Jaana Karjalainen

CARDIOVASCULAR AUTONOMIC FUNCTION IN CORONARY ARTERY DISEASE PATIENTS WITH AND WITHOUT TYPE 2 DIABETES

SIGNIFICANCE OF PHYSICAL ACTIVITY AND EXERCISE CAPACITY
JAANA KARJALAINEN

CARDIOVASCULAR AUTONOMIC FUNCTION IN CORONARY ARTERY DISEASE PATIENTS WITH AND WITHOUT TYPE 2 DIABETES
Significance of physical activity and exercise capacity

Academic dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 8 of Oulu University Hospital, on 13 December 2013, at 12 noon

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Abstract
Coronary artery disease (CAD) and type 2 diabetes (T2D) are associated with cardiovascular autonomic dysfunction, which is widely used as a predictor of mortality in cardiovascular diseases. The determinants of autonomic dysfunction in CAD patients with or without T2D are not well known. The aim of this thesis was to examine the determinants of cardiovascular autonomic function in healthy subjects and CAD patients with and without T2D. A second aim was to study the prognostic value of autonomic function in the patient population. A third aim was to study the effects of exercise prescriptions on physical activity and exercise capacity in the patient groups.

The association between heart rate (HR) variability and physical activity was examined by means of 24-hour recordings in 45 healthy adults. The determinants and prognostic value of autonomic function, measured by HR recovery, HR variability, and HR turbulence, were assessed in 1060 CAD patients (50% were patients with T2D). Physical activity was measured before and after a six-month exercise prescription in 44 CAD patients without T2D and 39 CAD patients with T2D.

In healthy patients, short-term HR variability indexes and the complexity properties of HR were influenced by physical activity, whereas long-term HR variability indexes remained relatively stable at various activity levels, making them robust indexes for assessment of autonomic function during ambulatory conditions. In CAD patients, exercise capacity was the most important determinant of autonomic function in addition to physical activity, age, presence of T2D, and left ventricular systolic function. During a 2-year follow-up, autonomic dysfunction predicted cardiovascular events only in CAD patients with T2D, but did not provide independent prognostic information after multivariate adjustment when high-sensitivity C-reactive protein, a marker of inflammation, remained as an independent predictor. CAD patients with T2D were physically less active than patients without T2D. Exercise prescription promoted a more active lifestyle and improved exercise capacity in both patient groups.

In conclusion, cardiovascular autonomic dysfunction in CAD patients with and without T2D is closely related to low exercise capacity and physical activity, which both can be increased by exercise prescriptions. Autonomic dysfunction predicts short-term cardiovascular events only in CAD patients with T2D, but is not as strong an independent predictor as low-grade inflammation.

Keywords: autonomic nervous system, coronary artery disease, exercise capacity, heart rate variability, physical activity, type 2 diabetes
Karjalainen, Jaana, Sydämen autonominen säätely sepelvaltimotautipotilailla ja sepelvaltimotautipotilailla, jotka sairastavat typpin 2 diabetesta. Fyysisen aktiivisuuden ja suorituskynyn merkitys
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta, Kliinisen lääketieteen laitos, Sisätaudit; Oulun yliopistollinen sairaala; Verve, Liikuntalääketieteen tutkimusyksikkö
Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

Tiivistelmä
Autonomisen hermoston toiminnan häiriö on yleinen komplikaatio sepelvaltimotaudissa ja typpin 2 diabeteksessa (T2D), ja sen tiedetään olevan itsenäinen kuolleisuutta ennustava tekijä. Autonomista säätelyä selittäviä tekijöitä ei kuitenkaan tunnettu hyvin. Tässä tutkimuksessa selvitettiin autonomisen hermoston toimintaa selittäviä tekijöitä kolmessa ryhmässä: osa tutkittavista oli terveitä, osalla oli sepelvaltimotauti ja T2D, ja osalla pelkästään sepelvaltimotauti. Lisäksi tutkittiin, miten autonominen säätely vaikuttaa sepelvaltimotautipotilaiden ennusteeseen sekä miten liikuntaohjelma vaikuttaa heidän fyysiseen aktiivisuuteensa ja suorituskynynsä.

Sykevaihtelun ja fyysisen aktiivisuuden välistä yhteyttä selvittyi 45 terveellä henkilöllä. Autonomisen hermoston toimintaa selittäviä tekijöitä ja sen ennustearvoa tutkittiin 1060 sepelvaltimotautipotilaalta, joista puolet sairasteli T2D:ta. Näistä potilaista valittiin satunnaisotannalla kuuden kuukauden liikuntaohjelmaan ja fyysisen aktiivisuuden mittauksiin 44 sepelvaltimotautipotilaasta, joilla ei ollut T2D:ta, ja 39 potilaasta, jotka sairastivat T2D:ta.


Tämän tutkimuksen tulokset osoittavat, että sepelvaltimotautipotilailla autonomisen hermoston toiminnan häiriö on yhteydessä vähäiseen fyysiseen aktiivisuuteen ja heikkoon fyysiseen kuntoon. Molempin tekijöihin voidaan vaikuttaa positiivisesti liikuntaohjelmalta. Poikkeava autonominen säätemysti ennustaa lyhyen aikavälin sydän- ja verisuonitautitapahtumia vain T2D:ta sairastavilla sepelvaltimotautipotilailla. Se ei kuitenkaan ole yhtä vahva itsenäinen ennustaja kuin tulehdusta kuvaava herkkä CRP.

Asiasanat: autonominen hermosto, fyysinen aktiivisuus, sepelvaltimotauti, suorituskyky, sykevaihtelu, typpin 2 diabetes
Acknowledgements

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Above all, I owe my most loving thanks to my dear husband Jouko for his endless love and support. Jouko, thank you for always listening to me and providing encouragement. You believed in me even in times when I did not believe in myself. I warmly thank our son Ville for providing joy into my life and making me laugh every day. You two mean the world to me.

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Oulu, October, 2013

Jaana Karjalainen
<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>α1</td>
<td>short-term scaling exponent of fractal-like correlation properties</td>
</tr>
<tr>
<td>AMI</td>
<td>acute myocardial infarction</td>
</tr>
<tr>
<td>ANS</td>
<td>autonomic nervous system</td>
</tr>
<tr>
<td>ApEn</td>
<td>approximate entropy</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>CI</td>
<td>confidence intervals</td>
</tr>
<tr>
<td>DFA</td>
<td>detrended fluctuation analysis</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiography</td>
</tr>
<tr>
<td>HbA1c</td>
<td>glycated hemoglobin</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>HF</td>
<td>high frequency</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>high-sensitivity C-reactive protein</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>LF</td>
<td>low frequency</td>
</tr>
<tr>
<td>LVEF</td>
<td>left ventricular ejection fraction</td>
</tr>
<tr>
<td>LVM</td>
<td>left ventricular mass</td>
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<tr>
<td>MET</td>
<td>metabolic equivalent</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>R-R</td>
<td>R-peak-to-R-peak interval</td>
</tr>
<tr>
<td>SampEn</td>
<td>sample entropy</td>
</tr>
<tr>
<td>SD1</td>
<td>beat-to-beat R-R interval fluctuation</td>
</tr>
<tr>
<td>SD2</td>
<td>long-term R-R interval fluctuation</td>
</tr>
<tr>
<td>SDANN</td>
<td>standard deviation of the average R-R intervals of analyzed segments</td>
</tr>
<tr>
<td>SDNN</td>
<td>standard deviations of all normal-to-normal R-R intervals</td>
</tr>
<tr>
<td>T2D</td>
<td>type 2 diabetes</td>
</tr>
<tr>
<td>TRIMP</td>
<td>training impulse</td>
</tr>
<tr>
<td>ULF</td>
<td>ultralow frequency</td>
</tr>
<tr>
<td>VLF</td>
<td>very low frequency</td>
</tr>
<tr>
<td>VPC</td>
<td>ventricular premature complex</td>
</tr>
</tbody>
</table>
List of original publications

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


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1 Introduction

Coronary artery disease (CAD) and type 2 diabetes (T2D) are leading health problems and causes of mortality throughout the world. In 2010, 8.3 million people died because of these diseases, which means one in six deaths worldwide. The prevalence of CAD and T2D has risen greatly in the last decades, partly because of physical inactivity and obesity, which characterize lifestyles especially in developing countries (Lozano et al. 2012).

Cardiovascular autonomic dysfunction, which is known to be associated with non-fatal and fatal cardiovascular events, is a common finding among patients with CAD (Bigger et al. 1995, Huikuri & Mäkikallio 2001) and T2D (Vinik & Ziegler 2007, Schönauer et al. 2008). It has been suggested that autonomic dysfunction among CAD patients may be explained by angiographic severity, the severity of left ventricular dysfunction, and inflammation (Hayano et al. 1990, Nolan et al. 1992, Ghaffari et al. 2011, von Kanel et al. 2011), whereas in patients with T2D, autonomic dysfunction may be related to poor glycemic control and duration of diabetes (Vinik & Ziegler 2007). However, patients with CAD and T2D typically have many shared complications, e.g., obesity and hyperlipidemia, which can be considered to be risk factors of clinical disease rather than its consequences and which are known to effect autonomic function in the general population (Kimura et al. 2006, Thayer & Fischer 2011, Poliakova et al. 2012).

Physical inactivity and low physical fitness are common among patients with CAD and T2D, despite the well-known health benefits of regular physical activity and exercise training (Wofford et al. 2007, Zhao et al. 2011). On the basis of previous studies we know that long-term physical activity and physical fitness have a positive effect on cardiovascular autonomic function, especially in the healthy population. Individuals with a high total level of physical activity and a higher fitness level have improved autonomic function compared with those with an inactive lifestyle and low physical fitness (Buchheit et al. 2005, Buchheit & Gindre 2006). However, the roles of physical inactivity, low exercise capacity, and other traditional cardiac risk factors in autonomic dysfunction in CAD patients with or without T2D are not well known.

The aim of this thesis was to provide new information about the determinants of cardiovascular autonomic function in healthy and CAD patients with and without T2D. Another aim of this thesis was to investigate the prognostic value of autonomic function and the effects of exercise prescription on physical activity and exercise capacity in the above-mentioned patient groups.
2 Review of the literature

2.1 Cardiovascular autonomic function

2.1.1 Physiological background of cardiovascular autonomic function

The autonomic nervous system (ANS) with neurochemical factors is responsible for regulating cardiovascular function by modulating the electrical and contractile activity of the myocardium and the internal opening of blood vessels. Complex mechanisms constantly cooperate to maintain a dynamic balance between systemic blood pressure and blood flow to tissues under different environmental influences. The ANS modulates heart rate (HR), cardiac output, and blood pressure through the interaction of the sympathetic and parasympathetic (vagal) pathways of the ANS. These two branches of the ANS operate in parallel, but differ from each other because of differences in their anatomical structure, neurotransmitter system, and physiological actions (McArdle et al. 2001).

The parasympathetic and sympathetic branches have opposite effects on regulation of the cardiovascular system. Parasympathetic modulation is dominant during rest by decreasing HR via acetylcholine released from efferent vagal nerve discharge. In contrast, sympathetic activation takes over the control of HR and myocardial contractility during the day or during exercise via release of epinephrine and norepinephrine. The sympathetic nerves innervate the entire heart including the atrial and ventricular myocardium, whereas parasympathetic fibers mainly innervate the atrial muscle (McArdle et al. 2001). However, it is suggested that the vagal nerves may innervate the ventricular myocardium, as well (Standish et al. 1994, Johnson et al. 2004).

Appropriate cardiovascular regulation is dependent on continuous feedback from peripheral receptors in the blood vessels and muscles. The wall of the aortic arch and the carotid sinuses contain pressure-sensitive baroreceptors which sense changes in arterial pressure. Stimulation of the baroreceptors because of increased blood pressure causes an increase in cardiac vagal outflow, which results in a reduction in HR and cardiac contractility and, thus, a drop in the blood pressure level. On the contrary, a decrease in blood pressure leads to an increase in HR and cardiac contractility via decreased activity of the vagus nerve. In addition, chemoreceptors and mechanoreceptors within the muscles monitor its chemical
and physical state. Activation of these sensory nerves modifies either sympathetic or parasympathetic outflow to achieve appropriate cardiovascular responses to various demands (McArdle et al. 2001).

The interplay between sympathetic and parasympathetic activations causes continuous beat-to-beat R-R interval fluctuations in HR, which is called HR variability (Levy & Martin 1979). HR variability has been used as a tool for investigating the cardiovascular control system and the balance between sympathetic and parasympathetic regulation (Task Force of ESC and NASPE 1996).

2.1.2 Analysis of cardiovascular autonomic function

Analyses of HR variability have become a widely used method for assessing cardiovascular autonomic function. The HR variability signal can be obtained from short-term electrocardiograph (ECG) measurements in laboratory conditions with different physiological autonomic tests or from long-term ambulatory ECG recordings during uncontrolled daily activities. HR variability can be analyzed by different methods which are categorized as time and frequency domain analyses and nonlinear analyses (Task Force of ESC and NASPE 1996). HR turbulence and HR recovery are also commonly used analyses of cardiovascular autonomic function (Bauer et al. 2008, Okutucu et al. 2011).

Time domain analyses of heart rate variability

The current view is that time domain methods, which measure the overall magnitude of HR fluctuation, are the simplest HR variability analysis, but they tend to provide less detailed information. The easiest and most widely used time domain variables are average HR and standard deviation of normal-to-normal R-R intervals (SDNN). SDNN reflects all the cyclic components responsible for variability in the period of recording. Variation in the SDNN is the standard deviation of the average normal-to-normal interval (SDANN) calculated over short periods of the entire recording, usually around five minutes (Task Force of ESC and NASPE 1996).
**Frequency domain analyses of heart rate variability**

Frequency domain analysis of HR variability measures the magnitude of HR fluctuation at some predetermined frequencies (Malliani *et al.* 1991). Methods based on nonparametric fast Fourier transformation and parametric autoregressive analysis are the most commonly used frequency domain methods which transform signals to the power spectrum. The power spectrum of HR variability contains three major spectral components: a high frequency (HF) component with a frequency rate between 0.15 and 0.4 Hz, a low frequency (LF) component with a frequency rate between 0.04 and 0.15 Hz, and a very low frequency (VFL) component with a frequency rate between 0.003 and 0.04 Hz (Task Force of ESC and NASPE 1996).

The HF component is known to be synchronous with respiration and it predominantly represents parasympathetic activation. It has been suggested that the LF component is affected by the mechanism that regulates blood pressure and it reflects both parasympathetic and sympathetic activation (Kamath & Fallen 1993). The ratio between LF and HF components has been used as an index of sympathovagal balance (Malliani *et al.* 1991). The physiological explanation for the VLF component is less understood, but it may be affected by thermoregulatory factors (Kitney & Rompelman 1977, Fleisher *et al.* 1996), the hormones of the renin-angiotensin system (Akselrod *et al.* 1981, Bonaduce *et al.* 1994), and physical activity (Bernardi *et al.* 1996).

In addition to the above-mentioned three main frequency components, the ultralow frequency (ULF) component with a frequency rate < 0.003 Hz has been included in frequency domain analyses (Task Force of ESC and NASPE 1996). Similar to VLF, the physiological background of the ULF component is not well known, but it is speculated that it is related to the circadian rhythm (Roach *et al.* 1998) as well as to physical activity (Serrador *et al.* 1999). The ULF component can be obtained only from long-term recordings (24 hours), whereas HF, LF, and VLF can be identified also in short-term recordings lasting from two to five minutes (Task Force of ESC and NASPE 1996).

**Nonlinear analyses of heart rate variability**

Analysis of HR variability based on nonlinear dynamics has become more and more common during the last couple of decades and has opened up a new approach for understanding the features of HR variability. Nonlinear analysis of
HR variability differs from traditional methods because these analyses typically detect qualitative rather than quantitative properties of HR variability and provide an estimate of the complexity of HR variability (Pincus & Goldberger 1994, Peng et al. 1995).

