The effect of physical exercise and resting heart rate on cardiovascular risk

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The effect of physical exercise and resting heart rate on cardiovascular risk

Het effect van lichamelijke inspanning en hartfrequentie in rust op cardiovasculair risico
(met een samenvatting in het Nederlands)

Proefschrift

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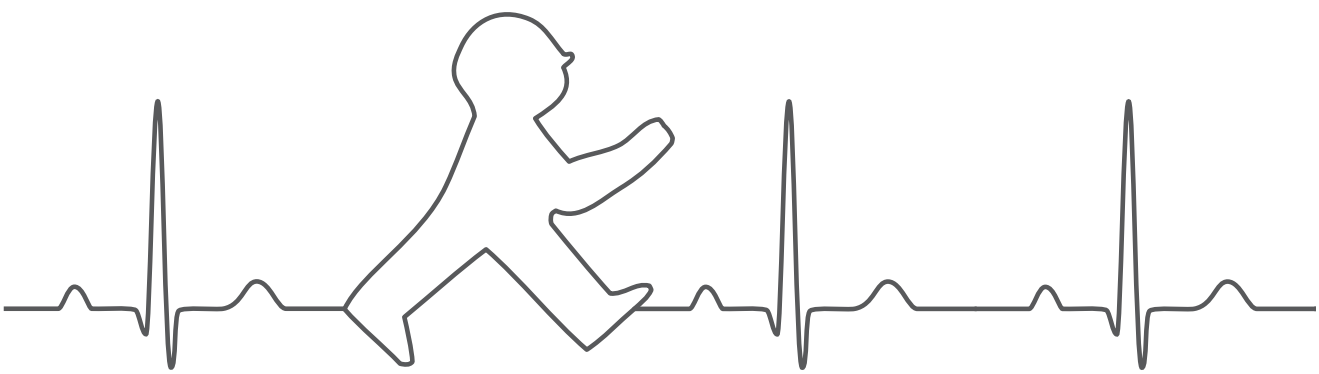
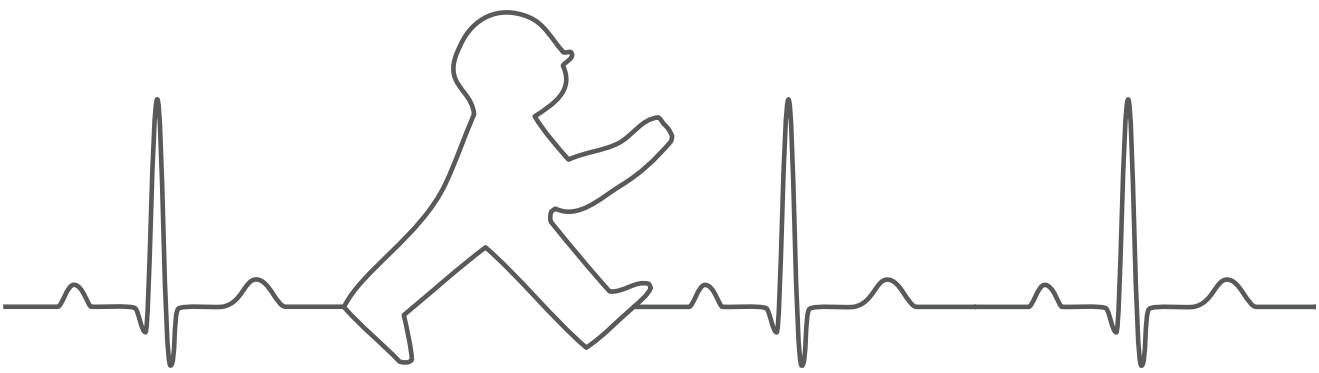
Voor Elvira
Voor Marcus

Contents



Chapter 1

General introduction



Since 1900, atherosclerotic cardiovascular disease (CVD) has been the leading cause of mortality in the United States every year but 1918 (1). In Europe, 4.3 million deaths per year, nearly half of all deaths annually, are caused by CVD (2). Moreover, globally, low and middle income countries contribute about 80% of all CVD-related deaths, and tripling of ischemic heart disease- and stroke mortality is expected in the next two decades in these countries (1;3). Besides causing premature mortality, this worldwide epidemic of CVD constitutes also an enormous burden of human morbidity, with huge social and economic costs to society (2).

Atherosclerosis

Atherosclerosis is currently perceived as a systemic disease, with simultaneous presence in multiple locations in the vascular tree (4), leading to slowly progressive plaque formation in the arterial wall, and narrowing of the lumen (5). Luminal narrowing causes chronic ischemic diseases such as intermittent claudication and angina pectoris, while plaque rupture with thrombosis causes acute ischemic diseases such as myocardial or cerebral infarction (5). Atherosclerotic disease is characterised by endothelial dysfunction, subendothelial lipid accumulation and a state of low-grade systemic inflammation (5).

Atherosclerotic risk factors and the metabolic syndrome

The traditional risk factors hypertension, hypercholesterolaemia, diabetes, obesity and tobacco use contribute to the bulk of the risk for developing atherosclerotic events, in both men and women and at all ages (3). These risk factors cluster in a condition often referred to as the metabolic syndrome, a constellation of metabolic abnormalities including abdominal obesity, insulin resistance, atherogenic dyslipidaemia, elevated blood pressure, a prothrombotic profile and low-grade inflammation (6;7). The prevalence of the metabolic syndrome has been increasing over the past decades and is now estimated to affect at least 25% of the US population (8;9) and about 50% of patients with clinically manifest vascular disease in the Netherlands (10). Crucial in the concept of the metabolic syndrome is the replacement of the classical perception of visceral adipose tissue as storage depot of fatty acids by the notion that visceral adipose tissue produces a large number of hormones and cytokines, e.g. tumor necrosis factor-α, interleukin-6, adiponectin, leptin and plasminogen activator inhibitor-1 (11). As visceral adipose tissue expands, macrophages infiltrate adipose tissue and the production of adipocytokines involved in glucose and lipid metabolism, haemostasis and inflammation increases, except for the production of adiponectin, an adipokine with beneficial effects on atherosclerosis and insulin resistance, which decreases (11). Subjects with the metabolic syndrome have a 3-8 times increased risk for the development of type 2 diabetes mellitus (12;13),

a 2-3 times increased risk for the development of cardiovascular disease (7;14;15) and a 2-3 times increased risk for premature all-cause and cardiovascular mortality (14). Increasing physical activity and weight reduction are two important lifestyle changes that independently reduce visceral obesity and insulin resistance and therefore play a central role in modifying adipose tissue dysfunction. Increasing physical activity, together with other lifestyle measures such as weight loss, is therefore widely advocated in guidelines as a first step in the treatment and prevention of cardiovascular disease (16-19).

Beneficial effects of physical exercise

Physical exercise has, just like weight loss, beneficial effects on virtually all aspects of cardiovascular disease (*Figure 1*). Exercise has an inverse dose-response relation with premature all-cause mortality (20). Besides beneficial effects on non-cardiovascular diseases such as depression (21) and osteoporosis (22), exercise also reduces the risk for cardiovascular disease and type 2 diabetes (23-27). Exercise training has shown to cause improvements in early measures of atherosclerotic disease, such as carotid intima media thickness (28) and flow mediated dilatation (29-33), a method of measuring endothelial function. These beneficial effects of exercise on early measures of atherosclerosis are caused by improvements of almost all classical cardiovascular risk factors. Intentional weight loss resulting from increased energy expenditure by physical exercise reduces the risk for cardiovascular mortality and morbidity; a 10 kg intentional weight loss was associated with a 33% decrease in all-cause mortality during 9-12 years of follow-up (34;35). But there are more advantageous effects of exercise independent of weight-loss. Exercise decreases blood pressure by lowering total peripheral vascular resistance as a consequence of decreased sympathetic nerve activity and improved insulin sensitivity (36). Insulin sensitivity is improved not only through weight loss, but also through an increase in GLUT4 glucose transporters, particularly in skeletal muscle (36;37). Increased skeletal muscle mass and capillary proliferation further improve insulin sensitivity (38). Serum triglycerides are decreased by up-regulated lipoprotein lipase activity as a result of exercise (39;40), predominantly in skeletal muscle and adipose tissue (41). Exercise increases HDL-cholesterol (HDL-c) by delaying particle clearance, increasing the production of nascent HDL particles and a higher cholesterol content per particle (39;40;42). LDL-cholesterol (LDL-c) plasma levels are typically unaffected by exercise; if decreases are reported, they are mostly caused by high intensity- or resistance exercise (39;43-46).

Besides all these well documented beneficial effects of exercise on cardiovascular risk factors, exercise may have even more beneficial effects which are still not completely unravelled, such as effects on atherosclerotic plaque stabilisation and reduction of systemic inflammation (47).

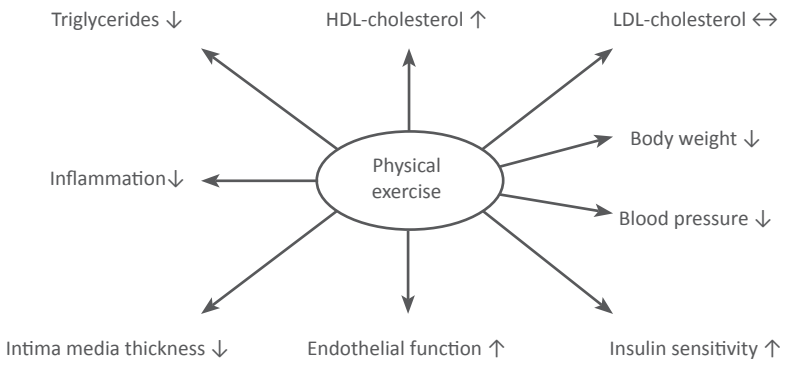


Figure 1. Beneficial effects of physical exercise on cardiovascular risk factors.

Exercise in a natural environment

Studies often focus on exercise under artificial conditions in training centres, using treadmills or ergometers, exactly controlling exercise intensity and duration (36;37;39;42;43). Studies investigating exercise in a natural environment, such as walking, cycling, running a marathon (40;48), or climbing the Mount Everest (49), are rarely published. Walking is one of the most accessible forms of physical exercise, and is, together with gardening, the major component of leisure time physical activity, often conducted in a natural environment (50). Walking parts of a pilgrim route, an ancient tradition, is increasingly popular. People from various cultural and religious backgrounds walk parts of pilgrim routes at various places in the world. It is estimated that over five million people walk pilgrim routes every year. For example, in 1984, 2,491 people registered arrival on foot in Santiago de Compostela, Spain, and this number increased to almost 150,000 in 2009 (51).

The effects of exercise intensity, specifically walking speed

Medical guidelines recommend a minimum weekly physical activity equal to 150 minutes of brisk walking, however, it is not specified at what intensity this exercise should be preferably conducted (16-18). Brisk walking or shorter periods of exercise at higher intensity (for example running) are considered equally effective for the treatment and prevention of cardiovascular disease (16-18). Studies evaluating the effects of exercise intensity on cardiovascular risk factors show conflicting results. Well-designed trials report no differences between various intensities of exercise and conclude that the total amount of exercise is more important than the exercise intensity (52-55). Other studies conclude that exercise at higher intensity results in more beneficial changes cardiovascular

risk factors compared to exercise at lower intensity (56-60). For example, high intensity aerobic exercise led to a decrease in diastolic blood pressure (-2.53 mmHg; 95% confidence interval (95%CI) -4.65 to -0.41) and weight (-0.88 kg; 95%CI -1.65 to -0.11) compared to low intensity aerobic exercise (58). Walking at high intensity/high frequency led to a significant increase in HDL-c (1.83±6.11 mg/dl) while walking at moderate intensity/high frequency did not (increase in HDl-c 0.54±6.43 mg/dl) (57). However, not all studies adequately control for differences in the total amount of exercise (57;58). Increased walking speed, an easy-to-measure parameter to express exercise intensity, has been related to a decreased risk for cardiovascular disease and diabetes (61-64). For example, women rating their walking speed as "brisk" had a relative risk of 0.44 (95%CI 0.33-0.52) for development of type 2 diabetes compared to subjects walking at an "easy" pace (61). Women walking at a self-estimated speed of 2-3, or >4 miles/hour had a relative risk of 0.86 and 0.58 respectively for developing a cardiovascular event compared to the reference category of women who never walked (relative risk =1) (63). In these studies, walking speed was not measured, but assessed by questionnaires, and these studies did not evaluate the effects of walking speed on cardiovascular risk factors.

Exercise in the treatment of the metabolic syndrome

Increasing physical activity constitutes, together with dietary changes to induce weight loss, the most important pillar in lifestyle modification programs, promoted by leading health organisations as the first-line strategy for the management of the metabolic syndrome (19;65;66). Lifestyle modification programs have shown to improve multiple metabolic risk factors and even resolution of the metabolic syndrome (67-70). The combination of diet and exercise interventions has shown to be more effective than either approach alone for the treatment of the metabolic syndrome (71). Despite healthy lifestyle advices routinely given to persons with diabetes or the metabolic syndrome, the majority of patients remain poorly controlled regarding the treatment targets of HbA1C, LDL-c and blood pressure and exhibit low adherence to diet and physical activity recommendations (72-74). Identification of patients with a higher chance on positive results of these lifestyle programs, before starting, would be of great advantage. Possibly, the amount of daily physical activity could help identifying these patients, as it can be hypothesized that patients with a higher baseline physical activity are more likely to increase their level of exercise resulting in better outcomes of a lifestyle modification program, as physical activity is already part of their daily behaviour. On the other hand, it can be argued that patients with a low baseline physical activity could more easily increase their daily physical activity level and profit more from the beneficial effects of it.

Physical exercise and autonomic function

During physical exercise, parasympathetic nerve activity is decreased and sympathetic nerve activity is increased, resulting in an increase in heart rate (75). During the period of recovery after exercise, autonomic tone and corresponding heart rate gradually return to the previous resting level, due to a combination of sympathetic withdrawal and parasympathetic reactivation (75). Endurance exercise results in an increase in heart rate variability, and as the ratio between parasympathetic and sympathetic tone is decreased the resting heart rate is also decreased (76;77).

Sympathetic nerve activation in cardiovascular disease and obesity

Increased sympathetic nerve activity is an important characteristic feature in patients with cardiovascular disease, such as heart failure, myocardial infarction or cerebrovascular disease, and in patients with cardiovascular risk factors such as diabetes mellitus or hypertension (78-81). Also in patients with the metabolic syndrome (82) or obesity, the sympathetic nerve system is activated, which is more pronounced in abdominal obesity than in peripheral obesity (83). The association between increased sympathetic nerve activity and obesity and insulin resistance is strong, however, the pathophysiologic relation is complex and not completely unravelled. Increased sympathetic nerve activity can be both the cause and the consequence of obesity and insulin resistance.

The relation between obesity, insulin resistance and sympathetic nerve activity

Abdominal obesity may have direct influence on sympathetic nerve activity, as abdominal obesity is associated with high serum levels of both insulin and leptin (84). The administration of insulin in humans increases muscle sympathetic nerve activity (MSNA), the reference standard of measuring sympathetic activity (85), by a direct effect on insulin-receptors in the central nervous system (86;87). Plasma leptin levels are independently associated with resting heart rate (RHR) in male subjects (88;89). In rats, the infusion of leptin increases the heart rate, via stimulation of leptin-receptors in the central nervous system (90;91).

There are also arguments that the causal relation between sympathetic nerve activity and insulin resistance could be the other way around. Sympathetic nerve activation is considered to play an important role in the pathogenesis of cardiovascular disease by increasing major risk factors such as blood pressure, insulin resistance and triglycerides (79;92;93). Acute reflex activation of the sympathetic nerve activation is reported to induce acute insulin resistance in humans (93). Chronically increased sympathetic nerve activity can precede the development of insulin resistance and obesity (94;95). RHR reflects sympathetic tone, and correlates with MSNA and noradrenaline serum levels (96). Increased

RHR at baseline is independently associated with an increased risk for developing type 2 diabetes in the healthy population (97-100) and in patients with obesity or impaired glucose tolerance (101), ranging from 10-46% increase per 1 standard deviation increase in RHR (7-12 beats/minute).

Resting heart rate

Among mammals, the total number of heart beats per lifetime is remarkably constant, despite wide variations in body size and RHR (102). This implies, that the RHR, which is determined by the ratio between body volume (generating heat) and body surface (heat loss), is strongly related to life expectancy (102). The only exception among mammals is the human species; it can be speculated that modern humans have stretched the boundaries of biology to achieve a life expectancy of 80 years through improvements in living conditions such as a better hygiene, safeguarding of clean water and food supply, secure accommodation, the prevention and treatment of diseases and many more (102). However, also in humans, RHR is related to mortality; an increase in RHR of 10 beats/minute is associated with around 10-30% increased risk for all-cause mortality (102-111).

RHR is easily obtained, noninvasive and nonexpensive. Besides being a reflection of sympathetic tone, elevated RHR is also a reflection of severe disease, such as heart failure. Finally, resting heart rate is an independent risk factor for cardiovascular disease and mortality. An increase in resting heart rate of 10 beats/minute is related to a 10-30% increased risk for cardiovascular events and mortality in the healthy population (104-108;112), in patients with cardiovascular risk factors such as diabetes (110) or hypertension (109) and in patients with coronary artery disease (*Figure 2*) (103;111).

Direct adverse effects of an increased RHR on cardiac and vascular function and morphology include endothelial dysfunction, direct stimulation of atherogenesis and atherosclerotic plaque rupture, increased susceptibility for ventricular arrhythmias and a negative influence on the balance between myocardial oxygen demand and supply (113;114).

Heart rate reduction, using beta-blockers or non-dihydropyridine calcium channel blockers, reduces the risk for all-cause and cardiac mortality with about 30% per 10 beats/minute heart rate reduction in patients with a recent myocardial infarction, although these agents have more mechanisms of action such as lowering blood pressure (115). Recently, ivabradine, a pure heart rate-lowering agent without other known effects on the cardiovascular system, has shown to reduce the risk for hospital admission for fatal and non-fatal myocardial infarction (hazard ratio (HR) 0.64; 95%CI 0.49-0.84) in patients with coronary artery disease (116) and to reduce the risk for cardiovascular death or hospital admission for worsening heart failure (HR 0.82; 95%CI 0.75-0.90) in patients with chronic heart failure on top

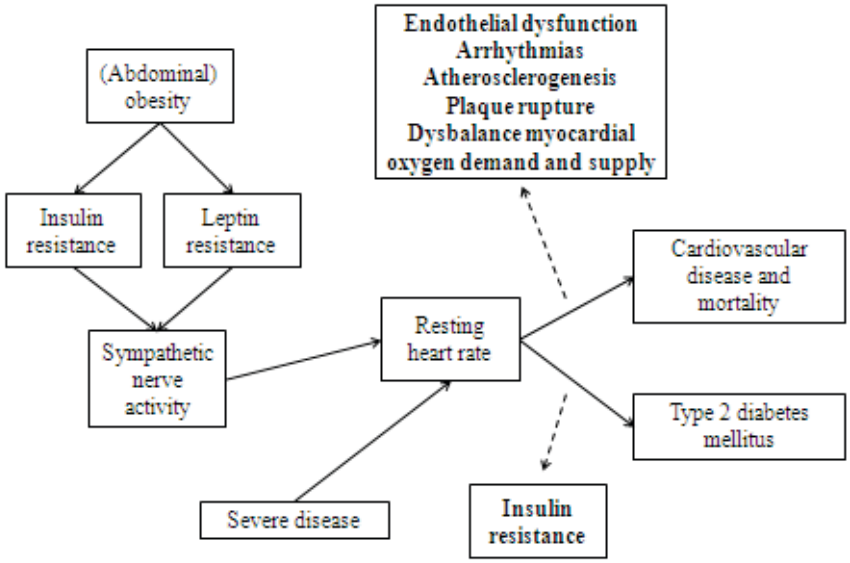


Figure 2. Relation between obesity, resting heart rate, cardiovascular disease and diabetes.

of β blocker therapy (117). This finding further emphasizes the pathophysiologic role of elevated RHR in the occurence of cardiovascular mortality and morbidity (116-118).

