73	0,137
LF/HF Ratio	60,00	2,23	1,71	0,085	127,00	1,44	2,25	0,194
VLF (ms^2)	88,00	175,57	183,53	0,667	124,00	174,41	184,36	0,246
RMSSD (ms)	98,00	35,02	31,51	1,000	115,00	30,19	35,51	0,454
SD (ms)	106,00	92,77	109,18	0,734	132,00	87,23	111,00	0,125
SDNN Index (ms)	93,50	58,51	54,84	0,839	128,00	50,03	61,55	0,178