Turun yliopisto
University of Turku

HEART RATE VARIABILITY IN YOUNG ADULTS

Reference values and associations with cardiometabolic risk factors and vascular properties

The Cardiovascular Risk in Young Finns Study

Tuomas Koskinen

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1134 | Medica - Odontologica | Turku 2014

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The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-5853-5 (PRINT)
ISBN 978-951-29-5854-2 (PDF)
ISSN 0355-9483
Painosalama Oy - Turku, Finland 2014

To my family

ABSTRACT

Tuomas Koskinen. Heart rate variability in young adults - Reference values and associations with cardiometabolic risk factors and vascular properties. The Cardiovascular Risk in Young Finns Study. From the Department of Clinical Physiology and Isotope Medicine, Institute of Clinical Medicine, Faculty of Medicine, Doctoral Programme of Clinical Investigation, and Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland and Turku University Hospital. Annales Universitatis Turkuensis, Medica-Odontologia, Turku, Finland, 2014.

Background: The function of the autonomic nervous system (ANS) can be evaluated with heart rate variability (HRV). Decreased HRV is associated with aging, the male sex, increased heart rate, and overall increased cardiometabolic risk. It has been hypothesised that early atherosclerotic vascular changes and ANS function are related.

Aims: The aims were to assess reference values on HRV in young adults, and examine associations with HRV and cardiometabolic risk factors and metabolic syndrome (MetS) and to study relations between HRV and ultrasonographically measured vascular properties.

Participants and methods: The present thesis is part of the Cardiovascular Risk in Young Finns Study. The thesis is based on the follow-up study in 2001, when the study individuals were 24-39 years of age. HRV data were available on 1 956 individuals.

Results: HRV was inversely associated with age and heart rate (for all $p<0.001$). High-frequency HRV (HF) was higher, and low-frequency HRV (LF) lower in women than men ($p<0.0001$ for both). MetS was associated with 11% decreased HF and 12% increased LF/HF-ratio in women, and 8% decreased HF and 4% increased LF/HF-ratio in men. Carotid artery distensibility was independently associated with HF and total HRV (for both $p<0.05$).

Conclusions: The reference values in young adults were generated. Decreased HRV was associated with age, the male sex and increased heart rate. Women had higher HF and lower LF variability than men. MetS was related to decrease in HRV. The observed associations between carotid elasticity and HRV, supports the hypothesis that reduction in carotid elasticity may lead to decrease in autonomic cardiac control.

Keywords: heart rate variability, autonomic nervous system, reference values, metabolic syndrome, atherosclerosis, autonomic cardiac control

TIIVISTELMÄ

Tuomas Koskinen. Sydämen sykevaihtelu nuorilla aikuisilla – viitearvot ja yhteys sydän- ja verisuonitautien riskitekijöihin sekä varhaisiin valtimomuutoksiin. From the Department of Clinical Physiology and Isotope Medicine, Institute of Clinical Medicine, Faculty of Medicine, Doctoral Programme of Clinical Investigation, and Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland and Turku University Hospital. Annales Universitatis Turkuensis, Medica-Odontologia, Turku, Finland, 2014.

Tausta: Autonomisen hermoston toimintaa voidaan arvioida mittaamalla sydämen syketaajuudessa tapahtuvaa jaksoittaista vaihtelua, sydämen sykevaihtelua. Vähentynyt sydämen sykevaihtelu on sydän- ja verisuonitautien itsenäinen riskitekijä. Sykevaihtelun väheneminen liittyy ikääntymiseen, miessukupuoleen ja kiihtyneeseen sydämen syketaajuuteen sekä useisiin valtimotaudin riskitekijöihin. Varhaisten valtimotautimuutosten sekä autonomisen hermoston toiminnan välillä on aiemmin esitetty olevan yhteys, jota voidaan tutkia sykevaihtelun ja ultraäänellä mitattujen varhaisten valtimomuuttujien avulla.

Tavoite: Tutkimuksen tavoitteena oli luoda sykevaihtelun viitearvot nuorille aikuisille sekä tutkia sykevaihtelun yhteyttä sydän- ja verisuonitautien riskitekijöihin ja metaboliseen oireyhtymään sekä selvittää onko varhaisilla valtimomuutoksilla yhteyttä sydämen autonomiseen säätelyyn.

Menetelmät: Väitöskirjatutkimus on osa Lasten Sepelvaltimotaudin Riskitekijät – tutkimusta ja perustuu vuonna 2001 toteutettuun seurantatutkimukseen, jolloin tutkittavat olivat iältään 24–39 vuotta. Tutkimukseen oli käytettävissä 1 956 tutkimushenkilön sykevaihtelumittaukset.

Tulokset: Kaikki sykevaihtelun muuttujat olivat yhteydessä tutkittavien ikään ja syketaajuuteen (molemmilla $p<0.001$). Naisilla korkeataajuuksinen sykevaihtelu oli miehiä runsaampaa ($p<0.0001$) ja matalataajuuksinen sykevaihtelu vähäisempää ($p<0.0001$). Metaboliseen oireyhtymään liittyi vähentynyt matalataajuuksinen sykevaihtelu (-11% naisilla ja -8% miehillä). Kaulavaltimon elastisuus oli itsenäisesti yhteydessä sydämen sykevaihteluun ($p<0.05$).

Johtopäätökset: Tutkimuksessa luotiin sykevaihtelun viitearvot terveille aikuisille. Sykevaihteluun vaikuttivat voimakkaimmin ikä, sukupuoli sekä syketaajuus. Metaboliseen oireyhtymään liittyi merkittävästi vähentynyt sykevaihtelu. Valtimoiden seinämän jäykistyminen saattaa olla yksi mekanismi sydämen autonomisen säätelyn muutosten taustalla.

Avainsanat: Sydämen sykevaihtelu, autonominen hermosto, viitearvot, metabolinen oireyhtymä, valtimonkovettumatauti

TABLE OF CONTENTS

ABSTRACT **4**

TIIVISTELMÄ **5**

ABBREVIATIONS **8**

LIST OF ORIGINAL PUBLICATIONS **9**

1. INTRODUCTION **10**

2. REVIEW OF THE LITERATURE **11**

2.1. Physiological cardiovascular control mechanisms and heart rate 11

2.2. Examination of heart rate variability 14

2.2.1. Time-domain analysis of heart rate variability 15

2.2.2. Frequency-domain analysis of heart rate variability 16

2.3. Heart rate variability in specific conditions 18

2.3.1. HRV and cardiometabolic non-modifiable risk factors 19

2.3.2. HRV and cardiometabolic biological risk factors 20

2.3.3. HRV and cardiometabolic lifestyle risk factors 24

2.3.4. HRV and vascular properties 25

2.4. Heart rate variability and the mechanism of disease 27

2.5. Clinical implications of heart rate variability analysis 27

3. AIMS OF THE STUDY **29**

4. PARTICIPANTS AND METHODS **30**

4.1. Description of the Cardiovascular Risk in Young Finns Study 30

4.2. Study design and participants 30

4.3. Examination of heart rate variability 32

4.4. Deep breathing test 32

4.5. Reproducibility study 33

4.6. Biochemical analyses 33

4.7. Definition of metabolic syndrome 33

4.8. Physical examination and questionnaires 34

4.9. Ultrasound studies 35

4.10. Statistical analyses 36

4.11. Ethics 38

5. RESULTS **39**

5.1. Characteristics of participants (Study I) 39

5.2. Reproducibility, office-hour variation and effect of time-series duration on heart rate variability 40

5.3. Correlation between time-domain and frequency-domain heart rate variability patterns....41
5.4. Effect of age, sex and heart rate on heart rate variability....43
5.5. Reference values of heart rate variability in young adults....46
5.6. Heart rate variability and metabolic syndrome (Study II)....48
5.7. Heart rate variability and vascular properties....52

6. DISCUSSION....56
6.1. Study participants....56
6.2. Effect of age, sex and heart rate on heart rate variability (Study I)....56
6.3. Reference values, reproducibility and office-hour variation of heart rate variability (Study I)....58
6.4. Heart rate variability and metabolic syndrome (Study II)....59
6.5. Heart rate variability and vascular properties (Study III)....61
6.6. Strengths and limitations....64
6.7. Future perspectives....65

7. CONCLUSIONS....66

ACKNOWLEDGEMENTS....67

REFERENCES....69

ABBREVIATIONS

ANS	Autonomic nervous system
ARIC	Atherosclerosis Risk in Communities study
ATRAMI	Autonomic Tone and Reflexes After Myocardial Infarction study
BMI	Body mass index
Cdist	Carotid artery distensibility
CHD	Coronary heart disease
cIMT	Carotid artery intima-media thickness
CV	Coefficient of variation
ECG	Electrocardiogram
EGIR	European Group for the Study of Insulin Resistance
FMD	Brachial artery endothelium-dependent flow-mediated dilatation
HDL	High-density lipoprotein
HF	High frequency-domain heart rate variability
HRV	Heart rate variability
IDF	International Diabetes Federation
LDL	Low-density lipoprotein
LF	Low frequency-domain heart rate variability
LF/HF	Ratio between low and high frequency heart rate variability
MAP	Mean arterial pressure
MetS	Metabolic syndrome
NCEP	National Cholesterol Education Program Expert Panel
PSD	Spectral density of HRV
RC	Reliability coefficient
RMSSD	Square root of the mean squared differences of successive R-R intervals
RSA	Respiratory sinus arrhythmia
SD	Standard deviation
SDANN	Standard deviation of the average R-R intervals calculated over short periods
SDNN	Standard deviation of R-R intervals
SVR	Systemic vascular resistance
TG	Triglycerides
TP	Total frequency-domain heart rate variability
VLDL	Very low-density lipoprotein
VLF	Very low frequency-domain heart rate variability

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to within the text by the Roman numerals I-III. In addition, previously unpublished data are presented.

I. Koskinen T, Kähönen M, Jula A, Laitinen T, Keltikangas-Järvinen L, Viikari J, Välimäki I, Raitakari OT. Short-term heart rate variability in healthy young adults: the Cardiovascular Risk in Young Finns Study. Auton Neurosci. 2009;145:81-8.

II. Koskinen T, Kähönen M, Jula A, Mattsson N, Laitinen T, Keltikangas-Järvinen L, Viikari J, Välimäki I, Rönnemaa T, Raitakari OT. Metabolic syndrome and short-term heart rate variability in young adults. The Cardiovascular Risk in Young Finns Study. Diabet Med. 2009;26:354-61.

III. Koskinen T, Juonala M, Kähönen M, Jula A, Laitinen T, Keltikangas-Järvinen L, Viikari J, Välimäki I, Raitakari OT. Relations between carotid artery distensibility and heart rate variability. The Cardiovascular Risk in Young Finns Study. Auton Neurosci. 2011;161:75-80.

1. INTRODUCTION

Arterial blood pressure is the driving force of circulation maintained by a spontaneously operating pump, the heart, which is under an instantaneous control of the autonomic nervous system (ANS). Studies during the last three decades have revealed relations between the function of ANS and cardiometabolic risk. Autonomic regulation of heart rate can be quantitatively examined by studying fluctuations in heart beat, i.e. heart rate variability (HRV). Decreased HRV has been found to be associated with multiple pathological cardiovascular conditions, including ischemic heart disease [1], heart failure [2] and hypertension [3], as well as metabolic disorders e.g. metabolic syndrome (MetS) [4] and diabetes [5]. A certain association between a sudden (cardiac) death and limited ANS activity has been reported since 1970s [6-8]. More recently it has been observed that decreased HRV after myocardial infarction is associated with an elevated risk of sudden cardiac death [9-11]. Reduced HRV is in additionally related to the extensiveness and progression of existing atherosclerosis [12;13]. Although the principle of the measurement of HRV in adults was already presented in the end of 1940s [14], and despite active research on its various aspects, it is still unclear why individuals with decreased HRV would be at increased risk of cardiovascular death.

The Cardiovascular Risk in Young Finns Study is an ongoing multi-centre study where participants have been followed since 1980, and both biological and lifestyle cardiometabolic risk factors have been regularly measured. In 2001, when the subjects of this project were 24 to 39 years old, the HRV analysis was included to the study protocol. The aim of this HRV study was to generate the normal reference values for HRV in healthy young adults and to examine associations between a standard set of cardiometabolic risk factors, ultrasound based vascular properties, especially arterial elasticity and HRV pattern in Finnish population.

2. REVIEW OF THE LITERATURE

2.1. Physiological cardiovascular control mechanisms and heart rate

The cardiovascular system is regulated by multiple mechanisms, including e.g. the nervous and hormonal pathways. The two divisions of the ANS, the parasympathetic, via vagus nerves, and the sympathetic system, including the adrenal medulla, play a major role in the control of cardiovascular system (Figure 1). The main controlled variable is the arterial blood pressure, which is monitored by multiple receptors and adjusted by modulating heart rate, cardiac contractility and systemic vascular resistance of circulation. In principle, this short-term regulatory mechanism forms a negative feed-back control loop with a time delay, which results in sinusoidal oscillations in the controlled variables of blood pressure and heart rate [15;16].

Cardiovascular ANS control is regulated by reflexes providing information to the central nervous system and vasomotor centre. These reflexes are modulated on a receptor level by baroreceptors, chemoreceptors, thermoreceptors and atrial volume receptors [17]. Baroreceptors are located in the sinus caroticus of the internal carotid artery and in the aortic arch. These receptors are regulated by the mean transmural pressure and the amplitude and the steepness of transmural pressure. Thus arterial receptors are continuously active during each cardiac cycle, monitoring oscillations in arterial blood pressure [17;18]. In normal conditions at rest there is a very close relation between instantaneous arterial blood pressure and the successive beat-to-beat interval provided by the baroreflex [19]. This fast control causes oscillations in the heart rate at the natural frequency around 0.1 Hz. The arterial baroreflex is an example of a negative loop feed-back system and is designed to buffer beat-to-beat oscillations in arterial blood pressure from the baseline. This sympatho-inhibitory reflex is stimulated by acute changes in arterial blood pressure. Afferent baroreceptor discharge evokes changes in efferent sympathetic and parasympathetic control to the heart and blood vessels that adjust cardiac output and vascular resistance to return blood pressure to its original baseline. The baroreceptor reflex can be described as a buffer, protecting the body, especially the brain, from excessive swings in systemic arterial pressure [20].

The carotid body chemoreceptors are stimulated by hypoxia, hypercapnia and acidosis. The circulatory effects are excitatory but the chemoreceptors are principally involved with respiratory control. Within normal conditions chemoreceptors have minimal cardiovascular effects though it has been proposed that during severe exercise the reflex may provide an additional excitatory boost [20]. Atrial volume receptors are located at the junction of the vena cava and right atrium, and around the pulmonary veno-atrial junctions, where they respond to changes in central blood volume [21;22]. The cardiovascular role of atrial volume receptors was shown in the 1950s when Henry et al. performed experiments with dogs in which they caused localized distension of the left atrium by inflating a balloon that partially obstructed

the mitral valve. This stretch of the atrial chamber resulted in a marked increase in urine flow. In a later study they showed that this response could be eliminated by cooling the cervical vagus nerves to block its nerve traffic [23;24]. These experiments demonstrated that a change (increase) in blood volume can elicit an increase in renal excretion. The afferent pathway of this reflex is via the vagus nerve and the response as a cardiac acceleration is mediated by sympathetic stimulation. The major role of atrial volume receptors is blood volume regulation through the reflex diuresis [20]. In addition, there are other simultaneous low-frequency oscillations in heart rate caused by vasomotor thermoregulatory control, mediated in part by the sympathetic control of the peripheral vascular bed [25]. With this negative loop feed-back reflex control, the ANS can function satisfactorily even if entirely separated from the control of the central nervous system [26].

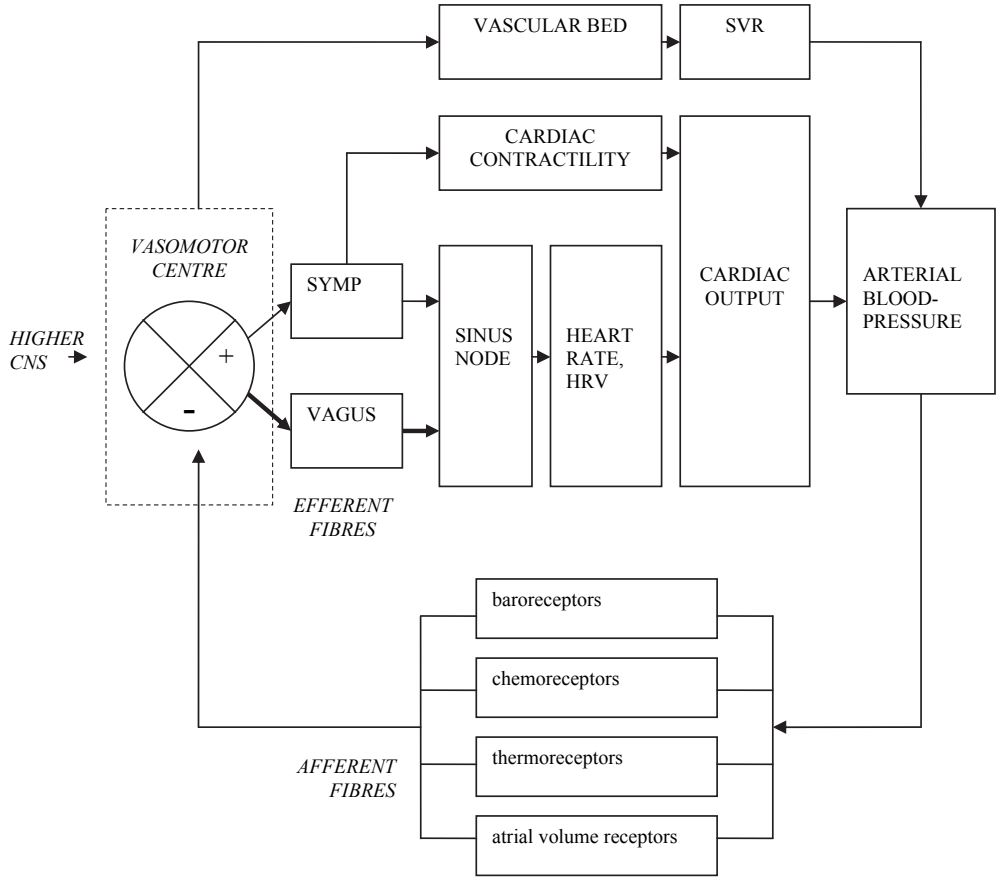

Figure 1. Block diagram of physiological control of the cardiovascular system influencing heart rate and blood pressure; notice negative feedback. CNS=central nervous system, HRV=heart rate variability, symp=sympathetic nervous system and SVR=systemic vascular resistance.

In humans, the cardiac rhythm is largely under the control of parasympathetic neurons influencing the sinus node via the vagus nerve and small number of blood vessels limiting their influence mainly to the control of cardiac function. The role of the vagal control is instantaneous adjustment of cardiac function at appropriately limited level. In addition to the heart, the sympathetic neural network innervates the peripheral vascular bed, adrenal glands, and kidneys providing for more widespread (direct and indirect) control of the cardiovascular system. The classical general actions of the sympathetic control are to prepare the individual for a 'fight or flight reaction'.

Acetylcholine is the principal neurotransmitter released from the parasympathetic nerve fibers on target muscarinic acetylcholine receptors. The effects of acetylcholine are local and short-lived due to high local concentrations of acetylcholinesterase, which very rapidly degrades the neurotransmitter and prevents its appearance in the bloodstream. Sympathetic fibres release norepinephrine, which has more prolonged and wider ranging effects than acetylcholine [21;26]. Efferent sympathetic and vagal activities directed to the heart via sinus node are characterized by discharge largely synchronous with each cardiac cycle that can be modulated by central vasomotor and respiratory centres as well as peripheral oscillation in arterial pressure and respiratory activity. The negative feedback control and resulting time delays generate rhythmic fluctuations in the heart period. Analysis of these variations may permit inferences on the state and function of the central oscillators, the sympathetic and vagal efferent activity, humoral factors and the sinus node itself.