Visceral adipose tissue and RHR

As discussed above, visceral adipose tissue is the predominant adipose tissue compartment producing various proinflammatory cytokines and adipokines (119). Therefore, visceral adipose tissue is considered to play a central role in the state of low-grade inflammation and clustering of metabolic disturbances seen in the metabolic syndrome (11). The presence of the metabolic syndrome is associated with an elevated RHR, however, in it unclear what the origin of the elevated RHR is. It can be hypothesized that it is specifically visceral adipose tissue which activates the sympathetic nerve system, via high insulin and leptin serum levels.

Elevated RHR and cardiovascular events and death in patients with vascular diseases.

The independent relation between elevated RHR and cardiovascular events and death and the beneficial effects of reducing heart rate are predominantly based on studies in patients with coronary artery disease. As RHR is a parameter reflecting heart function, and adverse effects of RHR include an increased susceptibility to cardiac events such as ventricular arrhythmias, the adverse pathophysiologic

effects of increased RHR could be predominantly present in patients with coronary artery disease. However, as elevated sympathetic nerve activity is also present in patients with vascular diseases at other locations and other adverse effects of RHR such as atherosclerotic plaque rupture and endothelial dysfunction are systemic, it can be hypothesized that the detrimental effects of an elevated RHR could affect patients with clinically manifest vascular diseases irrespective of the locations of vascular disease.

Elevated RHR and type 2 diabetes

Elevated RHR is independently associated with a 10-46% increased risk per standard deviation increase in RHR (7-12 beats/minute) for developing type 2 diabetes mellitus in healthy populations (97-100;120) and in subjects with obesity or impaired glucose tolerance (101), but this has not been demonstrated in patients with vascular diseases. As diabetes is a strong risk factor for cardiovascular morbidity and mortality (121;122), and patients with diabetes have a reduced quality of life (123), it is important to identify patients at high risk for diabetes. Patients with clinically manifest vascular diseases are at a particular high risk of developing new vascular events and type 2 diabetes, around 15% and 5% respectively per 5 years of follow-up (124;125), given shared pathophysiological pathways (the 'common soil' hypothesis) of diabetes and atherosclerosis such as insulin resistance and low-grade inflammation (126). As increased sympathetic nerve activity, reflected by an increased RHR, is associated with increased risk for vascular diseases and mortality on the one hand (80;103), and diabetes on the other hand (97-101;120), increased sympathetic tone may also be part of the shared pathophysiology in the development of vascular diseases and type 2 diabetes. Diabetes can be prevented with lifestyle interventions and medication (23). Patients with clinically manifest vascular diseases with a particular high risk for the development of diabetes are an ideal group for preventive measures, given their high risk and the fact that they are already receiving medication and lifestyle advices, in contrast to the general population.

Objectives

The main aim of this thesis is twofold: to determine the effects of physical exercise on cardiovascular risk factors (**Part 1**) and to investigate the relation between resting heart rate and obesity, diabetes risk and vascular risk in patients with clinically manifest vascular disease (**Part 2**). The objectives of this thesis are:

Part 1

- to establish the immediate and persisting effects of a 12-day pilgrimage, covering 280 km to Santiago de Compostela in Spain, on cardiovascular risk factors and vascular function in healthy middle-aged male and female subjects (**chapter 2**)
- to determine the influence of walking speed as a measure of exercise intensity on the changes in cardiovascular risk factors during the pilgrimage to Santiago de Compostela (**chapter 3**)
- to determine the relation between baseline amount of daily physical activity, and the change in daily physical activity during the study, and the effects of a lifestyle modification program on changes in weight, BMI, waist circumference and insulin resistance in patients with the metabolic syndrome (**chapter 4**)

Part 2

- to determine and quantify the relation between visceral adiposity and resting heart rate in patients with various clinical manifestations of vascular disease (**chapter 5**)
- to determine the relation between resting heart rate and cardiovascular events and mortality in patients with coronary artery disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm (**chapter 6**)
- to determine the relation between resting heart rate and incidence of type 2 diabetes mellitus during follow-up in a cohort of patients with clinically manifest vascular diseases at different locations (**chapter 7**)

Outline of this thesis

Part 1 of this thesis focuses on the effects of physical exercise on cardiovascular risk factors in various settings. In **chapter 2** the Santiago study is described, a study evaluating the effects on vascular function and cardiovascular risk factors of walking a 12-day, 280 km-long pilgrimage to Santiago de Compostela. Both the immediate and the persisting vascular and metabolic effects of walking the pilgrimage are studied in healthy middle-aged male and female participants compared to matched control persons. In **chapter 3**, we evaluated the relation of recorded walking speed, as a measure of exercise intensity, on the changes in plasma lipids, glucose, weight, waist circumference and blood pressure during the pilgrimage. Increasing physical exercise is an important first step treatment modality in patients with the metabolic syndrome and is part of most lifestyle modification programs. In **chapter 4**, we studied the relation between the baseline amount of daily physical activity and the effects of a 1-year intensive lifestyle modification program on body weight, BMI, waist circumference and insulin resistance in patients with the metabolic syndrome. Furthermore, we assessed whether a change in daily physical activity during the study was related to effects of the lifestyle modification program on weight, BMI, waist circumference and insulin resistance.

Part 2 of this thesis focuses on the role of resting heart rate in patients with clinically manifest vascular disease. In **chapter 5**, the hypothesis that specifically visceral adipose tissue is related to resting heart rate is tested. The relations between visceral adipose tissue, subcutaneous adipose tissue, BMI and waist circumference on the one hand and resting heart rate on the other hand are presented and compared. Increased resting heart rate has been related to increased mortality in healthy populations and patients with coronary artery disease, but it is unclear whether this relation is also present in patients with vascular diseases at other locations than the heart. Therefore, in **chapter 6**, we assessed and compared the relation between resting heart rate and cardiovascular events and mortality in patients with cardiovascular disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm. Increased resting heart rate has also been related to a higher risk for type 2 diabetes mellitus in different populations, but also this relation has not been studied in patients with cardiovascular disease. In **chapter 7**, we describe the relation between resting heart rate and incident type 2 diabetes during follow-up in a cohort of patients with vascular diseases at different locations. We also investigated whether this relation was different for patients with vascular diseases at different locations. In **chapter 8**, the main findings of the above studies are discussed. Finally, a summary of the results presented in this thesis is given in **chapter 9**.

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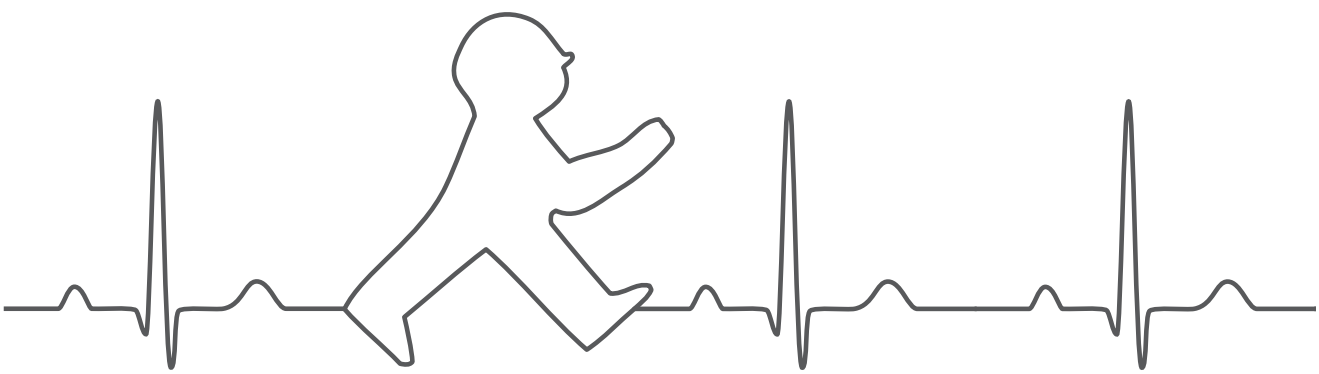
Part 1

Physical exercise



Chapter 2

Vascular and metabolic effects of 12 days intensive walking to Santiago de Compostela

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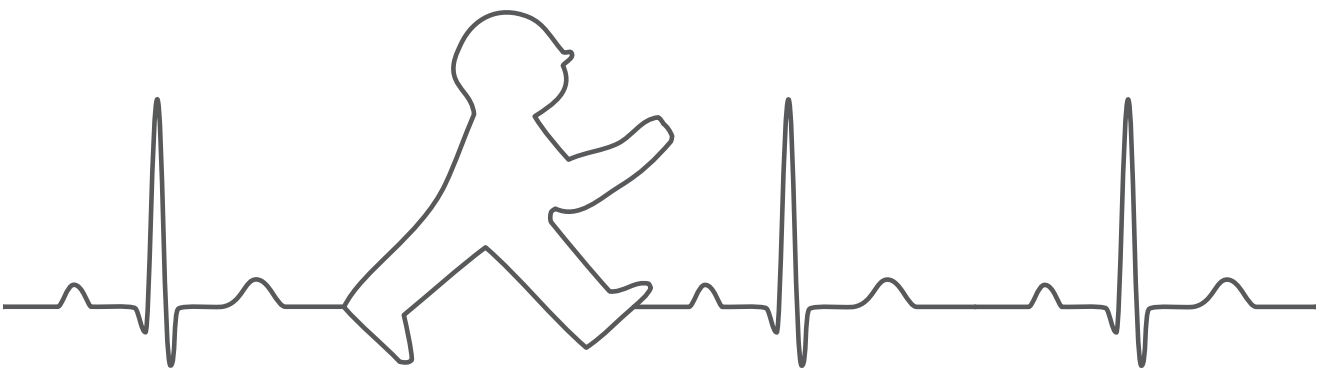
Atherosclerosis 2010;212(2):621-7



Abstract

Objective

Physical exercise has multiple beneficial health effects. Yearly, over 5 million persons walk a pilgrimage in various parts of the world, and this number is increasing. Here we report the effects on vascular function and cardiovascular risk factors of a 12-day pilgrimage to Santiago de Compostela in Spain.

Methods

Twenty-nine healthy male and female subjects between 40 and 70 years were included in the intervention group. The intervention consisted of walking the last 280 km of the pilgrim route to Santiago de Compostela. Twenty-nine control subjects were age- and gender-matched. Measures of endothelial function, vascular stiffness, autonomic function, and cardiovascular risk factors were measured 2 months and 2 weeks before the pilgrimage and 2 weeks and 2 months afterwards. During the pilgrimage cardiovascular risk factors, including weight, lipids, glucose and blood pressure were measured every other day.

Results

The mean daily walking distance during the pilgrimage was 23.42±0.80 km taking 5.39±0.36 hours/day. From start to end, HDL-cholesterol increased (0.20±0.30 mmol/L; +15%), while LDL-cholesterol (-0.6±0.6 mmol/L; -17%) and weight (-1.4±1.8 kg; -2%) decreased. After an initial rise, blood pressure came back to baseline. Two months after the pilgrimage a 2.0 kg weight loss persisted compared to the controls. There was no change in any vascular function parameter compared to the controls.

Conclusion

Walking a pilgrimage immediately influences major cardiovascular risk factors as a consequence of (strenuous) exercise and, likely, dietary changes. Two months after the pilgrimage these changes came back to baseline, except for weight loss. There was no effect on vascular function.

Introduction

In the past, walking was an inevitable and natural part of daily life. In modern Western societies and in urban parts of developing countries, physical exercise is not a self-evident part of every day activities anymore. Exercise has an inverse dose-response relation with all-cause mortality and is related to a lower risk of cardiovascular disease and type 2 diabetes (1;2). Therefore, physical exercise is widely recommended in guidelines for prevention of cardiovascular diseases (3).
The beneficial effects of exercise are caused by improvement of classical cardiovascular risk factors, partly caused by weight loss (4;5). Exercise decreases blood pressure by lowering total peripheral resistance as a consequence of decreased sympathetic nerve activity and improved insulin sensitivity (6). Serum triglycerides are decreased by up-regulated lipoprotein lipase activity as a result of exercise (4;7). Exercise increases HDL-cholesterol (HDL-c) by delaying particle clearance, increased production of nascent HDL-particles and higher cholesterol content per HDL particle (4;7;8). Plasma LDL-cholesterol (LDL-c) levels are typically unaffected by exercise (4;5). Furthermore, physical activity improves insulin sensitivity by increasing the amount of functional GLUT4 glucose transporters (6;9).
Evaluation of the metabolic effects of exercise often focuses on training programs using treadmills or controlled exercise intensity (4-6;8;9). In these studies, exercise programs range from 3 weeks to 1 year with moderate intensity exercise during 1-5 hours/week (4-6;8;9). Some studies investigate special forms of exercise such as climbing the Mount Everest or running or cycling a marathon (7;10;11). Furthermore, studies often report the immediate health effects of one exercise session (7;10), or long-term results of training programs (4;5;8;9).
People from various cultural and religious backgrounds walk parts of pilgrim routes at various places in the world. Walking pilgrim routes is an ancient tradition, which is regaining popularity. It is estimated that over 5 million people walk pilgrim routes every year. For example, in 1984, 2,491 people registered arrival on foot in Santiago de Compostela, Spain, and this number increased to almost 150,000 in 2009 (12). In the present study, we investigated both the immediate and persisting effects of a 12-day pilgrimage, covering 280 km, to Santiago de Compostela, on cardiovascular risk factors and vascular function in healthy middle-aged male and female subjects already intending to walk this pilgrimage, compared to healthy controls.

Subjects and methods

Subjects

The study was approved by the Medical Ethics Committee of the UMC Utrecht. All participants gave written informed consent before inclusion. Healthy male and female participants between 40 and 70 years of age were recruited by an announcement in the magazine of the Dutch Saint James Fellowship. The participants already intended to walk part of the Santiago de Compostela pilgrimage. Control subjects were physically capable of walking the pilgrimage but could not walk during the planned period due to logistical reasons and refrained from other types of strenuous exercise during the study. Controls were matched for age (±5 years) and gender to the subjects in the intervention group. Subjects diagnosed with diabetes mellitus, uncontrolled hypertension or a history of cardiovascular disease were excluded, as well as subjects using lipid lowering-medication.

Study design

This is a non-randomized intervention study with a control group. The intervention consisted of walking part of the Camino Francés, the classical pilgrimage route to Santiago de Compostela (13), from June 28th until July 10th 2009, covering 280 kilometers, between Hospital de Órbigo and Santiago de Compostela. All participants visited the research unit of the department of Vascular Medicine of the UMC Utrecht 2 months and 2 weeks before the pilgrimage and 2 weeks and 2 months afterwards for measurements of cardiovascular risk factors and vascular function (*Figure 1*). During the pilgrimage anthropometric and laboratory measurements in the intervention group were done every other day. In the analyses, 3 different periods are considered (*Figure 1*): the pilgrimage (V3 minus V2), the total exercise period (preparation and pilgrimage; V3 minus V1) and the whole study period (V4 minus V1).

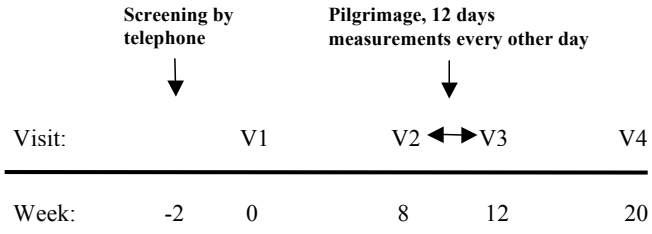


Figure 1. Study design

Measurements during clinic visits.
Prior to all visits at the UMC Utrecht, the subjects fasted for 12 hours, apart from drinking water, and refrained from smoking. All visits were planned during morning hours, for each individual at the same time as the preceding visit(s) to minimize within subject daytime-induced variation in vascular function.

Anthropometric measurements. During each visit blood pressure, resting heart rate, weight, waist- and hip circumference were recorded. Blood pressure was calculated as the mean of three recordings in seated position using an automated blood pressure device (Omron 705 IT, Hoofddorp, The Netherlands)).

Laboratory measurements. Fasting venous blood samples were collected for measurement of glucose, insulin, high-sensitivity C-reactive protein (hsCRP), total cholesterol, HDL-c, triglycerides (TG), and apolipoprotein B (ApoB) at each visit. At baseline thyroid stimulating hormone (TSH), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyltransferase (γGT), creatinin, hemoglobin, leucocytes and platelets were determined. Homeostatic model assessment of insulin resistance (HOMA IR), body mass index (BMI) and LDL-c were calculated.

Endothelial function measurements. Flow Mediated Dilatation (FMD) measures the increase in arterial diameter, a measure of endothelial function of conduit arteries, on reactive hyperaemia-induced shear stress. The procedure was conducted under standardized conditions. The brachial artery was visualized with a 5-14 MHz vascular transducer. After 1 minute of baseline recording, blood flow was obstructed for 5 minutes by cuff inflation. After deflation, reactive hyperaemia occurred and 3 more minutes were recorded. The sonographer ensured the exact transducer position. Images were analyzed using Brachial Analyzer 5.8.3 (Medical Imaging Applications, Coralville, Iowa, USA). Brachial FMD was calculated as (maximal – baseline diameter / baseline diameter) x 100%.
Peripheral Arterial Tonometry (PAT) measures the increase in finger blood volume with tonometry on reactive hyperaemia-induced shear stress, a measure of endothelial function of small vessels and microcirculation. PAT was recorded simultaneously with FMD. The Endo-PAT2000 (Itamar Medical, Caesarea, Israel) was used, connected to a laptop with installed Endo-PAT2000 software. The PAT data were uploaded and analyzed by Itamar Medical Ltd. PAT results are expressed as reactive hyperemia index (RHI): (maximal – baseline arterial tone occluded arm) / (maximal – baseline arterial tone control arm).
To assess intra- en inter-observer agreement of the FMD analyses, a random sample (10% and 5% respectively) of images was scored again by the same

observer (R.B.) and by another observer, blinded to the first result. There was no significant difference between the first and second analysis of the same observer (p=0.58) and between the different observers (p=0.11). The intraclass correlation coefficients were 0.89 and 0.97 respectively, indicating good intra- and interobserver agreement. Twenty-three FMD- and two PAT-measurements were of insufficient quality. These were replaced by measurements of the most nearby visit (both visits before or after the pilgrimage).