Several different nonlinear methods have been developed for evaluating nonlinear fluctuation of HR. Detrended fluctuation analysis (DFA) is a method which quantifies the presence or absence of fractal correlation properties in the R-R interval time series (Peng et al. 1995). With DFA it is possible to scale the long-term autocorrelation of non-stationary HR signals and quantify the complexity of signals by using the fractal property. Details of the fractal scaling method have been previously described by Peng et al. (1995). These fluctuations can be characterized by a scaling exponent α, which represents the autocorrelation properties of the signal. Scaling exponent values near 1.0 have been seen in healthy subjects, indicating fractal-like HR behavior, whereas altered fractal-like behavior has been reported in patients with cardiovascular diseases and with advancing age (Mäkikallio et al. 1999, Huikuri et al. 2000, Goldberger et al. 2002).

Other commonly used nonlinear measures are approximate entropy (ApEn) and sample entropy (SampEn), which both measure the amount of periodicity, regularity, and complexity of time series data. Larger values of ApEn and SampEn indicate high unpredictability in the time series, whereas a time series with high regularity provides smaller values (Pincus & Goldberger 1994, Richman & Moorman 2000). The Poincaré plot is also a useful method for visualizing the regularities or randomness that may occur in beat-to-beat HR variability. The Poincaré plot is a method in which each R-R interval is presented in the function of the preceding R-R interval (Huikuri et al. 1996, Brennan et al. 2001). The standard descriptors of the Poincaré plot are SD1 and SD2. SD1 measures the magnitude of vagally mediated instantaneous beat-to-beat HR variability, and SD2 measures the magnitude of continuous long-term R-R interval fluctuation (Huikuri et al. 1996, Tulppo et al. 1996, Brennan et al. 2001).

**Heart rate turbulence**

HR turbulence describes short-term fluctuations in sinus rhythm following spontaneous ventricular premature complexes (VPCs) (Schmidt et al. 1999). It consists of brief HR acceleration followed by more gradual HR deceleration before the rate returns to the baseline level. The initial HR acceleration is caused
by temporary vagal inhibition in response to a missed baroreflex afferent input triggered by hemodynamically incompetent ventricular contraction. A sympathetically mediated overshoot of arterial pressure is responsible for the following HR deceleration through vagal recruitment. Overall, the HR turbulence measurement provides an indirect assessment of baroreflex sensitivity (Bauer et al. 2008).

The early HR acceleration can be quantified by turbulence onset, which is defined as the difference between the mean of the first two sinus R-R intervals after a VPC and the last two sinus R-R intervals before the VPC divided by the mean of the last two sinus R-R intervals before the VPC. The value of turbulence onset is expressed as a percentage. The late deceleration of HR is quantified by the turbulence slope, which is defined as the maximum positive slope of a regression line assessed over any sequence of five subsequent sinus rhythm R-R intervals within the first 15 sinus rhythm intervals after the VPC (Schmidt et al. 1999, Bauer et al. 2008).

Heart rate recovery

Measures of HR after cessation of exercise are easy to obtain and are among the most commonly used techniques in evaluating cardiovascular autonomic function and the risk of mortality (Cole et al. 1999, Lauer & Froelicher 2002, Nissinen et al. 2003, Jouven et al. 2005, Tulppo et al. 2011). HR recovery is a combination of sympathetic withdrawal and parasympathetic reactivation. The decline of HR within the first minute after cessation of exercise is caused mainly by parasympathetic reactivation—sympathetic withdrawal being more prominent two minutes after exercise. Therefore, HR recovery immediately after exercise can be considered to reflect short-term vagal reactivity (Okutucu et al. 2011).

HR recovery is defined as the reduction in HR from the maximal rate during exercise to the rate during the recovery phase (Okutucu et al. 2011). HR recovery values during the first and second minutes after cessation of exercise have been the most commonly used prognostic measurements (Cole et al. 1999, Nishime et al. 2000, Shetler et al. 2001, Watanabe et al. 2001, Mora et al. 2003). However, decreased HR recovery even 5 min after exercise has been also shown to have a prognostic value independent of traditional cardiovascular risk factors (Cheng et al. 2003).
2.1.3 Prognostic value of cardiovascular autonomic function

There is strong evidence that altered cardiovascular autonomic function, measured by decreased HR recovery, HR variability, or HR turbulence, is a prognostic indicator for cardiovascular events, as well as for cardiac and all-cause mortality. Altered autonomic regulation indicates a poor prognosis especially in survivors of acute myocardial infarction (AMI), but also in patients with CAD or T2D and in the general population (Kleiger et al. 1987, Bigger et al. 1992b, Cole et al. 1999, Schmidt et al. 1999, Watanabe et al. 2001, Barthel et al. 2003, Cheng et al. 2003, Nissinen et al. 2003, Jouven et al. 2005, Mäkikallio et al. 2005, Chacko et al. 2008, Georgoulias et al. 2009, Huikuri et al. 2009, Huikuri et al. 2010, Barthel et al. 2011, Gayda et al. 2012). Table 1 summarizes the results of the main studies that have assessed the prognostic value of cardiovascular autonomic function measured by HR variability, HR turbulence, and HR recovery in various study populations.

Patients recovering from an AMI and with low values of HR variability or abnormal HR turbulence are two to seven times more likely to die prematurely than patients with high values of HR variability or normal HR turbulence (Kleiger et al. 1987, Bigger et al. 1992b, Schmidt et al. 1999, Huikuri et al. 2000, Barthel et al. 2003, Mäkikallio et al. 2005). Similarly, among the healthy population and patients with T2D, blunted HR recovery even after submaximal exercise is associated with a two to four times higher risk of cardiovascular and all-cause mortality (Cole et al. 1999, Cole et al. 2000, Watanabe et al. 2001, Cheng et al. 2003, Jouven et al. 2005). In most of the studies, the negative predictive value of decreased HR variability and HR recovery is independent of other well-known prognostic indicators and cardiac risk markers, e.g., low left ventricular ejection fraction (LVEF), history of cardiovascular diseases, presence of T2D, or low cardiorespiratory fitness (Kleiger et al. 1987, Bigger et al. 1992b, Cole et al. 1999, Schmidt et al. 1999, Watanabe et al. 2001, Cheng et al. 2003, Mäkikallio et al. 2005, Chacko et al. 2008, Georgoulias et al. 2009, Huikuri et al. 2009).

The studies that assessed the prognostic power of autonomic function have widely and diversely used various HR variability indexes and HR turbulence as measurements of cardiovascular autonomic regulation. Early studies mainly focused on measurement of time and frequency domain measures of HR variability, whereas more recent studies have used methods based on HR turbulence and the non-linear dynamic of R-R intervals (Huikuri et al. 1999, Billman 2011). Although all indexes of HR variability describe partly different
aspects of autonomic regulation and have different analysis methods, there are only slight differences in prognostic power between them. However, few studies have indicated that HR turbulence and some non-linear indexes are somewhat better predictors of cardiovascular events than other HR variability indexes. Mäkikallio et al. (2005) showed that SDNN, a traditional measure of global HR variability, failed to provide independent prognostic information about sudden cardiac death among survivors of an AMI, whereas HR turbulence has a strong predictive value. Moreover, a reduced short-term fractal scaling exponent $\alpha_1$ has been shown to be a more powerful predictor of all-cause mortality and recurrent nonfatal coronary events than other time and frequency domain measures of HR variability in post-AMI patients (Huikuri et al. 2000, Perkiömäki et al. 2008).

### 2.2 Coronary artery disease

CAD is a complex disease which is believed to be caused by lifestyle factors, genetic factors, as well as interactions among these factors. The generally accepted, traditional risk factors for CAD are older age, male sex, smoking, hypertension, hyperlipidemia, presence of T2D, obesity, systemic inflammation, and physical inactivity (Lusis 2000, Huikuri et al. 2001, Roger et al. 2011). Only a minority of the genetic variants associating with clinical CAD seems to act through traditional risk factors, while most variants appear to act through mechanisms that are independent of other risk factors (Schunkert et al. 2011). Since traditional risk factors together with genetic components do not explain all of the risk for CAD, there are some novel risk factors, such as coronary artery calcium scores, ankle-brachial index, and inflammation marker high-sensitivity C-reactive protein (hs-CRP), which have the potential to improve risk assessment for CAD among asymptomatic persons (U.S. Preventive Services Task Force 2009, Yeboah et al. 2012).
Table 1. Summary of the main studies that have assessed the prognostic value of cardiovascular autonomic function in various populations.

<table>
<thead>
<tr>
<th>Study</th>
<th>Measurement of autonomic function</th>
<th>Subjects</th>
<th>Mean follow-up time</th>
<th>Primary endpoint</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kleiger et al. 1987</td>
<td>HRV</td>
<td>Post-AMI patients, n = 808</td>
<td>34 months</td>
<td>All-cause mortality</td>
<td>5.3 times higher mortality risk if SDNN &lt; 50 ms</td>
</tr>
<tr>
<td>Bigger et al. 1992</td>
<td>HRV</td>
<td>Post-AMI patients, n = 715</td>
<td>2.5 years</td>
<td>All-cause, cardiac, and arrhythmic death</td>
<td>All frequency domain measures, especially VLF power (univariate RR 4.3–4.8), predicted the endpoints</td>
</tr>
<tr>
<td>Cole et al. 1999</td>
<td>HRR</td>
<td>Adults without CVD, n = 2428</td>
<td>6 years</td>
<td>All-cause mortality</td>
<td>Abnormal 1-min HRR was strongly predictive of mortality (univariate RR 4.0)</td>
</tr>
<tr>
<td>Schmidt et al. 1999</td>
<td>HRT</td>
<td>Post-AMI patients, n = 1191</td>
<td>21 months</td>
<td>All-cause mortality</td>
<td>Combination of abnormal HRT slope and onset was the most powerful multivariate risk stratifier (RR 3.2)</td>
</tr>
<tr>
<td>Cole et al. 2000</td>
<td>HRR</td>
<td>Adults without CVD, n = 5234</td>
<td>12 years</td>
<td>All-cause mortality</td>
<td>Abnormal 2-min HRR predicted death even after submaximal exercise (univariate RR 2.6)</td>
</tr>
<tr>
<td>Watanabe et al. 2001</td>
<td>HRR</td>
<td>Adults without CVD, n = 5438</td>
<td>3 years</td>
<td>All-cause mortality</td>
<td>Abnormal 1-min HRR predicted death even in the absence of a cool-down period (univariate hazard ratio 3.9)</td>
</tr>
<tr>
<td>Barthel et al. 2003</td>
<td>HRT</td>
<td>Post-AMI patients, n = 1455</td>
<td>22 months</td>
<td>All-cause mortality</td>
<td>Combination of abnormal HRT slope and onset was the strongest multivariate predictor (hazard ratio 5.9)</td>
</tr>
<tr>
<td>Cheng et al. 2003</td>
<td>HRR</td>
<td>Patients with T2D, n = 2333</td>
<td>14.9 years</td>
<td>All-cause and CV mortality</td>
<td>2.0 times higher mortality risk, if 5-min HRR &lt; 55 bpm vs. HRR &gt; 75 bpm</td>
</tr>
<tr>
<td>Jouven et al. 2005</td>
<td>HRR</td>
<td>Adults without CVD, n = 5713</td>
<td>23 years</td>
<td>Sudden and non-sudden cardiac death</td>
<td>The risk of sudden death was increased if 2-min HRR &lt; 25 bpm (univariate RR 2.2)</td>
</tr>
</tbody>
</table>
In past decades, cardiovascular diseases, including CAD and stroke, have been the leading disease group causing disability, morbidity, and mortality worldwide, especially in developing countries. In 2010, CAD was the top cause of the global burden of disease, measured in disability-adjusted life-years, compared to fourth place in global ranks 20 years earlier (Murray et al. 2012). In 2010, CAD killed 7 million people and stroke killed 5.9 million people, which together means one in four deaths worldwide compared with one in five deaths in 1990. CAD was the leading cause of death globally, and the number of deaths due to CAD has increased by 35% since 1990 (Lozano et al. 2012). It has been predicted that in 2020, CAD will still be the leading contributor to the burden of disease and mortality worldwide (Murray & Lopez 1997).

<table>
<thead>
<tr>
<th>Study</th>
<th>Measurement of autonomic function</th>
<th>Subjects</th>
<th>Mean follow-up time</th>
<th>Primary endpoint</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mäkikallio et al. 2005</td>
<td>HRV and HRT</td>
<td>Post-AMI patients, n = 2130</td>
<td>1012 days</td>
<td>Sudden and non-sudden cardiac death</td>
<td>All HRV measures, especially HRT slope and fractal α1 (univariate hazard ratio 3.4–6.7), predicted sudden and non-sudden cardiac death.</td>
</tr>
<tr>
<td>Chacko et al. 2008</td>
<td>HRR</td>
<td>Patients with T2D, n = 890</td>
<td>CV events, all-cause, and CV mortality</td>
<td>Abnormal 1- and 2-min HRR were significantly associated with endpoints</td>
<td></td>
</tr>
<tr>
<td>Georgoulas et al. 2009</td>
<td>HRR</td>
<td>Patients with T2D, n = 258</td>
<td>CV mortality, non-fatal MI, and late revascularization</td>
<td>Abnormal 1-min HRR was a predictor of both hard (coefficient −3.2) and soft cardiac events (coefficient −3.6)</td>
<td></td>
</tr>
<tr>
<td>Huikuri et al. 2009</td>
<td>HRV</td>
<td>Post-AMI patients, n = 312</td>
<td>2 years Ventricular fibrillation or ventricular tachycardia</td>
<td>Many HRV measures, especially VLF power (adjusted hazard ratio 7.0), predicted the endpoint</td>
<td></td>
</tr>
<tr>
<td>Gayda et al. 2012</td>
<td>HRR</td>
<td>Patients with stable CAD, n = 4097</td>
<td>14.7 years All-cause and CV mortality</td>
<td>3-min HRR &lt; 46 bpm associated with an increased risk of all-cause death (adjusted hazard ratio 1.15)</td>
<td></td>
</tr>
</tbody>
</table>

HRV, heart rate variability; AMI, acute myocardial infarction; SDNN, standard deviation of normal-to-normal R-R intervals; VLF, very low frequency; RR, relative risk; HRR, heart rate recovery; CVD, cardiovascular disease; HRT, heart rate turbulence; T2D, type 2 diabetes; CAD, coronary artery disease.
2.2.1 Pathogenesis of coronary artery disease

The development of CAD is thought to begin from minimal structural changes and micro-injuries in the arterial endothelium, which are perhaps caused by hypertension, inflammation, elevated cholesterol, smoking, free radicals, or differences in blood flow dynamics in, e.g., the bending points of arteries and areas near branching vessels. Damage in the inner wall of an artery and accumulation of low-density lipoprotein (LDL) activate a process called atherosclerosis, in which lipids, platelets, and macrophages aggregate on the artery wall, leading to development of fibrotic or lipid plaque. The plaque narrows the arteries and decreases blood flow to the heart muscles, causing ischemia and angina pectoris, the typical symptoms of CAD. In some cases, the surface of plaque can rupture, causing platelets to clump and blood to clot. This process obstructs blood flow completely and causes an AMI (Fuster et al. 1992, Lusis 2000).

Small accumulations of macrophages and macrophages containing lipid droplets—so-called foam cells—have been seen in the coronary arteries already in childhood and puberty. These initial and minimal changes develop over decades into plaques that obstruct arteries most often at late middle age. High levels of circulating LDL and its accumulation in the arterial subendothelial matrix is a primary initiating event in the progression of atherosclerosis over the years (Lusis 2000, Stary 2000). Since the development of CAD is often slow and most individuals with this chronic disease show no symptoms until in the advanced state of the disease, it can progress unnoticed until an AMI finally arises. However, the progression of early atherosclerotic lesions to clinically manifest atherosclerotic plaques and symptoms of angina is often more rapid in persons with many coronary risk factors (Fuster et al. 1992).