An interesting physiological phenomenon caused by the ANS control of the heart is respiratory sinus arrhythmia (RSA), heart rate variability linked to respiration. RSA is generated by autonomic reflexes and central autonomic influences [27]. During respiration pulmonary stretch receptors as well as cardiac mechanoreceptors and possibly baroreceptors contribute to regulating the pattern of HRV. Inspiration increases heart rate and expiration decreases it. RSA is used as an index of cardiac vagal control of the heart rhythm [28]. The RSA arises when the efferent cardiac vagal motoneurons in the medulla (nucleus ambiguous) at the central nervous system discharge during expiration, and silence during inspiration [29]. Two major mechanisms have been recognised for generating RSA: a direct modulation of the cardiac vagal preganglionic neurons by central respiratory drive; and inhibition of cardiac vagal efferent activity by lung inflation [30;31]. The physiological meaning of RSA is unclear but it has been hypothesised that RSA benefits pulmonary gas exchange by matching perfusion to ventilation within each respiratory cycle, and thus optimises oxygen uptake and carbon dioxide removal [32]. Another hypothesis is that RSA helps the heart do less work while maintaining healthy levels of blood gases [33]. RSA is an important determinant of the high-frequency HRV. RSA is an example that central factors, like the interactions between vasomotor and respiratory centres at medullar level also contribute to HRV. Metronome-controlled voluntary breathing induces heart rate entrainment and generates an RSA peak in the power spectrum of HRV analysis. For example, during metronome-controlled breathing at the

frequency of 15 respiratory cycles in minute, heart rate entrainment results in a peak of 0.25 Hz in the spectral curve (Figure 3). It has been estimated that about 80% of heart rate variability could be explained by thermoregulatory, blood pressure regulatory and respiratory oscillations of heart rate [34].

2.2. Examination of heart rate variability

Computer analysis of heart rate variability is an extensively used tool in contemporary biomedical and psychophysiological research of ANS control of cardiovascular function. As a result of interaction between sympathetic and vagal activity in the control loop, beat-to-beat heart rate shows periodic alterations over time, i.e. heart rate variability (HRV). Its analysis consists of signal acquisition (detection of R peaks), measurement of beat-to-beat intervals and quality control for a preselected duration of stationary ECG (Figure 2). The resulting 'signal' is a time-series, which can be conveniently used as source data for computing of first-order statistical indices of variability (also called *time-domain analysis* of HRV). However, the time series is not a continuous signal; there is no information between the consecutive heart beats. The R-R interval sequence has to be preprocessed to a continuous digital signal by linear interpolation and equispaced sampling for more advanced *frequency-domain analysis*. Furthermore, noise and non-stationarities, e.g. ectopic beats and global trends have to be eliminated for the analysis of HRV [16;19;35].

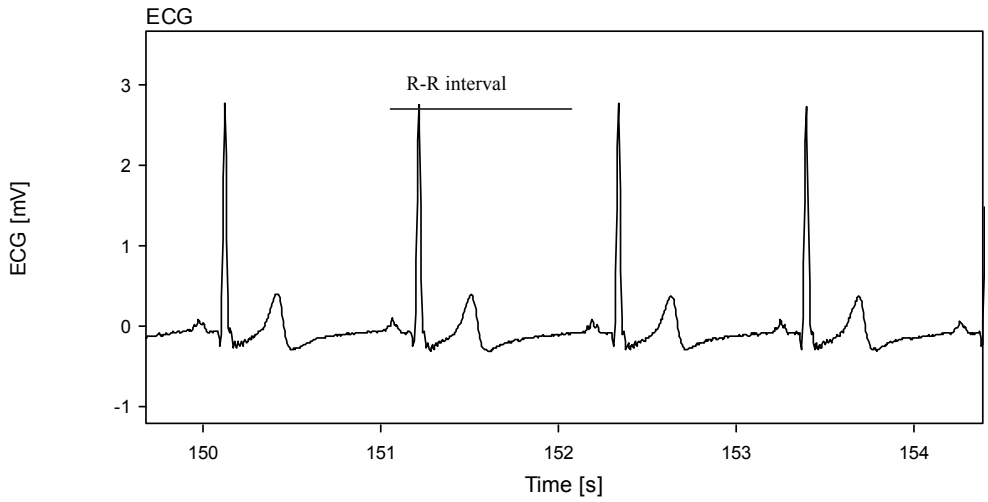

Figure 2. The length of successive R-to-R intervals are measured from ECG recordings.

The assessment of HRV can be classified also according to the length of the analysed electrocardiogram, to short-term and long-term recordings. In the short-term recordings, the ECG data sample is generally between 2 and 5 minutes and in the long-term recordings

usually 24 hours. In 1996, Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology published guidelines to standardise the clinical assessment of HRV [35].

2.2.1. Time-domain analysis of heart rate variability

Time-domain variables of HRV are simple to obtain because they are based on first-order statistics of variability. In these methods, the intervals between successive normal ECG complexes are used as source data. From continuous ECG, each QRS-complex is detected and R-R intervals in sinus rhythm are measured. The most common time-domain HRV variables include (Table 1); the standard deviation of R-R intervals (SDNN, index of total variation); the standard deviation of the average R-R intervals calculated over short periods (SDANN) used in long-term recordings; the square root of the mean squared differences of successive R-R intervals (RMSSD, index of beat-to-beat variation).

Time-domain analysis is convenient for both short-term and long-term HRV analyses. SDNN is an index of total HRV within the analysed ECG period. Thus, the SDNN is highly related to the length of the analysed period. In long-term recordings the effect of data length of the recording to HRV can be reduced by using SDANN, where recording is divided to multiple shorter samples of the ECG recording, typically 5 minutes in duration. SDANN provides information about the external effects on HRV, for example circadian variation and physical activity. RMSSD is a marker of beat-to-beat variation caused by vagal activity [35;36].

Table 1. Selected time-domain and frequency-domain variables of HRV

Time-domain variables	**Units**	**Description**
SDNN	ms	Standard deviation of R-R intervals
SDANN	ms	Standard deviation of the average R-R intervals calculated over short periods
RMMSD	ms	Square root of the mean squared differences of successive R-R intervals
Frequency-domain variables		
TP	ms^2	Total variance of R-R intervals, ≤0.4Hz
ULF	ms^2	Variance in the ultra low frequency range, ≤0.003 Hz
VLF	ms^2	Variance in the very low frequency range, 0.003-0.04 Hz
LF	ms^2	Variance in low frequency range, 0.04-0.15 Hz
HF	ms^2	Variance in high frequency range, 0.15-0.4 Hz
LF/HF-ratio		Ratio displaying balance between LF and HF

Modified from Task Force Report [35].

2.2.2. Frequency-domain analysis of heart rate variability

For the examination of sinusoidal oscillations of variability, the heart rate time-series is preprocessed to a continuous stationary signal (see 2.2) and then a power-spectral density of HRV is computed with the fast-Fourier-transform programme. The 'power' in the spectrum is a plot of variance (i.e. SDNN squared) of the HRV expressed as the area under the spectral curve (Figure 3). Frequency-domain HRV variables are computed by dividing heart rate signal into its (frequency) constituents and quantifying their relative intensity (power). Thus, the spectral analysis of HRV illustrates how the variance of the R-R interval sample (ms^2) distributes as a function of frequency (Hz). The peaks in the variability spectrum reflect the relative magnitude of the sinusoidal heart rate fluctuations present at different oscillation frequencies. These peaks are also named as spectral components of the HRV [16;22;35]. Computing of power-spectra of HRV can be done by either non-parametric or parametric methods. The non-parametric methods are based on the above-mentioned fast-Fourier transformation [16;35;37].

The four main spectral components of the area below the spectral curve are computed for the HRV analysis; ultra low frequency (ULF, ≤ 0.003 Hz) very low frequency (VLF, ≤ 0.04 Hz), low frequency (LF, 0.04-0.15 Hz) and high frequency (HF, 0.15-0.4 Hz) components (Table 1 and Figure 3). There appear to be three frequency components in the normal HRV spectrum which arise from the activity of normal physiological control systems: a temperature component in the range 0.05 Hz, a blood pressure component at around 0.1 Hz, and a respiratory component at about 0.25 Hz, depending on the respiratory rate [22].

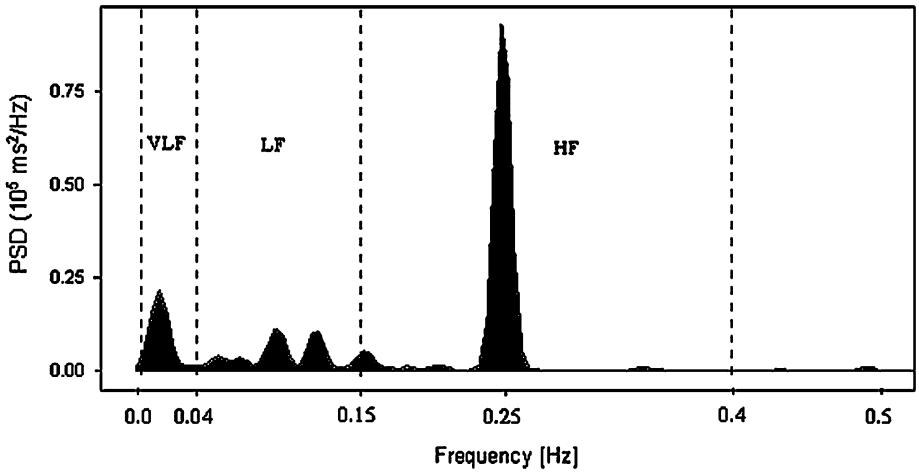

Figure 3. Power spectral density curve of heart rate variability.

The power (variability) spectral density of HRV (PSD) is computed for heart rate signal of a 3 minute ECG sample. The number of analysed R-R intervals of this sample is 196 and mean heart rate 65 bpm. The PSD shows three components of HRV; very low frequency (VLF, ≤ 0.04 Hz), low frequency (LF, 0.04-0.15 Hz) and high frequency (HF, 0.15-0.4 Hz) components. The high HF (0.25 Hz) component reflects respiratory entrainment caused by controlled breathing. [38].

HF variability is considered a marker of vagal cardiac control and is associated with respiratory sinus arrhythmia. The heart rate variations have been considered to arise from the peripheral reflex mechanisms (baroreceptors, chemoreceptors and atrial volume receptors), and in part to be of central origin. HRV is related to both of these control mechanisms. Based on the anatomical considerations (i.e. extra-central nervous system synapses and slow removal of noradrenaline) the time response of the sympathetic system is much longer than the parasympathetic nervous system, and too slow for HF variability in HRV [39]. To support this parasympathetic blockade, electrical vagal stimulation and vagotomy have been shown to abolish HF variability [39-43]. Furthermore, HF variability has been shown to increase in conditions known to increase vagal control, such as after admission of scopolamine [44]. An increase in HF variability is also induced by controlled respiration, cold stimulation of the face and rotational stimuli [42].

LF variability has been related to sympathetic cardiac control, and suggested to be compensatory heart rate oscillations for fluctuation in blood pressure mediated by the baroreceptors' reflex mechanism, to be related to the function of renin-angiotensin system and thermoregulation [17;45-47]. To support the sympathetic component in the LF variations of heart rate, the thoracic pregaglionic sympathetic outflow and LF variability are highly correlated in cats [48]. In dogs, the LF variability disappears after bilateral stellectomy, causing sympathetic cut-down [49]. LF variability has been observed to increase in situations where the sympathetic system activates, e.g. hypotension, during a 90-degree tilt, standing, mental stress and moderate exercise in healthy subjects [25;42;49]. In addition, a decrease in LF variability of heart rate has been observed in situations where vagal cardiac control is de-activated by i.e. atropine or parasympathectomy [40;43;50]. In resting conditions vagal control is the major contributor of HRV and the LF component of HRV seems to correlate with sympathetic activity [35;39]. However it is rather obvious that, at least in resting conditions, LF variability also contains influences of vagal cardiac control. The LF/HF-ratio is considered an index of the balance between sympathetic and vagal modulation. The role of ULF and VLF components is uncertain [35].

Frequency-domain measures are helpful especially for short-term analysis of HRV. In analysis of long-term (24h) recordings many confounding factors, especially external stimuli cause non-stationarities in the source signal and may interfere with the analysis. RMSSD is related to HF variability and SDNN is in fact equal to the total variability (Table 2) [35].

Table 2. Approximate relations between time-domain and frequency-domain variables of heart rate variability

Time-domain variables	Frequency-domain correlate
SDNN	TP
SDANN	ULF
RMMSD	HF

Modified from the Task Force Report [35]

Analysis of heart rate dynamics can be done with other methods in addition to time-domain and frequency-domain analysis. These HRV analysis methods are based on e.g. chaos theory and nonlinear system theory. Attempts to control the multiple factors assessing heart rate dynamics have been done with e.g. mathematical models, geometrical analyses, and several types of different fractal scaling measures, power law analyses, different complexity measures, and various symbolic measures. These methods have not been utilised in this study project [35;51].

2.3. Heart rate variability in specific conditions

Autonomic control disturbance of the cardiovascular system, characterised by a hyperactive sympathetic system and a hypoactive parasympathetic system, is associated with numerous pathological conditions. Over time, excessive energy demands on the cardiometabolic system related to ANS imbalance may lead to premature aging and diseases. Therefore, ANS failure may be one pathway to increased morbidity and mortality from a host of conditions and diseases, including cardiovascular disease [52]. The analysis of HRV may be used to assess cardiac autonomic function and its relation to diseases and mortality. The association with decreased HRV and mortality was first reported by Kleiger et al in 1987. When they studied 808 subjects after myocardial infarction, they found that decreased HRV was a significant independent risk factor for mortality in this high-risk group [10]. They found that individuals with low SDNN after acute myocardial infarction had a five times higher risk of death compared to those with high SDNN in the average of 31 month follow-up. The largest study to date investigating the association with HRV to mortality is the Atherosclerosis Risk in Communities study (ARIC) where 11,645 men and women with an average age of 54 year were studied. In the ARIC study, two minutes of supine resting beat-to-beat heart rate data were collected and both time-domain and frequency-domain variables of HRV analysed. The lowest quartile of HF variability was associated with incidents of myocardial infarction and coronary heart disease (CHD), fatal CHD, and fatal non-CHD deaths in those with diabetes with hazard ratios ranging from 1.27 to 2.03 over the eight-year follow-up period. In the non-diabetic individuals, the effects were much less consistent [53]. In the Autonomic Tone and Reflexes After Myocardial Infarction study, 1,284 subjects with a recent myocardial infarction were investigated using 24-h recordings. Low values of SDNN (<70 ms) were associated with a significant 3.2 greater risk of cardiac mortality in the two-year follow-up period [9]. Hartikainen et al observed that decreased HRV was associated with elevated risk for arrhythmic death after acute myocardial infarction during the 2-year follow-up of 575 post-infarction patients [11]. In the Framingham Heart Study, decreased HRV was independently associated with all-cause mortality in a sub-group of elderly subjects with the mean age of 72 [54]. Thus, multiple studies have revealed the association with decreased HRV and mortality.

During recent years numerous cardiovascular risk factors have been described. The National Heart, Lung, and Blood Institute of the US National Institutes of Health lists eleven risk factors for heart disease, nine of which are modifiable. Six of these modifiable risk factors are related to biological factors. They are hypertension, diabetes and prediabetes, and elevated LDL and triglycerides level and low HDL level. Three others listed as modifiable could be considered lifestyle factors and include smoking, physical inactivity and obesity. The three non-modifiable risk factors are age, sex and family history of early heart disease. The associations between HRV and these risk factors are presented in the following sections.

2.3.1. HRV and cardiometabolic non-modifiable risk factors

Age, sex and heritability: Cardiac autonomic function changes with increasing age and shows differences in men and women. Particularly, all HRV variables have strong association with age and sex [55-57]. Aging is associated with relevant decrease in HRV and it seems that with aging sympathovagal balance turns more to sympathetic dominance. Additionally it has been reported that the effect of aging on HRV is not linear during whole life span. Previous studies in children and adolescents have shown that there is a progressive increase in HRV up to age of 10 years, which may reflect a progressive development of the ANS [58]. The other possible explanations for increase in HRV during the first years of life may be related to mechanisms (reflexes) itself that produce heart rate oscillations. One possible explanation may be the change in cardiac volume which occurs with growth. Large changes in volume occur from infancy to adulthood, and the smaller infant heart may be more responsive to respiratory fluctuations in venous return and experience greater fluctuations of atrial volume receptors with increased influence on HRV [59]. Also differences in respiratory patterns may contribute to HRV analysis [59;60]. After childhood, the effect of aging on HRV seems linear until the age of 60 years, thereafter the age-related decrease in HRV seems to level off [61]. To investigate ANS function within the life cycle, Zulfiqar et al studied long-term records of HRV in 344 healthy individuals aged 10 to 99 years [62]. They found that in 80-99-year-old individuals HRV patterns, particularly RMSSD, increased instead of decreasing with age. This finding is supported by others [63;64]. The mechanism of this change in HRV, or vagal activity, with advanced age is uncertain. One possibility is that individuals with high HRV are clustered in the older population because of lifestyle modifications or genetically mediated changes in ANS function. The clustering hypothesis is supported by the report of Dekker et al where decreased HRV was associated with increased risk for all-cause mortality [65]. It is also possible that in old individuals the HRV analysis is biased by increasing prevalence of sinus arrhythmia that is not of respiratory origin [66].

Middle-aged women have lower LF variability, higher HF variability and lower LF/HF – ratio than men in respective age [55]. These differences may be the result of body fat distribution or hormonal differences between women and men [67-69]. Especially estrogens

seem to have significant effect on HRV [70]. Estrogens have certain electrophysiological effects through alterations in calcium and potassium flux in cardiac myocytes and trough changes in catecholamine uptake and release which may affect on ANS function and in relation to HRV [71]. Other factors that modulate or alter autonomic cardiac control, and may potentially influence gender differences, include obesity, changes in hormone levels, inflammation and psychological disorders (e.g. depression and work stress) [67;68;72-74].

There is some evidence that the pattern of HRV may have a genetic background. Singh et al studied spectral HRV in 517 sibling pairs and their 216 spouse control pairs [75]. They found that after adjusting for covariates, the correlations were consistently higher among siblings (0.21 to 0.26) when compared with spouses (0.01 to 0.19). They calculated that genetic factors accounted for 13% to 23% of the variation among HRV variables. Uusitalo et al reported in their study concerning healthy and chronically diseased (i.e. respiratory, cardiovascular and mental disease) male twins that genetic effects accounted for 31-57% of variance of the HRV patterns [76]. They found in a multivariate analysis that body mass index, percentile body fat, coffee consumption, smoking, all medication and chronic diseases were associated with different HRV variables, accounting only for 1–11% of their variance. In the genome-wide association study of the Framingham Heart Study, however, none of the HRV traits resulted in individually attained genome-wide significance [77]. In a recent study, we were able to demonstrate that a variant at *AGXT2* loci is associated with HF variability and LF/HF-ratio and this loci may have a role in the ANS control of the cardiac function [78].

2.3.2. HRV and cardiometabolic biological risk factors

Hypertension: Hypertension is a major risk factor for cardiovascular disease. The association of HRV with hypertension has been intensively studied, and the relation between cardiac autonomic function and hypertension can be considered to be rather well documented [79-81]. In the ARIC study, individuals in the lowest HRV quartile had a 2.44-fold greater risk of hypertension in a three-year follow-up. In the nine year follow-up of ARIC, the individuals in the lowest quartile of RMSSD at the beginning, adjusted for relevant covariates, were associated with a hazard ratio of 1.36 for the development of hypertension compared to individuals in the highest quartile [81]. In the Framingham Heart Study, Singh et al observed that both time-domain and frequency-domain HRV were decreased in hypertensive individuals. In men, decreased LF variability was also associated with the development of hypertension during four-year follow-up [80]. All these findings support the hypothesis that alteration in ANS function is present in hypertension and may even precede the onset of hypertension. There is growing evidence that sympathetic overactivity is implicated in the development of hypertension and its complications. Increased adrenergic activity in patients with hypertension is supported by various lines of evidence, including analysis of heart rate and catecholamine levels, and data obtained using microneurography [3;82]. Potential mechanisms leading to increased sympathetic activity in hypertension may be divided into two major categories, i.e. increased adrenergic activity resulting from disturbed

peripheral regulatory mechanisms such as arterial baroreceptors, cardiopulmonary mechanoreceptors, and chemoreceptors, and a primary increase of sympathetic activity within the central nervous system [83]. The mechanistic explanation on how ANS failure again may predispose to hypertension is that it triggers trophic factors, such as norepinephrine and beta-receptor mediated renin release and increased angiotensin II level, resulting to tissue hypertrophy and induces peripheral vasoconstriction leading to increase in systemic vascular resistance and elevated blood pressure (Figure 4) [84-86].

The role of antihypertensive medication on HRV pattern is important. Beta-blockers have naturally marked influence on HRV with possible increases in vagal cardiac control [87;88]. In addition, opposite results have been observed [89]. Whereas angiotensin-converting-enzyme inhibitors, calcium antagonist and diuretic hydrochlorothiazide seem not to have important effect on HRV, results on angiotensin-converting-enzyme inhibitors are mixed [90-92].