Heart Rate Variability (HRV), Pulse Wave Analysis (PWA) and Pulse Wave Velocity (PWV). HRV measures the variability in R-R intervals on a continuous ECG, reflecting the balance between sympathetic and parasympathetic activation. PWA measures vascular stiffness (Augmentation Index) and central blood pressures, by analysing the peripheral pulse wave form, while PWV measures the velocity of pulse waves, another measure of vascular stiffness. HRV, PWA and PWV were recorded using the Sphygmocor® system (AtCor Medical, West Ryde, Australia). Subjects were in supine position when HRV was measured with a 3 ECG lead during 5 minutes. The pressure tonometer was used to record PWA at the right radial artery and PWV at the right carotid and radial artery. PWA measurements were calibrated with the mean blood pressure.

Measurements during the pilgrimage

Measurements were conducted at the start of the pilgrimage and after arrival in Santiago de Compostela, and at every other day during the pilgrimage. All measurements were done early in the morning in fasted state, before the start of the walking distance that day. Subjects walked a very short distance to a mobile unit at a prespecified spot in the open air where all measurements were conducted. Measurements included weight, waist circumference, blood pressure and pulse rate. Furthermore, blood was obtained with a finger prick, for immediate analysis of total cholesterol, HDL-c, triglycerides, and glucose with a portable LDX analyzer (Cholestech Corporation, Hayward, USA). LDL-c was calculated.

To monitor the individual amount of exercise all participants carried a pedometer (Digiwalker SW-200, Yamax USA Inc., San Antonio, USA), measuring the number of steps during the walking route. The daily walked distance and time were recorded in a diary.

Sample size calculation

To detect a difference in change in FMD between intervention group and controls of 1.5% with a power of 90%, 22 study subjects were needed. Thirty subjects were included in each group to compensate for drop-out and missing data. The Type I error probability is 0.05. The power calculation was performed with the power calculator developed by Dupont and Plummer (Vanderbilt University, Nashville, USA).

Data analysis

Continuous variables are expressed as mean±standard deviation (SD) when normal distributed, and as median (interquartile range) in case of skewed distribution. Categorical variables are expressed as percentage (%). Baseline differences between the groups were tested using a two-tailed independent-samples t-test for normally distributed variables, a two-tailed Mann-Whitney U-test in case of skewed distributed variables, and a two-tailed Fisher's exact test for categorical variables. A p-value <0.05 was considered statistically significant. For these analyses SPSS version 15.0 was used.

For follow-up visits, the changes in all parameters within groups are expressed as mean changes±SD. The differences in mean changes between the groups were calculated (change intervention group – change control group). A 95% confidence interval (CI) was calculated using Confidence Interval Analysis (CIA) calculator version 2.2.0 (University of Southampton, Southampton, United Kingdom).

A mixed linear effects model was created to analyse the trend of change in cardiovascular risk factors during the pilgrimage. A model with random intercept was compared to a model with random intercept and fixed time-dependent variable. Differences between the models were tested with the log likelihood test statistic. These analyses were conducted using RCran 2.10.1.

Results

Subjects

The first 15 males and 15 females of the 52 subjects who responded to the advertisement for participation in the intervention group were selected. Thirty of the 37 persons who had applied for the control group were matched to the subjects in the intervention group. Due to one withdrawal before start of the pilgrimage, 29 persons in both groups (15 males, 14 females) completed the study and were analyzed.

Baseline characteristics

Baseline characteristics are shown in *Table 1* and the vascular parameters in *Table 2*. Apart from higher ASAT levels in the intervention group, there were no statistical significant differences between the groups at baseline.

Change in cardiovascular risk factors during the pilgrimage.

All subjects in the intervention group completed the 12-day pilgrimage. The amount of daily exercise is shown in *Figure 2*. Mean daily walking distance was 23.42±0.80 km, mean walking time 5.39±0.36 hours and mean steps/day 31,058±2,154. The shortest stage consisted of 14.02±8.39 km and the longest stage of 30.84±2.95 km. Counted steps were 17,993±11,347 and 40,281±6,945 respectively.

Table 1. Baseline characteristics.

	Pilgrims (n=29)	Controls (n=29)	P-value
Gender (M/F)	15/14	15/14	
Age (yr)	59.54 ± 5.31	59.70 ± 6.30	0.92
Currently smoking	5 (17%)	2 (7%)	0.42
Stopped smoking	14 (48%)	14 (48%)	1.00
Never smoked	10 (34%)	13 (45%)	0.59
Length (cm)	175 ± 12	174 ± 8	0.87
Weight (kg)	79.5 ± 12.1	75.6 ± 11.5	0.35
BMI (kg/m²)	25.7 ± 2.8	24.9 ± 3.3	0.31
Waist circumference (cm)	90 ± 9	88 ± 10	0.36
Hip circumference (cm)	101 ± 7	98 ± 8	0.21
Systolic blood pressure (mmHg)	135 ± 16	136 ± 15	0.95
Diastolic blood pressure (mmHg)	81 ± 10	81 ± 9	0.89
Pulse (beats/min)	63 ± 10	64 ± 9	0.47
Glucose (mmol/L)	4.9 ± 0.4	4.8 ± 0.6	0.68
Insulin (mU/L)	5.7 ± 4.4	6.7 ± 3.9	0.36
HOMA IR	1.2 ± 0.9	1.5 ± 1.0	0.34
HsCRP (mg/L)	2.4 ± 3.6	1.2 ± 0.9	0.09
Total cholesterol (mmol/L)	5.9 ± 0.8	5.5 ± 0.7	0.05
Triglycerides (mmol/L)	1.2 ± 0.6	1.2 ± 0.5	0.94
LDL-cholesterol (mmol/L)	3.9 ± 0.7	3.6 ± 0.6	0.09
HDL-cholesterol (mmol/L)	1.45 ± 0.43	1.32 ± 0.28	0.2
ApoB (g/L)	1.07 ± 0.19	1.01 ± 0.18	0.27
Creatinin (µmol/L)	78 ± 9	78 ± 9	0.8
TSH (mU/L)	1.3 (0.8 - 1.6)	1.4 (1.0 - 2.3)	0.12
ASAT (U/L)	29 ± 9	24 ± 5	0.02
ALAT (U/L)	27 ± 13	23 ± 11	0.14

Table 2. Vascular function tests at baseline.

	Pilgrims (n=29)	Controls (n=29)	P-value
PAT RHI	2.173 ± 0.761	2.013 ± 0.466	0.34
FMD (%)	5.87 ± 2.57	4.80 ± 3.15	0.16
BA diameter (mm)	4.07 ± 0.73	4.23 ± 0.62	0.42
PWV (m/s)	7.9 ± 0.9	8.2 ± 1.3	0.33
HRV LF (%)	61.37 ± 19.39	54.03 ± 23.16	0.21
HRV HF (%)	38.62 ± 19.39	45.97 ± 23.16	0.21
HRV LF/HF ratio	2.62 ± 2.38	2.10 ± 2.17	0.4
PWA central SBP (mmHg)	127 ± 15	127 ± 15	0.92
PWA central DBP (mmHg)	83 ± 10	83 ± 9	0.95
PWA AIx at HR 75	23.8 ± 9.1	23.5 ± 9.5	0.91

PAT RHI = Peripheral Arterial Tonometry Reactive Hyperaemia Index, FMD = Flow Mediated Dilatation, BA = Brachial Artery, PWV = Pulse Wave Velocity, HRV = Heart Rate Variability, LF = low frequency, HF = high frequency, PWA = Pulse Wave Analysis, SBP = systolic blood pressure, DBP = diastolic blood pressure, AIx at HR 75= Augmentation Index standardized for a heart rate of 75 beats per minute.

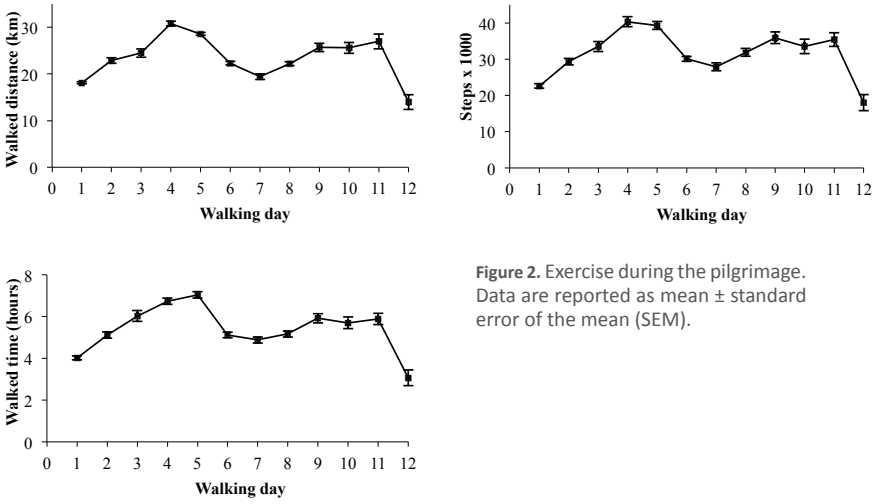


Figure 2. Exercise during the pilgrimage. Data are reported as mean ± standard error of the mean (SEM).

From start to end of the pilgrimage decreases in weight (-1.4±1.8 kg; -2%), waist circumference (-1.8±2.9 cm; -2%), LDL-c (-0.6±0.6 mmol/L; -17%) and triglycerides (-0.39±0.58 mmol/L; -30%) were observed, while HDL-c increased (0.20±0.30 mmol/L; +15%) (*Figure 3*). During the pilgrimage, systolic and diastolic blood pressure values were higher than the values obtained before the pilgrimage, but returned to baseline at the end of the pilgrimage. Maximum decreases in LDL-c during the pilgrimage were seen on day 10 (-0.75±0.70 mmol/L; -21%; p<0.01) compared to day 0. Compared to study baseline (V1), the decrease on day 10 was even larger (-1.1±0.7 mmol/L; -29%; p<0.01).

Change in cardiovascular risk factors during the whole study; intervention vs. control group.

The pilgrimage (V2-V3) led to an increase in HDL-c (difference in change between the groups 0.07 mmol/L; 95%CI 0.00 to 0.14; p<0.05), without significant changes in weight, waist circumference or LDL-c. Furthermore, the pilgrimage caused increases in serum glucose (difference in change between the groups 0.3 mmol/L; 95%CI 0.1 to 0.5; p<0.05) and HOMA IR (difference in change between the groups 0.5; 95%CI 0.2 to 0.8; p<0.05), and a decrease in resting heart rate (difference in change between the groups -5 beats per minute (bpm); 95%CI -9 to -1; p<0.05).

Preparation and pilgrimage (V1-V3) together led to a significant weight loss (difference in change between groups -1.8 kg; 95%CI -2.9 to -0.7; p<0.01) and increases in insulin (difference in change between the groups 2.3 mIU/L; 95%CI 0.3 to 4.3, p<0.05) and HOMA IR (difference in change between the groups 0.6; 95%CI 0.2 to 1.0, p<0.01). During the whole study period (V1-V4), a significant weight loss (difference between groups -2.0 kg; 95%CI -3.2 to -0.8; p<0.01) was seen.

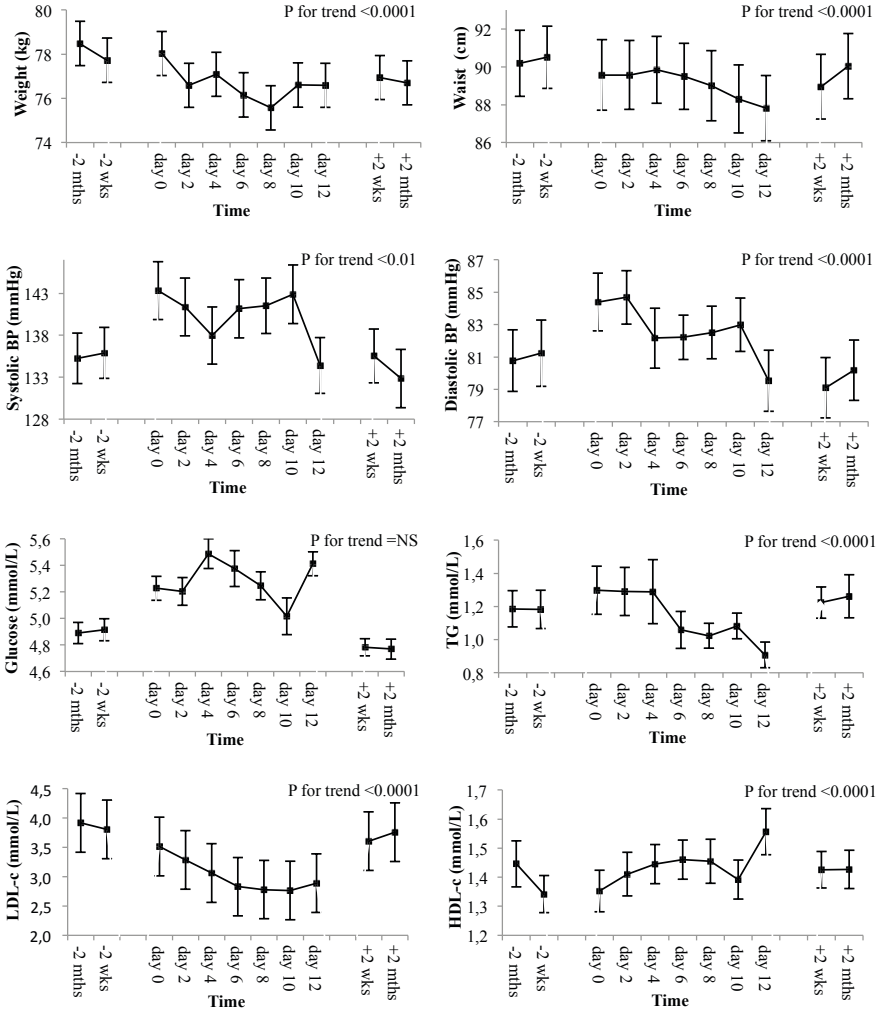


Figure 3. Changes in anthropometric and metabolic measurements during the pilgrimage.
P for trend is calculated from day 0 to day 12. Data are reported as mean ± standard error of the mean (SEM).

Effects of the pilgrimage on vascular function; intervention vs. control group.

Apart from an increase in augmentation index between the measurements V2-V3 in the intervention group (difference in change between the groups 4.4; 95%CI 1.3 to 7.5, p<0.01), there were no statistical significant differences in the changes in endothelial function, vascular stiffness and autonomic function parameters between the intervention and the control group (*Table 4*).

Table 3. Differences in changes in anthropometric and laboratory measurements between exercise and control group.

	Pilgrimage (V3 minus V2)			Pilgrimage and Preparation (V3 minus V1)			Whole study period (V4 minus V1)		
	ΔP	ΔC	ΔP - ΔC (95% CI)	ΔP	ΔC	ΔP - ΔC (95% CI)	ΔP	ΔC	ΔP - ΔC (95% CI)
Weight (kg)	-0.8 ± 1.9	-0.1 ± 1.4	-0.7 (-1.6 - 0.2)	-1.5 ± 1.9	0.3 ± 2.3	-1.8 (-2.9 - -0.7)**	-1.8 ± 2.1	0.2 ± 2.5	-2.0 (-3.2 - -0.8)**
BMI (kg/m²)	-0.3 ± 0.7	0.0 ± 0.5	-0.3 (-0.6 - 0)	-0.5 ± 0.7	0.1 ± 0.7	-0.6 (-1.0 - -0.2)**	-0.6 ± 0.7	0.1 ± 0.8	-0.7 (-1.1 - -0.3)**
Waist circ (cm)	-1.6 ± 3.0	-0.1 ± 3.0	-1.5 (-3.1 - 0.1)	-1.2 ± 4.4	0.2 ± 4.0	-1.4 (-3.6 - 0.8)	-0.2 ± 4.3	0.0 ± 4.6	-0.2 (-2.5 - 2.1)
Hip circ (cm)	-2.0 ± 2.2	-0.1 ± 2.8	-1.9 (-3.2 - -0.6)*	-2.2 ± 2.6	-0.9 ± 3.7	-1.3 (-3.0 - 0.4)	-3.1 ± 3.3	-1.6 ± 4.1	-1.5 (-3.5 - 0.5)
Systolic BP (mmHg)	0 ± 11	-1 ± 9	1 (-4 - 6)	0 ± 12	-3 ± 8	3 (-2 - 8)	-2 ± 13	-1 ± 11	-1 (-7 -5)
Diastolic BP (mmHg)	-2 ± 6	-1 ± 5	-1 (-4 - 2)	-2 ± 6	-2 ± 4	0 (-3 - 3)	-1 ± 7	-1 ± 6	0 (-3 -3)
Pulse (beats/min)	-2 ± 6	3 ± 8	-5 (-9 - -1)*	-2 ± 7	2 ± 7	-4 (-8 - 0)*	-2 ± 7	-1 ± 9	-1 (-5 - 3)
Glucose (mmol/L)	-0.1 ± 0.3	-0.4 ± 0.4	0.3 (0.1 - 0.5)*	-0.1 ± 0.5	-0.2 ± 0.6	0.1 (-0.2 - 0.4)	-0.1 ± 0.5	-0.1 ± 0.7	0.0 (-0.3 - 0.3)
Insulin (mIU/L)	0.5 ± 2.7	-1.0 ± 4.4	1.5 (-0.4 - 3.4)	2.1 ± 3.7	-0.2 ± 3.9	2.3 (0.3 - 4.3)*	2.6 ± 4.7	0.2 ± 2.1	2.4 (0.5 - 4.3)*
HOMA IR	0.1 ± 0.6	-0.4 ± 0.7	0.5 (0.2 - 0.8)*	0.4 ± 0.8	-0.2 ± 0.6	0.6 (0.2 - 1.0)**	0.5 ± 1.0	0.0 ± 0.5	0.5 (0.1 - 0.9)*
HsCRP (mg/L)	-0.6 ± 2.4	0.6 ± 2.9	-1.2 (-2.6 - 0.2)	-1.1 ± 3.1	0.6 ± 2.9	-1.7 (-3.3 - -0.1)*	-0.7 ± 3.1	0.9 ± 3.5	-1.6 (-3.3 - 0.1)
Tot chol (mmol/L)	-0.1 ± 0.6	0.0 ± 0.4	-0.1 (-0.4 - 0.2)	-0.3 ± 0.6	0.0 ± 0.6	-0.3 (-0.6 - 0.0)	-0.1 ± 0.6	0.0 ± 0.7	-0.1 (-0.4 - 0.2)
TG (mmol/L)	0.0 ± 0.3	0.0 ± 0.5	0.0 (-0.2 - 0.2)	0.0 ± 0.3	0.0 ± 0.5	0 (-0.2 - 0.2)	0.1 ± 0.4	-0.1 ± 0.4	0.2 (0.0 - 0.4)
LDL-c (mmol/L)	-0.2 ± 0.5	0.0 ± 0.3	-0.2 (-0.4 - 0.0)	-0.3 ± 0.6	0.0± 0.5	-0.3 (-0.6 - 0)	-0.2 ± 0.6	0.0 ± 0.5	-0.2 (-0.5 - 0.1)
HDL-c (mmol/L)	0.08 ± 0.16	0.01 ± 0.09	0.07 (0.00 - 0.14)*	-0.02 ± 0.21	0.04 ± 0.12	-0.06 (-0.15 - 0.03)	-0.02 ± 0.14	0.00 ± 0.20	-0.02 (-0.11 - 0.07)
ApoB (g/L)	-0.03 ± 0.23	-0.01 ± 0.10	-0.02 (-0.11 - 0.07)	-0.01 ± 0.20	-0.03 ± 0.11	0.02 (-0.07 - 0.11)	-0.05 ± 0.12	-0.03 ± 0.13	-0.02 (-0.09 - 0.05)

ΔP = mean change in pilgrim group, ΔC = mean change in control group, ΔP - ΔC: difference between ΔP and ΔC, tot chol = total cholesterol, BP = blood pressure
*= P <0.05, ** = P <0.01.