2.2.2 Cardiovascular autonomic function in coronary artery disease

Cardiovascular autonomic function has been shown to be altered among patients with ischemic heart disease (Airaksinen et al. 1987, Huikuri et al. 1994, Bigger et al. 1995). The measures of HR variability are significantly lower in patients with chronic or subacute CAD compared with healthy subjects at the same age (Bigger et al. 1995). It seems that autonomic dysfunction, which occurs after an AMI, recovers over time, especially during the first 3 months. HR variability measured late after an AMI is substantially higher than variability measured in the early
There is evidence that measures of HR variability differ between patients with uncomplicated stable CAD and those with CAD complicated by myocardial infarction (MI) (Huikuri & Mäkikallio 2001). Particularly high-frequency fluctuation of HR seems to be reduced in patients with stable CAD (Huikuri et al. 1994), whereas reduced overall HR variability has been observed in patients with a recent MI (Bigger et al. 1995). Moreover, in patients with a prior MI and impaired left ventricular function, HR fluctuations are typically decreased in the low-frequency area (Lombardi et al. 1996).

Similar to HR variability, HR recovery is also reduced significantly in patients with uncomplicated CAD, which indicates deceleration in autonomic nervous system responsiveness and blunted vagal reactivity after cessation of exercise (Evrengul et al. 2006). Abnormal post-exercise HR recovery has been found to be independently associated with the severity of CAD (Ghaffari et al. 2011) and the presence of T2D (Nonaka et al. 2007) in patients with suspected or clinically documented CAD.

### 2.3 Type 2 diabetes

Diabetes is a metabolic disorder characterized by chronic hyperglycemia, which results from impairment of insulin secretion or insulin action, or a combination of both. The classification of diabetes includes four clinical classes: type 1 diabetes, T2D, other specific types, and gestational diabetes. Type 1 diabetes—characterized by an absence of insulin due to extensive pancreatic β-cell destruction—typically occurs in young subjects, whereas T2D with a combination of decreased insulin secretion and decreased insulin sensitivity is more common after middle age. Other specific types of diabetes are caused by other rare causes, e.g., genetic defects in β-cell function or insulin action, and diseases of the exocrine pancreas such as cystic fibrosis. Gestational diabetes constitutes hyperglycemia of variable severity with onset or first recognition during pregnancy. T2D is the most common form of diabetes, constituting about 90% of the total diabetic population (WHO 1999).

T2D is a multifactorial disease involving a genetic component and lifestyle factors. Genome-wide association studies have identified nearly 75 genetic loci associated with T2D and related metabolic features (Sanghera & Blackett 2012), and the risk for T2D is increased if there is a positive family history of the
Lifestyle factors, particularly those related to obesity, contribute strongly to development of T2D. An inactive lifestyle with reduced physical activity and sedentary behavior is independently associated with an increased risk of T2D (Hu et al. 2001, Hu et al. 2003a, Hu et al. 2003b, Meisinger et al. 2005). The increased amount of adipose tissue seen in obesity can increase the circulating levels of free fatty acids and adipocytokines, which may contribute to the emergence of T2D (Boden 2011, Dunmore & Brown 2013). Also hypertension and hyperlipidemia—typical among obese individuals—have been listed as additional risk factors for T2D (American Diabetes Association 2011).

T2D is widely recognized as one of the leading risk factors of cardiovascular morbidity and mortality, and it has become a major public health problem throughout the world. It is estimated that T2D affected 366 million people in 2011 worldwide and this figure is expected to rise to 552 million by 2030 (Whiting et al. 2011). In 2010 there were 1.3 million deaths due to diabetes, twice as many as in 1990 (Lozano et al. 2012). Most people with T2D live in developing countries, and these countries will also see the greatest increase over the next years because of the aging of the populations, increasing urbanization, and lifestyle changes including reduced physical activity and dietary changes (Whiting et al. 2011).

### 2.3.1 Pathogenesis of type 2 diabetes

T2D is a complex metabolic disorder of glucose homeostasis, which is characterized by both impaired insulin secretion in pancreatic β-cells and inability of the muscles, fat cells, and liver to respond to insulin effectively, i.e., insulin resistance. Generally, insulin secretion from the β-cells reduces glucose output by the liver, improves glucose uptake by skeletal muscles, and restrains fatty acid release from fat tissue. In the normoglycemic state, β-cells can adapt to changes in insulin action and maintain the balance between insulin secretion and insulin action (Stumvoll et al. 2005).

The multifactorial pathogenesis of T2D is complex and its molecular mechanisms are not completely understood. Accumulating evidence suggests that the various factors, including genes, increased free fatty acid levels, inflammatory cytokines from fat, and oxidative factors, have been implicated in the pathogenesis of type 2 diabetes, affecting both insulin secretion from pancreatic β-cells and insulin action in the muscles, fat cells, and liver (Stumvoll et al. 2005). An increased level of free fatty acids and inflammatory cytokines
negatively impacts the function of β-cells, whereupon the less functional β-cell mass cannot adapt insulin secretion to compensate for increasing insulin resistance (Leahy et al. 2010). Reduced insulin secretion together with insulin resistance will decrease insulin signaling in its target tissues, leading to increased circulating fatty acids and hyperglycemia. The raised concentrations of fatty acids and hyperglycemia itself impair the function of β-cells and aggravate insulin resistance, leading to the cycle of hyperglycemia causing a worsening metabolic state (Stumvoll et al. 2005).

The impairment in insulin secretion is generally progressive. β-cell failure occurs already before the onset of clinical hyperglycemia and diagnosis of T2D, after which β-cells function continues declining progressively despite the treatment with antidiabetic medications (Stumvoll et al. 2005, Leahy et al. 2010). During the years, hyperglycemia can cause significant pathological and functional changes, which leads to organ damage and microvascular and macrovascular complications such as neuropathy, retinopathy, nephropathy, MI, and stroke (Stratton et al. 2000).

### 2.3.2 Cardiovascular autonomic function in type 2 diabetes

Most patients with T2D have various degrees of autonomic dysfunction depending mainly on the duration of the disease and the degree of glycemic control. In the first years of the disease, autonomic dysfunction can be asymptomatic, whereas symptomatic diabetic autonomic neuropathy usually occurs in more advanced stages of the disease and can affect the entire autonomic nervous system over time. Autonomic neuropathy usually starts in the skin’s neurovascular system and the cardiovascular system and can expand to the gastrointestinal and genitourinary systems, but this kind of multiple organ involvement occurs only rarely, however (Vinik et al. 2003, Rolim et al. 2008, Schönauer et al. 2008).

Cardiovascular autonomic neuropathy is the most studied and clinically important form of diabetic autonomic neuropathy. It results from damage to the peripheral autonomic nerve fibers that innervate the heart and blood vessels, causing disturbance in HR regulation and in central and peripheral vascular dynamics (Vinik & Ziegler 2007, Schönauer et al. 2008). Development of diabetic neuropathy is the result of a multifactorial process including poor blood glucose control with chronic hyperglycemia, increased oxidative stress, reduction in nerve growth factors, disorders in fatty acid metabolism, formation of
advanced glycosylation end products, and autoimmune damage including inflammatory processes (Schönauer et al. 2008). Also traditional cardiovascular risk factors, such as hypertension, hyperlipidemia, high body mass index (BMI), and smoking, play an important role in the development of cardiovascular autonomic neuropathy (Rolim et al. 2008).

Decreased HR variability is very often the earliest indicator of cardiovascular autonomic neuropathy, even in clinically asymptomatic patients (Vinik & Ziegler 2007, Schönauer et al. 2008). Primarily the vagus nerve is damaged. The parasympathetic dysfunction results in resting tachycardia because of unopposed increased sympathetic outflow. In advanced stages of the disease, regression of tachycardia appears due to damage in the sympathetic nerve fibers. Additionally, an increasing limitation in HR variability is the manifestation of advanced cardiovascular autonomic dysfunction (Schönauer et al. 2008). The main clinical manifestations associated with cardiovascular autonomic neuropathy include resting tachycardia, exercise intolerance, orthostatic hypotension, intraoperative cardiovascular instability, asymptomatic MI, and increased risk of mortality (Maser & Lenhard 2005, Vinik & Ziegler 2007).

The reported prevalence of cardiovascular autonomic neuropathy varies depending on the study populations, diagnosis criterion, and the type and number of autonomic tests performed (Vinik et al. 2003, Schönauer et al. 2008). Prevalence rates as low as 7.7% have been reported among newly diagnosed patients with type 1 diabetes when strict criteria for defining cardiovascular autonomic neuropathy were used (Ziegler et al. 1992), whereas 90% of patients with T2D and a scheduled pancreas transplant had abnormal HR variability (Kennedy et al. 1995).

2.4 Physical activity and exercise capacity

Physical activity has been defined as any bodily movement produced by contraction of skeletal muscles that increases energy expenditure above a basal metabolic rate (Caspersen et al. 1985). Physical activity can be categorized according to the context in which the activity occurs. Commonly used categories include occupational, leisure-time, household, self-care, and transportation or commuting activities (Physical Activity Guidelines Advisory Committee 2008). Exercise training is a component of physical activity that refers to planned, structured, and repetitive physical activity performed during leisure-time with the primary purpose of improving or maintaining physical fitness or health.
(Caspersen et al. 1985). In this study, physical activity included all activity performed during essential activities of daily living, occupation, leisure-time, exercise training, and transportation.

Physical activity has many health benefits, including improvement in physical fitness. Physical fitness has been defined as the ability to carry out daily tasks with vigor and alertness, without undue fatigue and with ample energy to enjoy leisure-time pursuits and to meet unforeseen emergencies. Physical fitness includes a number of components which fall into two groups: one related to public health and the other related to athletic performance. Health-related physical fitness includes cardiorespiratory fitness, muscular strength and endurance, body composition, flexibility, and balance. Cardiorespiratory fitness is the ability of the circulatory and respiratory systems to supply oxygen during sustained physical activity (Caspersen et al. 1985). Exercise capacity is part of cardiorespiratory fitness and can be defined as the maximum amount of physical exertion that a person can sustain (Goldstein 1990). In this study, exercise capacity describes the patient’s maximal effort tolerance and maximum capability to perform an exercise during a maximal exercise test.

2.4.1 Assessment of physical activity and exercise capacity

The methods of assessing free-living physical activity can be categorized as subjective methods (questionnaires and activity diaries), objective methods (accelerometers, pedometers, and HR monitoring), and criterion methods (direct observation, doubly labeled water, and indirect calorimetry), which all have their own strengths and weaknesses (Vanhees et al. 2005). Briefly, subjective methods are the most widely used and inexpensive methods for assessing physical activity in large study populations in a short time, in contrast to objective measures, which are more reliable and accurate but demand careful planning and extra study resources when used in large field-based research (Shephard 2003, Trost et al. 2005). Criterion methods are the most reliable and valid, but also expensive measurements against which all other physical activity assessment methods should be validated (Schoeller 1988). Self-report questionnaires provide valuable information about the type and number of activities, but they suffer from reporting bias, tending to overestimate physical activity levels compared with objective measures (Troiano et al. 2008, Boon et al. 2010). Accelerometers and pedometers provide a valid estimate of overall physical activity but have limited validity for energy expenditure estimation compared with criterion methods.
Since physical activity is a complex concept with different components (e.g., duration, intensity, frequency and number of activities), the use of various measurements simultaneously provides complemented information about total physical activity (Vanhees et al. 2005).

In this study physical activity was measured by an accelerometer which registers acceleration due to body movements. The most commonly used accelerometers are placed on the waist, hip, or wrist, depending on the type of accelerometer and the purpose for which it is used. Generally, accelerometers have piezoelectric sensors which measure acceleration in one (uniaxial), two (biaxial), or three (triaxial accelerometers) dimensions (Vanhees et al. 2005, Garatachea et al. 2010). Triaxial accelerometers are, in theory, able to monitor all vertical, medio-lateral, and anterior-posterior movements, but still it seems that uniaxial and triaxial accelerometers assess physical activity with the same accuracy (Macfarlane et al. 2006, Vanhelst et al. 2012). Vanhelst et al. (2012) demonstrated good reliability between uniaxial and triaxial accelerometers and showed that the differences between those two devices are low for light (1.4%) and moderate (0.7%) physical activity, which were the predominant physical activity levels for most of the patients of our study.

Regular physical activity and exercise training improve cardiorespiratory fitness, which can be objectively measured by an incremental exercise stress test either on a treadmill or a bicycle ergometer. Cardiorespiratory fitness can be assessed by measuring maximal oxygen uptake, either directly by respiratory gas exchange analysis in a maximal incremental exercise test or indirectly in a graded sub-maximal exercise test (Åstrand & Rodahl 1986, Fletcher et al. 2001, Vanhees et al. 2005). Indirect measure of maximal oxygen uptake is based on the assumption of a linear relationship between HR and oxygen uptake and extrapolation of a submaximal HR to a known or predicted maximal HR (Åstrand & Rodahl 1986). When an exercise test is performed according to a generally accepted incremental protocol, exercise capacity can be measured accurately from the duration, workload, or stage of exercise at the end of the test (Fletcher et al. 2001, Vanhees et al. 2005).

Both exercise capacity and physical activity intensity can be expressed in multiples of metabolic equivalents (METs) (Jette et al. 1990, Howley 2001). One MET is the rate of energy expenditure at rest (approximately 1 kcal per kilogram of body weight per hour), which equates to oxygen consumption of approximately 3.5 milliliters per kilogram of body weight per minute for an average adult (Howley 2001). Maximal exercise capacity of 12 METs is seen in moderately
active healthy men, while distance runners can have values as high as 18 to 24 METs (Fletcher et al. 2001). The minimum level of aerobic fitness compatible with independent living is about 15 ml/kg/min in women and 18 ml/kg/min in men, corresponding to 4 and 5 METs, respectively (Paterson et al. 1999, Shephard 2009). In CAD patients, exercise capacity less than 5 METs can be classified as low and exercise capacity more than 8 METs as high (Martin et al. 2013). Patients with cardiovascular disease or T2D are at high risk of mortality if their exercise capacity is less than 5 METs (Myers et al. 2002, Kokkinos et al. 2009).

Different MET categories correspond to different physical activity intensities. In healthy adults, physical activity at moderate intensity is defined as from 3.0 to 5.9 METs and at vigorous intensity as 6.0 METs or greater (Physical Activity Guidelines Advisory Committee 2008). However, the absolute MET values are not valid for elderly or physically unfit people in which the basal metabolic rate or maximal exercise capacity is usually lower (Kwan et al. 2004). For an activity of a given absolute intensity, e.g. walking at 5 kilometers per hour (3.3 METs), relative intensity varies inversely to the exercise capacity of the individual. Exercise intensity of 5 METs might be a warm-up for a healthy young person, but may require almost maximal effort by an older, less fit individual. Table 2 provides the classification of absolute physical activity intensities in METs for groups with different maximal exercise capacities (Howley 2001).

**Table 2. Classification of physical activity intensity in healthy adults with different maximal exercise capacities, modified from Howley (2001).**

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Maximal exercise capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 METs</td>
</tr>
<tr>
<td>Very light</td>
<td>&lt; 3.2</td>
</tr>
<tr>
<td>Light</td>
<td>3.2–5.3</td>
</tr>
<tr>
<td>Moderate</td>
<td>5.4–7.5</td>
</tr>
<tr>
<td>Hard</td>
<td>7.6–10.2</td>
</tr>
<tr>
<td>Very hard</td>
<td>≥ 10.3</td>
</tr>
</tbody>
</table>

MET = metabolic equivalent.

Since the patient populations of this study had moderate exercise capacity (on average from 6 to 8 METs), we defined physical activity at moderate intensity as 2 to 5 METs and high intensity as more than 5 METs in this study.
2.4.2 Physical activity, exercise capacity, and coronary artery disease

A sedentary lifestyle and low physical fitness increase the risk of CAD in the general population (Williams 2001), whereas regular physical activity and exercise training have a positive influence on the risk of CAD and many of its traditional risk factors, as well (Thompson et al. 2003, Swift et al. 2013). Both moderate and high levels of physical activity protect against the incidence of CAD, and there seems to be an inverse dose–response relation between baseline physical activity level and the incidence of CAD in later life. People with high amounts or intensity of physical activity have average risk reductions of 30–35% for incidence of CAD compared with people with low amounts or intensity of physical activity, whereas those with moderate amounts or intensity of physical activity had risk reductions of 20–25% (Sofi et al. 2008, Shiroma & Lee 2010). In that case, the currently recommended amount of physical activity—at least 150 min per week of moderate-intensity physical activity or 75 min per week of vigorous-intensity physical activity—is clearly associated with a reduced risk of CAD. However, among unfit people with very low levels of physical activity, even smaller amounts of activity may be enough to produce health benefits (Blair et al. 1992, Lee et al. 2003, Sattelmair et al. 2011).