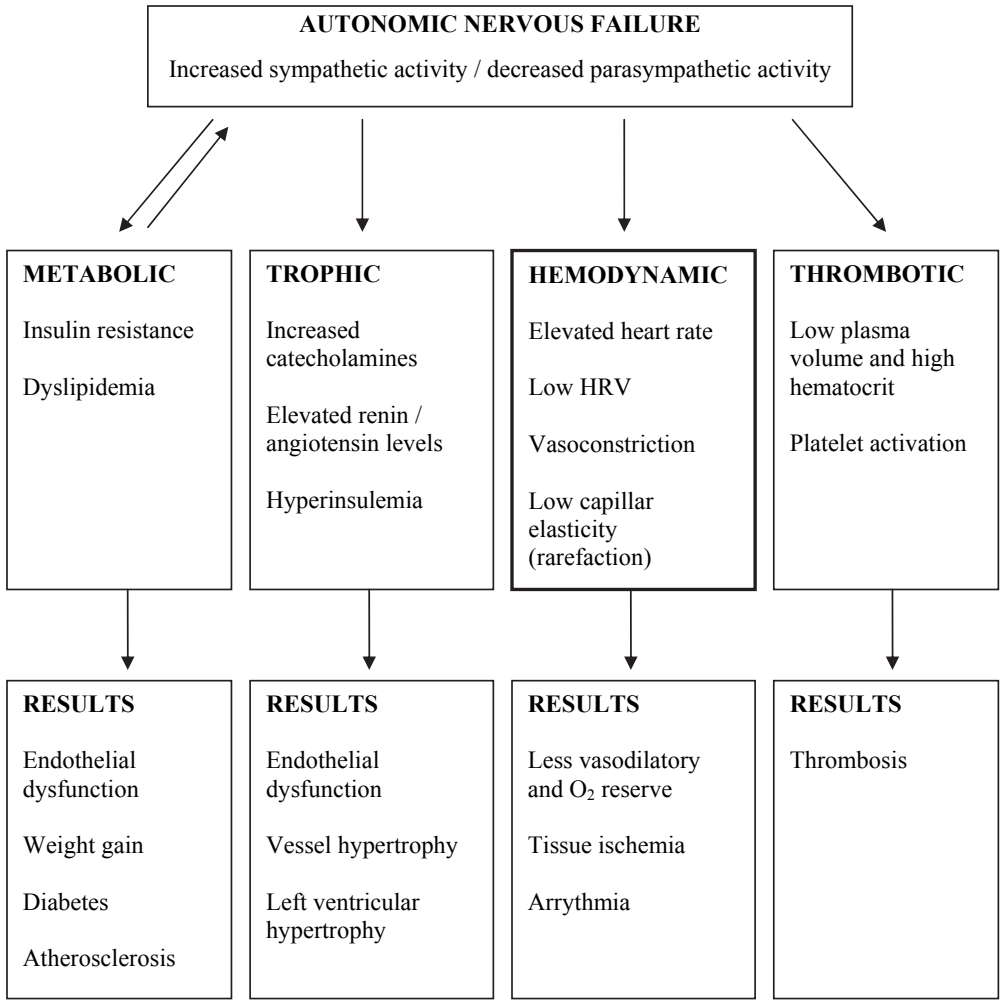

Figure 4. Effects of autonomic nervous failure on cardiovascular risk. Modified from Brook and Julius [86].

Diabetes and metabolic disturbances: Autonomic failure has been shown to be related to wide range of diabetic complications and to the progression of the disease. Individuals with diabetes and ANS dysfunction have increased mortality [93]. Previous work reveals significant reduction in both time-domain and frequency-domain HRV variables in subjects with diabetes or elevated fasting glucose concentration [94-96]. It is generally accepted that hyperglycemia causes degradation of the microvasculature that results peripheral and ANS neuropathy [97]. This is supported by the results from the Diabetes Control and Complications Trial in patients with type 1 diabetes which show that intensive glycaemic control can prevent ANS neuropathy, slowing the deterioration of autonomic dysfunction over time [98]. There are numerous pathways whereby autonomic dysfunction could in turn affect insulin action and glucose regulation (Figure 4). Two hypothesised mechanisms where by

autonomic imbalance predisposes for insulin resistance are; that epinephrine released by the sympathetic nervous system may reduce insulin mediated glucose uptake by receptor level; and that increased α-receptor–mediated vasoconstriction may decrease vascular lumen size and increase nutrient diffusion distances, lessen blood flow, and perhaps facilitate the shunting of glucose and insulin to less metabolically active skeletal muscle beds [86]. The latter hemodynamic hypothesis of insulin resistance is supported by the observation that β-blockers with partial β_2-agonistic and vasodilating properties improves glucose disposal compared to a selective β_1-blocker [99]. Several studies have also revealed an association with ANS function to serum insulin concentration and decreased insulin sensitivity independent of glucose levels, suggesting that ANS dysfunction may not only be the consequence but also a precursor of hyperglycemia [94;100;101]. This is supported by the prospective report of Carnethon et al, where two-minute HRV analyses were performed in 8,185 subjects in a mean follow up of 8.3 years [102]. They found that decreased LF variability and increased resting heart rate were associated with the development of type 2 diabetes during the follow-up regardless of baseline glucose concentration. This suggests that ANS dysfunction might be associated with the development of diabetes in healthy adults. In individuals with diabetes, early identification of cardiovascular autonomic neuropathy permits timely initiation of therapy. Early detection of autonomic impairment through HRV analysis in individuals with diabetes may be important for the risk stratification and subsequent therapeutic management and may be a helpful tool in follow-ups. In individuals with diabetes, examination of HRV appears to be an effective means for identifying patients at risk for asymptomatic myocardial ischemia and infarction so that adequate treatment may be instituted before a cardiac event occurs [97].

MetS is considered a group of atherosclerotic risk factors that tend to cluster and increase the risk of cardiovascular disease and the development of diabetes [103;104]. The clinical MetS criteria generally contain components of hypertension, dyslipidemia, obesity and impaired glucose metabolism. These risk markers are separately reported to be associated with decreased HRV [55;94;105]. In addition, MetS is associated with decreased HRV [4;106]. ANS function has a potentially relevant effect on metabolic status (Figure 4). The primary etiology of metabolic abnormalities is under discussion but autonomic imbalance seems important in the pathophysiology of glucose metabolic disturbances and dyslipidemia. Dyslipidemia is most commonly explained as secondary to insulin resistance which seems the major contributor. Autonomic imbalance may contribute to dyslipidemia with the enhanced catecholamine release and consequent increase in circulating free fatty acids and the above-mentioned potential effect on increased insulin resistance [86;107]. This is supported by observations that ANS function has an independent effect on lipid metabolism through alpha-adrenergic stimulation which may have an important role in suppressing adipose tissue lipase with secondary reduction of plasma high-density lipoprotein cholesterol and elevation of triglyceride concentrations [86;108]. An inverse association of plasma total-cholesterol and LDL concentrations to HRV has been observed in subjects with and without

a history of ischemic heart disease [109]. Similar associations have been seen in obese subjects, supporting the possible role of ANS function in dyslipidemia [110].

2.3.3. HRV and cardiometabolic lifestyle risk factors

Lifestyle: Smoking, physical inactivity and obesity all seem to be related to ANS function. Heavy smoking is associated with decreased vagal and increased sympathetic activity [111-114]. Most of the effects of smoking on ANS regulation are probably due to nicotine. Nicotine has certain acute and chronic cardiovascular effects via activation of sympathetic nervous system, likely as a consequence of enhanced release of catecholamines [115]. Furthermore, smoking elevates heart rate which may have an influence on HRV [116;117]. Smoking cessation seems to improve HRV which may primarily be attributable to the discontinuation of nicotine [118].

Endurance training improves HRV in both healthy subjects [119;120] and in patients with heart diseases and diabetes [110;121-123]. Regular physical activity is known to reduce the risk of morbidity and mortality from a variety of diseases [124]. The positive effect of exercise on health in general is most definitely multi-factorial, e.g. better cardiorespiratory performance (heart rate, stroke volume, cardiac output, oxygen uptake etc.) and metabolic fitness. Increase in HRV has been shown to be related to the cardiorespiratory fitness and exercise tolerance [125-127]. Also studies with no findings concerning the relation between leisure time physical activity, exercise and HRV patterns have been reported [76;128]. When Uusitalo et al randomised 140 male individuals, aged 53 years to 63 years, to a five-year controlled training intervention (30–60 minutes of regular exercise three to five times a week), they did not observe any significant positive effect of regular exercise to the 5-minute HRV variables. They discussed that the negative finding may be related to low compliance and the low intensity of the exercise program, and aging. The relation of physical activity and ANS function is well discussed in the recent article of Voulgari et al [110]. They conclude that regular physical activity is associated with an overall shift to increased vagal control of cardiovascular ANS function that may positively affect health and the prognosis of individuals with a variety of morbidities. The meta-analysis of Cornelissen and Fagart reveals a significant reduction in heart rate and systemic vascular resistance without change in cardiac output associated with chronic endurance training in healthy sedentary normotensive or hypertensive adults [129]. In their meta-analysis the blood pressure reduction associated with regular endurance training was based on a decrease in systemic vascular resistance, in which the sympathetic nervous system and the renin-angiotensin system appear to be involved (significant reduction in plasma norepinephrine and plasma renin activity). The fact that the decrease of heart rate is counterbalanced by an increase in stroke volume with unchanged cardiac output is compatible with the generally accepted effect of aerobic endurance training on resting hemodynamics. Better vascular compliance, including carotid sinus and aortic arch, is suggested to increase baroreceptor nerve traffic and thereby parasympathetic tone [110]. It has been even suggested that exercise training may lead to brain

stem cardiorespiratory center remodeling, resulting in the cardioprotective reduction of sympathetic and the enhancement of parasympathetic heart outflow [130]. The effect of regular exercise to HRV is probably mediated via both decreased heart rate and increased large artery compliance associated with better physical fitness.

Central obesity is considered important in the aetiology of MetS and is linked to increased cardiovascular mortality and the development of diabetes as well [131]. Many underlying mechanisms have been proposed; the release of free fatty acids from adipocytes, chronic activation of the immune system, disorders of the hypothalamic-pituitary-adrenal axis and cellural processes leading to formation of reactive oxygen species [132-135]. Obesity and MetS are characterized by sympathetic nervous system predominance in the basal state and reduced ANS responsiveness after various sympathetic stimuli, such as cold exposure, postural changes, mental effort, caffeine, alcohol and nicotine intake, and hypoglycemia [52;136]. Loss of weight contributes to the restoration of ANS function, estimated with HRV analysis [110;137;138].

2.3.4. HRV and vascular properties

Decreased HRV is observed to be associated with the severity and progression of coronary atherosclerosis. In 1991, Hayano et al investigated cardiac autonomic function by relating 5-min spectral HRV patterns to clinical and angiographic findings of 80 patients undergoing coronary angiography [13]. The age- and sex-adjusted magnitude of the HF variability showed a significant negative correlation with the extent of coronary atheromatosis ($r = -0.43$, $p < 0.0001$) and a less marked negative correlation with the severity of coronary stenosis ($r = -0.30$, $p = 0.0070$). These relationships were independent of previous myocardial infarction and left ventricular function. In 1999, Huikuri et al analysed long-term records of HRV on 265 patients with coronary artery disease. The baseline coronary angiography and follow-up angiography were applied on average 32 months later [12]. They observed that progression of coronary atherosclerosis was independently predicted by the baseline SDNN.

It has been hypothesised for a long time that a decrease in arterial elasticity, which also occurs trough aging and with cardiometabolic risk factors may partly be responsible for the decline in vagal cardiac control [139]. Carotid sinus baroreceptors are sensitive to the pulsating component of systemic arterial pressure [140]. The stretching of baroreceptors causes a decrease in sympathetic activity and an increase in vagal activity [141]. Therefore it has been suggested that changes in the elastic properties of the arterial wall may interfere with pressure transduction in baroreceptors which could lead to attenuation in the baroreflex modulation of the cardiovagal activity and subsequently to a decrease in HRV. This hypothesis is supported by small-scale clinical studies where the associations between baroreflex sensitivity and carotid artery elasticity have been observed [142-144].

The stiffness of the abdominal aorta (assessed with ultrasonography) has been found to be associated with vagal cardiac function during deep breathing test in women with diabetes

[145]. In the Ikaria study, there were association of 5-min HRV pattern and aortic artery distensibility, expressed by pulse pressure, studied in 469 elderly (mean 75 years) individuals [146]. They observed significant inverse association between the SDNN variable and aortic distensibility. They concluded that decreased HRV confers aortic stiffness in elderly non-hypertensive individuals in the absence of known cardiovascular disease. The Stockholm Diabetes Intervention Study found, in a group of 59 adults with type 1 diabetes, that all spectral HRV variables correlated with arterial wall stiffness of the right common carotid artery with the highest *r*-value for the correlation to HF variability [147]. In the SEARCH Cardiovascular Disease study, the cross-sectional relationships between HRV variables and vascular stiffness were explored (assessed with brachial distensibility and pulse wave velocity) in youth with (n = 344) and without (n = 171) type 1 diabetes. Decreased HRV (SDNN) was associated with increased vascular stiffness in both central and peripheral vascular beds, among youth with type 1 diabetes. These associations were independent of traditional cardiometabolic risk factors [148]. Thus, there is some data to support the hypothesis that arterial elasticity and HRV patterns are interrelated.

Endothelial cells play an important role in maintaining the structural and functional integrity of the vasculature. Inability of the endothelial cells to stimulate vasodilation properly is referred to as endothelial dysfunction which is believed to be one of the earliest stages of atherosclerosis and can be observed in healthy people with risk factors for heart disease [149]. It has been hypothesised that the endothelium and ANS work together to create a balance between the needs of local tissues and those of other systems by regulation between the release of vasodilating factors from the endothelium and vasoconstricting factors from sympathetic nerve terminals [150]. This hypothesis is supported by a number of small human case studies. Järvisalo et al found, in 12 healthy young men, that alterations in sympathetic nervous activity measured as serum 3,4-dihydroxyphenylglycol, an intraneuronal metabolite of noradrenaline and the substance most excessively released to the circulation from sympathetic nerve endings during activation, is a significant determinant of brachial artery endothelium-dependent flow-mediated dilatation variation [151]. Ieallamo et al found that healthy first-degree relatives of subjects with type 2 diabetes had a concomitant impairment in endothelial and cardiac autonomic control [152]. Similarly, it was found that endothelial function correlates with ANS activity, and ANS activity seemed to be one regulatory factor of endothelial function, in the study of 47 subjects with ischemic heart disease [153]. Sverrisdóttir et al showed that pulse amplitude measured by tonometry, as a surrogate marker of endothelial function, was related to the level of sympathetic nervous outflow to skeletal muscle, in a study of 10 healthy young adults [154]. Although there is an attractive theory and some evidence about the relation of endothelial and ANS dysfunction, it is also possible that they both are independent early signs of cardiovascular disease rather than linked to each other.

2.4. Heart rate variability and the mechanism of disease

A possible mechanism for increased cardiovascular risk, related to decreased HRV, is altered regulation of peripheral inflammation via an inflammatory reflex. Activation of efferent vagus nerve fibres leads to an increase in acetylcholine release that interacts with nicotinic receptors in the target organs. The activation of these receptors may inhibit the release of cytokines and thus suppress peripheral inflammation [72;155;156]. Increased vagal control also has a protective effect against ischemia-related arrhythmias and an effect on reducing blood pressure [157;158]. The protective mechanism of increased vagal cardiac control in myocardial ischemia is hypothesised to prevent coronary vasoconstriction by inhibiting sympathetic activity [159]. Furthermore, increased cardial vagal control decreases the amount of work and oxygen consumed by the heart via a reduction in resting heart rate and myocardial contractility which may be beneficial in heart diseases [160]. Marwah et al have proposed a hypothesis that atherosclerosis is a neurogenic phenomenon [161]. They base the hypothesis on that many risk factors for atherosclerosis, without well understood mechanistic explanations, are related to autonomic failure. They proposed that these disparate risk factors promote the development of atherosclerosis by increasing adventitial ANS dysfunction, which may e.g. increase peripheral inflammation. Adventitial dysfunction again may have further effects on the vascular wall, including changes affecting medial smooth muscle and endothelial cell biology [162]. Autonomic failure is also hypothesised to be related with an increased risk of thrombosis and clotting [86]. This hypothesis is based on the observations that high sympathetic activity results in the translocation of plasma into the interstitial space. Experimentally this has been demonstrated by infusions of norepinephrine and by unopposed alpha-activity generated by beta-blockers in human subjects [163;164]. The higher blood viscosity is again associated with cardiovascular risk [165]. In addition, epinephrine causes platelet aggregation in vitro by the alpha-2 receptor mechanism [166].

The mechanisms whereby risk factors predispose to cardiovascular events are many-sided, but diminished vagal control and the activation of the sympathetic nervous system, i.e. “chronic fight-response”, may be an important common pathway through which cardiovascular risk is conferred [167]. In metabolic disturbances it is proposed that increased sympathetic action leads to enhanced catecholamine release and consequent increase in circulating free fatty acids and the potential for increased insulin resistance (Figure 3) [86;107].

2.5. Clinical implications of heart rate variability analysis

Although the analysis of HRV has been a subject of numerous clinical and population based studies investigating wide spectrum of cardiovascular and non-cardiological diseases and clinical conditions, the practical use of HRV analysis has been applied in adult medicine only in two clinical scenarios. Decreased HRV can be used as a predictor of risk after acute

myocardial infarction and as an early warning sign of diabetic neuropathy [35]. HRV analysis in anesthesia and intensive care has been intensively studied and it has some prognostic value on critically ill individuals. Decreased HRV independently predicts haemodynamic events in high risk patients during general anesthesia, and both short-term and long-term postoperative mortality, mainly for cardiac events [168-170]. In the pre-operative period, the analysis of HRV can be used as a helpful, non-invasive, bedside, low-cost monitoring tool to evaluate the perioperative risk in patients with suspected autonomic failure, to select individuals who need further cardiac testing and to optimize pre-operative status [171]. The literature supports the role of HRV monitoring for early prognosis prediction and risk stratification in the critically ill patient, thus the decrease in HRV generally being associated with the severity of the illness and the restoration of HRV being associated with recovery. HRV analysis may provide additional diagnostic and prognostic information within the context of multiple confounding factors associated with critical illness [171-173].

The treatment of acute myocardial infarction has changed substantially since the Task Force recommendations in 1996. Although the prognostic value of HRV pattern after acute myocardial infarction is generally accepted [9;10;174], there are marked controversies in its clinical use. In the recent review by Liew, the prognostic value of reduced HRV after acute myocardial infarction is discussed [175]. Because HRV pattern is influenced by multiple variables such as age, sex and certain medication (eg, thrombolysis, antiarrhythmic drugs, beta-blockers and angiotensin-converting-enzyme inhibitors) and the fact that HRV cannot be evaluated in patients with atrial fibrillation or frequent arrhythmias, its use is limited as a sole determinant of increased risk in the patient with post-myocardial infarction [176].

For a long time there have been simple non-invasive cardiovascular reflex tests used especially in diabetics and neurological patients (i.e. the Valsalva maneuver, heart rate response to deep breathing, orthostatic heart rate with blood pressure response and blood pressure response to sustained handgrip). Once clinical manifestations of diabetic ANS neuropathy appear, the estimated natural 5-year mortality is approximately 50% [177]. Thus, early subclinical detection of autonomic nervous neuropathy is important for the risk stratification and management of diabetes. HRV analysis is a rather sensitive and early measurement to estimate the cardiovascular autonomic neuropathy in diabetics [97]. Due to the large percentage of diabetic patients with cardiovascular autonomic neuropathy complications, HRV testing is a helpful tool for identifying subjects at substantial risk of morbidity and mortality. It has even been recommended that a baseline determination of cardiovascular autonomic function should be performed upon diagnosis in type 2 diabetes and within 5 years of diagnosis for those with type 1 diabetes, followed by a yearly repeat test [178].

3. AIMS OF THE STUDY

This thesis is based on the Cardiovascular Risk in Young Finns Study, and the 21-year follow-up study performed in 2001. The purpose was to examine heart rate variability in the large sample of young healthy adults.

The major aims were as follows:

1. To reveal normal reference values of HRV variables in young adults and to identify factors affecting HRV (I).

2. To study the association between HRV variables and metabolic syndrome in young adults (II).

3. To study the relations between HRV patterns and vascular properties (FMD, IMT and Cdist), especially carotid distensibility, to test the hypothesis whether a better elasticity of the artery is associated with increased HRV (III).