Table 4. Differences in changes in vascular function measurements between exercise and control group.

	Pilgrimage (V3 minus V2)			Pilgrimage and Preparation (V3 minus V1)			Whole study period (V4 minus V1)		
	ΔP	ΔC	ΔP - ΔC (95% CI)	ΔP	ΔC	ΔP - ΔC (95% CI)	ΔP	ΔC	ΔP - ΔC (95% CI)
EndoPAT RHI	-0.031 ± 0.739	-0.207 ± 1.170	-0.240 (-0.753 - 0.277)	-0.021 ± 0.571	0.162 ± 0.992	-0.183 (-0.609 - 0.243)	0.097 ± 0.760	0.387 ± 0.968	-0.290 (-0.748 - 0.168)
FMD (%)	-0.37 ± 2.67	-0.95 ± 3.33	0.58 (-1.02 - 2.18)	-0.48 ± 2.78	-0.06 ± 3.25	-0.42 (-2.02 - 1.18)	0.41 ± 2.94	-0.22 ± 3.10	0.63 (-0.97 - 2.23)
BA diam (mm)	0.14 ± 0.36	-0.01 ± 0.54	0.15 (-0.11 - 0.41)	0.10 ± 0.49	-0.07 ± 0.51	0.17 (-0.12 - 0.46)	0.02 ± 0.60	-0.12 ± 0.29	0.14 (-0.12 - 0.40)
PWV (m/s)	-0.2 ± 1.2	-0.4 ± 1.5	0.2 (-0.5 - 0.9)	0.1 ± 1.1	0.0 ± 1.5	0.1 (-0.6 - 0.8)	0.4 ± 1.3	0.1 ± 1.4	0.3 (-0.4 - 1.0)
HRV LF (%)	3.21 ± 22.84	-2.97 ± 18.16	6.18 (-4.96 - 17.32)	-1.03 ± 21.12	-0.98 ± 22.47	-0.05 (-11.96 - 11.86)	-6.89 ± 13.91	1.36 ± 22.01	-8.25 (-18.25 - 1.75)
HRV HF (%)	-3.21 ± 22.84	2.97 ± 18.16	-6.18 (-17.32 - 4.96)	1.03 ± 21.12	0.98 ± 22.47	0.05 (-11.86 - 11.96)	6.89 ± 13.91	-1.36 ± 22.01	8.25 (-1.75 - 18.25)
HRV LF/HF	-0.82 ± 3.84	-1.44 ± 5.16	0.62 (-1.85 - 3.09)	-0.17 ± 2.28	-0.38 ± 2.31	0.21 (-1.04 - 1.46)	-0.44 ± 1.77	0.56 ± 2.18	-1.00 (-2.08 - 0.08)
CSBP (mmHg)	1 ± 11	-2 ± 9	3 (-2 - 8)	2 ± 11	-3 ± 9	5 (0 - 10)	0 ± 12	0 ± 11	0 (-6 - 6)
CDBP (mmHg)	-2 ± 6	-1 ± 6	-1 (-4 - 2)	-2 ± 6	-2 ± 4	0 (-3 - 3)	-1 ± 7	-1 ± 6	0 (-6 - 6)
AIx at HR 75	3.5 ± 4.9	-0.9 ± 6.4	4.4 (1.3 - 7.5) **	0.4 ± 6.5	-2.2 ± 5.5	2.6 (-0.6 - 5.8)	0.4 ± 5.6	-1.3 ± 6.9	1.7 (-1.6 - 5.0)

ΔP = mean change in pilgrim group, ΔC = mean change in control group, ΔP - ΔC: difference between ΔP and ΔC, PAT RHI = Peripheral Arterial Tonometry Reactive Hyperaemia Index, FMD = Flow Mediated Dilatation, BAdiam = Brachial Artery diameter, PWV = Pulse Wave Velocity, HRV = Heart Rate Variability, LF = low frequency, HF = high frequency, CSBP =central systolic blood pressure, CDBP =central diastolic blood pressure, AIx at HR 75= Augmentation Index standardized for a heart rate of 75 beats per minute, ** = P <0.01.

Discussion

In the present study, walking a 12-day pilgrimage to Santiago de Compostela led to rapid changes in cardiovascular risk factors, especially in LDL-c, in healthy middle-aged men and women compared to controls. Other immediate effects were a significant increase in HDL-c and decreases in weight, waist circumference, and triglycerides. Of these effects, only weight loss persisted for 2 months. Walking a pilgrimage had no effects on vascular function.

In this study, a remarkable steady drop in LDL-c of 0.17±0.13 mmol/L every 2 days during the first part of the pilgrimage was observed, reaching a maximal decrease of 1.1±0.7 mmol/L (-29%) after 10 days compared to study baseline (V1), which is similar to LDL-c lowering with statin treatment. Differential effects of exercise on LDL-c levels have been reported. High-intensity or resistance exercise during 2.5-9 months decreased LDL-c by up to 20% (14-16), while low or moderate intensity exercise programs generally have no or marginal effects on LDL-c (4;15-17). An increase in plasma volume (10;18), decrease in bodyweight or body fat composition (19), upregulated expression of LDL-receptors (20), increased cholesterol-transfer to HDL particles (17), and use of cholesterol for cellular metabolism and repair due to muscle damage immediately after intense exercise (7;18) are potential explanations for exercise-induced decrease in LDL-c. In this study it is unclear which proportion of LDL-c lowering is caused by exercise and which proportion by dietary changes. We consider the effect of changing from a Northern European diet in normal life to a Northern Spanish diet during the pilgrimage of minor importance compared to the effect of exercise on LDL-c levels. For example, the reported effect of a Mediterranean diet on LDL-c is small (between -0.25 to +0.05 mmol/L after 3 months) and usually takes several weeks to occur (21). In the present study, a return to normal exercise patterns and diet after the pilgrimage resulted in a quick increase in LDL-c to baseline levels.

The pilgrimage did not affect endothelial function and arterial stiffness. Exercise training is considered to ameliorate endothelial function and arterial stiffness, even within a few days (22), and with exercise quantities that are substantially less than in our study (22-24). The healthy participants in the present study had normal FMD, PAT, and PWV values at baseline, which may explain the lack of exercise-induced improvement (24-26). Nevertheless, maintaining optimal endothelial function and vascular stiffness during life is related to a lower risk on clinical vascular events (27). Changes in vascular function might have occurred but were not measured during the pilgrimage, due to the inability to perform high-quality measurements in daily changing environments. The rapid rebound to baseline levels of vascular risk factors could explain the absence of vascular function changes after 2 weeks. Exercise-induced improvements in vascular stiffness have been shown to return to baseline after the end of the training program within 1 month (23).

Structural arterial changes, particularly an increase in diameter, an adaptive mechanism to lower repetitive exercise-induced shear stress (28), were not observed in our study, and therefore likely did not contribute to the absence of changes in vascular function parameters. Changes in sympathetic nerve activity, also known to affect vascular function (29), were not consistently found in our study considering that no major HRV changes could be demonstrated. There was a statistical significant difference in resting heart rate shortly after the pilgrimage, which is of small clinical significance, and no change in blood pressure. Exercise is known to decrease blood pressure and resting heart rate, and exercise increases parasympathic activity represented by HRV-measured HF power (30). An explanation for the absence of effects of the pilgrimage on autonomic function might be the normal baseline values of resting heart rate and HRV and the time lag between the end of the pilgrimage and HRV measurements.

Walking a pilgrimage requires a considerable amount of time, a thorough preparation and a good physical and mental health. Our findings can be generalized to healthy middle-aged males and females who satisfy these conditions, and to other types of exercise, consisting of prolonged daily periods of moderate intensity. Subjects at older age and/or with a less favourable vascular risk factor profile may have lower levels of vascular function, which may immediately improve as a result of exercise. However, such subjects are unlikely to walk a pilgrimage.

A strength of our study is the evaluation of both immediate and persisting metabolic and vascular effects of exercise. We also acknowledge study limitations. We consider the 12-day exercise program the major component of the intervention contributing to metabolic changes, although other factors, such as diet, may also have contributed. This is inherent in a pragmatic study, evaluating the effects of a 'natural' exercise program, which was our primary study objective. Furthermore, there may have been inter-individual differences in relative exercise intensity which may have influenced study outcome, but aerobic capacity was not measured during the study. As this was a non-randomized intervention study, it can not completely be ruled out that differences in baseline characteristics between study groups may have influenced the results.

In conclusion, a 12-day pilgrimage covering 280 km, immediately lowers LDL-c, triglycerides, blood pressure and bodyweight and increases HDL-c. Changes in cardiovascular risk factors did not sustain for 2 months except for changes in bodyweight. There was no effect on vascular function probably due to short-lived changes in risk factors and the limited exercise duration.

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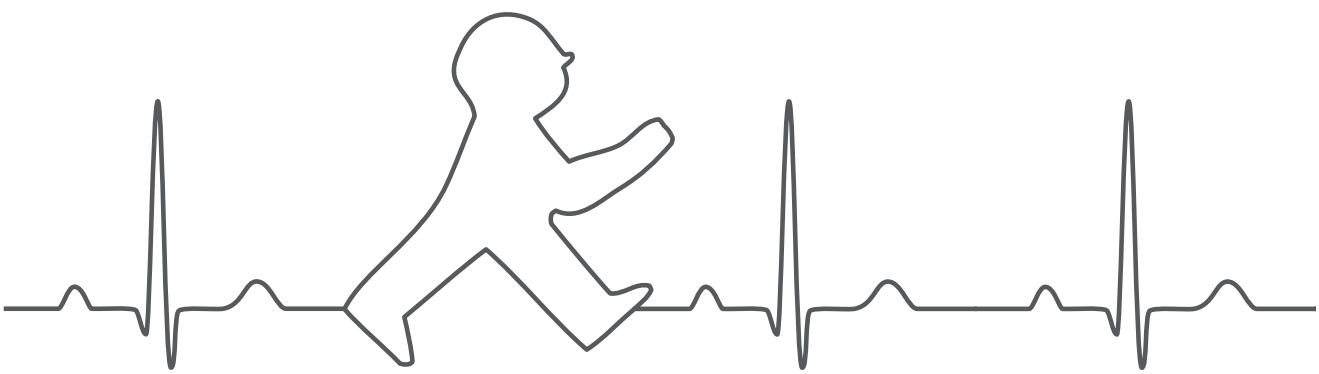

Chapter 3

The relation between walking speed and changes in cardiovascular risk factors during a 12-day walking tour to Santiago de Compostela

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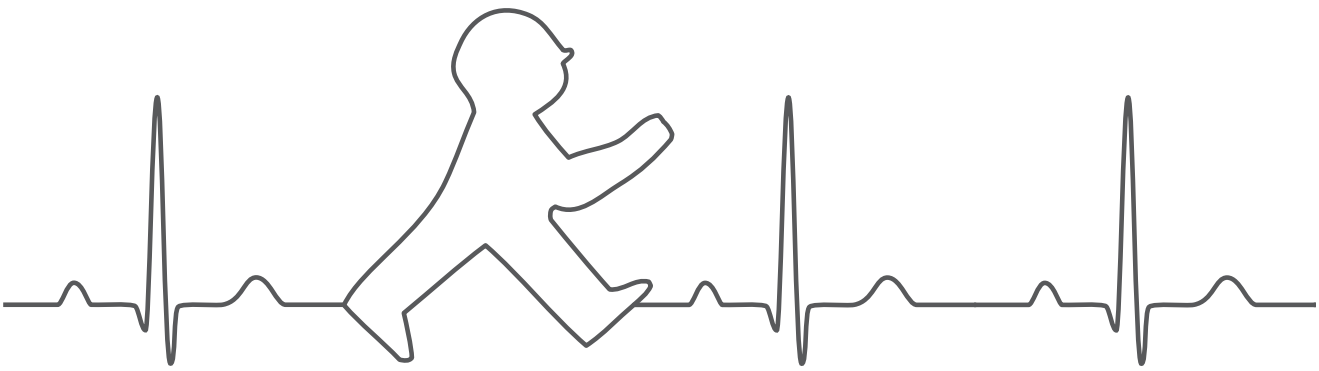


Abstract

Objectives

Physical exercise has beneficial effects on cardiovascular risk factors. Knowledge about the effect of exercise intensity, specifically walking speed, on cardiovascular risk factors is limited. We report the relation between walking speed and changes in cardiovascular risk factors in participants of a 12-day walking tour to Santiago de Compostela.

Design

Prospective cohort study

Setting

Single-centre study with healthy middle-aged volunteers.

Participants

Healthy middle-aged men (n=15) and women (n=14). Subjects using lipid-lowering medication were excluded.

Intervention

Participants walked 281±10 km of the classical route to Santiago de Compostela, in 12 days in 2009.

Primary and secondary outcome measures

Walking speed was recorded and blood pressure, weight, waist circumference, lipids and glucose were measured every other day. Changes in risk factors were compared between gender-pooled groups with faster and slower walking speed. Secondly, the relation between walking speed and changes in risk factors was quantified using a linear mixed effects model.

Results

In the faster walking speed (4.6±0.2 km/h) group HDL-c increased more than in the slower walking speed (4.1±0.2 km/h) group (difference in change between groups: 0.20; 95%CI -0.02-0.42 mmol/L) while LDL-c and total cholesterol decreased more in the slower walking speed group (differences in changes between groups: LDL-c: -0.50; 95%CI -0.88--0.12 mmol/L and total cholesterol: -0.75; 95%CI -1.19--0.31 mmol/L). A 1 km/h higher walking speed was related to an increase in HDL-c (0.24; 95%CI 0.12-0.30 mmol/L), LDL-c (0.18; 95%CI -0.16-0.42 mmol/L) and total cholesterol (0.36; 95%CI 0.12-0.60 mmol/L), adjusted for age, gender, smoking, BMI and heart rate, during the whole walking tour.

Conclusions

Walking the same distance faster improves HDL-c more, while LDL-c and total cholesterol decrease more with lower walking speed, independent of changes in body weight, in healthy middle-aged subjects.

Introduction

Exercise has an inverse dose-response relation with all-cause mortality and is related to a lower risk of cardiovascular disease and type 2 diabetes.(1,2) An important part of these long-term beneficial effects of exercise is caused by improvement of classical cardiovascular risk factors as physical activity lowers body weight, lowers blood pressure, decreases insulin resistance and glucose intolerance, lowers plasma triglycerides and increases high-density lipoprotein cholesterol (HDL-c).(3) For these reasons, physical exercise is widely recommended in guidelines for treatment and prevention of cardiovascular diseases.(4-6) Guidelines recommend a minimum weekly physical activity equal to 150 minutes of brisk walking, however, it is not specified at what intensity this exercise should be preferably conducted.(4-6) Brisk walking or shorter periods of exercise at a higher intensity (for example running) are considered equally effective.(4-6) However, the results from studies evaluating the effects of exercise intensity on cardiovascular risk factors are conflicting. Several randomised clinical trials report no differences between various intensities of exercise and conclude that the total amount of exercise is more important than exercise intensity.(7-10) Other studies conclude that exercise at a higher intensity results in more beneficial changes in cardiovascular risk factors compared to exercise at a lower intensity,(11-15) although not all studies adequately control for differences in the total amount of exercise.(12,13)

Walking is one of the most accessible forms of physical exercise and is, together with gardening, the major component of leisure time physical activity.(16) Walking speed is an easy parameter to express exercise intensity and can be measured outside a laboratory with limited resources. Results from large epidemiologic studies show a relation between increased walking speed and a decreased risk for cardiovascular disease and diabetes.(17-20) However, in these studies the walking speed was not measured, but assessed using questionnaires where study participants estimated their usual walking speed in broad categories such as ‘easy’, ‘average’ or ‘brisk’. Furthermore, these studies did not evaluate the effects of walking speed on cardiovascular risk factors.

In the Santiago study, 29 healthy, middle-aged men and women walked an equal distance consisting of 281 km at their own individual preferred speed during 12 days in Spain.(21) Marked inter-individual differences in changes in cardiovascular risk factors were observed, predominantly in plasma lipids.(21) In the present study, we evaluated the influence of the measured walking speed on changes in plasma lipids, blood pressure, weight, waist circumference and glucose.

Subject and methods

Subjects and exercise

Healthy male and female participants between 40-70 years of age were recruited by an announcement in the magazine of the Dutch Saint James Fellowship. The cohort size of 30 participants was based on a sample size calculation to detect a difference in endothelial function in the original Santiago study (21). Subjects diagnosed with diabetes mellitus, uncontrolled hypertension or a history of cardiovascular disease were excluded, as well as subjects using lipid lowering-medication. There were 49 subjects responding to the advertisement and applied for participation in the intervention group of the Santiago study. One subject was not eligible because of a history of diabetes mellitus, and 1 subjects was not eligible because of uncontrolled hypertension (systolic blood pressure >170 mmHg). From the remaining 47 eligible subjects, the first 15 males and 15 females were recruited for participation. After signing the informed consent form, but before the start of the intervention period, 1 female subject ended participation for personal reasons. The design of the santiago study is described in more detail elsewhere.(21) Briefly, the santiago study is a non-randomized intervention study on the immediate and longer-term effects of long daily periods of walking on vascular function and cardiovascular risk factors. Participants already intended to walk part of the Santiago de Compostela pilgrimage. The intervention consisted of walking part of the Camino Francés, the classical pilgrimage route to Santiago de Compostela,(22) from June 28th until July 10th 2009, covering 281 kilometers, between Hospital de Órbigo and Santiago de Compostela in Spain. Mean daily walked distance was 23±1 km, mean daily walked time 5.39±0.36 hours and mean steps per day 31,058±2,154. All participants completed the 12-day walking tour. For the present study, the data of the 29 persons (15 males, 14 females) in the intervention group were used. The santiago study was approved by the Medical Ethics Committee of the UMC Utrecht. All participants gave written informed consent before inclusion.