Physical activity has been shown to be an effective and beneficial strategy in treatment of CAD. Regular physical activity and exercise training reduce symptoms, hospitalization, the incidence of recurrent cardiac events, and mortality rates in patients with CAD (Belardinelli et al. 2001, Thompson et al. 2003). In statements from the American Heart Association and the European Association of Cardiovascular Prevention and Rehabilitation, 30–60 minutes of moderate-intensity aerobic physical activity daily (or at least 5 days per week) are recommended in patients with stable CAD. Additionally, it is recommendable to expand physical activity to include 3–5 sessions of 20–60 minutes of aerobic exercise training at 50–80% of maximal exercise capacity and also resistance training 2–3 days per week (Balady et al. 2007, Piepoli et al. 2010).

Although physical activity is a key element in secondary prevention and management of CAD, many patients with this chronic disease do not become or remain regularly active (Wofford et al. 2007). Moreover, CAD patients with T2D are less likely to comply with physical activity recommendations than their counterparts without T2D (Zhao et al. 2008). Cardiac rehabilitation programs including exercise counseling and/or supervised exercise training would be an
effective way of promoting physical activity and improving physical fitness among patients with CAD (Table 3) (Lavie & Milani 1995, Oberman et al. 1995, Verges et al. 2004, Oliveira et al. 2008, Svacinova et al. 2008, Shabani et al. 2010, Yohannes et al. 2010, Kim et al. 2011, Moholdt et al. 2012). Still, many cardiac patients do not attend recommended rehabilitation programs (Farley et al., 2003; Worcester et al., 2004; Higgins et al., 2008), and among patients who do attend programs the drop-out rate is as high as 40–50% (Sanderson et al. 2003, Sarrafzadeh et al. 2007).

Each 1 MET increase in exercise capacity lowers the risk of mortality by 13% in patients with cardiovascular disease, including CAD (Kokkinos et al. 2008, Martin et al. 2013). Improvement in exercise capacity is associated with decreased mortality, especially in patients whose baseline exercise capacity is lower than 5 METs. In those patients, overall mortality decreased up to 30% with each 1 MET increase in exercise capacity (Martin et al. 2013).
Table 3. Summary of the main studies that have examined the effect of cardiac rehabilitation programs and exercise interventions on cardiorespiratory fitness and exercise capacity in patients with coronary artery disease and type 2 diabetes.

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Intervention</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavie &amp; Milani 1995</td>
<td>patients with a major CAD event, n = 458</td>
<td>cardiac rehabilitation and exercise training program for 3 months</td>
<td>Exercise capacity improved 33% in women and 40% in men</td>
</tr>
<tr>
<td>Oberman et al. 1995</td>
<td>sedentary men with CAD, n = 186</td>
<td>high-intensity exercise training vs. low-intensity exercise training for 12 months</td>
<td>VO_{2\text{max}} improved after high-intensity exercise training (10%) and low-intensity exercise training (9%)</td>
</tr>
<tr>
<td>Banzer et al. 2004</td>
<td>patients with and without T2D enrolled in cardiac rehabilitation, n = 952</td>
<td>cardiac rehabilitation including supervised exercise training for 10 weeks</td>
<td>Exercise capacity improved equally in patients with (26%) and without T2D (27%)</td>
</tr>
<tr>
<td>Verges et al. 2004</td>
<td>diabetic and non-diabetic patients with an acute coronary event, n = 95</td>
<td>cardiac rehabilitation program with aerobic exercise training for 2 months</td>
<td>VO_{2\text{max}} improved significantly less in patients with T2D (13%) compared with those without T2D (30%)</td>
</tr>
<tr>
<td>Hindman et al. 2005</td>
<td>patients with and without T2D who participated in cardiac rehabilitation, n = 1505</td>
<td>cardiac rehabilitation including structured and supervised exercise training for 7–12 weeks</td>
<td>Exercise capacity improved equally (26%) in patients with and without T2D</td>
</tr>
<tr>
<td>Kadoglou et al. 2007</td>
<td>patients with T2D, n = 60</td>
<td>aerobic exercise training for 6 months</td>
<td>VO_{2\text{max}} improved significantly (25%) in patients assigned to the exercise group</td>
</tr>
<tr>
<td>Svacinova et al. 2008</td>
<td>diabetic and non-diabetic patients with an acute coronary event, n = 77</td>
<td>combined aerobic and resistance training for 3 months</td>
<td>VO_{2\text{max}} improved equally in patients with T2D (14%) and without T2D (11%)</td>
</tr>
<tr>
<td>Balducci et al. 2010</td>
<td>sedentary patients with T2D, n = 606</td>
<td>supervised exercise training plus exercise counseling alone for 12 months</td>
<td>VO_{2\text{max}} improved significantly in both groups: 4.5 ml/kg/min (17%) vs. 1.6 ml/kg/min (6%)</td>
</tr>
<tr>
<td>Larose et al. 2010</td>
<td>patients with T2D, n = 251</td>
<td>aerobic exercise training vs. resistance exercise training vs. combined aerobic and resistance training for 6 months</td>
<td>VO_{2\text{max}} improved significantly in the aerobic training group and the combined training group: 1.7 ml/kg/min and 1.9 ml/kg/min</td>
</tr>
<tr>
<td>Study</td>
<td>Subjects</td>
<td>Intervention</td>
<td>Main results</td>
</tr>
<tr>
<td>---------------</td>
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<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Kim et al. 2011</td>
<td>patients with acute coronary syndrome, n = 91</td>
<td>supervised exercise training vs. self-exercise training for 6 months</td>
<td>VO\textsubscript{2max} improved more after supervised exercise (21%) than self-exercise training (9%)</td>
</tr>
<tr>
<td>Balducci et al. 2012</td>
<td>sedentary patients with T2D, n = 303</td>
<td>supervised low-to-moderate-intensity vs. moderate-to-high-intensity aerobic and resistance training for 12 months</td>
<td>VO\textsubscript{2max} improved significantly and equally in both groups: 4.5 ml/kg/min (18%) and 4.6 ml/kg/min (17%), respectively</td>
</tr>
<tr>
<td>Moholdt et al. 2012</td>
<td>post-MI patients, n = 107</td>
<td>treadmill aerobic interval training vs. usual care aerobic group exercise training for 3 months</td>
<td>VO\textsubscript{2max} improved more after aerobic interval training (15%) than after usual care rehabilitation (8%)</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease; VO\textsubscript{2max}, peak oxygen uptake; T2D, type 2 diabetes; MI, myocardial infarction.

### 2.4.3 Physical activity, exercise capacity, and type 2 diabetes

Similar to CAD, regular long-term physical activity protects against development of T2D (Hu et al. 2003b, Laaksonen et al. 2005, Meisinger et al. 2005). Both moderate leisure-time and occupational physical activity decrease the risk of T2D by 30–35% (Hu et al. 2003a, Hu et al. 2003b). Similarly, more than 30 min of walking or cycling to and from work decreases the risk of T2D by 36% (Hu et al. 2003b). Sedentary behavior, such as watching television and overall sitting, is associated with increased risk of T2D independently of physical activity levels (Wilmot et al. 2012, Solomon & Thyfault 2013). The pooled relative risk for diabetes is 1.20 per each 2-hour increment in watching television daily (Hu et al. 2001, Hu et al. 2003a, Krishnan et al. 2009, Ford et al. 2010), whereas sitting at work more than 40 hours compared with less than 2 hours a week increases the relative risk of developing T2D by 48% (Hu et al. 2003a).

Long-term physical activity and regular exercise training are highly recommended as treatment for patients with T2D because of a positive influence on blood glucose control and insulin sensitivity (Boule et al. 2001, Snowling & Hopkins 2006, Thomas et al. 2006, Colberg et al. 2010). Moreover, exercise training increases cardiorespiratory fitness (Boule et al. 2003, Banzer et al. 2004, Kadoglou et al. 2007, Balducci et al. 2010, Larose et al. 2010, Balducci et al. 2012) and has a positive effect on co-morbidities associated with T2D such as obesity, hypertension, and hyperlipidemia, especially when physical activity is combined with other lifestyle changes (Colberg et al. 2010). Accumulating
evidence has demonstrated that physically active patients with T2D experience lower cardiovascular morbidity and all-cause mortality compared to their inactive counterparts (Tanasescu et al. 2003, Hu et al. 2004, Iijima et al. 2012, Blomster et al. 2013, Sone et al. 2013). Table 3 summarizes the results of the main studies that have examined the effect of cardiac rehabilitation programs and exercise interventions on cardiorespiratory fitness and exercise capacity in patients with CAD and T2D.

The American Heart Association, the American Diabetes Association and the European Association of Cardiovascular Prevention and Rehabilitation recommend physical activity for more than 30 minutes per day in addition to at least 150 minutes of moderate-intensity aerobic exercise training and/or at least 90 minutes of vigorous aerobic exercise per week for improvement of glycemic control, assisted with weight loss or maintenance, to reduce the risk of cardiovascular diseases in patients with T2D. Additionally, resistance training 3 times per week should be encouraged (Buse et al. 2007, Piepoli et al. 2010).

Despite the well-known health benefits of regular physical activity and exercise training, the majority of patients with T2D do not engage in recommended levels of physical activity and are physically more passive than the healthy population (Nelson et al. 2002, Thomas et al. 2004, Morrato et al. 2007, Zhao et al. 2011). Only 39% of adults with T2D are physically active compared with 58% of American adults without diabetes (Morrato et al. 2007). In Finland, on average 56% of middle-age patients with T2D engage in 20–30 min of moderate-intensity leisure-time physical activity at least 2–3 times per week (Karjalainen et al. 2008).

Maybe partly because of a sedentary lifestyle, diabetes-related co-morbidity, and physical disabilities, exercise capacity and cardiorespiratory fitness are also demonstrably lower in patients with T2D than in healthy subjects (Regensteiner et al. 1998, Fang et al. 2005). Low exercise capacity is associated with, e.g., poor blood glucose control and high BMI (Fang et al. 2005).

2.4.4 Physical activity, exercise capacity, and cardiovascular autonomic function

Previous studies have shown that long-term physical activity has a positive influence on cardiovascular autonomic function, especially in the healthy population. Individuals with a high level of total physical activity have significantly faster HR recovery (Carnethon et al. 2005) and higher HR variability.
indexes compared to those with reduced physical activity (Buchheit et al. 2005, Blom et al. 2009). It seems that physical activity intensity is more closely associated with improvement in HR variability than total physical activity time (Buchheit et al. 2004). Changes in physical activity level have evident influences on cardiovascular autonomic function. Carnethon et al. (2005) showed that during a 7-year follow-up period, HR recovery declined less among healthy subjects whose physical activity increased or remained stable than in those whose physical activity decreased.

Similar to physical activity, higher physical fitness levels are associated with higher levels of HR variability in healthy individuals (De Meersman 1993, Tulppo et al. 1998, Buchheit & Gindre 2006). The association between low exercise capacity and blunted HR recovery has also been found among patients with T2D (Fang et al. 2005) and with suspected or diagnosed ischemic heart disease (Georgoulias et al. 2003).

Exercise training has proved to be an effective way to improve autonomic function in healthy persons (Levy et al. 1998, Tulppo et al. 2003, Jurca et al. 2004), especially among those who are physically inactive at the baseline (Schuit et al. 1999). Regular aerobic exercise training affects autonomic nervous system function by increasing parasympathetic activity and decreasing sympathetic activity in the heart at rest (Carter et al. 2003). Moderate-intensity and low-volume (2–3 sessions weekly) exercise training did not produce a clinically meaningful increase in HR variability (Verheyden et al. 2006), whereas beneficial effects was seen in a moderate training volume (6 sessions weekly) (Tulppo et al. 2003). Increasing training duration from 30 to 60 min per session did not produce additional benefit (Tulppo et al. 2003). It has been found that each hour of high-intensity physical exercise training weekly is generally associated with a $\geq 2\%$ increase in HR variability in an aging population (Dietrich et al. 2006). Moderate-intensity exercise training for 3 months increases HR variability, but more prolonged and intensive training does not provide added positive benefit (Iwasaki et al. 2003). Also in cardiac patients, rehabilitation programs result in positive changes in cardiovascular autonomic function, which is observed in the form of an increase in HR recovery (Jolly et al. 2011), although CAD patients with T2D achieved less improvement in HR recovery after cardiac rehabilitation than their counterparts without diabetes (Soleimani et al. 2010).
3 Aims of the study

The main goal of the present study was to examine the determinants of cardiovascular autonomic function in the healthy population and in CAD patients with and without T2D. Another aim of this study was to investigate the prognostic value of autonomic function and the effects of exercise prescriptions on physical activity and exercise capacity in the above-mentioned patient groups. The specific aims of this serial study were:

1. to examine which HR variability parameters are affected by simultaneously measured physical activity during free-living conditions in 24-hour Holter recordings among healthy adults (I).
2. to examine the association between cardiovascular autonomic function assessed by HR recovery, HR variability, and HR turbulence, and clinical, demographic, and echocardiographic variables among CAD patients with and without T2D (II, III).
3. to investigate whether cardiovascular autonomic dysfunction predicts short-term cardiovascular events including cardiovascular death, acute coronary event, stroke, and hospitalization for heart failure among CAD patients with and without T2D (III).
4. to investigate the effects of individually tailored, home-based 6-month exercise prescriptions on physical activity and maximal exercise capacity among CAD patients with and without T2D (IV).
4 Materials and methods

4.1 Healthy population (I)

The subjects (n = 45) of study I were healthy males (n = 21, age 35 ± 3 years, BMI 25 ± 2 kg/m²) and females (n = 24, age 34 ± 4 years, BMI 24 ± 2 kg/m²). All smokers, those who had carried out regular physical exercise training > 3 h/week during the past month, and those with diabetes, asthma, or cardiovascular disorders were excluded. The subjects were requested to complete a health questionnaire to ascertain their medical history and level of physical activity. All the subjects provided written informed consent.

4.1.1 Analyses of heart rate variability

R-R intervals were recorded over a 24-h period with a Polar R-R recorder (Polar Electro Oy, Kempele, Finland) with an accuracy of one msec and saved on a computer for further analyses of HR variability with HEARTS software (Heart Signal, Kempele, Finland). To exclude all undesirable beats, R-R intervals were edited by visual inspection using a deletion method. HR variability indexes were calculated from the waking hours that represented active ambulatory daytime. Mean HR and SDNN were used as time-domain measures of HR variability. Average 30-min values of HR, LF power (0.04–0.15 Hz), and HF power (0.15–0.4 Hz) were calculated from blocks of 1,024 beats and VLF (0.0033–0.04 Hz) was analyzed in longer blocks of 4,096 beats. SDNN and VLF power were analyzed over the entire 30-min recording.

Poincaré plots were analyzed quantitatively using two-dimensional vector analyses in 30-min epochs. SD1 measures the magnitude of vagally mediated beat-to-beat variability of the data, and SD2 measures the magnitude of continuous long-term R-R interval fluctuation. SampEn and α1 were analyzed as nonlinear indexes of HR variability in 30-min epochs. In fractal analysis the root mean square fluctuations of integrated and detrended data are measured in observation windows of different sizes and then plotted against the size of the window on a log-log scale. SampEn was calculated using Kubios HR variability software (version 2.0, Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland). Two input
variables, \( m = 2 \) and \( r = 20\% \) of the SD of the data sets, were fixed to calculate SampEn.

### 4.1.2 Measurement of physical activity

Physical activity data were continuously collected with a customized Polar wrist watch equipped with a one-dimensional accelerometer (Polar Electro Oy, Kempele, Finland). The accelerometer was tuned to LF movements (0.3–1.5 Hz) and programmed to register movement if acceleration exceeded 0.1 g. The accelerometer used the pulse-filtering procedure presented in European Patent 1532924. The minute-by-minute movement count was transformed to intensity in MET values using a nonlinear relationship and a body height-based calibration factor. A 30-min average of the intensity level was calculated in METs.