4. PARTICIPANTS AND METHODS

4.1. Description of the Cardiovascular Risk in Young Finns Study

The present thesis is based on the Cardiovascular Risk in Young Finns Study, which is an ongoing, multi-centre follow-up study of atherosclerotic risk factors of Finnish children, adolescents and young adults [179]. The participants were randomly chosen from each participating centre (the cities of Helsinki, Turku, Kuopio, Oulu and Tampere) and from their respective rural communities. Two pilot studies were conducted in 1978 and 1979. At the beginning the original sample size was 4 320 healthy children and adolescents aged 3, 6, 9, 12, 15 and 18 years and where a total of 3 596 (83.2%) participated in the first cross-sectional study in 1980 [180]. The follow-up studies have been performed for the whole study group in 1983, 1986, 2001 and 2007 when 2 991 (83.2%), 2 779 (77.3%), 2 283 (63.5%) and 2 204 (61.3%) individuals from the original cohort have participated.

4.2. Study design and participants

In 2001, the HRV analysis was performed during the 21-year follow-up sessions to investigate cardiac ANS function and its associations with cardiometabolic risk factors in healthy young adults. During study visit an electrocardiogram (ECG) was recorded in 2 151 (94.2%) participants. The protocol included analysis of time-domain and frequency-domain HRV variables in rest during metronome-controlled breathing and during deep breathing.

Study I was designed to generate normal reference values for short-term HRV variables in healthy young adults. A total of 131 subjects with antihypertensive, lipid lowering, antidepressive or antipsychotic medications, seven subjects with previously diagnosed but non-medicated hypertension, 46 subjects with diabetes or impaired fasting glucose (>6.0 mmol/l), 51 pregnant women, and 48 breast-feeding women were excluded from analysis. In addition, we excluded the HRV data in subjects with three or more ectopic beats in their source ECG recordings (n=87). HRV data were also excluded in 108 subjects with technical or quality problems in the data recordings or saving. As a result there were total of 1 956 subjects with a complete HRV data set. This resulted, with the exclusion, a 1 780 individuals to the analysis of first (1) study.

In **Study II** the aim was to investigate the association of the metabolic syndrome and HRV variables and also the associations of the various components of the metabolic syndrome to the HRV pattern. Pregnant (n = 51) and breastfeeding (n = 48) women, subjects with type 1 diabetes (n = 12) and subjects using antihypertensive medication (n = 49) were excluded from Study 2. Subjects with type 2 diabetes, impaired fasting glucose or hypertension without

medication and subjects using psychiatric medication were included. The total number of subjects included for the final analysis after these exclusions was 1 889 (women 53.4%).

The aim of **Study III** was to assess associations of analysed HRV pattern and carotid distensibility that was measured with a carotid ultrasound. The exclusion criteria for Study 3 were identical to Study 2. Previously unpublished data about the relation of HRV variables and carotid intima-media thickness (IMT) and brachial artery flow-mediated dilatation (FMD) are also presented in this thesis. Both HRV and ultrasound studies were available for 1 802 subjects.

The study participants and the exclusion criteria are respectively presented in Figure 5.

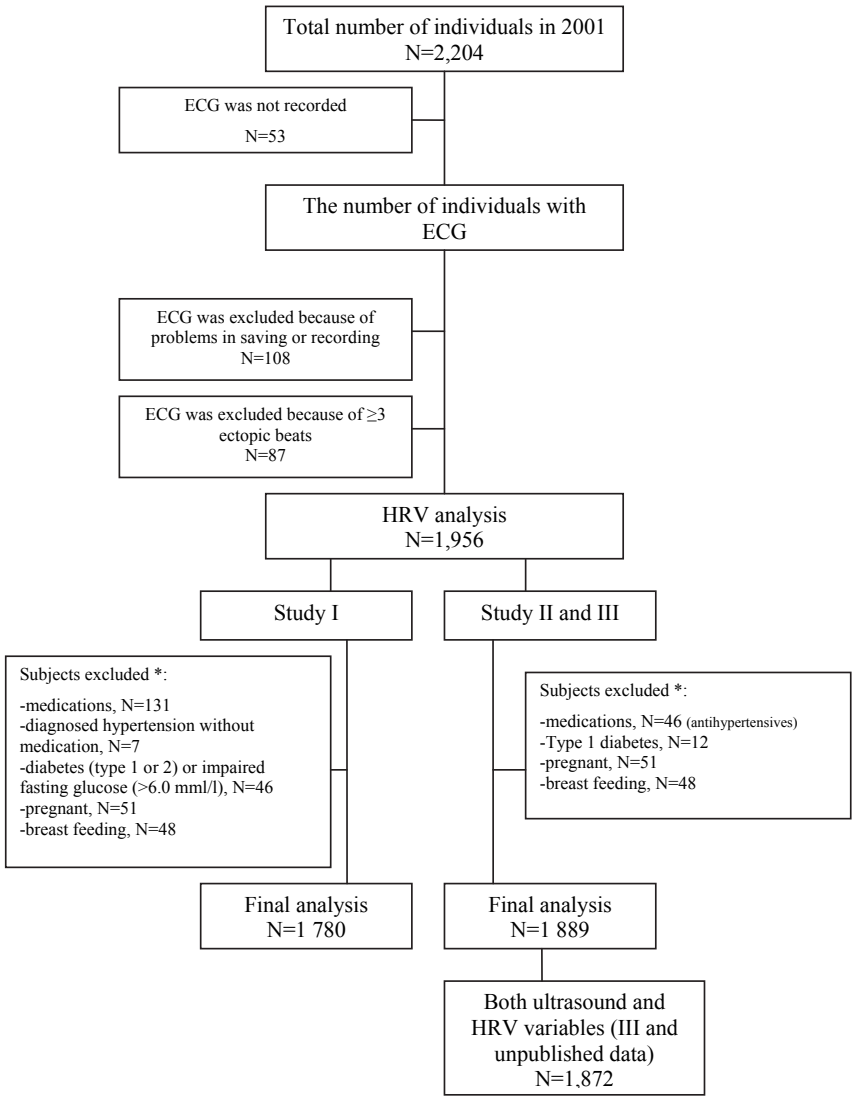

Figure 5. Participants and data assessed in studies I-III. *Subjects may be included in multiple subgroups (i.e. pregnant and fasting glucose >6.0 mmol/l).

4.3. Examination of heart rate variability

A single channel chest-lead ECG was recorded for a 3-minute period of metronome-controlled breathing at the frequency of 0.25 Hz, and during a one-minute period of regular deep breathing at the frequency of 0.1 Hz. The ECG-signal was recorded after the participants remained comfortably in the supine position for at least 15 minutes during ultrasound studies [181]. The signal was analogue-to-digital converted with a sampling rate of 200 Hz (resolution of 5 msec), and an automatic computer based trigger point was used to identify each R-peak. The time-series of R–R intervals were generated based on the respective R-peaks. Both the ECG signal and R-peak detection signal were visually revised by one operator before the analysis of HRV. The stationary period of R-R interval segment was identified during the 3-minute controlled breathing, and was used to compute the time-domain and frequency-domain HRV variables (chapter 2.2). The mean length of the analysed stationary period was 173 s (SD 12 s). The mean number of analysed R-R intervals was 196 (SD 34). Time-domain and frequency-domain HRV was analysed using a commercial WinCPRS program (Absolute Aliens, Turku, Finland), a program for general analysis of physiological data. The HRV spectrum was computed using a nonparametric fast-Fourier transform method [37]. Before the spectral HRV analysis the linear trend was removed from the R-R interval sequence and the signal was smoothed using the Hanning window filter and resampled to the frequency of 5 Hz [182].

Ectopic beats introduce a bias into the HRV analysis [183;184]. Therefore, ectopy correction is necessary, and deletion of ectopic beats is the simplest option [183]. We excluded HRV data in subjects with three or more ectopic beats, and manually interpolated all recordings including less than three ectopic beats by removing the compensatory R-R interval and then extrapolating the previous and following intervals.

4.4. Deep breathing test

The deep breathing test was performed with six cycles of 5 s of deep inspiration followed by 5 s of deep expiration, i.e. a rate of 6 breathing cycles in minute. In individuals with more than two ectopic beats during the 3-minute ECG recordings at a breathing frequency of 0.25 Hz, the deep breathing indices were not analysed (n=87), except in 7 subjects whose deep breathing test was successful and did not contain any ectopic beats. The deep breathing recordings were not analysed in 19 participants because of problems in data recordings (or saving). The subjects with ectopic beats during the deep breathing test were excluded (n=70). The deep breathing test failed due to inadequate co-operation in 35 individuals. In each breathing cycle, the longest R-R interval during expiration and shortest during inspiration were recognized. The mean ratio for these R-R intervals (MeanE/I) and the mean difference in instantaneous heart rate (MeanDBD) were estimated as markers of the parasympathetic function [185]. Deep breathing measures were available from 1 947

individuals. After applying the exclusion criteria (Study 1) 1 702 individuals were included in the final analysis.

4.5. Reproducibility study

To investigate the reproducibility of HRV variables, we recollected identical data sets of HRV in 51 subjects on average of 4.4 months (131±4.3 days) after the first measurement. After excluding subjects with diabetes, subjects using medication, and those with hypertension, pregnancy and breast feeding, 43 subjects with test–retest remained for the reproducibility study.

Short-term HRV analyses depend on the length of the time-series and shorter time-series may result in less variability. Five-minute recordings have been recommended [35]. The standard cut-off points for frequency-domain HRV variables were used (VLF variability ≤ 0.04 Hz, LF variability 0.04-0.15 Hz and HF variability 0.15-0.4 Hz). Spectral density is considered reliable if > 3 cycles of periodicity are included to the HRV analysis [35]. Maximum HF variability cut-off can be determined according to so called Nyqvist criterion, which states it is half of the sampling frequency (200 Hz in our study), thus here it would be 100 Hz [186]. Therefore, to investigate whether the duration of the time-series (3 min vs. 5 min) had significant effect on HRV variables, we recollected HRV on 75 individuals, and compared HRV variables based on 3 and 5 minute ECG-recordings. Breathing was metronome-controlled at the frequency of 0.25 Hz.

4.6. Biochemical analyses

All venous blood samples were drawn from the right antecubital vein after fasting for 12 hours. Standard enzymatic methods were used for serum cholesterol and triglycerides (Olympus System Reagent; Olympus Diagnostica GmbH, Hamburg, Germany) [187]. Serum HDL was measured from the serum supernatant after precipitation of VLDL and LDL with dextrane sulphate-$MgCl_2$ [188]. LDL was calculated using the Friedewald formula [189]. Plasma-glucose concentrations were analysed enzymatically (glucose dehydrogenase; Olympus Diagnostica GmbH). Serum insulin concentration was measured by a microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostics Division, Dainabot, Dublin, Ireland). All analyses were performed in the laboratory of the Research and Development Unit of the Social Insurance Institution in Turku.

4.7. Definition of metabolic syndrome

The MetS was defined using three criteria; the National Cholesterol Education Program Expert Panel (NCEP), the International Diabetes Federation (IDF) and the European Group

for the Study of Insulin Resistance (EGIR) [131;190-192] (Table 2). All these criteria were used and compared in Study II.

Table 2. The MetS definitions used in the present study.

	EGIR (Balkau and Charles 1999)	**Updated NCEP (Grundy et al. 2005)**	**IDF (Alberti et al. 2005)**
	Hyperinsulemia (highest quartile of fasting insulin in non-diabetic population) and ≥2 of the following:	≥3 of the following:	Central obesity (ethnicity specific) and ≥2 of the following:
Obesity	waist ≥94 cm in men, ≥80 cm in women	waist ≥ 102 cm in men, ≥ 88 cm in women	waist ≥94 cm in men, ≥80 cm in women
Blood pressure	≥ 140/90 mmHg*	≥130/85 mmHg*	≥130/85 mmHg*
Triglycerides	≥2.0 mmol/L	≥1.7 mmol/L*	≥1.7 mmol/L*
HDL-cholesterol	<1.0 mmol/L	<1.03 mmol/L in men*, <1.29 mmol/L in women*	<1.03 mmol/L in men*, <1.29 mmol/L in women*
Fasting plasma glucose	≥6.1 mmol/L	≥5.6 mmol/L*	>5.6 mmol/L or type 2 diabetes

* Medication for the condition is an alternative.

4.8. Physical examination and questionnaires

The physical examination contained measurements of height, weight, waist and hip circumferences, and systolic and diastolic arterial blood pressure. Height was measured with a Seca anthropometer and weight with a Seca weighting scale. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist (midway between the lowest rib and iliac crest in the midaxillary line) and hip (at the greater trochanters) circumferences were calculated as the average of two measures. An unstretchable plastic-covered cloth measuring tape with an accuracy of 0.1 cm was used. Blood pressure was measured using a random zero sphygmomanometer in a sitting position after 5 minutes of rest. Korotkoff's fifth sound was used as the sign of diastolic blood pressure and first sound as the sign of systolic blood pressure. Readings to the nearest even number of mmHg were obtained. The average of three readings of systolic and diastolic blood pressure was used as the measure of blood pressure in analyses.

Non-modifiable and biological cardiometabolic risk factors and lifestyle habits were assessed with questionnaires. These included questions about smoking habits, alcohol consumption, physical activity, hypertension or type 2 diabetes and medication used, socioeconomic status (number of parental school years in 1980 and number of own school years in 2001) and family history of premature coronary heart disease. Subjects smoking on a daily basis were considered as smokers. Family history of cardiovascular risk was considered positive if either the study subject's father or mother had been diagnosed with coronary heart disease at or before the age of 55 years.

Physical activity was assessed by a questionnaire. Participants were asked the frequency of participation in physical activity and its intensity during leisure time. In addition, commuting to work was assessed. When estimating the physical activity during commuting to the work-place, the length of the journey and whether it was travelled by foot or by bicycle was considered. A metabolic equivalent index for physical activity was calculated from the product of intensity x frequency x duration and commuting physical activity [193].

4.9. Ultrasound studies

Ultrasound studies were performed with similar Sequoia 512 ultrasound mainframes (Acuson, Mountain View, California), with a 13.0-MHz linear array transducers in all study centres. In 2001, ultrasound studies were performed for 2,264 subjects by physicians and ultrasound technicians similarly in all five study centres. Carotid artery intima-media thickness (cIMT) was measured from the left common carotid artery following a standardized protocol. The image was focused on the posterior (far) wall, and gain settings were used to optimize image quality. A resolution box function (zoom) was used to record an image 25 mm wide and 15 mm high. A magnified image was recorded from an angle that showed the greatest distance between the lumen-intima interface and the media-adventitia interface. A moving scan with duration of 5 seconds, which included the beginning of the carotid bifurcation and the common carotid artery, was recorded and stored in digital format on optical disks for subsequent off-line analysis. Digitally stored scans were manually analysed by a single reader blinded to participants' details, with analyses performed using ultrasonic callipers. From the 5-second clip image, the best-quality end-diastolic frame was selected (incident with the R wave on a continuously recorded electrocardiogram). From this image, at least 4 measurements of the common carotid far wall were taken approximately 10 mm proximal to the bifurcation to derive maximal cIMT. The three month between-visit coefficient of variation of cIMT measurements was 6.4%. [181].

To evaluate carotid artery elasticity, the best quality cardiac cycle was selected from the 5-second clip images. The common carotid diameter 10 mm from the carotid bifurcation was measured from the B-mode images using ultrasound callipers at least twice in end-diastole and end-systole, respectively. The mean of the measurements was used as the end-diastolic and end-systolic diameter. Ultrasound and concomitant brachial arterial blood pressure measurements (measured with an automated Omron device) were used to calculate carotid artery distensibility (Cdist) using the formula;

$$Cdist = ([Ds-Dd]/Dd)/(Ps-Pd),$$

where Ds is the systolic diameter, Dd is the diastolic diameter, Ps is systolic blood pressure and Pd is diastolic blood pressure.

The variable Cdist represents the ability of the arteries to expand as the response to pulse pressure caused by cardiac contraction and relaxation. The three month between-visit coefficient of variation for Cdist was 16.5 % [194].

To assess brachial flow-mediated dilatation (FMD) the brachial artery diameter was measured both at rest and during the reactive hyperemia in 2,109 subjects in 2001. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg for 4.5 minutes, followed by release. Three measurements of arterial diameter were performed at end-diastole at a fixed distance from an anatomic marker at rest and at 40, 60, and 80 seconds after cuff release. The vessel diameter in scans after the reactive hyperemia was expressed as the percentage relative to the scan at rest. The average of 3 measurements at each time point was used to derive the maximum FMD (the greatest value between 40 and 80 seconds). The three month between-visit coefficient of variation was 3.2% for brachial artery diameter measurements and 26.0% for FMD measurements [195].

4.10. Statistical analyses

Values are expressed as mean and standard deviation (SD) for continuous variables and as proportions for categorical variables unless stated otherwise. Inter-group comparisons were performed using the Student's t test for continuous variables and the $\chi 2$ test for categorical variables. Statistical analyses were performed using Statistical Analysis System software (version 9.0, SAS institute, Cary, NC, USA). The distributions of HRV variables and deep breathing tests (TP, HF, LF, LF/HF, SDNN, RMSSD, MeanE/I and MeanDBD) were not normal but all skewed to the right. The SAS procedure TRANSREG was used to find optimal transformation functions. Normal distributions of TP, HF, LF, LF/HF, SDNN and RMSSD were obtained by log-transformations. MeanDBD was square-root-transformed and MeanE/I was to 1/(MeanE/I)2-transformed to obtain normal distribution.

Study I:
The differences between sexes were tested using the t-test or $\chi 2$-test, as appropriate. The univariate relationships between heart rate, age, BMI, mean arterial pressure and HRV variables were examined using linear regression analysis. The multivariable analyses were done by linear regression analysis. To examine whether heart rate modifies the associations between sex and HRV-variables, we included a sex and HRV interaction term in the regression models.

To produce reference limits for HRV variables, we constructed sex-, age-, and heart rate-specific 95% reference limits by first applying the Box–Cox transformation and then using the technique described by Virtanen et al. [196]. This technique is based on computations using residual distribution of regression model where age, sex and heart rate are covariates. The regression model with age, sex and heart rate as the explanatory variables and transformed

HRV variable as the response variable was fitted. The regression model was formed to calculate age, sex and heart rate adjusted expected HRV. The 95% sex stratified reference limits for each age and heart rate can then be calculated using expected HRV and estimator of residual standard deviation (s); 95% reference limits=$HRV_{expected} \pm 1.96$ s.

For a special sample (n=43), which had duplicate measurements, the reliability coefficient (RC) and coefficient of variation (CV) were calculated by analysis of variance to obtain *test–retest reliability* [197]. $RC=SD^2_{between} / (SD^2_{between} + SD^2_{within}) \times 100\%$, RC less than 40% representing poor reproducibility and RC more than 75% representing good reproducibility [198]. $CV=SD_{within}/mean_{both\ measurements} \times 100\%$, with low values representing good reproducibility. Variation of HRV variables during office hours were analysed by dividing subjects in different study-hour groups. Subjects were study-hour grouped as follows: between 1) 7.00–8.00 a.m. (5.6%), 2) 8.00–9.00 a.m. (18.2%), 3) 9.00–10.00 a.m. (20.7%), 4) 10.00–11.00 a.m. (18.8%), 5) 11.00–12.00 a.m. (18.0%), 6) 12.00–13.00 p.m. (11.0%), and 7) after 13.00 p.m. (7.7%). Differences between study-hour groups were analysed with one-way ANOVA and Tukey's multiple comparison. Correlation of study-hour to HRV variables and deep breathing test was estimated by linear regression.

Study II:
Sex differences were tested with t-test and $\chi 2$ test, as appropriate. Linear trend of HRV variables with the increasing number of MetS components was analysed by regression analysis. The associations of the MetS components (waist circumference, triglycerides, glucose, insulin, HDL-cholesterol and systolic / diastolic blood pressure) with HRV were analysed using linear regression modelling. All components were first included as independent variables in the same model. Backward selection was then applied to determine the most parsimonious model.