Measurement of walking speed

All participants used a diary, to record their exact time of departure, time of arrival and resting time and the daily walking time was calculated. Participants walked at their individually preferred speed and were unaware that the effects of their walking speed would become subject of evaluation. All participants carried a pedometer (Digiwalker SW-200, Yamax USA Inc., San Antonio, USA), measuring the number of steps daily. The participants were instructed to wear the pedometer at their belt or waistband at the left or right side of the body. From these data, the walking speed was calculated in km/h by dividing the total distance covered during the study by the total walking time without including the resting time. Walking speed was also expressed in steps/h by dividing the total number of steps by the total walking time.

Measurement of cardiovascular risk factors

Measurements were conducted in Spain, at the start, after arrival and at every other day in between during the walking tour. All measurements were conducted in the fasted state, before the start of the walking distance that day. Measurements included weight, waist circumference and blood pressure. Weight was measured without shoes on the same balance during the whole study. Waist circumference was measured in standing position with a tape measure just above the iliac spine. Blood pressure was calculated as the mean of three recordings in seated position at the arm with the highest value at the baseline visit, using an automated blood pressure device (Omron 705 IT, Hoofddorp, The Netherlands). Furthermore, blood was obtained with a finger prick, for immediate analysis of total cholesterol, HDL-c, triglycerides and glucose with a portable LDX analyzer (Cholestech Corporation, Hayward, USA). LDL-cholesterol (LDL-c) was calculated. No information about dietary intake at baseline or during the study was obtained. The participants were not instructed on their diet.

Data analyses

Continuous variables are expressed as mean±standard deviation (SD) when normal distributed, and as median (interquartile range) in case of skewed distribution. Categorical variables are expressed as percentage (%). To analyze the role of walking speed on the change in cardiovascular risk factors, we first compared the changes in cardiovascular risk factors between participants walking with faster speed and participants walking with slower speed. As there is no generally accepted cut-off point for faster or slower walking speed, the study population was divided based on median walking speed, which also has the advantage of creating groups of equal size. To prevent overrepresentation of male participants in the high speed group, initially men and women were classified separately as walking with faster or slower speed according to the median speed of their gender. Thereafter, males and females classified as faster walking speed were pooled in the faster walking speed group, and males and females classified as slower walking speed were pooled in the slower walking speed group.

Secondly, a linear mixed effects model was used. In this model, the relation between walking speed and changes in cardiovascular risk factors was adjusted for differences in baseline values of cardiovascular risk factors (using a random intercept) and for changes in cardiovascular risk factors due to the progression of the walking tour (using a fixed time-dependent variable). To investigate the effect of walking speed, an interaction variable of walking speed and progression of the walking tour (represented by the fixed time-dependent variable) was added to the model. The β coefficients with 95% confidence intervals (95%CI) of this interaction terms are reported, denoting the change in the specific risk factor per 2 days which is related to an increase in walking speed of 1 km/h or 1000

steps/h. In model I the unadjusted relation between walking speed and changes in cardiovascular risk factors during the walking tour is presented. In model II, adjustments were made for the potential confounding variables age and gender. In model III additional adjustments were made for current smoking, heart rate at baseline as the best available measure for physical fitness and baseline body mass index (BMI). The main results are based upon this model. We conducted an exploratory analysis with additional adjustment for changes in body weight, to see if changes in body weight during the walking tour were in the causal pathway of the relation between walking speed and changes in blood lipids. In a sensitivity analysis, we additionally adjusted model III for baseline characteristics with large differences between the low and the high speed group: systolic and diastolic blood pressure, HDL-c, LDL-c and triglycerides.
For all analyses SPSS version 15.0.1 was used.

Results

Baseline characteristics

The faster walking speed group consisted of 8 men and 7 women, 60.9±3.5 years old, who walked with an average speed of 4.6±0.2 km/h, while the slower walking speed group comprised 7 men and 7 women, 58.1±6.6 years old (p-value for age between groups = 0.17), with a mean walking speed of 4.1±0.2 km/h (p-value for walking speed between groups < 0.01) (*Table 1*). The median speed of the men (n=8) in the faster walking speed group was 4.62 (IQR 4.57-4.92), of the women in the faster walking speed group (n=7) 4.52 (IQR 4.24-4.62), of the men in the slower walking speed group (n=7) this was 4.23 (IQR 4.01-4.33) and of the women in the slower walking speed group (n=7) this was 4.08 (IQR 3.94-4.10) km/h. Walking speed varied during the 12-day pilgrimage from 4.37 (IQR 4.21-4.80) to 5.01 (IQR 4.78-5.16) in the faster walking speed group, and from 3.77 (IQR 3.50-4.07) to 4.30 (IQR 4.29-4.51) in the slower walking speed group. Both groups walked a similar overall distance (284±7 and 278±11 km respectively, p = 0.13). At baseline the systolic and diastolic blood pressure (148±18/87±10 versus 138±8/81±9 mmHg, p-values respectively 0.16 and 0.11) and heart rate (69±10 versus 63±10 beats/minute, p=0.14) were higher in the faster walking speed group compared to the slower walking speed group, and BMI was lower (24.2±2.2 versus 27.0±2.7 kg/m^2, p < 0.01). The baseline lipid profile was more favorable in the faster walking speed group than in the slower walking speed group (HDL-c 1.45±0.39 versus 1.24±0.36 mmol/L, p=0.14, LDL-c 3.4±0.5 versus 3.7±0.8 mmol/L, p=0.22, and triglycerides 1.1±0.5 versus 1.5±0.9 mmol/L, p=0.12, respectively).

Table 1. Baseline characteristics for all participants and according to walking speed.

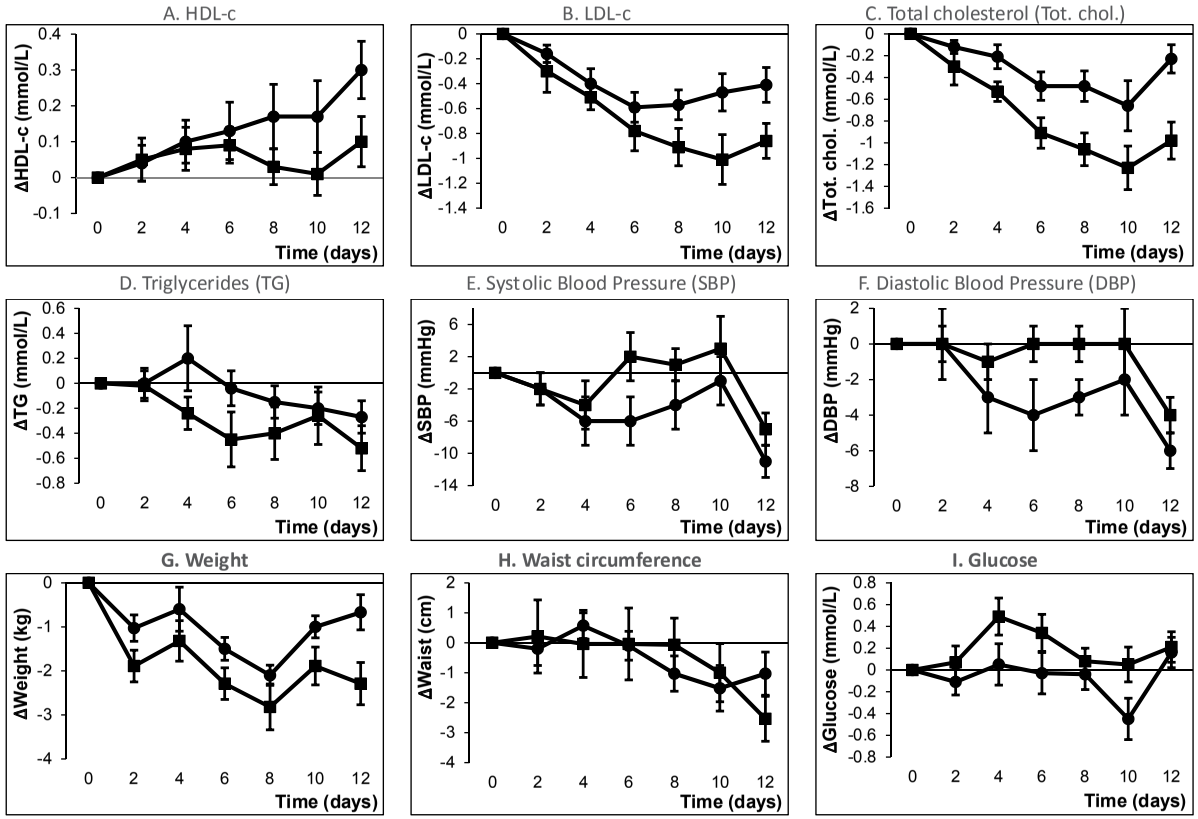
	faster walking speed group (n=15)	slower walking speed group (n=14)	all participants (n=29)
Mean walking speed (km/h)	4.6 ± 0.2	4.1 ± 0.2	4.4 ± 0.3
Walking speed range (km/h)	4.2-5.0	3.8-4.5	3.8-5.0
Number of steps/hour	6309 ± 582	5547 ± 437	5941 ± 639
Total walking time (hours)	62 ± 3	68 ± 3	65 ± 4
Total walking distance (km)	284 ± 7	278 ± 11	281 ± 10
Male subjects	8 (53%)	7 (50%)	15 (52%)
Age (years)	60.9 ± 3.5	58.1 ± 6.6	59.5 ± 5.3
Current smoking	3 (20%)	2 (14%)	5 (17%)
Systolic blood pressure (mmHg)	148 ± 18	138 ± 18	143 ± 19
Diastolic blood pressure (mmHg)	87 ± 10	81 ± 9	84 ± 10
Heart rate (beats/minute)	69 ± 10	63 ± 10	66 ± 11
BMI (kg/m^2)	24.2 ± 2.2	27.0 ± 2.7	25.5 ± 2.8
Waist circumference (cm)	88 ± 10	92 ± 11	90 ± 10
Glucose (mmol/L)	5.2 ± 0.6	5.2 ± 0.4	5.2 ± 0.5
Total cholesterol (mmol/L)	5.3 ± 0.7	5.6 ± 0.8	5.5 ± 0.8
LDL-cholesterol (mmol/L)	3.4 ± 0.5	3.7 ± 0.8	3.5 ± 0.7
HDL-cholesterol (mmol/L)	1.45 ± 0.39	1.24 ± 0.36	1.35 ± 0.38
Triglycerides (mmol/L)	1.1 ± 0.5	1.5 ± 0.9	1.3 ± 0.8
Total cholesterol/HDL-c ratio	3.8 ± 1.0	5.0 ± 2.1	4.4 ± 1.7
LDL-c/HDL-c ratio	2.5 ± 0.7	3.3 ± 1.5	2.9 ± 1.2

Baseline characteristics are shown according to walking speed and for all participants together. In order to avoid predominantly male subjects in the faster walking speed group, the faster walking speed group is gender-pooled and consists of the 8 men and 7 women with a walking speed high than the median speed for their gender. BMI= body mass index, LDL= low density lipoprotein, HDL= high density lipoprotein.

Changes in cardiovascular risk factors according to high or low walking speed

The whole study population together showed decreases in weight (-1.4±1.8 kg), waist circumference (-1.8±2.9 cm), LDL-c (-0.60±0.60 mmol/L), total cholesterol (-0.60±0.70 mmol/L), triglycerides (-0.39±0.58 mmol/L) and systolic (-9±9 mmHg) and diastolic (-5±4 mmHg) blood pressure during the walking tour, while HDL-c increased (0.20±0.30 mmol/L).(21) Most of these changes were short-lived; after two months, there was only a significant difference in change of weight (-2.0 kg; 95%CI -3.2 - -0.8) in the participants walking the pilgrimage compared to controls who did not walk the pilgrimage, while there were no differences in changes in the other cardiovascular risk factors between the groups (21). In *Figure 1A-I* the changes in cardiovascular risk factors for the faster and slower walking speed group during the walking period are shown. The HDL-c in the faster walking speed group increased more than in the slower walking speed group (difference in change between the groups 0.20; 95%CI -0.02-0.42 mmol/L) (*Figure 1A*). In the

Figure 1A-I. Changes in cardiovascular risk factors during the walking tour according to walking speed.



Changes in cardiovascular risk factors from baseline values during the walking tour for the faster walking speed group (–●–) and the slower walking speed group (–■–). Measurements were conducted at day 0, and every other day. Data are presented as mean with standard error of the mean.

slower walking speed group, the decreases in LDL-c and total cholesterol were larger than in the faster walking speed group (differences in changes in LDL-c between the groups -0.50; 95%CI -0.88--0.12 and for total cholesterol -0.75; 95%CI -1.19--0.31) (*Figure 1B* and 1*C*). Furthermore, weight decreased more in the slower walking speed group (difference in change between the groups -1.6; 95%CI -2.9--0.3 kg) (*Figure 1G*). The decreases in blood pressure were larger in the faster walking speed group compared to the slower walking speed group, although this difference was not statistically significant (difference in change between the groups -4; 95%CI -11-3 mmHg for systolic and -2; 95%CI -5-1 mmHg for diastolic blood pressure) (*Figure 1E* and 1*F*).

The quantitative influence of walking speed on the change in cardiovascular risk factors

A 1 km/h higher walking speed is related to an increase in HDL-c of 0.04 mmol/L (95%CI 0.02-0.05) per 2 days walking (*Table 2*). For the whole 12-day walking tour the increase in HDL-c related to a 1 km/h higher walking speed is then 6 times 0.04 mmol/L (0.24 mmol/L; 95%CI 0.12-0.30). Furthermore, a 1 km/h higher walking speed is related to an increase in LDL-c of 0.03 (95%CI -0.01-0.07) mmol/L per 2 days walking and for total cholesterol this is 0.06 (95%CI 0.02-0.10) mmol/L per 2 days walking. For the whole walking tour, a 1 km/h higher walking speed is related to a LDL-c increase of 0.18 mmol/L (95%CI -0.16-0.42) and an increase in total cholesterol of 0.36 mmol/L (95%CI 0.12-0.60) mmol/L. Lower or higher walking speed was not related to differences in blood pressure, weight, waist circumference, triglycerides or glucose (*Table 2*).

Similar analyses were performed with walking speed expressed in steps/hour in stead of km/h, with similar results. A 1000 steps/hour faster walking speed was associated with increases in HDL-c of 0.01 mmol/L (95%CI 0.00-0.02), LDL-c of 0.02 mmol/L (95%CI 0.00-0.04) and total cholesterol of 0.03 mmol/L (95%CI 0.00-0.05) per 2 days of walking (*Table 3*). Exploratory adjustment of the relation between walking speed and changes in total cholesterol, LDL-c, HDL-c and triglycerides for changes in body weight did not change the results. Adjusting all analyses for the total walked distance did not change the results, as expected, as differences in total walking distance between subjects were very small. In a sensitivity analysis, we additionally adjusted for baseline values of LDL-c, HDL-c, triglycerides and systolic and diastolic blood pressure, which did not change the results markedly.

Table 2. The effect of walking speed in km/h on the changes per 2 days in cardiovascular risk factors.

	HDL-cholesterol	LDL-cholesterol	Total cholesterol	Triglycerides	Systolic BP
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
model I	0.03 (0.02 - 0.05)*	0.02 (-0.02 - 0.06)	0.05 (0.01 - 0.09)*	-0.02 (-0.06 - 0.03)	0.03 (-0.74 - 0.80)
model II	0.04 (0.02 - 0.05)*	0.02 (-0.02 - 0.06)	0.05 (0.01 - 0.10)*	-0.01 (-0.06 - 0.03)	-0.07 (-0.84 - 0.70)
model III	0.04 (0.02 - 0.05)*	0.03 (-0.01 - 0.07)	0.06 (0.02 - 0.10)*	0.00 (-0.05 - 0.04)	-0.07 (-0.85 - 0.70)

	Diastolic BP	Weight	Waist circ.	Glucose
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
model I	0.01 (-0.43 - 0.45)	0.06 (-0.06 - 0.18)	0.15 (-0.25 - 0.56)	-0.01 (-0.05 - 0.03)
model II	-0.01 (-0.45 - 0.42)	0.05 (-0.07 - 0.18)	0.07 (-0.33 - 0.47)	-0.02 (-0.06 - 0.02)
model III	-0.03 (-0.47 - 0.41)	0.06 (-0.06 - 0.19)	0.18 (-0.21 - 0.57)	0.00 (-0.04 - 0.04)

The regression coefficient β (with 95% confidence interval (95%CI)) denotes the mean change in the risk factor per 2 days which is associated with a 1 km/h higher walking speed. For example, a 1 km/h higher walking speed is associated with an increase in HDL-cholesterol of 0.04 (95%CI 0.02-0.05) mmol/L (Model III) per 2 days, translating to 0.24 (95%CI 0.12-0.30) mmol/L during the whole 12-day walking tour. Model I= crude; model II= age and gender; model III = age, gender, current smoking, BMI and heart rate at baseline. *= p< 0.05, BP= blood pressure, Waist circ.= waist circumference, LDL= low density lipoprotein, HDL= high density lipoprotein.

Table 3. The effect of walking speed in 1000 steps/h on the changes per 2 days in cardiovascular risk factors.

	HDL-cholesterol	LDL-cholesterol	Total cholesterol	Triglycerides	Systolic BP
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
model I	0.01 (0.00 - 0.02)*	0.02 (0.00 - 0.04)*	0.02 (0.00 - 0.05)*	-0.01 (-0.03 - 0.01)	-0.41 (-0.81 - -0.01)*
model II	0.01 (0.00 - 0.02)*	0.02 (0.00 - 0.04)*	0.02 (0.00 - 0.05)*	-0.01 (-0.03 - 0.01)	-0.40 (-0.79 - -0.00)*
model III	0.01 (0.00 - 0.02)*	0.02 (0.00 - 0.04)*	0.03 (0.00 - 0.05)*	0.00 (-0.03 - 0.02)	-0.36 (-0.76 - 0.04)
	Diastolic BP	**Weight**	**Waist circ.**	**Glucose**	
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	
model I	-0.10 (-0.33 - 0.13)	0.01 (-0.05 - 0.08)	0.09 (-0.12 - 0.30)	0.00 (-0.03 - 0.02)	
model II	-0.09 (-0.32 - 0.14)	0.01 (-0.05 - 0.08)	0.09 (-0.12 - 0.30)	-0.01 (-0.03 - 0.02)	
model III	-0.06 (-0.29 - 0.17)	0.01 (-0.05 - 0.08)	0.12 (-0.08 - 0.32)	0.00 (-0.02 - 0.02)	

The regression coefficient β (with 95% confidence interval (95%CI)) denotes the mean change in the risk factor per 2 days which is associated with a 1000 steps/h higher walking speed. For example, a 1000 steps/h higher walking speed is associated with an increase in HDL-cholesterol of 0.01 (95%CI 0.00-0.02) mmol/L (Model III) per 2 days, translating to 0.06 (95%CI 0.00-0.12) mmol/L during the whole 12-day walking tour. Model I= crude; model II= age and gender;
model III = age, gender, current smoking, BMI and heart rate at baseline. *= p< 0.05, BP= blood pressure, Waist circ.= waist circumference, LDL= low density lipoprotein, HDL= high density lipoprotein.