### 4.2 ARTEMIS study population (II–IV)

The ARTEMIS (Innovation to Reduce Cardiovascular Complications of Diabetes at the Intersection) study was conducted in the Division of Cardiology of the Oulu University Hospital and was registered at ClinicalTrials.gov, Identifier NCT01426685. The study was conducted according to the Declaration of Helsinki, and the local research ethics committee of the Northern Ostrobothnia Hospital District approved the protocol.

The study population of study III consisted of 530 CAD patients and 530 CAD patients with T2D recruited from the consecutive series of patients who had undergone coronary angiography in the Oulu University Hospital between August 2007 and November 2011. The patient groups were matched in terms of age, sex, LVEF, and a history of MI. Patients with age < 18 years or > 85 years, impaired glucose tolerance or impaired fasting glucose, NYHA class IV, a permanent pacemaker or implantable cardioverter defibrillator, planned implantable cardioverter defibrillator implantation, or end-stage renal failure needing dialysis were excluded from this study, as were patients who had a life expectancy of < 1 year or who were psychologically or physically unfit for participation in the study (due to some other illness).
Sub-study: The ARTEMIS exercise prescription (II, IV)

The ARTEMIS exercise prescription sub-study is a randomized and controlled exercise prescription trial, which was conducted in the Department of Exercise and Medical Physiology at Verve. Patients were selected from the ARTEMIS database while observing the following exclusion criteria: advanced age (> 75 years), BMI > 40 kg/m², NYHA class III or IV, LVEF < 40%, scheduled cardiac revascularization therapy, heart failure, unstable angina pectoris, severe peripheral atherosclerosis, severe diabetic retinopathy or neuropathy, or other inability to perform regular home-based exercise, e.g., due to musculoskeletal problems. Altogether 105 voluntary patients (55 CAD patients and 50 CAD patients with T2D) were randomly selected for study II. After exclusion of patients who did not carry out the physical activity measurement and did not participate in the 6-month measurement, 83 patients (44 CAD patients and 39 CAD patients with T2D) were included in the follow-up analyses of exercise prescription (IV).

In study IV a controlled 6-month exercise prescription was based on current guidelines (Piepoli et al. 2010) and consisted of home-based, HR-controlled endurance training and strength training (Table 4). The intensity of the endurance training was stated as the target level of HR reserve, using a HR recorder (Polar F1, Polar Electro Oy, Kempele, Finland). Exercise prescription was introduced after baseline laboratory and physical activity measurements and the patients got a daily diary in which was marked training days, the target duration of exercise sessions, and the actual training HR zones. The patients also got oral and written instructions on how to use the HR recorder and how to mark the duration and the average HR of every training session in the diary.

<table>
<thead>
<tr>
<th>Exercise period</th>
<th>Endurance training</th>
<th>Strength training</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3 months</td>
<td>50–60% of heart rate reserve, 30 min per session, 3 times per week.</td>
<td>Home-based circuit training, 30 min per session, once a week.</td>
</tr>
<tr>
<td>4–6 months</td>
<td>50–60% of heart rate reserve, 30 min per session, 2 times per week. 60–70% of heart rate reserve, 30 min per session, 2 times per week.</td>
<td>Home-based circuit training, 30 min per session, once a week.</td>
</tr>
</tbody>
</table>

Weekly training load was calculated as the mean training impulse (TRIMP) using the following formula: TRIMP = ABC, in which A is exercise time (min), B is
HR (proportional to HR reserve), and C is $e^{1.92B}$ for men and $e^{1.67B}$ for women (Morton et al. 1990). The target TRIMP per week for endurance training and strength training was calculated using the same formula. In calculating target TRIMP for endurance training B was stated as an average of target HR (55% or 65% of HR reserve) and in strength training B was defined as 90% of the target HR of light endurance training based on our previous experience (Hautala et al. 2006). All the patients were included in the analysis regardless if their realized TRIMP was under or over the target TRIMP.

4.2.1 Measurement of cardiovascular autonomic function

Heart rate recovery

Recovery of HR was measured in a supine position immediately after cessation of a maximal exercise test and no cool-down period was allowed. HR recovery was calculated as the reduction in HR from the rate at maximal exercise to the rate one minute after cessation of exercise using a 15-lead ECG (GE Healthcare, CAM-14, Freiburg, Germany) (III) and R-R intervals (II). R-R intervals were recorded with a Polar R-R Recorder (Polar Electro Oy, Kempele, Finland) at a sampling frequency of 1,000 Hz. The maximal HR and HR recovery values were calculated as the mean value of 5 R-R intervals before cessation of exercise and from the R-R interval data around the time point 60 seconds after cessation of exercise. R-R intervals were converted to maximal HR in beats per minute with the following equation: maximal HR (beats · min$^{-1}$) = 60 · (mean R-R interval in msec · 1000$^{-1}$)$^{-1}$. In the ECG, HR recovery was calculated as the reduction in HR from the rate at maximal exercise to the rate 60 seconds after cessation of exercise.

Heart rate variability

In study III a twenty-four-hour ambulatory ECG was recorded with a digital Holter recorder (Medilog AR12, Huntleigh Healthcare, UK) with an accuracy of four msec and saved on a computer for further analysis with HEARTS software (Heart Signal, Kempele, Finland). From the total of 1060 patients, 27 patients did not undergo the 24-hour ECG recording and 66 patients were excluded from the analysis due to a large amount of technical and biological disturbances, e.g.,
periods of atrial fibrillation. In total, 496 CAD patients and 471 CAD patients with T2D were included in the analyses of 24-hour ECG recordings. To exclude all undesirable beats, the R–R intervals were edited by visual inspection using the interpolation method. This method replaces the edited, removed R-R interval with a local average of the previous accepted normal R-R intervals (Salo et al. 2001). SDNN was used as time-domain measures of HR variability.

**Heart rate turbulence**

In study III, post-ectopic HR turbulence was analyzed from the twenty-four-hour ECG recording (Medilog AR12, Huntleigh Healthcare, UK) according to a previously published method (Schmidt et al. 1999). The HR turbulence slope was defined as the maximum slope of the regression line assessed over any sequence within five subsequent sinus R-R intervals during the first 15 sinus beats following an ectopic beat, as described earlier (Huikuri et al. 2010). HR turbulence was measured from 423 CAD patients and 414 CAD patients with T2D.

### 4.2.2 Measurement of physical activity

In study IV, physical activity was measured over five consecutive days, including weekend days, during waking hours with a wristwatch equipped with a one-dimensional accelerometer (Polar AW200, Polar Electro Oy, Kempele, Finland). The accelerometer uses algorithms which distinguish the intensity of the acceleration and classifies physical activity time per day into five intensity zones corresponding to slow walking (≥2 METs), walking (>3 METs), brisk walking (>5 METs), running (>8 METs), and fast running (>12 METs). For the statistical analyses, the different intensities of physical activity were dichotomized as moderate (intensity levels 1 and 2, METs = 2–5) and high (intensity levels 3–5, METs > 5) intensity. Patients were advised to wear the accelerometer during all waking hours and to carry on their normal daily routine. Physical activity was reassessed during five consecutive days after the completion of the 6-month exercise period. The wrist-worn accelerometer technology has been compared with energy expenditure measured by indirect calorimetry and doubly labeled water technique, and it appeared to be a reliable and valid method for assessing physical activity (Brugniaux et al. 2010, Kinnunen et al. 2012).
4.2.3 Measurement of exercise capacity and oxygen uptake

In studies II–IV the patients performed an incremental symptom-limited maximal exercise test on a bicycle ergometer (Monark Ergomedic 839 E, Monark Exercise AB, Vansbro, Sweden) for assessment of maximal exercise capacity. The test was started at 30 W, and the work rate was increased by 15 W in men and 10 W in women every minute until voluntary exhaustion (II–IV) or ST segment depression > 0.2 mV in ECG (II, IV). Maximal workload was calculated as the average workload during the last minute of the test and maximal exercise capacity was then calculated in METs from the maximal workload. In studies II and IV, ventilation and gas exchange (M909 Ergospirometer, Medikro, Kuopio, Finland) were monitored continuously during the test. The highest 1-min mean value of oxygen consumption was taken to express peak oxygen uptake.

4.2.4 Measurement of cardiac function

All the patients were diagnosed as having CAD, which had been documented previously by coronary angiography. The severity of CAD was assessed by measurement of the Syntax score in coronary angiography (II, IV) (Sianos et al. 2005). Left ventricular systolic function was assessed by two-dimensional echocardiography (Simpson method) and diastolic function by tissue Doppler echocardiography (Vivid 7, GE Healthcare, Wauwatosa, WI, USA). Left ventricular mass (LVM) was calculated using the following equation (Devereux et al. 1986):

$$LVM(g) = 0.8 \times \{1.04 [(LVIDd + PWTd + SWTd)^3 - LVIDd^3] \} + 0.6,$$

in which LVIDd is left ventricle internal diameter, PWTd is posterior wall thickness, and SWTd is septum wall thickness measured during end-diastole. The LVM index was calculated by dividing LVM with body surface area which was determined by the Dubois equation (Du Bois & Du Bois 1989).

4.2.5 Laboratory analyses

All laboratory measurements were done after a 12-hour overnight fast by using standardized methods. Fasting blood samples were obtained for analysis of plasma glucose and glycated hemoglobin (HbA1c) levels, blood lipids, and
inflammation markers. Urine samples were obtained for analysis of renal function and microalbuminuria.

T2D was verified according to the current criteria of the World Health Organization (WHO 1999). Patients who had a fasting capillary plasma glucose level \( \geq 7.0 \text{ mmol/l} \) or 2-h plasma glucose \( \geq 12.2 \text{ mmol/l} \) during an oral glucose tolerance test or were on antihyperglycemic medication based on a prior diagnosis of T2D were classified as having T2D. Patients with normal glucose tolerance must be normoglycemic, defined as plasma capillary glucose levels < 6.1 mmol/l in the fasting state and a 2-hour post-load value < 8.9 mmol/l in the oral glucose tolerance test. Patients with prediabetes, i.e. impaired fasting glucose (two-hour plasma glucose < 8.9 mmol/l and fasting plasma glucose \( \geq 6.1 \text{ but } \leq 6.9 \text{ mmol/l} \)) or impaired glucose tolerance (two-hour plasma glucose \( \geq 8.9 \text{ but } \leq 12.1 \text{ mmol/l} \) and fasting plasma glucose < 7.0 mmol/l) were excluded.

4.2.6 Follow-up

In study III the follow-up was 2 years after the patient’s first measurement in the ARTEMIS study in the Oulu University Hospital. The endpoint was defined as a combination of cardiovascular complications including cardiovascular death, acute coronary event, stroke, and hospitalization for heart failure. The follow-up data were collected from patient records of Oulu University Hospital and mortality statistics of Statistics Finland and the Causes of Death Register. The patients who were alive were also contacted by telephone after 2 years. The follow-up was completed for 427 CAD patients without T2D and for 525 patients with T2D until January 2013 at the time the query was run.

4.3 Statistical analysis

SPSS software (SPSS Inc., Chicago, IL, USA), version 12.0.1 (I), 19.0 (II–IV) and 21.0 (III) was used for the statistical analysis. Continuous variables are presented as means ± standard deviations and categorical variables as the number of cases followed by the corresponding percentage within brackets. All \( p \) values were two-tailed and \( p < 0.05 \) was considered statistically significant.

A Shapiro-Wilk’s test (I) and a Kolmogorov-Smirnov Z-test (II–IV) were used to examine the Gaussian distribution of the data. Comparisons between patient groups were done using a t-test for independent samples for normally distributed data and a nonparametric Mann-Whitney U-test for unequally
distributed data. The chi-square test was used to compare categorical variables. Since some of the data were skewed, all the correlation analyses in studies I–III were performed using Spearman’s nonparametric test.

In study I the sign test was performed to complement the statistical approach of the study (GrapPad Software, La Jolla, CA). ANOVA was used to compare if the baseline characteristics and mean values of HR variability differed between men and women.

In studies II and III, linear regression analyses with stepwise regression analyses were used to construct the predictive regression models for cardiovascular autonomic function in the patient groups. All the significant demographic, laboratory, and echocardiographic variables which correlated with the measures of cardiovascular autonomic function in the Spearman’s correlation analyses \( p < 0.05 \) and medication were included as covariates in the linear regression analyses. The variables which have non-Gaussian distribution were transformed into natural logarithms before the parametric statistical tests.

In studies II and IV, ANCOVA was used to compare the differences in HR recovery and physical activity variables between the patient groups including daily physical activity time, exercise capacity, and/or BMI as covariates. In study IV the effects of the exercise prescription were analyzed by two-factor ANOVA with time and interventions. When significant time × intervention interaction was observed, a post hoc analysis was performed using a paired t-test between pre- and post-prescription values within each group and a t-test for independent samples for between-group comparisons at pre- and post-prescription conditions.

In study III an optimal cutoff value for each of three measures of autonomic function was defined from receiver operating characteristic (ROC) curves as the maximum sum of sensitivity and specificity below the median with sensitivity at least 20% using a composite of cardiovascular complications as the endpoint. Univariate Cox regression analysis was used to obtain values for the hazard ratio with 95% confidence intervals (CI) for categorized autonomic markers. Thereafter, multivariate Cox regression analysis was performed including covariates which were associated with the composite cardiovascular endpoint. Cox regression analyses were also performed for the sub-groups of CAD patients with and without T2D.
5 Results

5.1 Physical activity and heart rate variability in healthy adults (I)

In the first study (I), HR variability and physical activity were measured simultaneously during waking hours among 45 healthy adults. The mean physical activity time was 15:44 ± 1:01 h, and the mean MET was 1.91 ± 0.14. HR and SampEn, but not the other measures of HR variability, was significantly related to physical activity, as shown by both correlation analyses ($r = 0.64$, $p = 0.021$ and $r = -0.55$, $p = 0.022$, respectively) and sign tests ($p < 0.0001$ for both). Beat-to-beat R-R interval fluctuation—expressed as SD1—also demonstrated a significant relation to physical activity according to the sign test ($p = 0.037$) and a trend of association according to the correlation analysis ($r = -0.40$, $p = 0.129$). All the mean values of within-individual correlation analyses between physical activity and HR variability and the results of the sign tests are shown in Table 5.

Table 5. Mean values of within-individual Spearman correlation coefficients and $p$ values between concurrently analysed physical activity and heart rate variability of the study group.

<table>
<thead>
<tr>
<th>HR variability variables</th>
<th>Spearman’s rho</th>
<th>Sign Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$ value</td>
<td>$p$ value</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>0.64</td>
<td>0.021</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>0.05</td>
<td>0.368</td>
</tr>
<tr>
<td>HF power, ln ms$^2$</td>
<td>$-0.39$</td>
<td>0.144</td>
</tr>
<tr>
<td>LF power, ln ms$^2$</td>
<td>$-0.14$</td>
<td>0.306</td>
</tr>
<tr>
<td>VLF power, ln ms$^2$</td>
<td>$-0.03$</td>
<td>0.410</td>
</tr>
<tr>
<td>LF-to-HF ratio</td>
<td>0.35</td>
<td>0.164</td>
</tr>
<tr>
<td>SD1, ms</td>
<td>$-0.40$</td>
<td>0.129</td>
</tr>
<tr>
<td>SD2, ms</td>
<td>0.07</td>
<td>0.357</td>
</tr>
<tr>
<td>$\alpha_1$</td>
<td>0.40</td>
<td>0.135</td>
</tr>
<tr>
<td>SampEn</td>
<td>$-0.55$</td>
<td>0.022</td>
</tr>
</tbody>
</table>

HR, Heart rate; SDNN, standard deviation of all R-R intervals; HF, high frequency; LF, low frequency; VLF, very low frequency; SD1, beat to beat R-R interval fluctuation; SD2, long term R-R interval fluctuation, $\alpha_1$, short-term fractal scaling exponent; SampEn, sample entropy. The sign test requires that at least 29 of the 45 subjects demonstrate a significant relation, i.e., correlation with $p$ value to the mean.
5.2 Determinants of cardiovascular autonomic function in coronary artery disease patients with and without type 2 diabetes (II, III)

First, in study II, the determinants of cardiovascular autonomic function were examined using measurement of HR recovery separately in 50 CAD patients with T2D and 55 patients with CAD alone. Physical activity was measured only in study II. After that, in study III, the determinants of cardiovascular autonomic function were examined in more detail using measurements of HR recovery, HR variability, and HR turbulence in a larger study population of 1060 CAD patients (50% were patients with T2D). Detailed patient characteristics are shown in Table 6.