Study III and unpublished data:
The associations of the HRV to cIMT, FMD and Cdist were analysed using linear regression modelling. To compare the relative strengths of the associations between various predictors and Cdist in the regression models, we calculated regression coefficients also as standardised units. To examine the effect of multiple risk factors to HF and TP variability according to Cdist, individuals were defined to have a risk factor if their values of systolic blood pressure, glucose or triglyceride concentration or waist circumference exceeded age- and sex-specific 80th percentile, or HDL-cholesterol was below the age- and sex-specific 20^{th} percentile. The risk factors were also defined according to the recent joint interim statement guidelines [199]. Low and high elasticity represents age- and sex-specific 10th and 90th percentiles for distensibility. We used regression modelling to examine if the relation of HF and TP variability and risk score is different between subjects with various levels of Cdist, i.e. to examine whether distensibility modifies the association between HF and TP variability and risk score. This was done by including distensibility and risk score interaction term in the regression model. Similar results were seen when using risk scores

calculated on the basis of percentile or clinical cut-points, or when using either continuous or categorical distensibility variables in the product term. Both carotid ultrasound and HRV variables were available for 1 872 individuals, and both FMD and HRV variables for 1 755 individuals.

4.11. Ethics

The investigation was approved by the Ethics Committee of the University of Turku, and all individuals gave their written informed consent before participation. The investigation conforms with the principles outlined in the Declaration of Helsinki [200]. The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin Originality Check service.

5. RESULTS

5.1. Characteristics of participants (Study I)

The clinical data of study participants are presented in Tables 3 and 4. The mean heart rate was higher in women than in men. Women had statistically higher HF and lower LF variability than men, and significantly lower LF/HF-ratio. In all, women had more favourable cardiometabolic risk profile than men, with lower blood pressure, better lipid profile and lower fasting glucose concentration. Furthermore, women smoked less frequently than men. Because of a marked sex difference in clinical features, the statistical analyses were mainly performed stratified by sex.

Table 3. Clinical data of study participants in the 2001 follow-up examination of the Young Finns study.

	Men (n=831)	Women (n=949)	p for difference
Age, yr	31.5±5	31.6±5.0	0.50
Height, cm	179.5±6.6	166.1±6.0	<0.0001
Weight, kg	82.5±14.3	66.6±12.6	<0.0001
Body mass index, kg/m^2	25.6±3.9	24.2±4.4	<0.0001
Systolic blood pressure, mmHg	121±12	113±12	<0.0001
Diastolic blood pressure, mmHg	73±11	69±10	<0.0001
Mean arterial blood pressure, mmHg	89±10	83±10	<0.0001
Daily smoking, % (n)	31 (249)	21 (195)	<0.0001
Total cholesterol, mmol/l	5.2±1.0	5.0±0.9	<0.0001
LDL-cholesterol, mmol/l	3.4±0.9	3.1±0.7	<0.0001
HDL-cholesterol, mmol/l	1.2±0.3	1.4±0.3	<0.0001
Triglycerides, mmol/l	1.5±1.0	1.1±0.6	<0.0001
Glucose, mmol/l	5.1±0.4	4.9±0.4	<0.0001

Numerals represent mean ±standard deviation. Differences between sexes were tested using t tests, and χ^2 test for smoking. HDL=high-density lipoprotein and LDL=low-density lipoprotein,

Table 4. Heart rate and heart rate variability data in study participants distributed by sex.

	Men (n=831)	Women (n=949)	p for difference
Heart rate, bpm	65.0±10.0	69.7±10.8	<0.0001
SDNN, ln(ms)	3.86±0.42	3.84±0.44	0.25
RMSSD, ln(ms)	3.70±0.58	3.74±0.63	0.20
HF, ln(ms^2)	6.18±1.10	6.44±1.17	<0.0001
LF, ln(ms^2)	5.97±0.89	5.52±0.93	<0.0001
TP, ln(ms^2)	7.40±0.86	7.34±0.91	0.16
LF/HF, ln(%)	4.39±0.94	3.69±0.98	<0.0001
MeanE/I $^{-2}$	0.57±0.13	0.58±0.12	0.26
MeanDBD, bpm$^{-½}$	4.26±0.90	4.34±0.87	0.09

Numerals represent mean ±standard deviation. Differences between sexes were tested using t test. HF=high-frequency frequency-domain heart rate variability (0.15-0.4 Hz), LF=low-frequency frequency-domain heart rate variability (0.04-0.14 Hz), MeanDBD=the mean difference in instantaneous heart rate, MeanE/I=the mean ratio for longest and shortest R-R-interval during deep breathing cycle, SDNN=the standard deviation of all R-R intervals, TP=total frequency-domain heart rate variability and RMSSD=square root of mean squared differences of successive R-R intervals.

5.2. Reproducibility, office-hour variation and effect of time-series duration on heart rate variability

Deep breathing tests had a sufficient reproducibility (for both CV<14% and RC>78%). The reproducibility of short-term HRV variables were satisfactory (CV=5.3–11.5% and RC=62.8–77.5%) (Table 5). There were no statistically significant hour-to-hour differences between the HRV variables, deep breathing test patterns or mean heart rate (p for pair-wise study-hour group differences always ≥0.17). Study-hour correlated weakly and inversely with TP (r=−0.053 and p=0.03) and SDNN (r=−0.055 and p=0.02), when the linear trend was tested. There were no statistically significant linear trend correlations between study-hour and other HRV variables, deep breathing test data or mean heart rate (HF, LF, LF/HF and RMSSD, all p≥0.05).

Table 5. Reproducibility of HRV analysis.

	n	Measurement 1 (X_1)	Measurement 2 (X_2)	(X_1-X_2)	p*	CV (%)	RC (%)
TP, ms^2	43	7.46 (0.62)	7.33 (0.69)	0.13	0.25	5.9	60.1
HF, ms^2	43	6.46 (0.68)	6.25 (0.83)	0.21	0.09	6.8	69.7
LF, ms^2	43	5.80 (0.77)	5.62 (0.89)	0.17	0.16	8.1	71.5
LF/HF, %	43	3.94 (0.83)	3.98 (1.03)	-0.035	0.79	11.5	77.5
SDNN, ms	43	3.88 (0.30)	3.79 (0.34)	0.09	0.11	5.3	64.1
RMSSD, ms	43	3.82 (0.38)	3.63 (0.42)	0.19	**<0.01**	7.1	62.8
MeanE/I	40	0.60 (0.10)	0.60 (0.10)	0.001	0.96	8.0	78.2
MeanDBD, bpm	40	3.99 (0.75)	4.08 (0.82)	-0.09	0.36	13.9	84.7

Values are expressed as means (SD). TP, HF, LF, LF/HF, SDNN and RMSSD were obtained by log-transformations. MeanDBD was square-root–transformed and MeanE/I was to $1/(MeanE/I)^2$–transformed. CV=coefficient of variation, HF=high frequency heart rate variability, LF=low frequency heart rate variability, MeanDBD=the mean difference in instantaneous heart rate, MeanE/I=the mean ratio for longest and shortest R-R-interval during deep breathing cycle, SDNN=standard deviation of all R-R-intervals, RC=reliability coefficient, RMSSD=square root of mean squared differences of successive R-R-intervals, SEM=standard error of mean and TP=total frequency heart rate variability. *P for difference, tested with t test.

The effect of time-series duration on HRV variables was studied on 75 subjects making use of 3-minute and 5-minute recordings. The coefficient of variation between 3- and 5-minute time-series was 2.2% for HF variability, 4.4% for LF variability, 5.4% for LF/HF, 2.5% for TP variability, 1.2% for RMSSD and 1.7% for SDNN. Mean values for the 3-minute vs. 5-minute time-series were 6.1 lnms2 for both HF variability (p=0.60, for difference), 5.8 lnms2 for both LF variability (p=0.22, for difference), 4.3 lnms2 for both LF/HF (p=0.43, for difference), 7.3 vs. 7.4 lnms2 for TP variability (p=0.04, for difference), 3.6 vs. 3.7 lnms for RMSSD (p=0.42, for difference) and 3.8 lnms for both SDNN (p=0.01, for difference). Thus, the effect of time-series duration (3-minute vs. 5-minute) was not significant on HRV variables, except for the variables reflecting total HRV, SDNN and TP (p<0.05 for both).

5.3. Correlation between time-domain and frequency-domain heart rate variability patterns

Time-domain and frequency-domain HRV variables were naturally highly correlated in both sexes. Pearson correlation coefficients between HF variability and RMSSD were 0.9 on both sexes; TP variability and SDNN also correlated highly with coefficients of 0.9 for both women and men; LF/HF-ratio was correlated with LF and HF variability when expressed as normalised units (LFnu, HFnu) coefficients -0.9 for HFnu in both sexes and 0.9 for LFnu in both sexes (Table 6).

Table 6. Pearson coefficients between time-domain and frequency-domain variables of HRV in healthy women (n = 949, deep breathing tests n = 905) and men (n = 831, deep breathing tests n = 788).

Women	**TP**	**HF**	**HFnu**	**LF**	**LFnu**	**LF/HF-ratio**	**SDNN**	**RMSSD**	**MeanE/I**	**MeanDBD**
TP	1.00									
HF	**0.85**	1.00								
HFnu	0.25	0.67	1.00							
LF	0.77	0.58	-0.16	1.00						
LFnu	-0.30	-0.58	**-0.86**	0.30	1.00					
LF/HF-ratio	-0.29	-0.64	**-0.94**	0.25	**0.97**	1.00				
SDNN	**0.95**	**0.88**	0.36	0.68	-0.39	-0.40	1.00			
RMSSD	**0.84**	**0.96**	0.61	0.58	-0.54	-0.59	**0.90**	1.00		
MeanE/I	0.53	0.55	0.28	0.42	-0.23	-0.26	0.54	0.51	1.00	
MeanDBD	0.37	0.40	0.19	0.33	-0.14	-0.17	0.37	0.32	**0.94**	1.00

Men	**TP**	**HF**	**HFnu**	**LF**	**LFnu**	**LF/HF-ratio**	**SDNN**	**RMSSD**	**MeanE/I**	**MeanDBD**
TP	1.00									
HF	**0.82**	1.00								
HFnu	0.18	0.65	1.00							
LF	0.80	0.57	-0.21	1.00						
LFnu	-0.23	-0.56	**-0.88**	0.33	1.00					
LF/HF-ratio	-0.21	-0.63	**-0.96**	0.28	**0.96**	1.00				
SDNN	**0.93**	**0.84**	0.27	0.71	-0.31	-0.30	1.00			
RMSSD	**0.81**	**0.95**	0.58	0.58	-0.51	-0.57	**0.86**	1.00		
MeanE/I	0.48	0.55	0.31	0.35	-0.27	-0.29	0.50	0.50	1.00	
MeanDBD	0.35	0.41	0.23	0.27	-0.20	-0.22	0.35	0.32	**0.95**	1.00

Coefficients of MeanE/I are Spearman's correlations. TP, HF, LF, LF/HF-ratio, SDNN and RMSSD are log-transformed. LFnu and MeanDBD are square-root transformed. HF = high frequency heart rate variability, HFnu = high frequency heart rate variability in normalized units, LF = low frequency heart rate variability, LFnu = low frequency heart rate variability in normalized units, MeanDBD = the mean difference in instantaneous heart rate, MeanE/I = the mean ratio for longest and shortest R-R interval during deep breathing cycle, SDNN = standard deviation of all R-R intervals, RMSSD = square root of mean squared differences of successive R-R intervals and TP = total heart rate variability. All $p < 0.0001$.

5.4. Effect of age, sex and heart rate on heart rate variability

In univariate analysis, heart rate, age, BMI and mean arterial pressure (MAP) were inversely associated with all HRV variables, except the LF/HF (Table 7). The association of heart rate was stronger than the association of age; both associations were highly significant (for all $p<0.0001$). BMI and MAP were significantly associated with all HRV variables ($p<0.05$), except BMI did not associate with LF, TP, RMSSD and SDNN in women. The relations between heart rate and HRV variables were similar between sexes (heart rate by sex interaction terms, always $p>0.05$). Age, BMI and MAP were inversely associated with MeanE/I in both sexes, except MAP in men. Age and BMI were inversely associated with MeanDBD in both sexes ($p<0.01$).

The multivariable determinants of HRV and deep breathing patterns are shown in Table 8. Heart rate and age were highly significantly associated with all HRV variables ($p<0.0001$). BMI and MAP were generally not statistically significant determinants of HRV variables in the multivariable models, except for weak relations between BMI and TP variability in men, and between MAP and SDNN in women (both $R^2 \leq 0.005$). In the multivariable analysis, age was significantly associated with MeanE/I and MeanDBD ($p<0.0001$). BMI was associated with both deep breathing patterns (all $p \leq 0.05$), except to MeanE/I in women.

HF and LF variability, and LF/HF, were significantly different between sexes (all $p<0.0001$). Mean HF variability was markedly higher, and mean LF variability and LF/HF lower in women but there was no difference in TP variability (Figure 6 and Table 4). In time-domain analysis there were no significant differences between sexes. Both deep breathing patterns were also similar in men and women (Table 4).

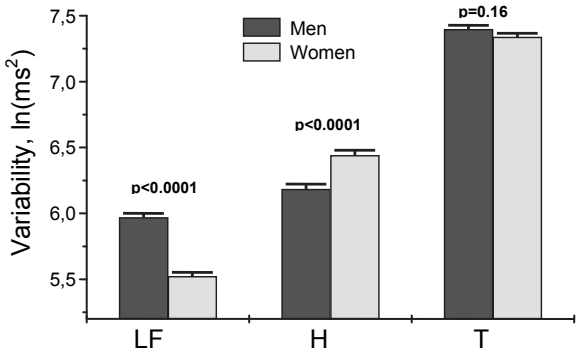

Figure 6. Mean HF, LF and TP components of HRV in men and women.

TP, HF and LF are log-transformed. HF = high frequency heart rate variability, LF = low frequency heart rate variability and TP = total heart rate variability. Standard error of mean (SEM) is marked on columns. p represents inter-group difference.

Table 7. Univariate relations of study variables with HRV variables and deep breathing test patterns

		Men (n = 831)			Women (n = 949)		
		Regression coefficient (SE)	R^2	p value	Regression coefficient (SE)	R^2	p value
HF	HR	-0.050 (0.004)	0.212	***	-0.059 (0.003)	0.296	***
	Age	-0.057 (0.007)	0.067	***	-0.061 (0.007)	0.070	***
	BMI	-0.045 (0.010)	0.026	***	-0.020 (0.009)	0.005	*
	MAP	-0.022 (0.004)	0.043	***	-0.031 (0.004)	0.069	***
LF	HR	-0.026 (0.003)	0.084	***	-0.031 (0.003)	0.127	***
	Age	-0.028 (0.006)	0.024	***	-0.029 (0.006)	0.026	***
	BMI	-0.027 (0.008)	0.014	***	-0.000 (0.007)	0.000	0.99
	MAP	-0.011 (0.003)	0.015	***	-0.012 (0.003)	0.018	***
TP	HR	-0.036 (0.003)	0.171	***	-0.041 (0.002)	0.241	***
	Age	-0.032 (0.006)	0.035	***	-0.039 (0.006)	0.047	***
	BMI	-0.035 (0.008)	0.025	***	-0.007 (0.007)	0.001	0.34
	MAP	-0.017 (0.003)	0.040	***	-0.020 (0.003)	0.048	***
LF/HF	HR	0.025 (0.003)	0.070	***	0.028 (0.003)	0.100	***
	Age	0.029 (0.006)	0.024	***	0.032 (0.006)	0.027	***
	BMI	0.019 (0.008)	0.006	*	0.020 (0.007)	0.007	**
	MAP	0.012 (0.003)	0.017	***	0.018 (0.003)	0.035	***
RMSSD	HR	-0.036 (0.002)	0.388	***	-0.040 (0.001)	0.470	***
	Age	-0.026 (0.004)	0.049	***	-0.029 (0.004)	0.053	***
	BMI	-0.025 (0.005)	0.028	***	-0.008 (0.005)	0.003	0.08
	MAP	-0.014 (0.002)	0.064	***	-0.018 (0.002)	0.085	***
SDNN	HR	-0.020 (0.001)	0.225	***	-0.022 (0.001)	0.286	***
	Age	-0.014 (0.003)	0.028	***	-0.019 (0.003)	0.048	***
	BMI	-0.017 (0.004)	0.025	***	-0.004 (0.003)	0.001	0.28
	MAP	-0.009 (0.001)	0.047	***	-0.011 (0.001)	0.060	***
MeanE/I [a]	Age	0.005 (0.001)	0.037	***	0.005 (0.001)	0.035	***
	BMI	0.003 (0.003)	0.011	**	0.002 (0.000)	0.007	*
	MAP	0.001 (0.000)	0.002	0.25	0.001 (0.000)	0.010	**
MeanDBD	Age	-0.037 (0.006)	0.042	**	-0.033 (0.006)	0.037	**
	BMI	-0.022 (0.008)	0.009	***	-0.019 (0.007)	0.009	***
	MAP	0.003 (0.003)	0.001	0.31	-0.000 (0.003)	0.000	0.91

MeanE/I is $1/(\text{MeanE/I})^2$ -transformed, i.e. the associations of back-transformed MeanE/I to age, BMI and MAP are inverse. TP, HF, LF, LF/HF–ratio, SDNN and RMSSD are log-transformed, and MeanDBD is square-root transformed. BMI = body mass index, HR = heart rate, HF = high frequency heart rate variability, LF = low frequency heart rate variability, MAP = Mean arterial pressure, MeanDBD = the mean difference in instantaneous heart rate, MeanE/I = the mean ratio for longest and shortest R-R interval during deep breathing cycle, R^2 = coefficient of determination, SDNN = standard deviation of all R-R intervals, SE = standard error, RMSSD = square root of mean squared differences of successive R-R intervals and TP = total heart rate variability*** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$.

Table 8. Multivariate relations between study variables with HRV variables and deep breathing test.

		Men (n=831)			Women (n=949)		
		Regression coefficient (SE)	Partial model R^2	p value	Regression coefficient (SE)	Partial model R^2	p value
HF, ms^2	HR	-0.051 (0.003)	0.212	***	-0.057 (0.003)	0.297	***
	Age	-0.058 (0.007)	0.072	***	-0.061 (0.006)	0.073	***
	BMI	-0.017 (0.009)	0.003	0.06	-0.002 (0.007)	0.000	0.77
	MAP	0.001 (0.004)	0.000	0.69	-0.006 (0.003)	0.003	0.06
LF, ms^2	HR	-0.026 (0.003)	0.084	***	-0.030 (0.003)	0.126	***
	Age	-0.028 (0.006)	0.026	***	-0.031 (0.006)	0.027	***
	BMI	-0.014 (0.008)	0.003	0.09	0.007 (0.007)	0.001	0.33
	MAP	0.003 (0.003)	0.001	0.43	-0.001 (0.003)	0.000	0.83
TP, ms^2	HR	-0.035 (0.003)	0.172	***	-0.040 (0.002)	0.241	***
	Age	-0.032 (0.006)	0.039	***	-0.039 (0.005)	0.049	***
	BMI	-0.015 (0.007)	0.005	*	0.005 (0.006)	0.001	0.42
	MAP	-0.001 (0.003)	0.000	0.84	-0.004 (0.003)	0.001	0.15
LF/HF, %	HR	0.025 (0.003)	0.070	***	0.026 (0.003)	0.100	***
	Age	0.030 (0.006)	0.027	***	0.030 (0.006)	0.027	***
	BMI	0.003 (0.009)	0.000	0.75	0.009 (0.007)	0.005	0.23
	MAP	0.001 (0.004)	0.000	0.74	0.006 (0.003)	0.001	0.09
RMSSD, ms	HR	-0.036 (0.002)	0.389	***	-0.039 (0.001)	0.472	***
	Age	-0.027 (0.003)	0.057	***	-0.029 (0.003)	0.056	***
	BMI	-0.007 (0.004)	0.002	0.09	0.001 (0.003)	0.000	0.75
	MAP	0.000 (0.002)	0.000	0.81	-0.003 (0.001)	0.002	0.08
SDNN, ms	HR	-0.020 (0.001)	0.226	***	-0.021 (0.001)	0.286	***
	Age	-0.014 (0.003)	0.032	***	-0.020 (0.002)	0.051	***
	BMI	-0.007 (0.004)	0.004	0.06	0.003 (0.003)	0.001	0.33
	MAP	-0.001 (0.001)	0.000	0.71	-0.003 (0.001)	0.002	*
MeanE/I [a]	Age	0.005 (0.001)	0.037	***	0.004 (0.001)	0.034	***
	BMI	0.003 (0.001)	0.006	*	0.001 (0.001)	0.002	0.24
	MAP	-0.000 (0.000)	0.001	0.40	0.001 (0.000)	0.006	*
MeanDBD, bpm	Age	-0.039 (0.007)	0.042	***	-0.032 (0.006)	0.036	***
	BMI	-0.025 (0.009)	0.010	**	-0.019 (0.007)	0.006	*
	MAP	0 011 (0 003)	0 007	*	0 004 (0 003)	0 002	0 17

MeanE/I is $1/(MeanE/I)^2$ -transformed, i.e. the associations of back-transformed MeanE/I to age and BMI are inverse. TP, HF, LF, LF/HF, SDNN and RMSSD are log-transformed and MeanDBD is square-root transformed. BMI = body mass index, HR = heart rate, HF = high frequency heart rate variability, LF = low frequency heart rate variability, MAP = Mean arterial pressure, MeanDBD = the mean difference in instantaneous heart rate, MeanE/I = the mean ratio for longest and shortest R-R interval during deep breathing cycle, R^2 = coefficient of determination, SDNN = standard deviation of all R-R intervals, SE = standard error, RMSSD = square root of mean squared differences of successive R-R intervals and TP = total heart rate variability. ***$p<0.001$ and *$p\leq0.05$

5.5. Reference values of heart rate variability in young adults

Because the effects of age, heart rate and sex on HRV appeared significant, the reference values were adjusted for these variables. The generated statistical function enables calculating 95% reference limits for each HRV variable according to age, sex and heart rate (Table 9). For example, a 24-year-old man with a heart rate of 60 bpm would have an expected HF of:

$$\ln(HF) = 12.714 - 0.062(24) - 0.055(60) - 0.519(2) = 6.888$$
$$HF(ms2) = e^{6.888} = 980$$

The unbiased estimator of residual standard deviation(s) for an observation was equal to 0.933 for ln(HF). In our example, for an interval of ±1.96 s, we would have central 95% reference limits for an HF variability of 160– 6100 ms2. The estimators of residual standard deviation are presented in Table 9. The lower 2.5% reference values of frequency-domain HRV variables are graphically presented in Figure 7. The normative percentual reference values of deep breathing test patterns are shown in Figure 8.