Discussion

In the present study, it is shown that walking speed significantly relates to changes in the lipid profile in healthy middle-aged men and women walking 12 days to Santiago de Compostela. A higher walking speed was related to a higher increase in HDL-c and attenuated decrease in LDL-c and total cholesterol, a relation that was not explained by changes in body weight. Differences in walking speed were not related to changes in blood pressure, weight, waist circumference, triglycerides or glucose.

Several well designed randomized controlled trials, controlling for exercise volume, report no effects of exercise intensity on plasma lipoproteins, or on other cardiovascular risk factors.(7-10) These trials describe long-term changes (after 3-8 months) in cardiovascular risk factors and the total weekly amount of exercise is limited (not more than 3 hours or 1000-1200 calories per week).(7-10) The present study describes changes in cardiovascular risk factors during exercise, and the daily amount of exercise in the current study was almost twice the amount of weekly exercise in the trials described above (5.39±0.36 hours daily in the present study).

Possibly, the changes in lipoproteins related to the walking speed described in the current study are present for a limited time span shortly after very large bouts of exercise and are therefore not seen in the studies described above. Other randomized trials report larger decreases in weight, waist circumference and diastolic blood pressure,(13) or larger increases in HDL-c,(12) for higher compared to lower intensity exercise, but these studies did not control for differences in the total amount of exercise, so the reported effects could be due to the higher exercise volume instead of the higher intensity. In the present study all participants walked almost the same distance and in addition we adjusted the analyses for the small differences in total walking distance, which did not change the results.

There is no doubt that physical exercise should be advised to everyone who is capable to exercise, as physical exercise has multiple beneficial health effects.(1-3) Furthermore, more exercise is better, as there is a clear inverse dose-response relation between exercise and all-cause mortality.(2) However, what walking speed is optimal for improving the lipid profile is not sure. Should we advise people to walk with high speed or with low speed when the goal is improvement of the lipid profile? In the present study, walking with higher speed increases HDL-c more, but at the expense of less LDL-c decrease, and walking with lower speed leads to less HDL-c increase but a more profound LDL-c decrease. Does the extra increase in HDL-c related to a higher walking speed outweighs the less decrease in LDL-c? This question cannot be answered with the results of the current study. In general, the primary lipid target in the prevention and treatment of cardiovascular disease

is LDL-c, which is best reached with lower walking speed, according to the results of the present study. However, in large prospective cohort studies in the healthy population, an increased walking speed assessed by a questionnaire has been related to a lower risk for coronary heart disease and diabetes, independent of walking volume.(17-20) This finding can lead to the speculation that the extra increase in HDL-c related to a higher walking speed could be more important than the less decrease in LDL-c. However, drawing conclusions from the combined findings of these two completely different types of studies is a step to far.

Several physiological mechanisms can be considered to explain the exercise-induced and intensity-independent changes in LDL-c and HDL-c. Exercise-induced changes in LDL-c may be due to dilution as a result of an increase in plasma volume,(23) a decrease in body weight or a change in body fat distribution,(24) an up-regulated expression of hepatic LDL-receptors,(25) an increased cholesterol transfer from apoA-containing particles (LDL-c, VLDL) to HDL particles,(26) and the use of cholesterol for cellular metabolism and repair due to muscle damage immediately after intense exercise.(23) Exercise-induced HDL-c changes may be explained by the increased acceptance of free cholesterol from peripheral tissues by nascent HDL-particles,(27) increased HDL particle maturation by cholesterol esterification due to increased lecithin:cholesterol acyltransferase (LCAT),(28) increased breakdown of triglyceride-rich particles resulting from an increased lipoprotein lipase activity, leading to uptake of the cholesterol content by HDL-c particles,(29) which could lead to prolonged HDL-particle survival,(30) and finally a decrease in cholesteryl ester transfer protein (CETP) leading to a reduced shift of cholesterol esters from HDL to non-HDL lipoproteins.(31) Which of these mechanisms is responsible for the observed increases in HDL-c and LDL-c related to higher walking speed in the present study is unknown. We did not measure (markers of) plasma volume changes, which could possibly be of influence on the results. However, as the reported results are linear during 12 days, and the measurements were conducted early in the morning, more than 12 hours after the ending of the previous walking stage, we believe the influence of changes in plasma volume on the results to be small. Furthermore, we showed in an exploratory analysis that the relation between walking speed and changes in blood lipids were not explained by changes in body weight. As the differences between the slower and faster walking speed groups occurred rapidly, within several days, and the amount of daily exercise was large, it is conceivable that consumption of cholesterol, from both HDL and LDL particles, for cellular metabolism and cellular repair due to muscle damage contributes to the observed changes. This explanation is more likely than other, more long term metabolic adaptations. The overall duration of exercise could have a higher impact than the small differences in intensity of this exercise on the amount of cholesterol needed for cellular

metabolism and repair of muscle damage, leading to less increase in HDL-c and more decrease in LDL-c with longer exercise at a lower walking speed.

Walking a pilgrimage requires a considerable amount of time, a thorough preparation and a good physical and mental health. Our findings can be generalised to healthy middle-aged males and females who satisfy these conditions, and possibly to other types of exercise, consisting of prolonged daily periods of moderate intensity. However, the results of the present study are based on a relatively small group of subjects walking 281 km in 12 days. Therefore, no statistical interaction tests and no subgroup analyses could be performed. Whether the relation between walking speed and the change in lipoproteins can be extrapolated to smaller amounts or other types of exercise is not known. The current study reports pragmatic research about exercise in real life, however, more research needs to be done in a controlled lab-based setting in order to fully explore and understand the results of this study. A strength of this study is the equal amount of exercise, in this case the total walking distance, for all participants, eliminating this factor as a possible confounder in the relation between walking speed and changes in cardiovascular risk factors. Furthermore, walking speed was measured and not assessed with a questionnaire like in many cohort studies, and the consistent results for walking speed expressed in km/h and steps/h strengthen our findings.

We also acknowledge study limitations. Participants walking with slower speed were metabolically unhealthier at baseline than subjects walking with faster speed. Whether the worse baseline metabolic profile (such as higher BMI) is the cause of the slower walking speed achieved, or the consequence of for example a lower physical fitness which also results in a slower walking speed, is unclear and cannot be determined from the present study. Therefore, we adjusted the mixed linear effect models for baseline differences between the faster and slower walking speed groups, which did not change the results. Furthermore, we were not able to adjust for differences in the dietary pattern or cardiorespiratory fitness level of the participants, as these variables were not measured. However, by adjusting for the heart rate at baseline as a proxy for cardiorespiratory fitness and for other variables related to cardiorespiratory fitness or unhealthy dietary intake such as age, gender, BMI and smoking, residual confounding of cardiorespiratory fitness or dietary intake is unlikely.

In conclusion, during a 12-day walking tour to Santiago de Compostela with long daily walking stages, walking the same distance with a higher walking speed was related to a more pronounced increase in HDL-c, but to less decrease in LDL-c and total cholesterol, independent of changes in body weight, in healthy middle-aged men and women.

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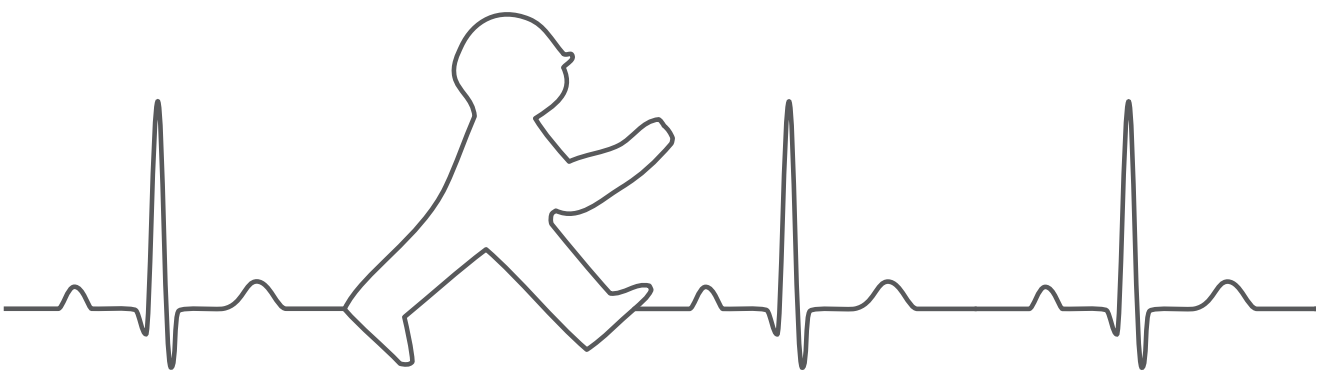

Chapter 4

The effect of physical exercise during lifestyle modification on changes in bodyweight and insulin resistance in patients with the metabolic syndrome

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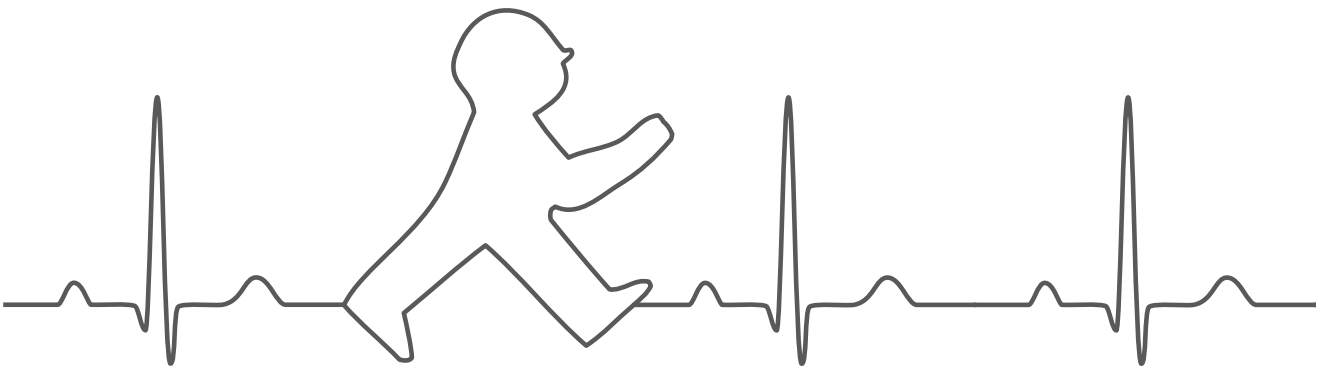

Abstract

Introduction

Patients with the metabolic syndrome are at increased risk for type 2 diabetes, vascular diseases and premature mortality. First treatment step consists of lifestyle modification including increasing physical exercise and changing dietary habits. We investigated the relation between baseline daily physical activity or changes in daily physical activity and changes in bodyweight and insulin resistance during a lifestyle modification program in patients with the metabolic syndrome.

Methods

Overweight patients (BMI>25 kg/m²) with the metabolic syndrome (n=76) willing to change their lifestyle were included in a 1-year nurse-led lifestyle modification program to increase physical exercise and change dietary habits. The relation between physical activity levels, measured with SenseWear Armband, and 3-monthly changes in bodyweight and insulin resistance during 1-year follow-up were evaluated using a linear mixed effects model.

Results

Patients hardly changed their daily physical activity during the study (change in METs 0.04; 95%CI -0.01-0.08), but weight after 1 year changed -2.30 kg (95%CI -3.82--0.79). A 1 metabolic equivalent (MET) higher baseline physical activity level was related to changes in weight (-0.69 kg; 95% confidence interval (CI) -1.77-0.40), waist circumference (-0.51 cm; 95%CI -1.65-0.63), BMI (-0.18 kg/m²; 95%CI -0.53-0.17) and HOMA-IR (-0.78; 95%CI -1.62-0.07) per 3 months during the intervention. A 1 MET increase in physical activity during 1 year was related to changes in weight (-2.31 kg; 95%CI -3.92--0.70), BMI (-0.80 kg/m²; 95%CI -1.32--0.28), waist circumference (-2.75 cm; 95%CI -4.37--1.14) and insulin resistance (-0.34; 95%CI -1.39-0.71) per 3 months, adjusted for age, gender, smoking, diabetes, history of vascular disease and baseline level of physical activity.

Conclusions

Not only an increase in physical activity during a 1-year lifestyle modification program, as expected, but also the level of baseline physical activity was related to decreases in bodyweight and insulin resistance in overweight patients with the metabolic syndrome.

Introduction

The metabolic syndrome is a combination of obesity-related metabolic abnormalities including insulin resistance, atherogenic dyslipidemia, elevated blood pressure, a prothrombotic profile and systemic low-grade inflammation (1). Patients with the metabolic syndrome are at increased risk for type 2 diabetes mellitus (2), cardiovascular disease (1), and mortality (3). The prevalence of the metabolic syndrome has been increasing over the past decades and is now estimated to affect at least a quarter of the US population, particularly older adults (4;5) and about 50% of patients with clinically manifest vascular disease (6). Leading health organisations promote the use of lifestyle modification programs, predominantly based on the combination of increasing physical activity and changing dietary habits to induce weight loss, as the first-line therapeutic strategy for the management of the metabolic syndrome (7-9).

In subjects with the metabolic syndrome, lifestyle modification programs focussing on increasing physical activity and changing dietary habits have been shown to lead to improvements in multiple metabolic risk factors and even resolution of the metabolic syndrome (10-13). The combination of diet and exercise interventions are more effective than either approach alone for the treatment of the metabolic syndrome (14). However, a wide range of different lifestyle interventions is described in the literature but there is no consensus about a specific ideal or advised program.

In clinical practice, despite the fact that the advice to adopt a healthy lifestyle is routinely given to persons with diabetes or the metabolic syndrome, the majority of patients remain poorly controlled regarding the treatment targets of HbA1C, LDL-c and blood pressure and exhibit low adherence to dietary and physical activity recommendations (15-17). Identifying patients with a higher likelihood on positive results of these lifestyle programs, before starting, would be of great advantage. We hypothesize that patients with a higher baseline physical activity level are more likely to increase their level of exercise resulting in better outcomes of a lifestyle modification program, as physical activity is already part of their daily behaviour. On the other hand one could argue that patients with low baseline physical activity could more easily increase their daily physical activity level and could benefit more.

In the present study, we investigated if the amount of daily physical activity measured at baseline, and the increase in daily physical activity is related to the effects of a lifestyle modification program on changes in weight, BMI, waist circumference and insulin resistance in overweight patients with the metabolic syndrome. We focused primarily on weight, physical activity and insulin resistance because these are crucial pathophysiological factors in the causal pathway of the metabolic syndrome and associated complications, and are less likely influenced by concomitant medication than, for example, blood pressure or lipid levels.

Subjects and methods

Study design and population

This is a non-randomized, single arm study without a control group, comparing measures of bodyweight measures and insulin resistance before and after a 1-year nurse-led lifestyle modification program. Between 2006 and 2010, overweight patients with the metabolic syndrome referred to the University Medical Center Utrecht for treatment of the metabolic syndrome were offered to participate in a comprehensive lifestyle modification program. Those who were motivated to change their lifestyle, were eligible to participate in the lifestyle modification program. According to the NCEP/ATP III (2001) criteria (18), for the diagnosis of the metabolic syndrome at least 3 of the following criteria are required: abdominal obesity (waist circumference >102 cm (men) or >88 cm (women)), elevated blood pressure (≥130 mmHg systolic and/or ≥85 mmHg diastolic and/or use of blood pressure-lowering medication), hypertriglyceridemia (fasting serum triglycerides ≥1.70 mmol/l), low high density lipoprotein-cholesterol (HDL-c) (serum HDL-c <1.04 mmol/l (men) or <1.29 mmol/l (women)) or high fasting glucose (fasting serum glucose ≥6.1 mmol/l and/or use of glucose-lowering medication). Patients using systemic corticosteroids or highly active anti-retroviral therapy (HAART) were excluded from participation.

As this lifestyle modification program was seen as part of regular care for those who were motivated to extra intense follow-up, no approval of the medical ethics committee was required and no written informed consent had to be obtained. Patients could participate voluntarily in this program after being thoroughly informed. In total, 132 patients were recruited for participation, 4 withdrew their participation before the program started and the remaining 128 were included in the lifestyle modification program (*Figure 1*). Subjects (n=22) who entered the lifestyle modification program before the start of measurement of physical activity in May 2007 and subjects with missing baseline physical activity measurements for other reasons (n=5) were excluded for the current analyses. Furthermore, subjects who stopped participation in the lifestyle modification program within the first 6 months (n=25) were excluded for the current analyses, as they did not have follow-up measurements of the outcome variables.