Table 6. Demographic characteristics of the study populations.

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>ARTEMIS study population (n = 1060)</th>
<th>ARTEMIS sub-study CAD patients with T2D (n = 50)</th>
<th>p value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with T2D</td>
<td>530 (50%)</td>
<td>50 (100%)</td>
<td></td>
</tr>
<tr>
<td>Duration of T2D, months</td>
<td>103 ± 105</td>
<td>62 ± 85</td>
<td></td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>731 (69%)</td>
<td>44 (84%)</td>
<td>0.595</td>
</tr>
<tr>
<td>Age, years</td>
<td>67 ± 8</td>
<td>62 ± 6</td>
<td>1.000</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.69 ± 0.09</td>
<td>1.71 ± 0.08</td>
<td>0.491</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81 ± 16</td>
<td>79 ± 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.3 ± 4.7</td>
<td>30.3 ± 3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of acute myocardial infarction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSTEMI</td>
<td>277 (26%)</td>
<td>21 (38%)</td>
<td>0.269</td>
</tr>
<tr>
<td>STEMI</td>
<td>167 (16%)</td>
<td>13 (24%)</td>
<td>0.965</td>
</tr>
<tr>
<td>Revascularization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCI</td>
<td>624 (59%)</td>
<td>33 (60%)</td>
<td>1.000</td>
</tr>
<tr>
<td>CABG</td>
<td>251 (24%)</td>
<td>16 (29%)</td>
<td>0.902</td>
</tr>
<tr>
<td>Cardiac function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF, %</td>
<td>65 ± 9</td>
<td>66 ± 8</td>
<td>0.281</td>
</tr>
<tr>
<td>E/E'</td>
<td>10.9 ± 4.0</td>
<td>9.1 ± 2.6</td>
<td>0.364</td>
</tr>
<tr>
<td>Maximal exercise test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>max exercise capacity, METs</td>
<td>6.0 ± 1.8</td>
<td>7.8 ± 1.8</td>
<td>0.006</td>
</tr>
<tr>
<td>max heart rate, bpm</td>
<td>123 ± 22</td>
<td>133 ± 18</td>
<td>0.349</td>
</tr>
</tbody>
</table>
Clinical features ARTEMIS study population (n = 1060) ARTEMIS sub-study CAD patients (n = 55) CAD patients with T2D (n = 50) p value between groups

**Laboratory analyses**

<table>
<thead>
<tr>
<th></th>
<th>ARTEMIS</th>
<th>ARTEMIS sub-study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycated hemoglobin, %</td>
<td>6.4 ± 1.1</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/l</td>
<td>6.5 ± 1.9</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.0 ± 0.8</td>
<td>3.9 ± 0.7</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>2.3 ± 0.7</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.4 ± 0.8</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>hs-CRP, mg/l</td>
<td>2.2 ± 4.5</td>
<td>2.0 ± 4.2</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>93.2 ± 34.8</td>
<td>97.8 ± 22.3</td>
</tr>
</tbody>
</table>

**Medication for T2D**

<table>
<thead>
<tr>
<th></th>
<th>ARTEMIS</th>
<th>ARTEMIS sub-study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral medication</td>
<td>297 (28%)</td>
<td>37 (74%)</td>
</tr>
<tr>
<td>Insulin</td>
<td>146 (14%)</td>
<td>5 (10%)</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease; T2D, type 2 diabetes; NSTEM, no-ST segment elevation myocardial infarction; STEMI, ST segment elevation myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery by-pass grafting; LVEF, left ventricular ejection fraction; E/E', ratio of early transmitral flow velocity to early diastolic mitral annulus velocity; MET, metabolic equivalent; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein.

### 5.2.1 Determinants of heart rate recovery

In study II, HR recovery had the closest univariate correlation with daily physical activity time and exercise capacity ($r = 0.38$, $p = 0.006$ and $r = 0.37$, $p = 0.008$, respectively) in CAD patients with T2D. Daily physical activity time was a stronger predictor of HR recovery than exercise capacity when those two variables were included in the same linear regression model without any other confounding variables ($R = 0.30$, $p = 0.034$ and $R = 0.13$, $p = 0.354$ for physical activity and exercise capacity, respectively). Additionally, HR recovery was positively correlated with high-density lipoprotein (HDL) cholesterol level ($r = 0.31$, $p = 0.018$) and negatively correlated with age ($r = −0.36$, $p = 0.010$) and LVEF ($r = −0.29$, $p = 0.042$) in CAD patients with T2D. In the CAD patients without T2D, there was a clear positive correlation between HR recovery and the amount of high-intensity physical activity ($r = 0.44$, $p = 0.001$), while a significant negative correlation was found between HR recovery and the depression score ($r = −0.39$, $p = 0.003$). In CAD patients with T2D, the linear regression analyses showed that age, exercise capacity, and HDL cholesterol level
explained 30% of HR recovery ($R = 0.55, p = 0.001$), whereas physical activity at high intensity was the only predictor of HR recovery in patients with CAD alone, with $R$ values of 0.35 ($R^2 = 0.12, p = 0.010$).

In study III, HR recovery had the closest univariate correlation with exercise capacity, age, fasting plasma glucose, and left ventricular diastolic function ($r = 0.55, p < 0.001$; $r = −0.41, p < 0.001$; $r = −0.29, p < 0.001$; and $r = −0.29, p < 0.001$, respectively) when CAD patients with and without T2D were combined (Table 7, Figure 1). When all factors correlated with HR recovery were entered into a stepwise linear regression model, exercise capacity ($R = 0.34, R^2 = 0.12, p < 0.001$), age ($R = −0.23, R^2 = 0.05, p = 0.001$), and presence of T2D ($R = 0.09, R^2 = 0.01, p = 0.001$) proved to be the three most significant predictors of HR recovery for the total study population (Table 8).

5.2.2 Determinants of heart rate variability

In study III, SDNN had the closest univariate correlation with exercise capacity, fasting plasma glucose, and HbA1c level ($r = 0.40, p < 0.001$; $r = −0.32, p < 0.001$; and $r = −0.26, p < 0.001$, respectively) in the total study population (Table 7, Figure 1). In linear regression analyses, exercise capacity ($R = 0.29, R^2 = 0.08, p < 0.001$) and the presence of T2D ($R = 0.15, R^2 = 0.02, p = 0.001$) and triglycerides ($R = −0.10, R^2 = 0.01, p = 0.002$) were the most significant predictors of SDNN in CAD patients with and without T2D (Table 8).

5.2.3 Determinants of heart rate turbulence slope

In study III there was a clear positive correlation between HR turbulence slope and exercise capacity ($r = 0.22, p < 0.001$) in the total study population. Additionally, clear negative correlations were found between HR turbulence slope, age, fasting plasma glucose, and HbA1c ($r = −0.18, p < 0.001$; $r = −0.18, p < 0.001$; and $r = −0.17, p < 0.001$) (Table 7, Figure 1). When all factors correlated with HR turbulence slope were entered into a stepwise linear regression model, exercise capacity ($R = 0.12, R^2 = 0.01, p = 0.003$) and left ventricular systolic ($R = 0.18, R^2 = 0.03, p < 0.001$) and diastolic function ($R = −0.11, R^2 = 0.01, p = 0.002$) were the most significant predictors of HR turbulence slope in CAD patients with and without T2D (Table 8).
Fig. 1. Correlation between relative exercise capacity (METs) and heart rate recovery 60 s after cessation of exercise (A, n = 1060), heart rate variability (B, n = 967), and heart rate turbulence (C, n = 837) among coronary artery disease patients with and without type 2 diabetes.
Table 7. Significant correlations of heart rate recovery, heart rate variability and heart rate turbulence slope to demographic characteristics, features of type 2 diabetes, laboratory measurements and echocardiographic parameters in coronary artery disease patients with and without type 2 diabetes.

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>HR recovery (n = 1060)</th>
<th>SDNN (n = 967)</th>
<th>HR turbulence slope (n = 837)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
<td>r value</td>
</tr>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>−0.41</td>
<td>&lt;0.001</td>
<td>−0.07</td>
</tr>
<tr>
<td>BMI</td>
<td>−0.22</td>
<td>&lt;0.001</td>
<td>−0.19</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>−0.13</td>
<td>&lt;0.001</td>
<td>0.04</td>
</tr>
<tr>
<td>Exercise capacity</td>
<td>0.55</td>
<td>&lt;0.001</td>
<td>0.40</td>
</tr>
<tr>
<td>Depression score</td>
<td>−0.15</td>
<td>&lt;0.001</td>
<td>−0.17</td>
</tr>
<tr>
<td>Features of T2D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>−0.29</td>
<td>&lt;0.001</td>
<td>−0.32</td>
</tr>
<tr>
<td>HbA1c</td>
<td>−0.24</td>
<td>&lt;0.001</td>
<td>−0.26</td>
</tr>
<tr>
<td>Albuminuria</td>
<td>−0.20</td>
<td>&lt;0.001</td>
<td>−0.15</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>0.21</td>
<td>&lt;0.001</td>
<td>−0.06</td>
</tr>
<tr>
<td>Albumin-creatinine ratio</td>
<td>−0.24</td>
<td>&lt;0.001</td>
<td>−0.19</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs-CRP</td>
<td>−0.22</td>
<td>&lt;0.001</td>
<td>−0.16</td>
</tr>
<tr>
<td>Lipid profile</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.11</td>
<td>0.001</td>
<td>0.14</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.15</td>
<td>&lt;0.001</td>
<td>−0.17</td>
</tr>
<tr>
<td>Cardiac function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of AMI</td>
<td>−0.09</td>
<td>0.005</td>
<td>−0.07</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.03</td>
<td>ns</td>
<td>0.03</td>
</tr>
<tr>
<td>LVEDD</td>
<td>0.05</td>
<td>ns</td>
<td>0.14</td>
</tr>
<tr>
<td>E/E'</td>
<td>−0.29</td>
<td>&lt;0.001</td>
<td>−0.18</td>
</tr>
<tr>
<td>LVMI</td>
<td>−0.11</td>
<td>&lt;0.001</td>
<td>0.06</td>
</tr>
</tbody>
</table>

HR, heart rate; SDNN, standard deviation of all R-R intervals; BMI, body mass index; BP, blood pressure; T2D, type 2 diabetes; HbA1c, glycated hemoglobin; hs-CRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein; AMI, acute myocardial infarction; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic diameter; E/E', ratio of early transmitral flow velocity to early diastolic mitral annulus velocity; LVMI, left ventricular mass index.
Table 8. Linear regression analyses with heart rate recovery (n = 1060), SDNN (n = 967), and heart rate turbulence slope (n = 837) as the dependent variables in coronary artery disease patients with and without type 2 diabetes.

<table>
<thead>
<tr>
<th>Linear regression models</th>
<th>(Partial) R</th>
<th>(Partial) R^2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model of HR recovery</td>
<td>0.60</td>
<td>0.36</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Exercise capacity</td>
<td>0.34</td>
<td>0.12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.23</td>
<td>0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>0.09</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>-0.10</td>
<td>0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E/E'</td>
<td>-0.07</td>
<td>0.01</td>
<td>0.007</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>-0.07</td>
<td>0.01</td>
<td>0.010</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.06</td>
<td>0.01</td>
<td>0.027</td>
</tr>
<tr>
<td>Model of SDNN</td>
<td>0.48</td>
<td>0.23</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Exercise capacity</td>
<td>0.29</td>
<td>0.08</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>0.15</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.10</td>
<td>0.01</td>
<td>0.002</td>
</tr>
<tr>
<td>LVEDD</td>
<td>0.09</td>
<td>0.01</td>
<td>0.002</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>-0.09</td>
<td>0.01</td>
<td>0.005</td>
</tr>
<tr>
<td>Depression score</td>
<td>-0.07</td>
<td>0.01</td>
<td>0.018</td>
</tr>
<tr>
<td>Albuminuria</td>
<td>-0.06</td>
<td>0.01</td>
<td>0.050</td>
</tr>
<tr>
<td>Model of HR turbulence slope</td>
<td>0.38</td>
<td>0.14</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Exercise capacity</td>
<td>0.12</td>
<td>0.01</td>
<td>0.003</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.18</td>
<td>0.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E/E'</td>
<td>-0.11</td>
<td>0.01</td>
<td>0.002</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>-0.07</td>
<td>0.01</td>
<td>0.033</td>
</tr>
<tr>
<td>Depression score</td>
<td>-0.09</td>
<td>0.01</td>
<td>0.006</td>
</tr>
<tr>
<td>Age</td>
<td>-0.12</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.10</td>
<td>0.01</td>
<td>0.004</td>
</tr>
</tbody>
</table>

HR, heart rate; hs-CRP, high-sensitivity C-reactive protein; E/E', ratio of early transmitral flow velocity to early diastolic mitral annulus velocity; SDNN, standard deviation of all R-R intervals; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; HDL, high-density lipoprotein.
5.3 Cardiovascular autonomic function as a predictor of cardiovascular events in coronary artery disease patients with and without type 2 diabetes (III)

In study III a total of 127 patients (13%) reached a composite endpoint, including 16 patients (2%) with cardiovascular death, 78 (8%) with acute coronary events, 29 (3%) with strokes, and 16 (2%) patients hospitalized for heart failure during the follow-up period. In the ROC analysis, areas under the curve were 0.606 \( (p = 0.001) \), 0.552 \( (p = 0.072) \), and 0.564 \( (p = 0.020) \), respectively for HR turbulence, SDNN, and HR recovery. In univariate analyses with optimal cutoff points, both blunted HR turbulence slope (< 3.4 ms/RR), SDNN (< 110 ms), and HR recovery (< 21 bpm) were powerful predictors of a composite endpoint (hazard ratio 2.1, 95% CI 1.4–3.2, \( p < 0.001 \); hazard ratio 1.9, 95% CI 1.3–2.7, \( p = 0.001 \); hazard ratio 1.6; 95% CI 1.1–2.2, \( p = 0.012 \), respectively) in the total population. However, after multivariate adjustment, none of the autonomic markers remained predictive of a composite endpoint (Table 9).

Among CAD patients with T2D, cardiovascular endpoints occurred in 80 (15%) patients, including 10 (2%) cardiovascular deaths, 43 (8%) acute coronary events, 23 (4%) strokes, and 14 (3%) hospitalizations for heart failure. A composite endpoint was predicted by blunted HR turbulence slope, SDNN, and HR recovery in univariate analyses (hazard ratio 2.1, 95% CI 1.3–3.4, \( p = 0.003 \); hazard ratio 2.0, 95% CI 1.2–3.1, \( p = 0.005 \); hazard ratio 1.7; 95% CI 1.1–2.6, \( p = 0.020 \), respectively, Figure 2), but after multivariate adjustment hs-CRP remained as the only independent predictor of a composite endpoint (Table 9).