Table 9. Age, sex and heart rate adjusted regression based reference limits of HRV variables.

Expected HRV	s	95% RL
ln(HF)=12.714–0.062(age)–0.055(HR)–0.519(sex)	0.933	$e^{\ln(HF) \pm 1.96s}$
ln(LF)=8.113–0.030(age)–0.028(HR)+0.312(sex)	0.849	$e^{\ln(LF) \pm 1.96s}$
ln(LF/HF)=0.004+0.032(age)+0.026(HR)+0.831(sex)	0.909	$e^{\ln(LF/HF) \pm 1.96s}$
ln(TP)=11.303–0.037(age)–0.038(HR)–0.123(sex)	0.768	$e^{\ln(TP) \pm 1.96s}$
ln(SDNN)=5.910–0.018(age)–0.021(HR)–0.075(sex)	0.362	$e^{\ln(SDNN) \pm 1.96s}$
ln(RMSSD)=7.483–0.029 (age)–0.038 (HR)–0.215(sex)	0.436	$e^{\ln(RMSSD) \pm 1.96s}$

Age=age in years, HR=heart rate rounded to nearest 5 bpm, sex=1 for women and 2 for men, e=Napier's constant (~2.718) and s=unbiased estimator of residual standard deviation. HF=high-frequency heart rate variability (0.15-0.4 Hz), LF=low-frequency heart rate variability (0.04-0.14 Hz), SDNN=the standard deviation of all R-R-intervals, TP=total heart rate variability, RL=reference limits and RMSSD=square root of mean squared differences of successive R-R-intervals.

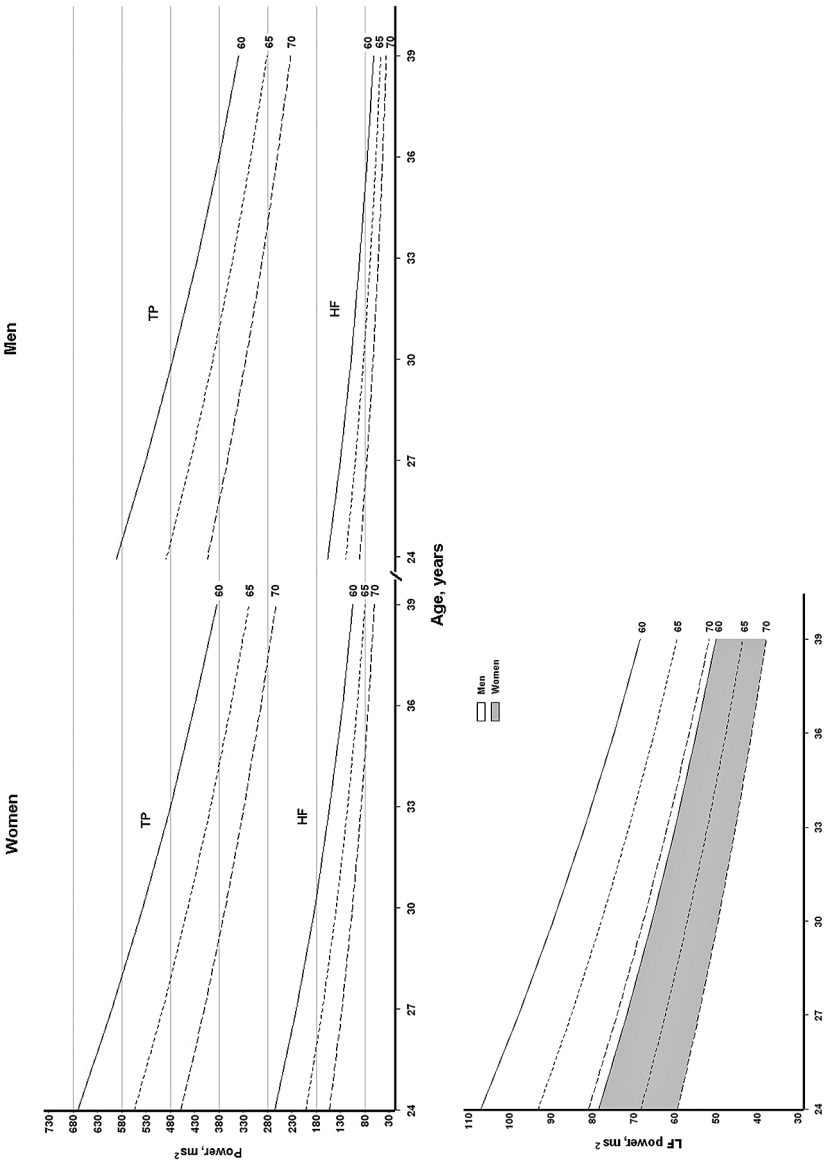

Figure 7. Lower 2.5 % reference limits of HF, TP and LF variability distributed by age, sex and heart rate.
60, 65 and 70 bpm represents mean heart rate during ECG recording. In women, numbers of individuals stratified by heart rate (60, 65 and 70) were 131, 180 and 205, respectively. In men, numbers of individuals stratified by heart rate (60, 65 and 70) were 166, 179 and 115, respectively. HF=high-frequency heart rate variability, power = variability of heart rate, TP=total heart rate variability and LF= low-frequency heart rate variability.

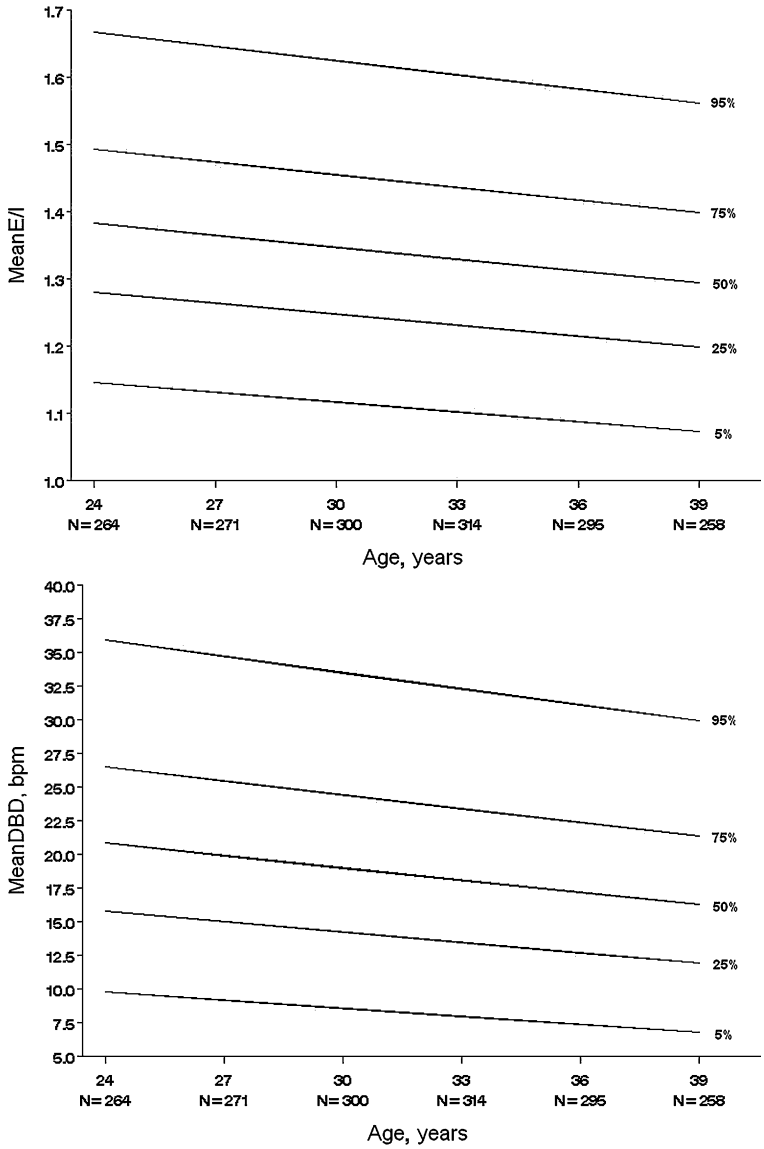

Figure 8. Percentiles of Mean E/I and MeanDBD in a deep breathing test presented by age.

MeanE/I=the mean ratio for longest and shortest R-R-interval during a deep breathing cycle, MeanDBD=the mean difference in instantaneous heart rate.

5.6. Heart rate variability and metabolic syndrome (Study II)

The associations between HRV patterns and MetS were studied in 1 889 subjects. In unadjusted analyses, significantly decreased HF variability, LF variability and TP variability were observed in both men and women with MetS based on the NCEP criteria. On the average, the presence of MetS was associated with 11% lower HF variability in women and 8% lower HF variability in men. The LF/HF ratio was 12% higher in women and 4% higher

in men with the MetS (Figure 9). All differences were statistically significant, except for the LF/HF ratio in men (p = 0.06). Essentially similar results were observed when the IDF and EGIR criteria for MetS were used.

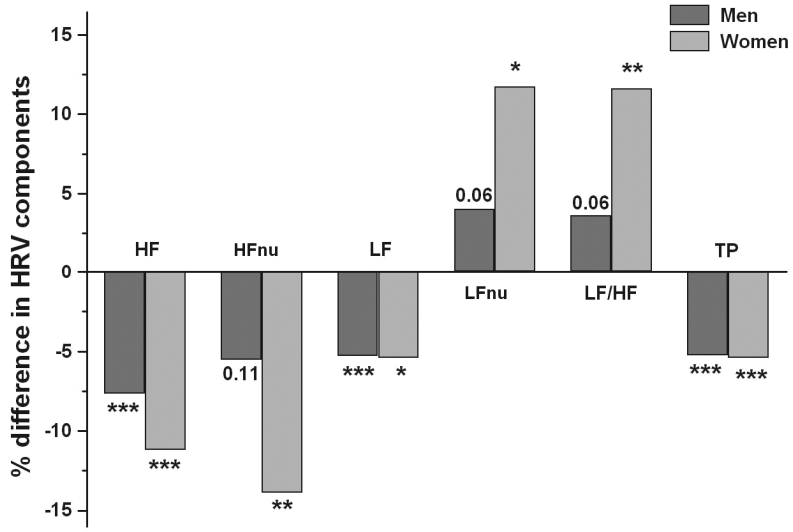

Figure 9. Percentage difference in HRV variables in individuals with the MetS compared to those without the MetS. Negative values indicate that the values are decreased in individuals with the MetS.

Metabolic syndrome is defined by NCEP-criteria. HF, LF, LF/HF and TP are log-transformed and LFnu square-transformed to obtain normal distribution; differences were computed from mean values. Mean difference is the difference in heart rate variability component between subjects with and without the MetS [(HRV in subjects with the MetS) − (HRV in subjects without the MetS)]. p for difference was tested with the t test. Number of subjects with all MetS components available is 1,864; women n=995 (85 with the MetS) and men n=869 (144 with the MetS). HF=high frequency component of spectral HRV, HFnu=HF in normalized units, LF= low frequency component of spectral HRV, LFnu=LF in normalized units, LF/HF=ratio between low and high frequency components and TP=total spectral HRV. *p < 0.01, **p < 0.001 and ***p ≤ 0.0001.

The age adjusted differences in HRV between subjects with and without the MetS, as defined by the NCEP criterion are presented in Table 10. After adjustment for age, significant differences persisted for all HRV components (all p<0.05), except for HFnu, LFnu and LF/HF-ratio in men (all p> 0.1). After further adjustment for heart rate, a significant difference persisted in women for HF (p=0.02) and HFnu (p=0.05) and in men for TP and LF (both p<0.05). When the IDF criterion was applied in the age and heart rate adjusted model, the MetS was significantly associated with HF, HFnu, LFnu and LF/HF-ratio in women (p<0.05), and with TP in men (p=0.02). When the EGIR criterion was used, the age and heart rate adjusted differences were not statistically significant, except for TP in men (p<0.05).

Table 10. Age adjusted differences of mean heart rate variability variables between individuals with and without the MetS (NCEP criteria).

	Men (n=869)		Women (n=995)	
	Mean difference (95% Cl)	**p**	**Mean difference (95% Cl)**	**p**
HF, *ln*ms^2	−0.406 (−0.597 – (−)0.215)	***	−0.621 (−0.873 – (−)0.369)	***
HFnu, *%*	−2.0 (−1.4 – 5.4)	0.25	−7.7 (−12.0 – (−)3.5)	**
LF, *ln*ms^2	−0.285 (−0.444 – (−)0.127)	**	−0.250 (−0.456 – (−)0.044)	*
LFnu, *sqrt%*	0.200 (−0.465–0.065)	0.14	0.516 (0.170 – 0.863)	*
LF/HF, *ln%*	0.121 (−0.287–0.044)	0.15	0.369 (0.152 – 0.586)	**
TP, *ln*ms^2	−0.352 (−0.504 – (−)0.199)	***	−0.333 (−0.53 – (−)0.134)	**

The MetS is defined according to NCEP criterion. Number of subjects with all MetS components available is 1,864. Cl=confidence limits, HF=high-frequency heart rate variability, HFnu=HF in normalized units, LF= low-frequency heart rate variability, LFnu=LF in normalized units, LF/HF=ratio between low- and high-frequency heart rate variability, Mean difference=difference in heart rate variability component between subjects with and without the MetS [(HRV in subjects with the MetS) − (HRV in subjects without the MetS)], P=probability value for difference and TP=total heart rate variability. *$p < 0.05$, **$p \leq 0.001$ and ***$p < 0.0001$.

Age and heart rate adjusted statistically significant associations between MetS components (treated as continuous variables) and HRV variables are presented in Table 11. All the MetS components (waist circumference, triglycerides, glucose, insulin, HDL-cholesterol and systolic/diastolic blood pressure) were first in the same regression model. After adjustment of age (Model 1) the backward selection was applied to come up with the most parsimonious model. The elevated blood pressure was associated with decreased HF, LF and TP variability, and higher LF/HF-ratio in both sexes (all $p<0.05$). In men, waist circumference was inversely related with HF, LF and TP variability (all $p<0.05$). In women, fasting glucose was associated with decreased HF variability, and fasting insulin with increased LF/HF-ratio. To study whether these associations remained independent after further adjustment the model with heart rate (Model 2), only waist circumference remained significantly associated with decreased HF, LF and TP variability (all $p<0.01$), in men. Whereas in women, there remained a significant association with triglycerides and decreased HF variability ($p=0.02$) and with systolic blood pressure and increased LF/HF-ratio ($p=0.02$). Age and heart rate were statistically significant in all models (all $p<0.001$) (Table 11).

Table 11. Age and heart rate adjusted multivariable associations between metabolic syndrome components and HRV in men and women.

HF	**Men (n=880)**		**Women (n=1,009)**	
	β (SE)	**p**	**β (SE)**	**p**
Model 1				
Waist circumference, cm	-0.012 (0.004)	**		
Diastolic BP, mmHg	-0.013 (0.004)	***		
Glucose, mmol L^{-1}			-0.237 (0.086)	**
Systolic BP, mmHg			-0.020 (0.003)	***
Model 2				
Waist circumference, cm	-0.010 (0.003)	**		
Triglycerides, mmol L^{-1}			-0.164 (0.071)	*
LF	**β (SE)**	**p**	**β (SE)**	**p**
Model 1				
Waist circumference, cm	-0.008 (0.003)	*		
Diastolic BP, mmHg	-0.006 (0.003)	*		
Systolic BP, mmHg			-0.009 (0.002)	***
Model 2				
Waist circumference, cm	-0.006 (0.003)	*		
LF/HF –ratio	**β (SE)**	**p**	**β (SE)**	**p**
Model 1				
Systolic BP, mmHg	0.008 (0.003)	**	0.012 (0.003)	***
Insulin, mU L^{-1}			0.133 (0.060)	*
Model 2				
Systolic BP, mmHg			0.006 (0.002)	*
TP	**β (SE)**	**p**	**β (SE)**	**p**
Model 1				
Waist circumference, cm	-0.010 (0.003)	***		
Diastolic BP, mmHg	-0.011 (0.003)	***		
Systolic BP, mmHg			-0.014 (0.002)	***
Model 2				
Waist circumference, cm	-0.009 (0.003)	***		

First associations were adjusted with age (Model 1), and then further adjusted with heart rate (Model 2). β=parameter estimate indicating the change in spectral HRV components caused by one unit change in the explaining variable (e.g. for one mmol L^{-1} in glucose), SE=standard error. HRV variables, insulin and triglycerides are log-transformed. HF=high-frequency heart rate variability, LF= low-frequency heart rate variability, LF/HF=ratio between low- and high-frequency heart rate variability and TP=total heart rate variability. *p<0.05, **p<0.01 and ***p≤0.001.

The associations between the number of MetS components (NCEP) and HRV variables are presented in Figure 10. The number of components was inversely related to HF, LF and TP and directly to the LF/HF-ratio. After adjustments for heart rate and age, the inverse relation to HF and the direct relation to LF/HF-ratio persisted in women.

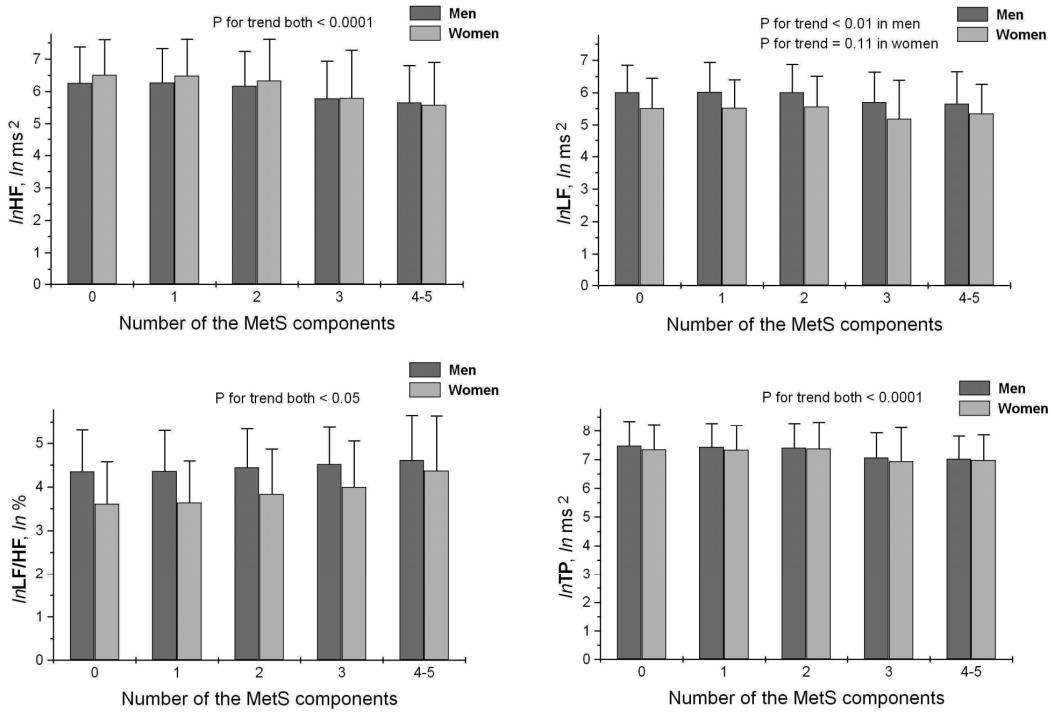

Figure 10. Mean values for heart rate variability component versus increasing number of metabolic syndrome components.