The lifestyle modification program

The lifestyle modification program was guided by specifically trained nurses and supervised by an internist specialized in Vascular Medicine. The primary goal was to induce weight loss by changing dietary habits, reducing calorie intake and increasing physical activity and to stop smoking by means of education, stimulation of self-efficacy, motivation and biofeedback. In the first 6 months of the intervention, the patients visited the nurse monthly, and during the second 6

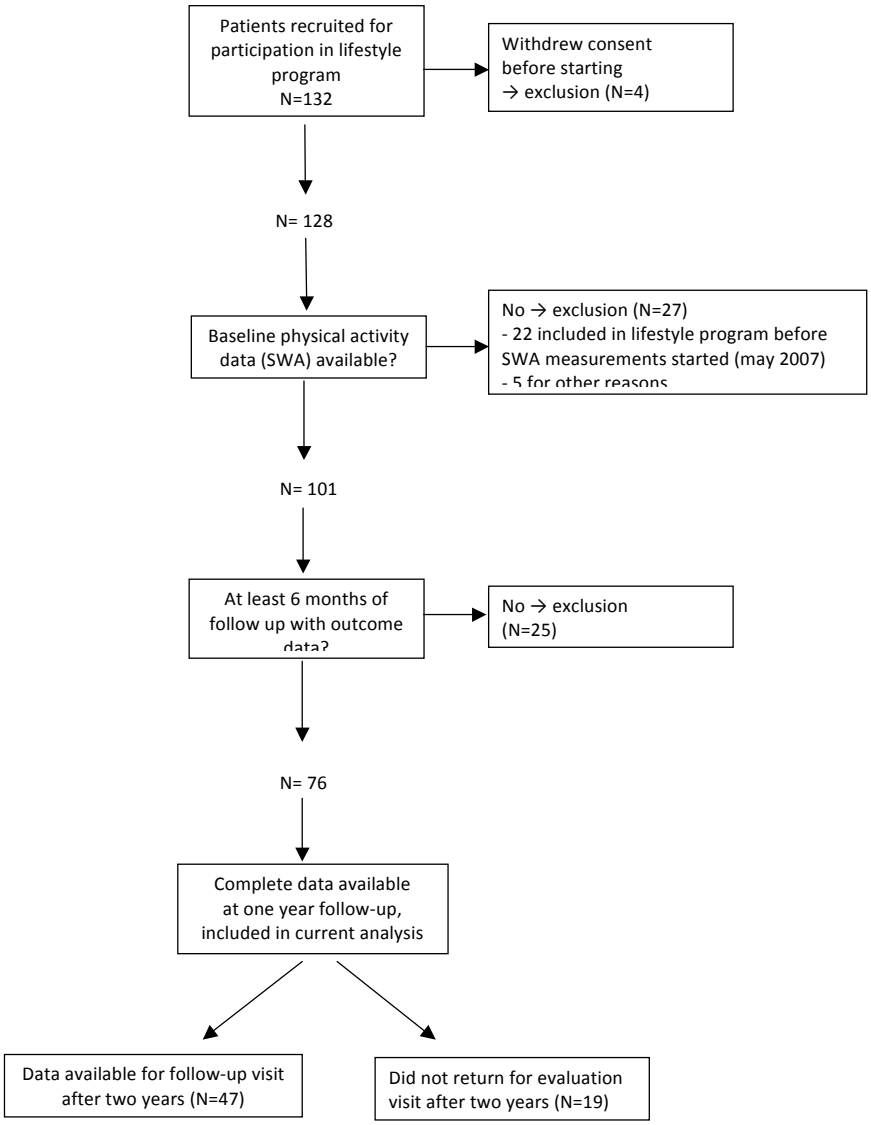

Figure 1. Study flow chart

months every other month. After 1 year the lifestyle modification program stopped and patients returned to regular care. Two years after the start of the intervention an evaluation visit was scheduled.

During the intervention period patients were counseled on healthy dietary habits according to guidelines (19), but without describing a specific diet or a specific low-calorie diet. The exercise recommendation of a daily minimum of 30 minutes of moderate-intensity physical activity was given, according to guidelines (20).

At baseline and during all follow-up visits all participants were extensively advised on lifestyle modification by the nurse, and introducing or changing pharmacotherapeutic interventions was optional after 6 months if treatment goals for lipids or blood pressure were not reached. At baseline and during the follow-up visits at 6, 12 and 24 months, anthropometric, laboratory and physical activity measurements were conducted.

Anthropometric and laboratory measurements

Weight was measured without heavy clothing and shoes. Body mass index (BMI) was calculated as weight divided by squared height. Waist circumference was measured with a tape measure halfway between the lower rib and the iliac crest. Blood pressure was calculated as the mean of three recordings in seated position at the arm with the highest value at the baseline visit, using an automatic blood pressure device (Omron 705 IT, Hoofddorp, The Netherlands).

Fasting venous blood samples were drawn to determine plasma levels of glucose and insulin. Homeostatic model assessment of insulin resistance (HOMA-IR) was used as quantitative estimate of the degree of insulin resistance, calculated by the formula: HOMA-IR = (fasting serum glucose (mmol/L) x fasting serum insulin (mIU/L)/ 22.5).

Physical activity measurements

For the assessments of physical activity, participants wore the SenseWear Armband (SWA) (BodyMedia Inc., Pittsburgh, Pennsylvania) for one or more consecutive days. The SWA is a lightweight physical activity monitor worn on the upper right arm, using a tri-axial accelerometer, a thermistor-based skin sensor, a proprietary heat flux sensor and a galvanic skin response sensor in combination with demographic characteristics to assess energy expenditure, physical activity intensity and duration, number of steps, and on body wear time. This device has been validated against indirect calorimetry and doubly label water technique in resting, exercise, and free-living conditions (21;22). The SWA also reports the number of metabolic equivalents (MET), defined as the ratio of the work metabolic rate to a standard resting metabolic rate of 1.0 $kcal \cdot kg^{-1} \cdot h^{-1}$ (23). A value of 1 MET is considered the energy cost of a person at rest (quiet sitting).

Outcome measures

Weight, BMI, waist circumference and insulin resistance were measured at baseline, 6 and 12 months during the 1-year lifestyle modification program, and at the evaluation visit at 24 months, 1 year after the lifestyle modification program had stopped. Changes from baseline values in measures of bodyweight and insulin resistance, in measures of physical activity and in the number of subjects smoking

are presented graphically, expressed as mean changes with standard error of the mean (SEM). A 95% confidence interval (95%CI) was calculated around each change from baseline value, using confidence interval analysis calculator version 2.2.0 (University of Southampton, Southampton, United Kingdom).

Data analyses

Continuous variables are expressed as mean±standard deviation (SD) when normal distributed, and as median (interquartile range) in case of skewed distribution. Categorical variables are expressed as percentage (%). The baseline characteristics are described per tertile of daily physical activity, expressed as daily average METs. To prevent overrepresentation of male subjects in the higher METs tertiles, the data from men and women were ranked separately into tertiles and then combined in gender-pooled tertiles resulting in an almost even distribution of gender over the tertiles.

A linear mixed effects model was used to investigate the relation between both the amount of physical activity at baseline or the change in physical activity and changes in measures of bodyweight and insulin resistance. In this model, the relation between physical activity and changes in measures of bodyweight and insulin resistance was adjusted for differences in baseline values (using a random intercept) and for changes in body weight and insulin resistance due to the progression of the lifestyle modification program (using a fixed time-dependent variable). To investigate the effect of physical activity on measures of bodyweight and insulin resistance, an interaction variable of physical activity and the progression of the intervention period (represented by the fixed time-dependent variable) was added to the model. The β-coefficients with 95%CI of the interaction terms are reported to quantify the relation between a specific increase in physical activity (for example 1 MET) and the changes in bodyweight and insulin resistance per 3 months during the lifestyle modification program. In model I the unadjusted relation between physical activity and changes in bodyweight and insulin resistance is presented. In model II, adjustments were made for the potential confounding variables age and gender. In model III additional adjustments were made for current smoking, type 2 diabetes mellitus and a history of vascular disease. The main results are based upon this model. To study the relation between change of physical activity and changes in bodyweight and insulin resistance we conducted a sensitivity analysis with additional adjustment for baseline physical activity (Model IV). To make sure that changes in HOMA-IR were not excessively influences by patients with diabetes or using glucose-lowering medication, the analyses for the effects on HOMA-IR were repeated after exclusion of patients with type 2 diabetes (n=44), which also excludes all patients with changes in glucose-lowering medication during the intervention period (n=11). For all analyses SPSS version 15.0.1 was used.

Results

Baseline characteristics

Baseline characteristics of the study population are presented in gender-pooled tertiles of daily physical activity, expressed as daily average METs (*Table 1*), ranging from 1.0±0.1 in tertile 1 to 1.5±0.2 in tertile 3. The differences in daily total energy expenditure between the tertiles (2631±458 calories for tertile 1, compared to 3249±952 calories for tertile 3) were largely explained by the difference in daily physical activity with an intensity of >3 MET energy expenditure (258±187 calories in tertile 1 versus 858±548 calories in tertile 3). Patients in tertile 3 were younger than patients in tertile 1 (45±11 versus 57±9 years), had a lower BMI (32.4±4.6 versus 37.9±6.6 kg/m²) and were less insulin resistant (HOMA-IR 5.8±4.4 versus 7.7±3.3).

Results of the lifestyle modification program

The results of the lifestyle modification program are presented in *Figure 2A-H*. The average amount of daily physical activity did hardly change during the study, whether expressed in METs (0.04; 95%CI -0.01-0.08)(*Figure 2A*), total energy expenditure (25 calories; 95%CI -58-107)(*Figure 2B*) or number of steps (564; 95%CI -174-1301)(*Figure 2C*) during the 1-year intervention period. However, the total study population decreased in weight (-2.30 kg; 95%CI -3.82--0.79)(*Figure 2D*), BMI (-0.76 kg/m²; 95%CI -1.25--0.27)(*Figure 2E*), waist circumference (-3.42 cm; 95%CI -5.00--1.84)(*Figure 2F*) and HOMA-IR (-0.33; 95%CI -1.58-0.91)(*Figure 2G*). The most prominent decreases were seen in the first 6 months, but were attenuated to a large extent in the second year of the study (dashed lines).

Relation between baseline physical activity level and bodyweight and insulin resistance

Compared to tertile 1 of baseline physical activity, patients in tertile 3 had a change per 3 months in weight (-0.58; 95%CI -1.23-0.07 kg), waist circumference (-0.66; 95%CI -1.34-0.02 cm), BMI (-0.18; 95%CI -0.39-0.04 kg/m²), and HOMA-IR (-0.34; 95%CI-0.82-0.14), based on the fully corrected model (*Table 2*). A 1 MET higher baseline physical activity was related to a change per 3 month in weight (-0.69; 95%CI -1.77-0.40 kg), waist circumference (-0.51; 95%CI -1.65-0.63 cm), BMI (-0.18; 95%CI -0.53-0.17 kg/m²) and HOMA-IR (-0.78; 95%CI -1.62-0.07) (*Table 3*). When baseline physical activity was expressed in number of steps (per 1000) of calories of energy expenditure (per 1000), the results were comparable, although the point estimates were smaller, as 1000 steps, 1000 calorie energy expenditure and 1 MET are not equivalent (*Table 3*). Higher baseline physical activity was related to an increase in physical activity during the lifestyle modification program: 0.12 (95%CI 0.09-0.14) METs per 3 months more for tertile 3 versus tertile 1 (*Table 2*) and an increase of 0.26 (95%CI 0.21-0.30) METs per 3 months for a 1 MET higher baseline physical activity (*Table 3*).

Table 1. Baseline characteristics according to gender-pooled tertiles of daily average METs of patients with the metabolic syndrome (n=76).

	Tertile 1 (n=24)	**Tertile 2** (n =32)	**Tertile 3** (n =20)
Daily average METs	1.0±0.1	1.3±0.1	1.5±0.2
Daily average METs (range)	(0.8-1.2)	(1.1-1.4)	(1.3-1.9)
Daily exercise parameters			
Total energy expenditure (calories)	2631±458	2935±654	3249±952
Number of steps	4892±2217	8687±2105	12299±4451
Calorie expenditure during intensity >3METs	258±187	559±324	858±548
Duration of physical activity >3METs (min)	36±24	82±33	134±68
Patient characteristics			
Age (years)	57±9	54±9	45±11
Male gender, n (%)	12 (50)	19 (59)	11 (55)
Current smoking, n (%)	6 (25)	9 (28)	6 (30)
Weight (kg)	111±17	99±17	94±22
Body mass index (kg/m^2)	37.9±6.6	32.2±3.7	32.4±4.6
Waist circumference (cm)	123±13	110±12	109±12
Diabetes mellitus type 2, n (%)	15 (63)	11 (34)	6 (30)
Vascular disease, n (%)	8 (33)	8 (25)	3 (15)
Systolic blood pressure (mmHg)	150±20	149±17	141±12
Lipid-lowering medication, n (%)	16 (67)	19 (59)	11 (55)
Glucose-lowering medication, n (%)	11 (46)	4 (13)	3 (15)
Blood pressure-lowering medication, n (%)	19 (79)	26 (81)	8 (40)
Laboratory parameters			
eGFR (ml/min/1.73m^2)	83±19	79±16	86±14
Triglycerides (mmol/l)	1.9 (1.4-3.1)	2.4 (1.6-3.9)	2.0 (1.3-3.7)
HDL-cholesterol (mmol/l)	1.04±0.21	1.07±0.33	0.94±0.25
LDL-cholesterol (mmol/l)	2.9±1.2	2.7±1.1	3.2±1.0
HOMA-IR	7.7±3.3	5.0±3.0	5.8±4.4
Components of metabolic syndrome ¶			
Central obesity, n (%)	24 (100)	30 (94)	18 (90)
Hypertension, n (%)	24 (100)	30 (100)	18 (90)
Low HDL-cholesterol, n (%)	18 (75)	22 (69)	16 (80)
Hypertriglyceridemia, n (%)	16 (67)	23 (72)	12 (60)
Hyperglycemia, n (%)	18 (75)	20 (63)	7 (35)

The study population is presented in gender-pooled tertiles according to their daily average METs. Data are expressed as means±SD, median (interquartile range) or numbers (percentage). ¶= using the NCEP/ATPIII 2001 definition. MET= metabolic equivalent of task, expressing the energy cost of activities relative to the resting metabolic rate set by convention at 1 kcal·kg^{-1}·h^{-1}, eGFR=estimated glomerular filtration rate, HDL= high density lipoprotein, LDL= low density lipoprotein, HOMA-IR= homeostasis model of assessment-insulin resistance.

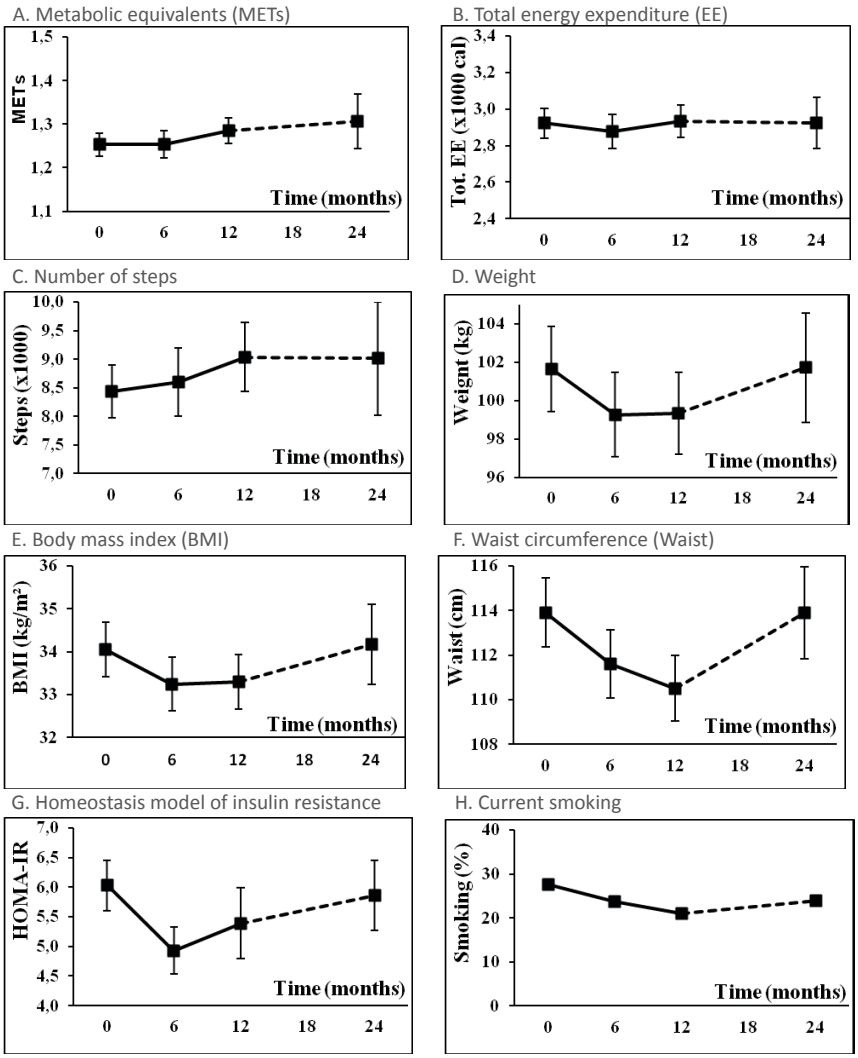

Figure 2 A-H. Results of the 1-year lifestyle program in patients with the metabolic syndrome. Changes in physical activity parameters (METs (A), total energy expenditure (B) and number of steps (C)) and body weight (D), BMI (E), waist circumference (F), HOMA-IR (G) and smoking (H) during the 1-year lifestyle program (0-12 months, solid line) and after a year of follow-up (24 months, dashed line). Data are means with standard error of the mean (SEM).

Table 2. Relation between gender-pooled tertiles of baseline physical activity and measures of obesity and insulin resistance per 3 months of the lifestyle program.

		Tertile 1 (n= 24)	**Tertile 2** (n= 32)	**Tertile 3** (n = 20)
Daily average METs		1.0±0.1	1.3±0.1	1.5±0.2
Daily average METs	(range)	(0.8-1.2)	(1.1-1.4)	(1.3-1.9)
	Model	β (95%CI)	β (95%CI)	β (95%CI)
Δ Weight (kg)	I	0 (reference)	-0.45 (-1.03 - 0.13)	-0.52 (-1.17 - 0.13)
	II	0 (reference)	-0.51 (-1.08 - 0.07)	-0.59 (-1.24 - 0.06)
	III	0 (reference)	-0.49 (-1.07 - 0.09)	-0.58 (-1.23 - 0.07)
Δ Waist circ. (cm)	I	0 (reference)	-0.71 (-1.32 - -0.11)	-0.66 (-1.34 - 0.02)
	II	0 (reference)	-0.75 (-1.36 - -0.15)	-0.67 (-1.35 - 0.01)
	III	0 (reference)	-0.74 (-1.34 - -0.13)	-0.66 (-1.34 - 0.02)
Δ BMI (kg/m^2)	I	0 (reference)	-0.18 (-0.37 - 0.01)	-0.18 (-0.39 - 0.03)
	II	0 (reference)	-0.18 (-0.37 - 0.01)	-0.19 (-0.40 - 0.03)
	III	0 (reference)	-0.17 (-0.36 -0.01)	-0.18 (-0.39 - 0.04)
Δ HOMA-IR	I	0 (reference)	-0.08 (-0.51 - 0.35)	-0.32 (-0.79 - 0.15)
	II	0 (reference)	-0.12 (-0.55 - 0.31)	-0.39 (-0.89 - 0.11)
	III	0 (reference)	-0.08 (-0.49 - 0.33)	-0.34 (-0.82 - 0.14)
Δ MET	I	0 (reference)	0.07 (0.04 - 0.10)	0.10 (0.06 - 0.13)
	II	0 (reference)	0.08 (0.05 - 0.10)	0.12 (0.09 - 0.14)
	III	0 (reference)	0.07 (0.05 - 0.10)	0.12 (0.09 - 0.14)

Results are expressed as beta coefficients (β) with 95% confidence intervals (95%CI), denoting the increase in outcome variable per 3 months compared to patients in the reference tertile 1. For example, tertile 3 increases -0.2 (-0.4 - 0.0) kg/m² in BMI per 3 months compared to tertile 1 (Model III). MET: Metabolic equivalent, Δ= change per 3 months, BMI= body mass index, HOMA-IR= Homeostasis model of insulin resistance. Model I: crude; model II: age and gender; model III: age, gender, current smoking, history of vascular disease, type 2 diabetes mellitus.