Among patients with CAD alone, cardiovascular complications occurred in 47 (11%) patients \( (p = 0.05 \) compared with CAD patients with T2D), including 6 (1%) cardiovascular deaths, 35 (6%) acute coronary events, 6 (1%) strokes, and 2 (1%) hospitalizations for heart failure. None of the autonomic markers predicted cardiovascular endpoints in univariate or multivariate analyses in CAD patients without T2D (Figure 2). In multivariate analyses, exercise capacity together with LVEF remained as the independent predictors of a composite endpoint (Table 9).
Table 9. Heart rate recovery, heart rate variability, and heart rate turbulence slope as predictors of a composite endpoint of cardiovascular death, acute coronary event, stroke, and hospitalization for heart failure during a 2-year follow-up in coronary artery disease patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>Univariate</th>
<th>Multivariate*</th>
<th>Significant covariates</th>
<th>Hazard ratio (CI)</th>
<th>p value</th>
<th>Hazard ratio (CI)</th>
<th>p value</th>
<th>Hazard ratio (CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Hazard ratio (CI)</td>
<td>p value</td>
<td>Hazard ratio (CI)</td>
<td>p value</td>
<td>Hazard ratio (CI)</td>
<td>p value</td>
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<tr>
<td>All patients</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>HRR &lt; 21 bpm</td>
<td>325/952</td>
<td>1.57 (1.11–2.23)</td>
<td>0.012</td>
<td>0.91 (0.59–1.41)</td>
<td>0.679</td>
<td>Exercise capacity</td>
<td>0.82 (0.70–0.96)</td>
<td>0.014</td>
<td>hs-CRP</td>
<td>1.03 (1.00–1.06)</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>HbA1c</td>
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<tr>
<td>hs-CRP</td>
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<tr>
<td>CAD patients with T2D</td>
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</tr>
<tr>
<td>HRR &lt; 21 bpm</td>
<td>239/525</td>
<td>1.69 (1.09–2.64)</td>
<td>0.020</td>
<td>1.29 (0.75–2.23)</td>
<td>0.360</td>
<td>hs-CRP</td>
<td>1.03 (1.01–1.06)</td>
<td>0.006</td>
<td>HbA1c</td>
<td>1.20 (1.03–1.40)</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>HbA1c</td>
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<td>CAD patients without T2D</td>
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</tr>
<tr>
<td>HRR &lt; 21 bpm</td>
<td>86/427</td>
<td>1.05 (0.52–2.11)</td>
<td>0.888</td>
<td>0.53 (0.23–1.20)</td>
<td>0.126</td>
<td>Exercise capacity</td>
<td>0.69 (0.54–0.89)</td>
<td>0.005</td>
<td>Ejection fraction</td>
<td>0.96 (0.93–1.00)</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>HbA1c</td>
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<td></td>
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</tr>
</tbody>
</table>

n, number of subjects whose value was below the cutoff value/number of subjects who were included in the analyses; CI, confidence intervals; HRR, heart rate recovery; SDNN, standard deviation of all R-R intervals; HRT, heart rate turbulence; hs-CRP, high-sensitivity C-reactive protein; CAD, coronary artery disease; T2D, type 2 diabetes; HbA1c, glycated hemoglobin; *Adjusted for sex, age, presence of T2D (HbA1c in CAD patients with T2D), hs-CRP, triglycerides, albuminuria, left ventricular systolic and diastolic function, and exercise capacity.
Fig. 2. Heart rate recovery (HRR) (A), heart rate variability (SDNN) (B), and heart rate turbulence slope (HRT) (C) as predictors of a composite endpoint of cardiovascular death, acute coronary event, stroke, or hospitalization for heart failure in Kaplan-Meier survival analysis. Red describes the coronary artery disease patients with type 2 diabetes (CAD, T2D+, n = 525) and blue describes the coronary artery disease patients without type 2 diabetes (CAD, T2D-, n = 427).
5.4 Effects of exercise prescription on physical activity and exercise capacity in coronary artery disease patients with and without type 2 diabetes (IV)

In study IV, six-month exercise prescriptions were introduced in 44 CAD patients and 39 CAD patients with T2D. At the baseline, total physical activity time per day was lower in the CAD patients with T2D than in the patients with CAD alone (2.7 ± 1.4 versus 3.5 ± 1.3 h/d, \( p = 0.010 \)). Divided separately into moderate- (2–5 METs) and high-intensity levels (> 5 METs), the CAD patients with T2D engaged in less moderate-intensity physical activity than the CAD patients (\( p = 0.014 \)) and they exhibited a non-significant trend to reduced high-intensity physical activity (\( p = 0.091 \)) (Table 10a, Figure 3). However, differences in physical activity variables between groups did not remain statistically significant after adjustment for BMI and exercise capacity. At the baseline the CAD patients with T2D had lower exercise capacity than the patients without T2D, but the differences between groups did not remain statistically significant after adjustment for BMI (Table 10a).
Fig. 3. Changes in total (A) and high-intensity (B) physical activity between pre- and post-prescription measurements in coronary artery disease patients with (CAD, T2D+, n = 39) and without type 2 diabetes (CAD, T2D-, n = 44). Error bars indicate mean ± SE.
Table 10a. Physical activity and maximal exercise test of the study groups before exercise prescription.

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>CAD (n = 44)</th>
<th>CAD+T2D (n = 39)</th>
<th>Adj. for BMI</th>
<th>Adj. for METsmax</th>
<th>Adj. for BMI+METsmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA at moderate intensity, h/d</td>
<td>3.4 ± 1.3</td>
<td>2.7 ± 1.4*</td>
<td>0.118</td>
<td>0.165</td>
<td>0.193</td>
</tr>
<tr>
<td>PA at high intensity, min/d</td>
<td>5.0 ± 9.3</td>
<td>2.1 ± 3.0</td>
<td>0.820</td>
<td>0.403</td>
<td>0.308</td>
</tr>
<tr>
<td>Maximal exercise test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>max workload, W</td>
<td>159 ± 47</td>
<td>142 ± 36</td>
<td>0.348</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HRmax, bpm</td>
<td>139 ± 19</td>
<td>128 ± 19*</td>
<td>0.125</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>METsmax</td>
<td>8.1 ± 2.0</td>
<td>6.5 ± 1.6**</td>
<td>0.198</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VO2max, l/min</td>
<td>2.11 ± 0.6</td>
<td>1.92 ± 0.5</td>
<td>0.181</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VO2max, ml/kg/min</td>
<td>27.2 ± 7.2</td>
<td>21.6 ± 5.7**</td>
<td>0.085</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

There were no significant changes in total or moderate-intensity physical activity in either group after exercise prescription. However, high-intensity physical activity increased in both patient groups from 2.1 ± 3.0 to 6.2 ± 10.3 min/d and from 5.0 ± 9.3 to 10.0 ± 15.1 min/d for the CAD patients with and without T2D, respectively (main effect for time \( p = 0.001 \)) (Figure 3). Maximal exercise capacity increased in both patient groups from 8.1 ± 2.0 to 8.4 ± 1.9 METs and from 6.5 ± 1.6 to 6.9 ± 1.7 METs for the CAD patients with and without T2D, respectively (main effect for time \( p < 0.001 \)), but there was no main effect for time \( \times \) group interaction in CAD patients with or without T2D (Table 10b).

Table 10b. Physical activity and maximal exercise test of the study groups after exercise prescription.

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>CAD (n = 44)</th>
<th>CAD+T2D (n = 39)</th>
<th>Time effect</th>
<th>Group effect</th>
<th>Interaction effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA at moderate intensity, h/d</td>
<td>3.7 ± 1.4</td>
<td>2.6 ± 1.1</td>
<td>0.418</td>
<td>&lt;0.001</td>
<td>0.204</td>
</tr>
<tr>
<td>PA at high intensity, min/d</td>
<td>10.0 ± 15.1</td>
<td>6.2 ± 10.3</td>
<td>0.001</td>
<td>0.048</td>
<td>0.473</td>
</tr>
<tr>
<td>Maximal exercise test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>max workload, W</td>
<td>162 ± 46†</td>
<td>151 ± 36†</td>
<td>&lt;0.001</td>
<td>0.101</td>
<td>0.041</td>
</tr>
<tr>
<td>HRmax, bpm</td>
<td>139 ± 18</td>
<td>131 ± 19</td>
<td>0.217</td>
<td>0.015</td>
<td>0.189</td>
</tr>
<tr>
<td>METsmax</td>
<td>8.4 ± 1.9</td>
<td>6.9 ± 1.7</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.193</td>
</tr>
<tr>
<td>VO2max, l/min</td>
<td>2.16 ± 0.6</td>
<td>2.04 ± 0.5†</td>
<td>&lt;0.001</td>
<td>0.190</td>
<td>0.039</td>
</tr>
<tr>
<td>VO2max, ml/kg/min</td>
<td>28.1 ± 6.8</td>
<td>23.2 ± 6.6</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.188</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease; T2D, type 2 diabetes; BMI, body mass index; METsmax, maximal exercise capacity; PA, physical activity; HR, heart rate; VO2max, peak oxygen uptake; * \( p < 0.05 \) and ** \( p < 0.01 \) between the groups without adjustments; † \( p < 0.05 \) between pre- and post-measurements.
Adherence to the tailored exercise prescription did not differ between the CAD patients with and without T2D at any condition. In both patient groups, the weekly realized TRIMP of endurance training was significantly higher than the prescription during the first 3 months (232 ± 131 vs. 146 ± 35 and 212 ± 112 vs. 141 ± 38 for CAD patients with and without T2D, respectively), but did not differ from the planned training TRIMPs during the last 3 months.
6 Discussions

6.1 Physical activity and cardiovascular autonomic function in healthy (I)

The main finding of study I was that physical activity, measured simultaneously with HR variability during waking hours, has a significant influence on HR, SD1, and the complexity properties of HR. However, long-term HR variability indexes remained relatively stable at various activity levels, which indicates that they reflect an intrinsic regulatory system that is not disturbed by physical activity. Since altered HR variability has been shown to be an independent predictor of mortality in both healthy subjects and various patient groups, the present results may provide important information for understanding and considering the contribution of physical activity that underlies the regulation of cardiac autonomic function.

Reduced HR variability during ambulatory monitoring has been shown to be associated with the occurrence of various clinical events. The results of study I indicate that individual differences in the extent of physical activity affect short-term and non-linear indexes of HR variability. Our results are in line with previous reports, which have shown that markers of cardiac sympathovagal modulation of HR, i.e., short-term HR variability indexes, not only reflect individual differences in sympathovagal regulation but also variations in daily PA patterns (Grossman et al. 2004, Grossman & Taylor 2007). Therefore, it seems important to consider concurrent monitoring of physical activity, especially when assessing vagally mediated short-term indexes of HR.

In study I we did not observe a marked relation between long-term indexes of HR variability and physical activity. Also previous studies have shown that HR variability, when analyzed in the frequency band ranging from 0.0035 to 0.1 Hz, is independent of behavioral effects, including those in usual daily physical activity (Aoyagi et al. 2003). This is an important note, since it can be suggested that long-term spectral indexes of HR variability reflect an intrinsic regulatory system that is not disturbed by PA. In this respect, long-term indexes of HR variability may be more suitable for clinical studies assessing cardiac risk from ambulatory ECG recordings. This observation is in line with a previous study showing that long-term indexes are strong predictors of mortality (Bigger et al. 1992a, Huikuri et al. 2009).
In summary, physical activity at the time of ambulatory HR monitoring has an influence on short-term HR variability indexes and the complexity properties of HR. However, long-term HR variability indexes remain relatively stable at various activity levels, making them robust indexes when HR variability is measured during free-running ambulatory conditions for prognostic purposes.

6.2 Determinants of autonomic function in coronary artery disease patients with and without type 2 diabetes (II, III)

In our studies, cardiovascular autonomic function was measured by three different methods which describe partly different aspects of autonomic regulation. The associations between autonomic function and potential determinants have also been studied earlier in various study populations. However, most previous association studies have usually used only one measure of the autonomic nervous system—either HR recovery or HR variability—whereas the determinants of HR turbulence are less well studied. Moreover, previous studies have mainly examined the association between autonomic function and only a few associated factors, whereas we have taken into account many potential determinants of autonomic function at the same time.

The results of studies II and III indicate that abnormalities in HR recovery and HR variability strongly correlate with low exercise capacity in CAD patients with and without T2D. Also previous studies have found an association between autonomic function and exercise capacity or physical fitness, especially in the healthy population (De Meersman 1993, Buchheit & Gindre 2006). Moreover, Fang et al. (2005) showed that patients with diabetes who had abnormal HR recovery 1 minute after peak exercise had a lower exercise capacity than their counterparts with normal HR recovery. Similarly, Georgoulas et al. (2003) found that patients with suspected or diagnosed ischemic heart disease and a normal value of HR recovery had higher values of exercise capacity than patients with abnormal HR recovery. However, we also found a similar association between HR turbulence and exercise capacity, which has not been reported earlier.

Not only exercise capacity and physical fitness but also physical activity and exercise training may have a positive influence on autonomic function (Buchheit et al. 2005, Carnethon et al. 2005, Buchheit & Gindre 2006). We also examined the association between HR recovery and physical activity (II), and observed that physical activity at high intensity was a strong predictor of HR recovery in CAD patients with moderate exercise capacity (on average 8 METs). Moreover, we
found that daily physical activity time was a stronger predictor of HR recovery than exercise capacity when those two variables were included in the same regression model without any other confounding variables. Similar to our results, Buchheit and Gindre (2006) found that HR recovery appears to be more closely associated with training load than with cardiorespiratory fitness. Also a previous population-based study showed that self-reported participation in physical activity is associated with faster HR recovery, so that participants who reported a high level of total physical activity had significantly faster HR recovery than those with reduced physical activity (Carnethon et al. 2005). Thus, our data support the concept that the autonomic dysfunction observed in CAD patients with or without T2D is related not only to low exercise capacity, but also to a low level of daily physical activity, which is potentially a modifiable lifestyle factor.

Autonomic dysfunction has been shown to be associated with an increased level of fasting glucose or HbA1c in the general population (Singh et al. 2000) and in patients with CAD (Gayda et al. 2011) or T2D (Fang et al. 2005). We also found in study III, as expected, a significant association between autonomic function, fasting plasma glucose, and HbA1c levels among CAD patients with and without T2D. Presence of T2D was one of the strongest predictors of HR recovery and SDNN after exercise capacity and age, but it did not predict HR turbulence. Therefore, our study confirmed the relationship between autonomic dysfunction, increased glucose level, and presence of T2D also among patients with stable CAD.

The results of study II showed that blunted HR recovery was more common among CAD patients with T2D than in those without, and similar to our results, Nonaka et al. (2007) demonstrated that abnormal HR recovery is associated with T2D in patients with suspected CAD. However, there were no significant differences in HR recovery between the patients with and without T2D after adjustment for physical activity, exercise capacity, and BMI. Based on the results of study II, with a limited number of patients, we may assume that although impaired HR recovery is related to the presence of T2D, reduced exercise capacity and daily physical activity are even stronger determinants of autonomic dysfunction than the duration or metabolic features of T2D, including glucose control.

We did not find a correlation between HR recovery and angiographic severity measured by the Syntax score (II) and we observed only a weak correlation between a history of MI and all three measurements of autonomic function (III). Previous studies have suggested that autonomic dysfunction in CAD patients is
associated with the angiographic severity and degree of coronary occlusion, but not with a history of a previous MI (Hayano et al. 1990, Ghaffari et al. 2011). In our study population, left ventricular diastolic function correlated weakly with all three measures of autonomic function, whereas systolic function correlated only with HR turbulence. The previous study results regarding the association between left ventricular function and autonomic markers have been variable. Szydlo et al. (1996) found an association between lower HR variability and left ventricular systolic dysfunction, which also seems to be the key factor influencing HR turbulence parameters in CAD patients (Cygankiewicz et al. 2003). However, some recent studies did not find significant correlations between decreased HR recovery and left ventricular systolic dysfunction (Nonaka et al. 2007, Ghaffari et al. 2011). The findings of our studies suggest that the presence of a previous MI, the severity of CAD, and abnormalities in cardiac function are not as strong predictors of autonomic function as exercise capacity, daily physical activity habits, and presence of T2D in patients with CAD.

In our studies a combination of various clinical, demographic, and echocardiographic risk variables explained from 14% to 36% of the cardiovascular autonomic function assessed by either HR recovery, HR variability, or HR turbulence. It has been investigated previously that also genetic and environmental factors, which were not taken into account in our analysis, may contribute to cardiovascular autonomic regulation, as well. According to family and twin studies, HR variability measures are highly heritable traits and estimates of heritability have ranged from 13% to 65% (Boomsma et al. 1990, Snieder et al. 1997, Busjahn et al. 1998, Sinnreich et al. 1999, Singh et al. 2001, Kupper et al. 2004, Snieder et al. 2007, Uusitalo et al. 2007). Moreover, it seems that psychosocial stressors such as work stress, depression, and anxiety are negatively associated with HR variability (Gorman & Sloan 2000, Jarczok et al. 2013).