The MetS components are defined according to the NCEP criterion. Error bars represent standard deviation. P values describe the decreasing or increasing (LF/HF-ratio) trend according to the number of the Mets components. HF=high-frequency heart rate variability, LF=low-frequency heart rate variability and LF/HF=ratio between low- and high-frequency heart rate variability and TP=total heart rate variability.

5.7. Heart rate variability and vascular properties

There were significant differences in men and women in cIMT, Cdist and FMD (p for all <0.001) which are presented in Table 12. When relations of cIMT, Cdist and FMD to frequency-domain HRV variables were studied in the univariate model (Model 1); cIMT was significantly associated with HF variability, TP variability and LF/HF-ratio (all p <0.05) but not with LF variability (p=0.8); Cdist was significantly associated with all frequency-domain HRV variables (all p<0.0001); and FMD was significantly associated with LF variability, TP variability and LF/HF-ratio (all p<0.05) but not with HF variability (p=0.8). After adjustments for heart rate, sex and age (Model 2) associations remained significant only for Cdist with HF variability, TP variability and LF/HF-ratio (all p<0.05). After further adjustments for carotid wall thickness (intima-media thickness), and several risk factors including systolic blood pressure, body mass-index, LDL-cholesterol, triglycerides, glucose and insulin (Model 3), Cdist remained significantly associated with HF variability and TP variability (both p<0.05, Figure 11).

Table 12. Ultrasound measurements of vascular properties in men and women.

	Men (SD)	Women (SD)	p for difference
cIMT, mm	0.63 ±0.10	0.61 ±0.09	<0.001
Cdist, %	2.0 ±0.66	2.3 ±0.77	<0.001
FMD, %	6.9 ±4.0	8.7 ±4.5	<0.001

Both HRV variables and cIMT and Cdist were measured for 1.002 women and 870 men. Both FMD and HRV were measured for 949 women and 806 men. cIMT=carotid intima-media thickness, Cdist=carotid distensibility, FMD=flow mediated dilatation of brachial artery, SD=standard deviation. The difference is tested with a *t* test.

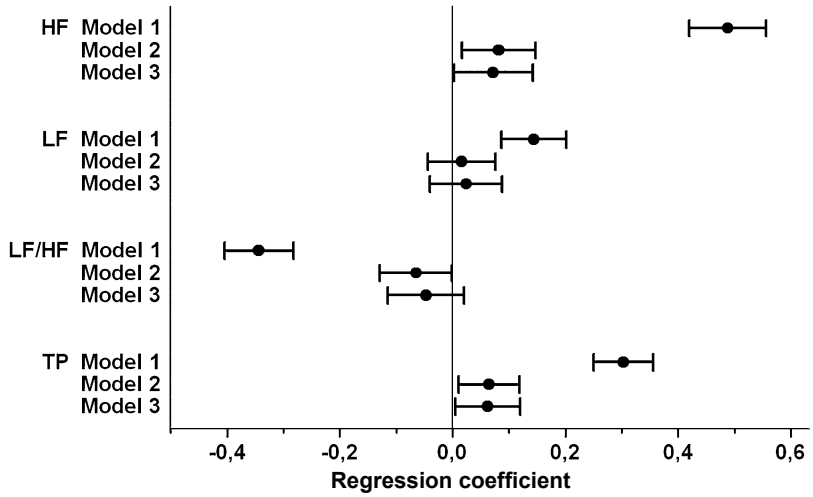

Figure 11. Unadjusted and adjusted relations between HRV variables and distensibility. The regression coefficients and their 95% confidence are shown.

Model 1 was unadjusted and Model 2 was adjusted for sex, age and heart rate. In Model 3 components were further adjusted for carotid wall thickness (intima-media thickness), systolic blood pressure, body mass –index, LDL-cholesterol, triglycerides, glucose and insulin concentration. Regression coefficients represent the effect of one unite increase in Cdist to the HRV components. HF=high-frequency heart rate variability, LF=low-frequency heart rate variability and LF/HF=ratio between low- and high-frequency heart rate variability and TP=total heart rate variability. N=1,872.

The relative contributions of the predictor variables for HF variability and TP variability are shown in Table 13 that displays the full multivariate models. The predictor variables are sorted by standardized beta regression coefficients so that the relative strength of the various predictors within can be assessed. Heart rate was the strongest predictor for HF variability, followed by age, the male gender and Cdist. The other risk factors were not significantly associated with HF variability in the model. Results were similar with TP variability, except for gender which was not statistically significantly related with TP variability.

Table 13. Standardised regression coefficients (STβ) and regression coefficients (β) of HF and TP variability with risk factors and vascular properties in multivariate analysis.

HF			
	STβ	**β ± SE**	**p**
Heart rate, bpm	−0.50	−0.05 ± 0.002	<0.0001
Age, years	−0.26	−0.06 ± 0.005	<0.0001
Male sex	−0.19	−0.43 ± 0.05	<0.0001
Cdist, %/10mmHg	0.05	0.07 ± 0.04	<0.05
BMI, kg/m^3	−0.04	−0.01 ± 0.01	0.1
LDL-C, mmol L^{-1}	−0.02	−0.03 ± 0.03	0.3
IMT, mm	0.02	0.28 ± 0.26	0.3
Insulin, *log*mU L^{-1}	0.02	0.04 ± 0.05	0.5
TG, *log*mmol L^{-1}	−0.02	−0.04 ± 0.06	0.5
Glucose, mmol L^{-1}	−0.01	−0.03 ± 0.05	0.6
SystBP, mmHg	−0.01	−0.00 ± 0.00	0.6
TP			
	STβ	**β ± SE**	**p**
Heart rate, bpm	−0.45	−0.04 ± 0.00	<0.0001
Age, years	−0.19	−0.03 ± 0.00	<0.0001
Cdist, %/10mmHg	0.05	0.06 ± 0.03	<0.05
BMI, kg/m^3	−0.03	−0.01 ± 0.01	0.2
LDL-C, mmol L^{-1}	−0.03	−0.04 ± 0.02	0.1
Male sex	−0.03	−0.05 ± 0.04	0.2
Insulin, *log*mU L^{-1}	0.03	0.05 ± 0.04	0.3
SystBP, mmHg	−0.01	−0.00 ± 0.00	0.6
IMT, mm	−0.01	−0.09 ± 0.21	0.7
TG, *log*mmol L^{-1}	−0.01	−0.02 ± 0.05	0.7
Glucose, mmol L^{-1}	0.00	0.01 ± 0.4	0.9

All variables are introduced in the same regression model. To compare the relative strength of the various predictors within the model the standardized regression coefficients were computed (STβ). BMI=body mass index, C=cholesterol, Cdist=carotid artery distensibility, IMT=carotid wall thickness (intima-media thickness), SystBP=systolic blood pressure, TG=triglycerides and β ± SE=regression coefficient ± standard error. Insulin and triglycerides are log-transformed to obtain normal distribution. N=1,824.

To study whether the carotid artery distensibility (Cdist) modifies the associations between cardiometabolic risk factors and vagal outflow, estimated with HF variability, we divided the individuals of Study 3 into three subgroups according to the Cdist. Low and high elasticity represents age- and sex-specific 10th and 90th percentiles for Cdist. The relation between the number of risk factors and HF variability across the distensibility subgroups is shown in Figure 11. In individuals with low and intermediate distensibility, there was an inverse trend between the number of cardiometabolic risk factors and HF (both $p<0.01$). In individuals with high carotid distensibility there was no such trend ($p=0.41$). The inverse association between the number of risk factors and HF was stronger in individuals with low Cdist than in individuals with intermediate Cdist (p for interaction=0.01). Similar results were seen between the number of cardiometabolic risk markers and TP variability (Figure 12). These results were identical if the carotid strain (relative change in diameter) variable was used instead of Cdist. The associations of HF and TP in Cdist groups with risk factors were practically identical when clinical cut-points were used instead of percentile based cut-offs, except for the interaction term risk factor*HF which was not significant (p for interaction=0.07).

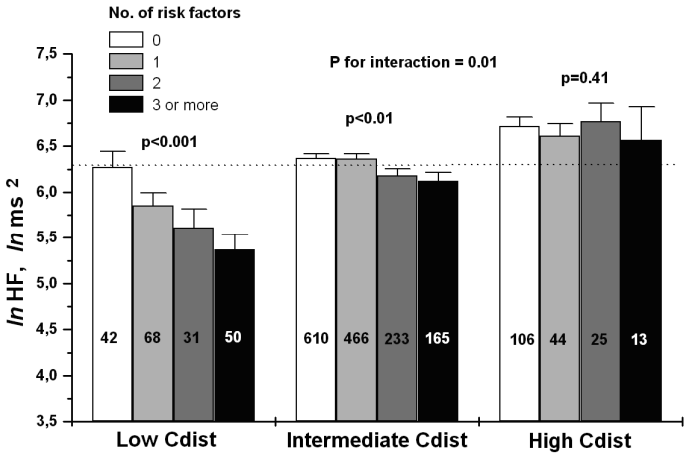

Figure 11. Trends in high frequency heart rate variability in relation to low, intermediate and high carotid distensibility and according to increasing number of risk factors in young adults.

The columns representing mean HF and bars are standard errors of mean (SEM). Probability values are derived from linear regression models adjusted for age and sex, testing a linear trend between HF and number of risk factors. Subjects were defined to have a risk factor if their current values of systolic blood pressure, waist circumference, fasting glucose or triglyceride concentration exceeded the age- and sex-specific 80th percentile, or HDL-cholesterol was below the age- and sex-specific 20th percentile. The low and high distensibility represents age- and sex-specific 10th and 90th percentiles. The dashed line indicate the population mean for HF. The numbers of subjects are shown in columns. N=1,853. Cdist=carotid artery distensibility, HF=high frequency spectral HRV.

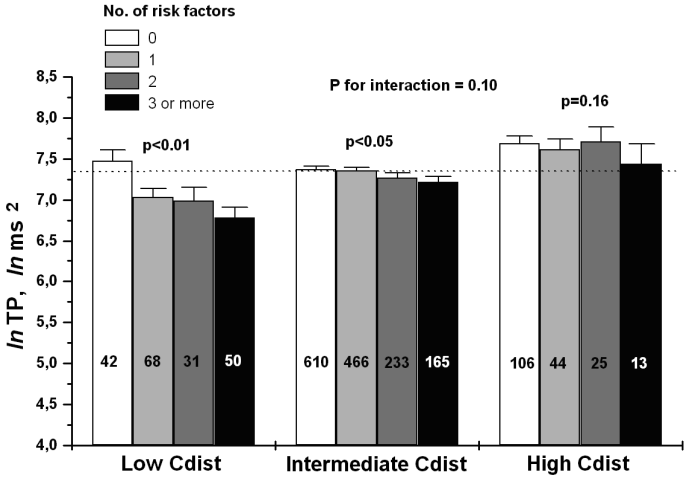

Figure 12. The trends in total heart rate variability in relation to low, intermediate and high carotid distensibility and according to increasing number of risk factors in young adults.

The columns representing mean TP and bars are standard errors of mean (SEM). Probability values are derived from linear regression models adjusted for age and sex, testing a linear trend between TP and number of risk factors. Subjects were defined to have a risk factor if their current values of systolic blood pressure, waist circumference, fasting glucose or triglyceride concentration exceeded age- and sex-specific 80th percentile, or HDL-cholesterol was below the age- and sex-specific 20th percentile. The low and high distensibility represents age- and sex-specific 10th and 90th percentiles. The dashed line indicate the population mean for TP. The numbers of subjects are shown in columns. N=1,853. Cdist=carotid artery distensibility, TP=total spectral HRV

6. DISCUSSION

6.1. Study participants

This thesis is a novel part of the on-going long-term epidemiological Cardiovascular Risk in Young Finns study. Many studies into lifetime cardiovascular risk profiles in this study population have been reported previously. In 2003, Raitakari et al demonstrated that cardiometabolic risk profile assessed in 12-year to 18-year-old adolescents predicts adult carotid artery intima-media thickness (cIMT) independently of contemporaneous risk factors. These findings suggested that exposure to cardiometabolic risk factors early in life may induce changes in arteries that actually contribute to the development of atherosclerosis [181]. In the current thesis assessing the links between HRV and cardiometabolic risk profile, the participants were 24 to 39 years old. This thesis project is based on the cross-sectional follow-up investigation in 2001, where a total of 2 283 individuals (63.5% of participants in first study in 1983) participated. The source data were collected with single channel ECG from 2 151 individuals. Previously, no population-based studies of this magnitude on HRV have been conducted in healthy young adults. Two similar studies have been done in older individuals. In the Atherosclerosis Risk in Communities study (ARIC), 2-minute recordings of ECG were analysed for the HRV variables in 1 984 individuals aged 45 to 64 years [100]. The other large population-based study on HRV is the Framingham Heart Study where 2 722 subjects aged 55 years on average were examined; it included 442 individuals younger than 40 years [201]. In the Framingham Heart Study, the pattern of HRV was analysed from the first 2 hour ambulatory ECG collected during the study visit in 1982 to 1987, thus it was not necessarily in stable conditions. In the Young Finns study, the participants were younger than in the ARIC and the Framingham studies and ECG was recorded during a 3-minute controlled breathing. Because our study participants were healthy and sufficiently young in 2001 we were able to generate reference values of HRV for a younger population, to study determinants of HRV in this population based sample and to study their associations with cardiometabolic risk factors and with vascular properties assessed using ultrasound.

6.2. Effect of age, sex and heart rate on heart rate variability (Study I)

The patterns of HRV are known to depend on age and sex [55;56;202-204]. Our findings on strong associations with HRV variables to age and sex are in line with the previous reports. The effect of age in this 24 to 39 year population seemed rather linear, demonstrating decreasing trends in both the time-domain and frequency-domain variables HF, LF, RMSSD, TP, SDNN and a related increasing trend in the LF/HF-ratio. Previously it has been reported that the effect of aging on HRV may not be linear during whole life cycle. The studies in children and adolescents have shown that there is a progressive increase in HRV up to the

age of 10 years, which may reflect a progressive development of the ANS [58]. After childhood, the effect of aging on HRV seems linear up to the age of 60 years. Thereafter the age-related decrease in HRV stabilises [61]. Zulfiqar et al found that in 80-99 year old individuals, HRV patterns, particularly RMSSD, increased instead of decreasing with aging [62]. This finding was previously observed by others [63;64]. It is possible that individuals with high HRV are common in older populations because of lifestyle habits or genetically mediated changes in the ANS function. This validation hypothesis can be supported by the previous report of Dekker et al. They followed 40 to 60 year old individuals at the beginning of the survey for 30 years and observed that decreased HRV was associated with risk of death from all causes (coronary heart disease and cancer) in both middle-aged and elderly men. They suggest that decreased HRV may be an indicator of poor general health [65]. In addition, it is possible that in senior subjects, the HRV results are influenced by an increasing prevalence of non-respiratory sinus arrhythmia, i.e. the higher prevalence of erratic rhythms, especially in long-term recordings [66]. It is also possible that advanced age contributes to nerve conduction, and as a consequence, interferes with the negative feed-back control loop [15;16]. This may actually result to increased sinusoidal oscillations in blood pressure and heart rate, and thus an increase in the HRV.

Our data demonstrate that women have lower LF variability and LF/HF-ratio and higher HF variability than men. This may indicate lower sympathetic and higher vagal ANS control. In the other HRV variables, including total heart rate variability (TP), there were no significant sex differences. Similar sex differences have been previously reported in middle-aged individuals [205]. On the other hand in the ARIC study, there was no significant difference between women and men in the HF variability [56]. In the Young Finns study, the subjects were much younger (24–39 years vs. 45–64 years in the ARIC study) and HRV was examined during controlled breathing. These differences in study protocol and age group may explain differences between the reports in question. These sex related differences may also be explained by different body fat distribution or hormonal differences between women and men [67-69]. It is hypothesised that estrogen inhibits the release of norepinephrine in the heart and potentiates cholinomuscarinic activity in the heart and central nervous system [70;71]. Airaksinen et al have shown that there are sex-related differences in the heart rate control during coronary occlusion. Women have more often evidence of vagal activation, bradycardia or increase in HRV, during coronary occlusion indicating a different type of ANS response to myocardial ischemia from men [206]. Other factors that modulate or alter ANS control, and may potentially influence gender differences in HRV, include inflammation and psychological aspects [72;73;207]. Interestingly also the gender differences seem to disappear after the age of 60 years [61]. Although the observed gender differences in LF variability and HF variability are statistically significant in our study, the differences are minor and might have been found because of a large study population. The observation that there was no significant sex difference in variables of total heart rate variability (TP and SDNN) further indicates that caution is needed for assumptions to clinical relations of sex

differences in the cardiac control of ANS. There was significant sex-difference in resting heart rate which may contribute to the observed sex-differences in HRV variables.

It is expected that several previous studies have found strong relations between heart rate and HRV [201]. In the analysis of HRV, the adjustment for heart rate can be problematic, as it may create an over-adjusted model when the ANS control of the heart rate is the target of the analysis. However, because the baseline heart rate has mathematical relation to HRV [117], the mean heart rate needs to be considered when HRV is analysed. In this report, as assumed, we found significant association with heart rate to both all HRV and deep breathing variables. The inverse association between heart rate and LF variability, which may mirror sympathetic cardiac modulation, seemed weaker than the inverse association between heart rate and HRV variables associated with parasympathetic cardiac control (HF and RMSSD). This finding is consistent with the observation that increased heart rate commonly indicates increased sympathetic action and might therefore be expected to decrease LF variability to a lesser degree. In middle-aged subjects, the influence of heart rate on HF variability and LF variability has been observed to be more pronounced in women than men [55]. In our study, the effect of heart rate on HRV variables was statistically similar in both sexes, as assumed. The cardiovascular ANS has an important effect on heart rate (through i.e. arterial and cardiopulmonary baroreflexes and chemoreflexes, skin thermoregulatory reflexes and renin-angiotensin-aldosterone system) [21;25;208;209]. But observations between HRV data and heart rate support the accepted concept that the mean heart rate is still subjected to other control mechanisms in addition to ANS, i.e. genetics and sex [55;210]. Therefore, heart rate alone cannot be used as a reliable estimator of autonomic activity.

6.3. Reference values, reproducibility and office-hour variation of heart rate variability (Study I)

As discussed above HRV is significantly related to age, sex and heart rate. Thus, unless the physiological effects of aging, heart rate and sex are not recognised, individuals may be incorrectly attributed to having an abnormal ANS balance. The HRV reference values of this study are based on regression analysis, where sex, age and heart rate are covariates. The study population was aged 24 to 39 years old. Because of the age range of the study population, the presented reference values may be considered valid between the ages of 20 to 40 years. Although, we found that calculated regression based HRV values were inside the confidence limits of mean HRV reported in previous studies in 20 to 60 year-old subjects [55;211].

In the reproducibility studies, the deep breathing tests were highly reproducible. The reference values and reproducibility of deep breathing tests presented are comparable to the results previously reported in the thesis of Piha concerning 143 healthy individuals [202]. Furthermore, the reliability of short-term HRV data was relatively good (CV% 6-12). In the

previous studies, when reproducibility of short-term HRV variables was analysed during spontaneous breathing, the reproducibility was not as good and it greatly varied from study to study [57;197;212]. Our data suggest that patterns of short-term HRV can be fairly well measured in standardised manner and the results are reproducible. Decreased HRV is related to increased risk for sudden cardiac death in individuals with chronic heart failure, and both arrhythmic cardiac and non-cardiac death after myocardial infarction, and is a marker of early diabetic neuropathy [10;11;53;54;94-96;213]. The Diabetes Control and Complications Trial showed that intensive glycaemic control can prevent ANS dysfunction, slowing the deterioration of autonomic dysfunction over time, in individuals with type 1 diabetes [98]. Thus the assessment of HRV has potential in the prediction of arrhythmias, non-arrhythmic cardiac events, and autonomic neuropathy in association with cardiac disease and diabetes [35;214]. The advantages of this technique are its non-invasiveness, ease of use and availability. In particular the deep breathing tests may be a helpful tool in follow-up studies because of its high reproducibility, and thus because it allows repeat comparison of personal values. In the follow-up, should alterations in HRV be compared to the each individual base-line sample, because of a distinctive nature of HRV patterns.

In middle-age subjects, HRV follows a circadian pattern. In long-term analysis HF variability and RMSSD decrease during the before-noon hours and reach the lowest levels in the afternoon (03:00–06:00 after noon), then turn upwards in the evening and reach again the highest oscillation in the early morning (3:00–5:00 before noon). The LF/HF-ratio show an opposite behaviour, reaching the peak in the afternoon (02:00-04:00 after noon), then decreasing until the first hours after midnight [215]. This circadian rhythm of the cardiovascular ANS is related mainly to the sleep-wake rhythm. The circadian variations of blood pressure and heart rate reflect an increase of sympathetic activity, which is clinically related with e.g. morning peak of ischaemic events [216]. Because of a marked circadian variation in HRV patterns, we studied whether the office-hour is related to HRV variables in our young healthy adults. The time of the day correlated weakly and inversely with the variables of total HRV, i.e. TP variability ($r=-0.053$ and $p=0.03$) and SDNN ($r=-0.055$ and $p=0.02$) but there were no statistically significant correlations between study-hour and other HRV variables, deep breathing test or mean heart rate. Thus even in stationary conditions total HRV is slightly influenced by time of the day. Within office-hour (7.00 a.m.-15.00 p.m.) there was no significant circadian variation in other time-domain or frequency-domain short-term HRV variables in our study.

6.4. Heart rate variability and metabolic syndrome (Study II)

Previous studies have shown that hypertension, dyslipidemia, obesity, diabetes and impaired glucose metabolism may generate decrease in HRV [55;94;105;217]. In middle-aged and elderly subjects, the MetS has been found to be associated with reduced HRV [4;106]. Interestingly, in our young adult sample, the MetS was similarly associated with decreased HRV in both

sexes, regardless of the criteria used for MetS. Our findings are in part confirmatory to previous observations, but the strength of the present study is that a similar effect can be shown in young age. It appears that the process affecting cardiac autonomic control starts at a young age, as would be suspected. Furthermore, our data are based on large sample size representative for the general population [187].

In women, the MetS was independently (after adjustments for age and heart rate) associated with decreased HF variability, probably indicating decreased vagal cardiac control. In men, MetS was independently associated with decreased TP and LF variability. In studies concerning ANS function, especially decreased vagal control has been associated with the development of coronary heart disease [1]. A proposed explanatory mechanism is that the activation of efferent vagus nerve fibres and related activation of nicotinic receptors in target organs may inhibit the release of cytokines and thus suppress peripheral inflammation, and may slow the progression of atherosclerosis [72;155]. Previous studies have observed that MetS has a greater impact on coronary heart disease and all-cause mortality in women than in men [218;219]. Our data suggest that in women, especially vagal cardiac control may be affected more by MetS than in men.

In men, when the effects of MetS components on HRV variables were separately studied, waist circumference was the only MetS component that was independently associated with decreased HRV. This association was not seen in women, whose strongest associations were with systolic blood pressure and with triglycerides. It may be that central obesity in men is associated with significant influence on the cardiovascular control of ANS. A similar gender difference has previously been reported in middle-aged individuals [55]. Central obesity is considered important in the aetiology of MetS and is linked to increased cardiovascular mortality and the development of diabetes as well [131]. Many underlying mechanisms have been proposed; the release of free fatty acids from adipocytes, chronic activation of the immune system, disorders of the hypothalamic-pituitary-adrenal axis and cellular processes leading to formation of reactive oxygen species [132-135]. The observation that the waist circumference and HRV are related in men may be explained by the previously observed difference in fat accumulation between sexes. Pre-menopausal women more frequently develop peripheral obesity with subcutaneous fat accumulation and have a smaller intra-abdominal fat depot, whereas men are more prone to central obesity [220]. It has even been proposed that differences in fat distribution may in part explain the reduced risk of cardiovascular disease in women compared to men, although the reasons are most definitely multifactorial [218]. In the Woman's Ischemia Syndrome Evaluation Study, 780 women were followed up for 3 years after coronary angiography applied for suspected myocardial ischemia. The study showed that MetS and BMI were strongly associated as assumed, but only MetS, not BMI, predicted future cardiovascular risk in women [221]. Previously Grassi et al. compared peripheral versus central obesity on sympathetic, metabolic and reflex function in 36 lean individuals, 20 individuals with peripheral obesity and in 26 individuals with central obesity [69]. They measured postganglionic muscle sympathetic nerve traffic at rest and

during baroreceptor stimulation and deactivation induced by stepwise intravenous infusions of phenylephrine and nitroprusside. They reported that central obesity was characterised by a higher degree of sympathetic activation than peripheral obesity. In young men, the fat accumulation to the central body may be more pronounced than in young women, which may explain the relation with HRV to waist-circumference observed only in men. This is also supported by the observation that in post-menopausal women central obesity (waist-circumference) seems to be related to decreased RMSSD [222]. In addition, it is possible that associations between waist circumference and HRV may be more difficult to observe in young women, as waist measures may vary depending on the menstrual phase, potentially causing a bias in women [223].

HRV and blood pressure are related because of physiology (negative feed-back control loop). The marked association has been shown also in previous research and may be considered well-documented [79-81]. One would suspect that blood pressure is a most significant MetS component related to HRV. As suspected, we found that in age adjusted models blood pressure (systolic- or diastolic-component) and HRV variables were significantly related in both sexes. After further adjusting with heart rate, the relation with HRV and blood pressure remained only in women with LF/HF-ratio and systolic blood pressure. Although the prevalence of MetS in this young population study (mean age 32 years) may considered quite high (9% in women and 17% in men) there were less hypertensive individuals (men 10% and women 4%), and thus the effect of blood pressure to HRV may not be as strong as in older age.

6.5. Heart rate variability and vascular properties (Study III)

In the Young Finns study the vascular properties were assessed using ultrasonography. The measurements of carotid intima-media thickness (cIMT), carotid artery distensibility (Cdist) and brachial artery endothelium-dependent flow-mediated dilatation (FMD) were examined.

Vascular ultrasound examination can display both mechanical and functional properties of arteries. The cIMT represents an important marker of carotid atherosclerosis and relates to the severity and extent of coronary artery atherosclerosis [224]. Increased cIMT also predicts the likelihood of future cardiovascular events, such as non-fatal and fatal acute myocardial infarction [225]. FMD is widely used as a marker of systemic arterial endothelial function. Previous studies have shown that the measurement of FMD closely associates with structural and functional coronary artery atherosclerosis [226]. It has also been hypothesised that the endothelium and ANS work together to create a balance between the needs of local tissues and those of other systems by sharing a functional antagonism to maintain blood vessel tone [150]. Thus a tonic balance is achieved between the release of vasodilating factors from the endothelium and vasoconstricting factors from sympathetic nerve terminals. Altered ANS function, assessed via HRV analysis, is also observed to be associated with an increased risk

of coronary heart disease and with angiographic progression of coronary atherosclerosis, the HRV patterns being a supplementary predictor of cardiovascular prognosis [1;12;167;227].

To study possible relations of HRV variables and ultrasonographically measured vascular properties we linked both measured data sets. In univariate models, we found significant associations between HRV and cIMT and FMD. After adjusting the model (where HRV was explained variable) for age, sex and heart rate, there remained no significant associations with HRV and cIMT or FMD. Previously the extent and progression of CHD has been shown to be associated with decreased HRV [12;13]. These studies were done in an older population than our study. The individuals participating in these studies already had CHD whereas in the Young Finns Study in 2001 the participants were considered overall healthy. It is possible that associations between HRV and cIMT cannot be found until a marked progression of CHD, even in large samples of individuals. Similar findings were found by Eller et al when they studied associations with HRV and cIMT, in 78 healthy subjects aged 34 to 64 years [228]. HRV was negatively associated with cIMT but these associations were not statistically significant. Fakhrzadeh et al found that cIMT was associated with decreased TP variability (analysed from 10-minute ECG recording), in both diabetic and non-diabetic individuals aged between 30 and 65 years [229]. This association was independent of conventional risk factors such as hypertension, BMI, and dyslipidemia, but after further adjustment for age and sex, the association became non-significant. Gottsäter et al studied relation between HRV and progression of cIMT in 39 male type-2-diabetics for median age of 59 years during three year follow-up [230]. They observed significant inverse correlation between LF variability and cIMT at baseline. Furthermore, the baseline LF variability inversely correlated with the increase in cIMT during the follow-up. Based on moderately mixed results on literature concerning early atherosclerosis and HRV patterns in healthy young individuals, it seems that HRV examination cannot reliably discover early atherosclerosis. Nevertheless, ANS failure (increased sympathetic and decreased parasympathetic control), estimated with HRV analysis, may be related to the progression of atherosclerosis [1;12;53;227;230]. This may be associated to the suppressed prevention of local inflammation by the inflammatory reflex, i.e. the reflex where efferent vagal activity leads to inhibition of the release of peripheral cytokines, in which normal ANS function may have an important role [72].

It has been hypothesised for long that a decrease in arterial elasticity, which also occurs by aging and in the presence of cardiometabolic risk factors, may partly explain the decline in cardiac vagal function. Randall et al hypothesised already in the end of 1970's that stiffer vessels might cause less firing for cardiovascular ANS through the diminished activation of baroreceptors [139]. The elasticity of an artery can be estimated by measuring its distensibility. In the Young Finns study, we found that HF variability and TP variability of HRV were associated with carotid artery distensibility (Cdist) independently of cardiometabolic risk factors. Observed associations remained significant after further adjustments with carotid wall thickness (intima-media thickness) suggesting arterial distensibility itself may be involved in regulating vagal cardiac control. A similar finding was seen in the Stockholm Diabetes

Intervention Study, where 59 adults with type 1 diabetes were studied. They found that all spectral HRV variables correlated with the arterial wall stiffness of the right common carotid artery and the highest *r*-value was for the correlation with HF variability [147]. Our observations are in line with the idea that a reduction in carotid artery wall elastic properties may interfere with baroreceptor function that could lead to diminished neuronal discharges of vagal nerves. If decreased HRV reflects an attenuated baroreceptors function partly due to stiff arteries, the association between decreased HRV and increased mortality found in studies of post-infarction patients could be due to the fact that the patients with decreased HRV had a more advanced atherosclerotic disease. This hypothesis is supported by the finding that decreased HRV seem to be associated with the severity of coronary atherosclerosis [13]. The relation with baroreceptors and arterial elasticity is supported by the reports from small scale clinical studies. Bonyhay et al. observed a statistically significant correlation between baroreflex sensitivity, measured during phenylephrine infusion, and ultrasonographically measured carotid artery elasticity in 19 healthy subjects [142]. Similarly, Monahan et al. observed a significant correlation between baroreflex sensitivity and arterial compliance in 47 healthy men [143]. Kaushal and Taylor examined the relations between mechanostructural and neural components of baroreflex function in 24 subjects, including young and older volunteers, and observed that a decrease in carotid elasticity explained reduced baroreflex function with age [144]. In the study of Kaushal and Taylor, the association between decreased carotid elasticity and decreased vagal control was non-significant indicating that vascular structural changes may not directly affect resting vagal tone. Previously associations of arterial elasticity with baroreflex sensitivity has been published in a population-based Rotterdam Study by Mattace-Raso et al [231]. They observed an independent association between carotid distensibility and baroreflex sensitivity in older adults (mean age 72 years). Another, maybe more likely explanation is that carotid artery stiffness could be closely related to the function of the autonomic nervous system via the whole negative feed-back control loop.

The arterial elasticity may be involved with the relation between cardiometabolic risk factors and HRV patterns, because the risk factors correlated with the HF and TP variability among individuals with less elastic arteries but not in individuals with more elastic arteries (age- and sex-specific 10^{th} and 90^{th} percentiles for distensibility). One explanation for this may be that carotid arterial elasticity lies in the causal pathway between risk factors and HRV. Thus, in the individuals who are prone to develop reduced carotid elasticity when exposed to risk factors (e.g. genetic propensity for atherosclerosis), the loss of elasticity may also influence the function of the negative feed-back control loop and hence HF and TP variability of HRV. On the other hand, in individuals who have risk factors but are not sensitive to their harmful effects on elasticity this association between risk factors HRV patterns cannot be observed. Together these findings support the hypothesis of the physiological relation between diminished elasticity and related lesser neuronal discharging of the vagal nerves. Further studies on this subject are needed.

In the Young Finns population we were unable to observe significant association between HRV and endothelial function, evaluated with brachial artery endothelium-dependent flow-mediated dilatation (FMD). The hypothesis introduced by Harris and Matthews that the endothelium and ANS work together to generate vascular tone balance (see above), is supported only by small scale studies [150;152-154]. It is more probable that both ANS and endothelial dysfunction are independent early signs of cardiovascular disease rather than linked to each other. Additionally, there are indirect routes to link ANS control and endothelial function together, such as angiotensin II and inflammation [153]. Further studies on this subject are needed.

6.6. Strengths and limitations

An important strength of the current thesis is the large population-based sample of individuals. Physical, laboratory, heart rate variability and ultrasound examinations were carried out using well-established methods in a standardised manner. Our study design was cross-sectional and further longitudinal studies may be conducted to confirm and explore the effect of age on HRV data. HRV variables were analysed from 3-minute ECG-signal on average which is less than recommended 5-minute by the Task Force. The recording should last 3 to 10 times the wavelength of the lowest frequency bound of the component investigated [35;232]. Due to time restriction in our population study, we collected 3-minute ECG recordings. However, we could demonstrate that these gave practically identical results compared to 5-minute recordings. The standard cut-off points were used for frequency-domain HRV components [35]. Results presented here may considered essentially similar when values mathematically created according to the length of recording would be used [232]. All ECG recordings and HRV spectra were visually revised by the single operator to assess requirements of signal stationarity. To reduce the potential effect of different respiratory frequencies we used metronome controlled breathing during ECG-recording. This thesis was based on a cross-sectional study and thus is unable to sort out the temporal relations between arterial distensibility, CVD risk factors and MetS to HRV.

Because of metronome-controlled breathing, the recordings showed a stationary respiratory sinus arrhythmia. The analysis of HRV and estimation of vagal cardiac control may be considered reliable, but the effect of the sympathetic nervous system cannot be reliably estimated in resting HRV analysis. In these resting supine conditions with metronome controlled breathing, the LF variability is created by the effect of both sympathetic and vagal cardiac control, and thus cannot be solely used as a marker of sympathetic cardiac ANS control.

When associations between vascular properties and HRV were studied, the pulse pressure used in the equations to calculate carotid distensibility was measured from the brachial artery. It would be more ideal to study the pulse pressure from the artery in question,

because the use of brachial pressures overestimates pulse pressure in central arteries [233]. However, the difference between central and peripheral pulse pressure is likely to be similar between study subjects within a narrow age range, as in our study. Borow and Newburger have shown an excellent correlation of r=0.98 between systolic blood pressures and r=0.97 between diastolic pressures measured invasively from ascending aorta and non-invasively from brachial artery [234].

6.7. Future perspectives

The autonomic nervous system is a net of connections with multiple effects on homeostasis, metabolism and hemodynamics. The cardiac control via autonomic nervous system can be evaluated non-invasively and, in stable conditions, with minimal repeat variability with HRV analysis. The associations between HRV patterns and multiple risk factors are observed because of the widespread effects of ANS. Although several studies have reported the clinical and prognostic value of HRV analysis in the assessment of patients with cardiometabolic diseases (i.e. after myocardial infarction and as an early diagnostic tool for diabetic neuropathy), this technique has not been widely incorporated into clinical practice. This might be due to the fact that HRV patterns are influenced by multiple factors and in clinical practice the conditions can rarely be standardised. In addition, interpretation problems remain which are related to the quality control of recordings and to the intrinsic complexity of regional pathologies which complicates their prediction, control and interpretation. The analysis of HRV is not always feasible in subjects using certain medication (e.g. beta-blockers or ACE-inhibitors) or the presence of cardiac arrhythmias. Furthermore, despite a number of analytical systems, evaluation of HRV for each individual subject may still require proof-reading and manual elimination of artefacts. The development of more simple techniques for on-line computing of HRV components is needed.

Before introducing HRV analysis in everyday clinical use, further research is mandatory, including prospective, randomized studies focusing on the clinical utility of HRV analysis as a means of assessing cardiometabolic risk both in primary prevention and adequacy of therapy in secondary prevention. The mechanisms which underlie HRV are not fully understood, i.e. the peripheral mechanism mediating HRV at different frequency components and the physiological components especially in LF variability. In research, the analysis of HRV provides interesting physiological information on body ANS function related to disease, recovery and prognosis. An attractive area of research is the use of HRV analysis to further explore the role of ANS alterations in disease mechanisms and possibly to study the actions of specific medications on ANS in pharmacological studies. HRV analysis offers also usable tool for physiological impact studies, where baseline cardiovascular signal(s) are interfered with standardised manoeuvres.

7. CONCLUSIONS

1. Decreased HRV is associated with age, male gender and increased heart rate. Women have higher HF variability and lower LF variability than men. Women also have a higher resting heart rate. Reproducibility of HRV variables and deep breathing test are considered satisfactory and short-term analysis of HRV and deep breathing tests are feasible for clinical work. Furthermore there was no significant office-hour variation within these measurements.

2. MetS is associated with decreased HRV in young adults. In women, the MetS seems to carry a more pronounced risk for decreased vagal cardiac control and a possible increase in sympathetic predominance of the ANS than in men. The individual components of MetS are differentially associated with HRV in young men and women.

3. Vagal control, assessed via HF variability, and total HRV were independently associated with carotid elasticity but not with carotid artery intima-media thickness and not with brachial artery endothelium-dependent flow-mediated dilatation. This may indicate that decreased carotid elasticity and cardiovascular ANS control are related. Furthermore, carotid distensibility seemed to modify the relation between risk factors and HRV.

ACKNOWLEDGEMENTS

This study was carried out in the Department of Clinical Physiology and Isotope Medicine University of Turku, and in the Research Centre of Applied and Preventive Cardiovascular Medicine University of Turku, Finland during the years 2001-2014.

I owe my deepest gratitude to my supervisor Professor Olli Raitakari who has believed in me and my research for several years. I have been privileged to be able to closely follow his successful and enthusiastic work within the Cardiovascular Risk in Young Finns Study. The understanding of life events, the patience and support I have been experienced by Professor Raitakari during this project has made this work possible.

I am especially grateful to Professor emeritus Ilkka Välimäki who taught me in cardiovascular physiology with great experience and enthusiasm and helped me to understand the measurement methods of heart rate variability. Professor emeritus Välimäki reviewed this thesis with great patience and his valuable critical and supporting comments made this thesis a lot better.

I express my sincere gratitude to Professor emeritus Jorma Viikari, who is one of the founders of the Cardiovascular Risk in Young Finns study. The passion and devotion of Professor emeritus Viikari has made it possible for several young researchers to be introduced to scientific work, including me. During the decades the Cardiovascular Risk in Young Finns study has developed as a unique study world wide and it has been honour to be a member of this project.

I sincerely want to thank the reviewers of my thesis, Professor emeritus Martti Hakumäki and Docent Arja Uusitalo.

The atmosphere in the Research Centre of Applied and Preventive Cardiovascular Medicine has been warm and friendly for all these years. I have always felt myself welcome during my visits in CAPC despide living elsewhere for many years. I want to especially thank my co-researchers Markus Juonala, Noora Mattsson, Liisa Saarikoski and Kristiina Pälve for the fun moments throughout the years. I deeply thank Irina Lisinen for helping me with the statistical analyses. I also wish to thank Nina Ruotsalainen, Anni Pakarinen, Nina Aalto, Marja Piippo and Ville Aalto for their help. All the people in CAPC have been able to create warm and supporting atmosphere for this work, and I express my greatest gratitude for this.

I warmly thank my co-authors: Tapani Rönnemaa, Mika Kähönen, Terho Lehtimäki, Tomi Laitinen, Liisa Keltikangas-Järvinen and Antti Jula for their help and comments during these years.

I also wish to express my gratitude to the volunteer study participants who made this study possible.

I want to express special thanks to my family. I want to thank my parents Eija and Seppo for their support throughout my life. Finally, I want to express my deepest gratitude to my wife Suvi, for her patience and neverending support during these years. Without her help many things including this thesis would not been possible. I especially want to thank her for the love and care she has given to our lovely children Lauri, Lotta and Iida, and also to me.

This work was financially supported by the Academy of Finland, the Finnish Cultural Foundation, the Research Foundation of Turku University, the Paolo Foundation, the Research Foundation of Orion Corporation, the Lydia Maria Julin Foundation and the Juho Vainio Foundation.

Pori, August 2014

Tuomas Koskinen

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