In conclusion, studies II and III showed that cardiovascular autonomic function in stable CAD patients is associated with several variables, including exercise capacity, physical activity, age, presence of T2D, and left ventricular systolic function. In multivariate models, low exercise capacity is the most important determinant of autonomic dysfunction assessed by either HR recovery, HR variability, or HR turbulence.
Prognostic value of cardiovascular autonomic function in coronary artery disease patients with and without type 2 diabetes (III)

In study III the univariate analysis showed that autonomic function assessed by either HR recovery, HR variability, or HR turbulence predicted cardiovascular complications in CAD patients with T2D, but not in their counterparts without T2D. This emphasizes the role of autonomic markers as predictors of cardiovascular events, especially in patients with T2D and stable CAD. Also previous studies have indicated that cardiovascular autonomic function is an independent prognostic indicator for cardiovascular events as well as for cardiac and all-cause mortality. This negative predictive value has been found among the healthy population, but also in patients with CAD or T2D and survivors of an AMI (Cole et al. 1999, Schmidt et al. 1999, Cheng et al. 2003, Nissinen et al. 2003, Jouven et al. 2005, Mäkikallio et al. 2005, Chacko et al. 2008, Georgoulias et al. 2009, Huikuri et al. 2009, Huikuri et al. 2010, Barthel et al. 2011, Gayda et al. 2012). Moreover, our results indicated that HR turbulence is a somewhat better predictor of cardiovascular events than HR recovery or SDNN. This is in agreement with a previous study of a post-infarction population, which showed that SDNN failed to provide independent prognostic information about sudden cardiac death, whereas HR turbulence has a strong predictive value (Mäkikallio et al. 2005).

Although autonomic dysfunction predicted short-term cardiovascular events among CAD patients with T2D, it did not provide independent short-term prognostic information after adjusting for clinical, demographic, and echocardiographic risk variables. Also in previous studies of patients surviving an AMI, the prognostic value of autonomic function weakened after adjusting by traditional cardiovascular risk factors (Bigger et al. 1992b, Schmidt et al. 1999, Huikuri et al. 2000, Barthel et al. 2003, Barthel et al. 2011). In our study, only a marker of low-grade inflammation remained as the independent prognostic marker in CAD patients with T2D. Biasucci et al. (2009) have investigated a prognostic role for hs-CRP in patients with acute coronary syndrome during a 1-year follow-up. Interestingly, their findings were opposite to the results of our study, which suggested that hs-CRP is strongly associated with death and AMI, especially in patients without T2D, but not in patients with T2D. However, Biasucci et al. (2009) had enrolled a relatively small number of patients with unstable angina (n = 251), and hard events were observed in only 7 patients with
T2D, whereas we report the prognostic power of hs-CRP for cardiovascular events in a larger population with T2D and stable CAD. The definite role of hs-CRP in risk stratification of CAD patients with T2D needs further investigation.

In our study population, autonomic function did not have short-term prognostic value in patients with CAD alone. In multivariate analyses exercise capacity together with left ventricular systolic function remained as independent prognostic markers in that patient group. The association between low exercise capacity or cardiopulmonary fitness and increased risk of all-cause and cardiovascular mortality in CAD patients has been established also in previous studies (Vanhees et al. 1994, Martin et al. 2013).

In study III we concentrated on studying the short-term prognostic value of autonomic markers in patients with a stable CAD, whereas previous studies have mainly focused on long-term prognostic value for post-infarction patients. It can be speculated that risk profiles and their predictive value may change over time, especially after an acute coronary event. Recent studies have shown that autonomic function measured late after an AMI predicts mortality better than measurements performed in the early post-infarction phase (Exner et al. 2007, Huikuri et al. 2010). Therefore, analysis of factors predicting cardiovascular complications after stabilization of an acute coronary event may in fact provide more relevant clinical information.

CAD patients with blunted autonomic function are often considered to have an overall poor prognosis, but the reasons for this risk may be unclear. As study II and III showed, autonomic dysfunction is associated with many factors and co-morbidities, which may suggest that future unfavorable outcomes are related to such co-morbid conditions, not only the blunted autonomic function. The results of study III strengthen the role of inflammation and physical fitness in the prevention of future cardiovascular events in stable CAD patients with and without T2D.

6.4 Effects of exercise prescription on physical activity and exercise capacity in coronary artery disease patients with and without type 2 diabetes (IV)

Study IV showed that objectively measured daily physical activity time is lower in CAD patients with T2D than in patients with CAD alone. This difference is largely a result of obesity, i.e., a higher BMI in patients with diabetes than in their non-diabetic counterparts. In agreement with our observations, a previous study
has shown that the prevalence of achieving the recommended physical activity level is significantly lower among CAD patients with T2D compared with their counterparts without T2D (Zhao et al. 2008). In the present study, the average total physical activity time per day was almost 50 min less in CAD patients with T2D than in patients with CAD alone.

The recommendation for moderate-intensity aerobic physical activity is at least 30 minutes per day in patients with CAD or T2D (Piepoli et al. 2010). If physical activity at moderate intensity is defined as ≥ 3 METs (Physical Activity Guidelines Advisory Committee 2008), only 22% of the patients in study IV engaged in the recommended amount of moderate-intensity physical activity daily. In addition, the recommendation for vigorous aerobic exercise is at least 90 minutes per week in patients with T2D (Buse et al. 2007, Piepoli et al. 2010), whereas patients with stable CAD are recommended to exercise at the intensity of brisk walking or higher 20–60 minutes daily or at least 3–5 days per week (Balady et al. 2007). In study IV, the average physical activity time per day at high intensity (> 5 METs) was approximately 2 and 5 min for CAD patients with and without T2D, respectively, which is very low and far from the recommendations in both patient groups.

Previous studies have showed that in patients with T2D, older age, female gender, a higher level of perceived disability, obesity, and CAD are associated with lower physical activity levels and likelihood of meeting physical activity recommendations (Plotnikoff et al. 2006, Zhao et al. 2011). Moreover, many patients in our study described a fear of exercise after a cardiac event and they had decreased physical activity at high intensity, in particular, because of a fear of cardiac complications. In patients with a lower exercise capacity, an activity requiring a high-intensity physical activity level required almost maximal effort, which may feel inconvenient. Thus, physically unfit individuals find it more difficult to be physically active. Our results suggest that it is necessary to develop more intensive physical activity counseling, especially for CAD patients with T2D and other co-morbidities such as obesity and low physical fitness. Practice related to physical activity promotion for patients with CAD and T2D should better take into account the specific psychological factors associated with physical activity, such as a lack of motivation and a fear of cardiac complications during exercise training.

In study IV, a carefully tailored, controlled 6-month exercise prescription based on maximal exercise tests and individual HR zones increased particularly high-intensity physical activity among CAD patients with and without T2D.
Similarly, increased levels of high-intensity physical activity have been observed during a 3-month home-based cardiac rehabilitation program (Blanchard et al. 2010). Previous studies have shown that both low- and high-intensity exercise training improve physical fitness in sedentary healthy men and in men with CAD. However, it seems that high-intensity exercise is more effective in increasing cardiorespiratory fitness and exercise capacity than low-intensity exercise, even regardless of equal energy cost (Oberman et al. 1995, O’Donovan et al. 2005). In patients with diabetes, regular exercise training has a clinically significant effect on cardiorespiratory fitness and HbA1c levels, but higher-intensity exercise training seems to produce greater improvement (Boule et al. 2003). Moreover, moderate- and high-intensity physical activities are associated with a lower incidence of acute coronary events, whereas low-intensity physical activity has no significant association with development of acute coronary events in patients with T2D (Makrilakis et al. 2004). Therefore, our findings that CAD patients with and without T2D increased their high-intensity physical activity by at least 50% during exercise prescription can be considered clinically relevant. However, changes in high-intensity physical activity were rather small when expressed as actual minutes. This indicates that promotion of physically active lifestyle among patients with CAD and T2D is challenging and there is a need to develop a more intensive physical activity counseling strategy for cardiac and diabetic patients.

Our study showed that similar to physical activity level, also exercise capacity was lower in CAD patients with T2D than in their counterparts without diabetes. A recent systematic review and meta-analysis has concluded that an over-six-month exercise intervention started soon after a cardiac event is most effective in improving maximal oxygen uptake in patients with CAD (Valkeinen et al. 2010). In our study, cardiorespiratory fitness, defined as exercise capacity and maximal oxygen uptake, improved significantly among both patient groups during the six-month exercise prescription. However, on average, the improvement in maximal oxygen uptake was smaller (1 ml/kg/min) than in previous studies (~2 ml/kg/min) (Valkeinen et al. 2010) even though the CAD patients exercised according to current guidelines. This is may be partly explained by the differences between the baseline exercise capacities of the CAD patients in the present study (27 ml/kg/min) and studies in meta-analysis (~20 ml/kg/min) (Valkeinen et al. 2010). It has been shown that low baseline fitness results in higher improvement in exercise capacity after exercise intervention compared with high-fitness subjects at the baseline (Hautala et al. 2006).
Several studies have demonstrated that, among patients with CAD or cardiovascular disease, both a relatively high degree of exercise capacity at the baseline and an improvement in exercise capacity over time produce marked reductions in the risk of mortality (Myers et al. 2002, Kokkinos et al. 2008, Martin et al. 2013). It seems that improvement in exercise capacity is associated with decreased mortality most strongly in patients whose baseline fitness is less than 5 METs (Martin et al. 2013). In our study, CAD patients had high fitness (on average 8.1 METs) and CAD patients with T2D had moderate fitness (on average 6.5 METs) at the baseline when compared to the classification created by Martin et al. (2013). The average improvement in exercise capacity was 0.4 and 0.3 METs in CAD patients with and without T2D, whereas every 1 MET increase in exercise capacity would be associated with about a 10% and 16% reduction in mortality risk among patients with cardiovascular disease and T2D, respectively (Myers et al. 2002, Kokkinos et al. 2008, Kokkinos et al. 2009).

6.5 Study limitations

There are a few limitations in the present studies that have to be taken into account when evaluating the overall results. First, a high number of patients were excluded from the ARTEMIS exercise prescription sub-study (II, IV) due to advanced age or serious co-morbidities. The exercise prescription was home-based and rather intensive, and it excluded patients who may not be able to perform the prescription because of contraindication for high-intensity exercise, for example, musculoskeletal problems and severe chest pain or arrhythmias during or after exercise. Moreover, about 30% of the patients were not willing to participate in the exercise training prescription even though they met the inclusion criteria. Typically, patients who have significant obesity and very low exercise capacity, for example, or with other serious co-morbidities, are not willing to participate in exercise interventions. There may also be a lack of motivation or a fear of exercise after a cardiac event. In our study population, patients who refused to participate in the exercise intervention had a lower exercise capacity than the participants and they described unwillingness and a lack of motivation to take part in a long-term intervention. In the sub-study population, the mean exercise capacity was above 7 METs, whereas in the entire ARTEMIS population it was 6 METs. Heart rate recovery was also higher in the sub-study patient sample compared with the whole ARTEMIS population (37 vs. 26 bpm). These findings and other health measures tell something about the differences between
the sub-study population and the total study population. Therefore, the patient sample in the exercise prescription sub-study was partly selected and may not be representative of the whole CAD population.

Secondly, in the exercise prescription sub-study (IV), physical activity was measured as time spent at different absolute intensity levels, expressed as moderate (2–5 METs) and high intensity (> 5 METs). Our physical activity measurements did not take into account variations in the subjects’ individual fitness levels. An activity requiring a high-intensity physical activity level may require almost maximal effort among patients with a lower fitness level. Moreover, the clinical significance of our study would be greater if we could have measured physical activity objectively also in the total, unselected study population of 1060 CAD patients with and without T2D. However, exercise capacity, which is to some extent determined by physical activity levels over the past months, was measured in the total study population. Generally, regular physical activity produces increases in physical fitness, although the genetic component determines the magnitude of adaption in fitness to the exercise dose (Blair et al. 2001). Since it is difficult to develop or maintain a level of physical fitness that is consistent with good health without appropriate physical activity, physical fitness and physical activity are quite often used interchangeably (Blair et al. 2001, Warburton et al. 2006). Physical fitness even seems to be the clinically more important predictor of cardiovascular disease and mortality than physical activity (Williams 2001, Lee et al. 2011). Therefore, we can assume that CAD patients with lower exercise capacity also have lower levels of daily physical activity in the total study population.

In study III we took into account many potential associated factors of autonomic function at the same time, but still there are some missing factors which deserve some comments. First, in the statistical analyses T2D was treated as a group variable and we did not consider the duration of the disease. The newly diagnosed patients with T2D and patients with advanced stages of the disease were treated equally, although autonomic dysfunction has been described to be progressive and patients with advanced stages of the disease may also have other diabetic complications. Moreover, in study II the severity of CAD was assessed by measurement of the Syntax score from coronary angiography, but unfortunately the data from all the patients were not available for the statistical analysis of study III. However, in the analysis of the sub-group of ARTEMIS patients, neither the duration of T2D nor the severity of CAD were associated with autonomic function, measured by HR recovery.
The prognostic part of study III was limited by a relatively short follow-up and a mixture of various endpoints. The low number of endpoints in the CAD patients without T2D limits the generalizability of the results in terms of the negative predictive value of the autonomic markers. Similarly, the negative predictive value of these markers in multivariate analysis in patients with T2D may be partly due to the small sample size and the short follow-up. However, we wanted specifically to study the short-term prognostic values of risk markers which could provide insight into the therapeutic targets of the well-defined population of CAD patients who had recently undergone coronary angiography and most of who were treated with a recent coronary intervention.

Finally, the measurements in studies II–IV were performed under continued prescribed medication, for ethical reasons and because of the well-known withdrawal effect of beta-blocker cessation. Moreover, we did not control respiratory activity during the ambulatory measurements in studies I and III. Indeed, short-term HR variability indexes are associated with physical activity due to the fact that during low to moderate physical activity, the respiratory pattern changes not only in terms of frequency but also in terms of volume. Obviously, controlling breathing rate might have given additional information, since ambulatory respiratory sinus arrhythmia magnitude is associated with physical activity and vagally mediated indexes of HR variability (Grossman et al. 2004). It is clear, however, that the results will have more practical implications if the study setups mimic ambulatory ECG measurement outside the clinic and if the analyses are performed at a time when the patients are on their normal medication.
7 Conclusions

The following conclusions can be drawn on the basis of the results discussed previously:

1. Physical activity at the time of ambulatory HR monitoring has an influence on short-term HR variability indexes and the complexity properties of HR. However, long-term HR variability indexes remain relatively stable at various activity levels, making them robust indexes when HR variability is measured during free-running ambulatory conditions for prognostic purposes.

2. Cardiovascular autonomic function in stable CAD patients is associated with several variables, including exercise capacity, physical activity, age, presence of T2D, and left ventricular systolic function. Of these factors, low exercise capacity and physical activity are the most important determinants of autonomic dysfunction assessed by either HR recovery, HR variability, or HR turbulence in multivariate models.

3. Cardiovascular autonomic dysfunction predicts short-term cardiovascular events among CAD patients with T2D, but not in their counterparts without T2D. However, autonomic dysfunction does not provide independent short-term prognostic information after adjusting for clinical, demographic, and echocardiographic risk variables. This strengthens the role of inflammation and physical fitness in the prevention of future cardiovascular events in stable CAD patients with and without T2D.

4. CAD patients with T2D are physically less active than CAD patients without diabetes in their daily life. Individually tailored home-based exercise prescriptions are an effective way to promote more active lifestyles and improve exercise capacity in both patient groups.
References


Original publications


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CARDIOVASCULAR AUTONOMIC FUNCTION IN CORONARY ARTERY DISEASE PATIENTS WITH AND WITHOUT TYPE 2 DIABETES

SIGNIFICANCE OF PHYSICAL ACTIVITY AND EXERCISE CAPACITY.