Relation between change in physical activity during the study and body weight and insulin resistance

An increase in physical activity of 1 MET during the intervention program was related to statistically significant changes per 3 months in weight (-2.31 kg; 95%CI -3.92--0.70), waist circumference (-2.75 cm; 95%CI -4.37--1.14), BMI (-0.80 kg/m²; 95%CI -1.32--0.28), while there was no change in HOMA-IR (-0.34; 95%CI -1.39-0.71) (*Table 4*). Further adjustment for the baseline physical activity level (model IV) did not markedly change the results.

Sensitivity analyses

Results were not essentially different when analyses for the effects on HOMA-IR were repeated for patients without diabetes (n=44), which also excludes all patients with changes in glucose-lowering medication during the intervention period (n=11).

Table 3. Relation between more baseline physical activity and measures of obesity and insulin resistance per 3 months of the lifestyle program.

		Δ Weight (kg)	Δ Waist (cm)	Δ BMI (kg/m²)	Δ HOMA-IR	Δ MET
Physical activity	Model	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
	I	-0.44 (-1.51 - 0.64)	-0.32 (-1.45 - 0.81)	-0.19 (-0.54 - 0.16)	-0.46 (-1.24 - 0.32)	0.31 (0.27 - 0.35)
1 MET	II	-0.69 (-1.76 - 0.40)	-0.49 (-1.64 - 0.65)	-0.18 (-0.53 - 0.17)	-0.79 (-1.68 - 0.09)	0.26 (0.21 - 0.31)
more	III	-0.69 (-1.77 - 0.40)	-0.51 (-1.65 - 0.63)	-0.18 (-0.53 - 0.17)	-0.78 (-1.62 - 0.07)	0.26 (0.21 - 0.30)
	I	-0.14 (-0.20 - -0.08)	-0.14 (-0.20 - -0.08)	-0.05 (-0.07 - -0.03)	-0.04 (-0.08 - 0.01)	0.01 (0.01 - 0.01)
1000 steps	II	-0.15 (-0.20 - -0.09)	-0.14 (-0.20 - -0.08)	-0.05 (-0.07 - -0.03)	-0.04 (-0.09 - 0.00)	0.01 (0.01 - 0.01)
more	III	-0.14 (-0.20 - -0.09)	-0.14 (-0.20 - -0.08)	-0.05 (-0.06 - -0.03)	-0.03 (-0.08 - 0.01)	0.01 (0.01 - 0.01)
	I	-0.47 (-0.81 - -0.14)	-0.09 (-0.45 - 0.27)	-0.15 (-0.26 - -0.04)	-0.05 (-0.29 - 0.20)	0.01 (0.00 - 0.01)
1000 calories	II	-0.53 (-0.87 - -0.20)	-0.15 (-0.51 - 0.21)	-0.14 (-0.25 - -0.03)	-0.14 (-0.43 - 0.15)	0.01 (-0.01 - 0.03)
more	III	-0.53 (-0.86 - -0.19)	-0.14 (-0.50 - 0.22)	-0.15 (-0.25 - -0.04)	-0.15 (-0.43 - 0.13)	0.01 (-0.01 - 0.03)

Results are expressed as beta coefficients (β) with 95% confidence intervals (95%CI), denoting the increase in outcome variable per 3 months associated with a 1 MET, 1000 steps or 1000 calories more baseline physical activity. For example, 1000 calorie total energy expenditure more at baseline is related to an increase in weight of -0.5 (-0.9 - -0.2) kg per 3 months (Model III). MET: Metabolic equivalent, Δ= change per 3 months, BMI= body mass index, HOMA-IR= Homeostasis model of insulin resistance. Model I: crude; model II: age and gender; model III: age, gender, current smoking, history of vascular disease, type 2 diabetes mellitus.

Table 4. Relation between change in physical activity and change in measures of obesity and insulin resistance.

		Δ Weight (kg)	Δ Waist (cm)	Δ BMI (kg/m^2)	Δ HOMA-IR
Physical activity	Model	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
1 MET more	I	-2.35 (-3.97 - -0.73)	-2.84 (-4.47 - -1.22)	-0.81 (-1.33 - -0.29)	-0.65 (-1.74 - 0.44)
	II	-2.34 (-3.96 - -0.73)	-2.86 (-4.48 - -1.25)	-0.81 (-1.33 - -0.29)	-0.66 (-1.75 - 0.43)
	III	-2.31 (-3.92 - -0.70)	-2.75 (-4.37 - -1.14)	-0.80 (-1.32 - -0.28)	-0.34 (-1.39 - 0.71)
	IV	-2.54 (-4.16 - -0.94)	-3.10 (-4.69 - -1.50)	-0.85 (-1.37 - -0.33)	-0.62 (-1.67 - 0.44)
1000 steps more	I	-0.02 (-0.11 - 0.07)	-0.11 (-0.20 - -0.02)	-0.01 (-0.04 - 0.02)	-0.03 (-0.09 - 0.03)
	II	-0.03 (-0.12 - 0.06)	-0.11 (-0.20 - -0.02)	-0.01 (-0.04 - 0.02)	-0.04 (-0.10 - 0.02)
	III	-0.03 (-0.12 - 0.06)	-0.11 (-0.20 - -0.02)	-0.01 (-0.04 - 0.02)	-0.03 (-0.09 - 0.02)
	IV	-0.03 (-0.12 - 0.06)	-0.11 (-0.20 - -0.02)	-0.01 (-0.04 - 0.02)	-0.04 (-0.09 - 0.02)
1000 calories more	I	1.23 (0.43 - 2.02)	0.28 (-0.54 - 1.09)	0.37 (0.12 - 0.63)	-0.03 (-0.56 - 0.50)
	II	1.22 (0.43 - 2.01)	0.28 (-0.53 - 1.10)	0.37 (0.12 - 0.63)	-0.04 (-0.57 - 0.49)
	III	1.23 (0.44 - 2.02)	0.31 (-0.50 - 1.12)	0.38 (0.12 - 0.63)	0.01 (-0.49 - 0.51)
	IV	1.43 (0.65 - 2.22)	0.48 (-0.34 - 1.29)	0.39 (0.14 - 0.65)	-0.02 (-0.55 - 0.50)

Results are expressed as beta coefficients (β) with 95% confidence intervals (95%CI), denoting the increase in outcome variable per 3 months associated with a 1 MET, 1000 steps or 1000 calories increase in physical activity during the intervention program. For example, an increase of 1 MET is related to an decrease in weight of -2.3 (-3.9 - -0.7) kg per 3 months (Model III). MET: Metabolic equivalent, Δ= change per 3 months, BMI= body mass index, HOMA-IR= Homeostasis model of insulin resistance. Model I: crude; model II: age and gender; model III: age, gender, current smoking, history of vascular disease, type 2 diabetes mellitus; model IV (exploratory): model III and additional adjustment for baseline amount of physical exercise.

Discussion

In the present study, overweight patients with the metabolic syndrome followed a 1-year lifestyle modification program. Both the amount of baseline daily physical activity, and the increase in daily physical activity during the study were related to decreases in weight, BMI, waist circumference and insulin resistance.

In the present study, an average body weight loss of 2.5% after 1 year was achieved by patients with the metabolic syndrome participating in a lifestyle modification program, a result that is comparable to other studies (24;25). However, other lifestyle modification programs in patients with the metabolic syndrome, testing various dietary regimens with or without physical exercise, report larger decreases in body weight, of up to 5-10% during a follow-up of 5-24 months (10;11;13). However, in these studies, patients with diabetes (10;11) and patients with cardiovascular disease (13) were excluded, whereas 42% of the patients in the present study had type 2 diabetes and 25% had a history of cardiovascular disease. Patients with diabetes or cardiovascular disease may have more difficulties losing weight or increasing their physical activity, because of medication use causing weight gain (sulfonylurea derivatives or insulin) or limiting exercise capacity (beta-blockers). Furthermore, exercise capacity can be restricted due to (fear of) hypoglycemia, symptoms of cardiovascular disease (angina, intermittent claudication or fatigue) or physical impairments as a result of cerebrovascular disease. Besides differences in study population, the lifestyle modification programs in various studies included diets tailored to provide a ~500-calorie/day deficit (10;11), whereas patients in the current study did not receive a hypocaloric diet.

There was no control group in the present study, but it seems reasonable to state that a population of patients with the metabolic syndrome will normally stay on the same weight or even gain weight during 1 year, as was also seen in the second year of follow-up after the lifestyle modification program had stopped. In clinical practice, a large proportion of patients with obesity and diabetes are reported not to reach dietary and physical exercise recommendations and remain poorly controlled (15-17). Because any weight loss in this population at high risk for cardiovascular disease is beneficial, we consider our lifestyle modification program, resulting in 2.5% (-2.3 kg; 95%CI -3.8--0.8) weight loss during 1 year, to be a successful strategy.

In the present study, a higher baseline physical activity level was consistently related to decreases in measures of bodyweight and insulin resistance during the 1-year lifestyle modification program, both analysed continuously and per tertile, and expressed in METs, calorie expenditure or number of steps. A possible explanation for this association is that physically active subjects are more likely to increase their physical activity when stimulated, which is also confirmed in the

present study. Due to limited statistical power, the results did not always reach statistical significance, but as all the effect estimates point in the same direction, we consider the results of these analyses to be valid. On the other hand, very large differences in daily baseline physical activity, such as an increase of 1 MET, or the comparison between the highest versus the lowest tertile of physical activity, are associated with only modest decreases in measures of bodyweight and insulin resistance, whereas the effects of 1000 steps per day more at baseline on these parameters are small. This is comparable to what is reported in another study from our group investigating the effects of prolonged daily walking (280km in 12 days), where only a small decrease in body weight (-1.4±1.8 kg) is reported and no change in insulin resistance (26). Therefore, the clinical significance of the positive relation between baseline physical activity and decreases in measures of bodyweight and insulin resistance is limited, implying that patients with limited physical activity at baseline can also achieve beneficial results with lifestyle modification.

An increase in daily physical activity of 1 MET during the study was related to clinically significant decreases in measures of bodyweight and insulin resistance. However, an increase of 1 MET is very large. For example, 1 MET increase in average daily physical activity is equal to walking 8 hours at 4 km/h (3 MET/h) or jogging for 3,5 hours (8 MET/h). Patients in the lowest tertile had a physical activity level of 1.0 MET compared to 1.5 MET in the highest tertile. Increasing 1000 steps per day is hardly related to any measurable weight loss, which means that also the effect of implementing the guideline recommendation of 30 minutes of moderate-intensity exercise per day (20), equivalent to around 2500-3000 steps more, on weight loss will be very modest. More weight loss, between 1-3% of body weight, is reported in trials of supervised exercise therapy in patients with the metabolic syndrome (14;24;25), although calorie intake was sometimes reduced as well (14). Furthermore, also trials with negative results from exercise therapy on body weight are reported (27). Overall, the effect of increasing physical activity on body weight loss seems to be limited, with the best results obtained with supervised programs.

The present study has several strengths. Unlike other studies, the present study population comprised patients with the metabolic syndrome with and without complications such as type 2 diabetes mellitus and vascular diseases, and therefore better represents the patient population in clinical practice. As the lifestyle modification program was not conducted in a typical research setting, but was part of regular medical care, the current results represent the clinical situation as well. Physical activity parameters were measured with a validated device, instead of, for example, assessed by questionnaire, and measurements comprised physical exercise patterns of several days.

Some limitations need to be considered. The population size was limited, but using a mixed linear effect model enabled us to use all follow-up data and increase statistical power. There was a considerable dropout rate of the lifestyle program of 28%, however, again, this resembles clinical practice very much in this population. The results in this study can thus only be generalised to patients who are compliant to a lifestyle modification program. Sensitivity analyses excluding all patients with diabetes did not show different results. Finally, we report results of a versatile lifestyle modification program, and we calculate which parts of the results are related to physical exercise measures. Causality is not sure in this relation, as a person increasing physical activity might also improve in other healthy behaviour such as compliance to dietary advices, which may have influenced the results.

In conclusion, in overweight patients with the metabolic syndrome participating in a lifestyle modification program, a mean weight loss of 2.5% of baseline body weight was achieved after 1 year of follow-up. Both baseline physical activity and increases in the daily physical activity level during the study were related to decreases in weight, BMI, waist circumference and insulin resistance.

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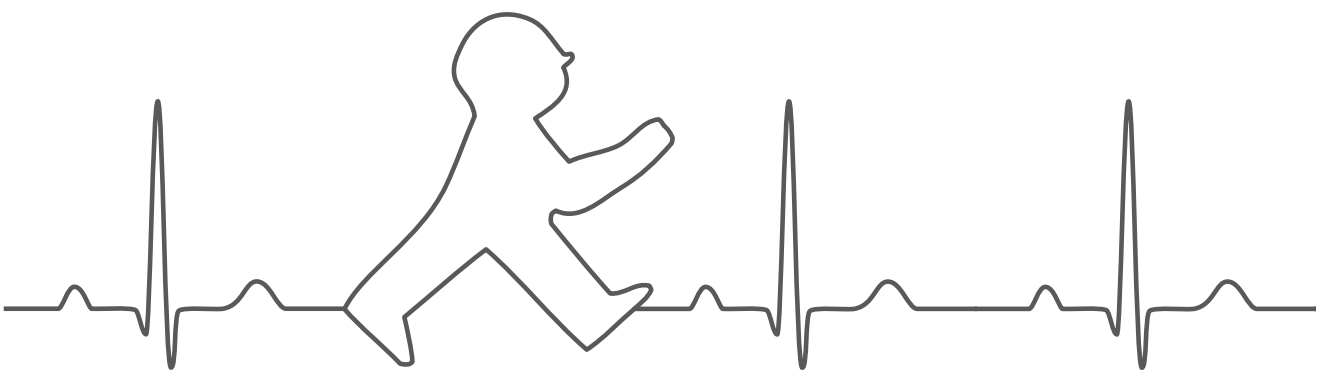
Part 2

Resting heart rate



Chapter 5

Increased visceral adipose tissue is associated with increased resting heart rate in patients with manifest vascular disease

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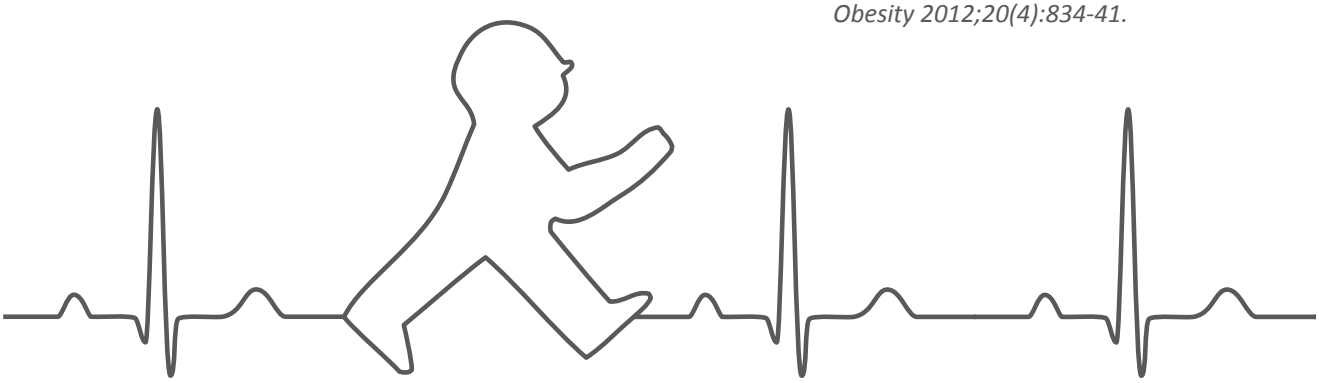
Obesity 2012;20(4):834-41.



Abstract

Abdominal obesity is characterized by sympathetic nerve activation (SNA), probably mediated by elevated insulin and leptin levels. Resting heart rate (RHR) is a marker of sympathetic tone, and independently associated with cardiovascular events and death in various populations. We investigated and quantified the relation between visceral adipose tissue (VAT) and RHR in patients with vascular disease.

In 3,723 patients with manifest vascular disease, visceral and subcutaneous fat tissue was measured with ultrasonography. RHR was obtained from an electrocardiogram (ECG). The association between quartiles of VAT and RHR was quantified using linear regression analysis with adjustments for potential confounding factors. Separate analyses were performed for men and women and for location of vascular disease.

Visceral fat was categorized into sex-pooled quartiles (Q) ranging from 2.7 to 8.0 cm in Q1 (reference) to 9.4 to 20.6 cm in Q4. High visceral fat thickness was associated with increased RHR, in men (Q4 versus Q1, β = 4.36; 95% confidence interval (CI) 3.11 to 5.61) and women (β = 1.48; 95%CI -0.70 to 3.66), after full adjustment. Waist circumference and BMI had a significant relation with RHR in men (β = 3.51; 95%CI 2.21 to 4.81 and β = 2.80; 95%CI 1.51 to 4.08 respectively) but these relations were smaller and not significant in women (β = 0.71; 95%CI -1.44 to 2.85 and β = 0.24; 95%CI -1.90 to 2.37 respectively). There was no relation between subcutaneous fat and RHR in men and women. The relation between visceral fat and RHR was similar in patients with different locations of vascular diseases.

Increased visceral fat is associated with increased RHR in male and female patients with vascular disease, independent of the location.

Introduction

Obesity, and in particular abdominal obesity, is an independent risk factor for the development of cardiovascular diseases (1), and strongly related to the development of metabolic complications such as dyslipidemia, insulin resistance and diabetes mellitus (2). Subjects with abdominal obesity have an increased volume of visceral adipose tissue (VAT) (3). VAT is the predominant adipose tissue compartment producing various proinflammatory cytokines and adipokines (4). Therefore, VAT is currently considered to play a central role in the state of low-grade inflammation and clustering of metabolic disturbances often referred to as metabolic syndrome (5).

Another characteristic feature of obesity is sympathetic nerve activation (SNA), which is more pronounced in abdominal obesity than in peripheral obesity (6). Central obesity is associated with high serum levels of both insulin and leptin which may affect SNA (7). In humans, administration of insulin increases muscle sympathetic nerve activity (MSNA), the reference standard of measuring sympathetic activity (8), by a direct effect on insulin-receptors in the central nervous system (9;10). Plasma leptin levels are independently associated with resting heart rate (RHR) in male subjects (11;12). Furthermore, the infusion of leptin in rats increases the heart rate, via leptin-receptors in the central nervous system (13;14). Thus, VAT may directly influence SNA. SNA is increased in subjects with cardiovascular disease or cardiovascular risk factors (15;16). Moreover, it is considered to play an important role in the pathogenesis of cardiovascular disease by increasing major risk factors such as blood pressure, insulin resistance and triglycerides (16-19).

RHR is a measure of sympathetic tone, and correlates with MSNA or noradrenalin serum levels (20). In contrast to MSNA, RHR is easily obtained, noninvasive and nonexpensive and thus better applicable in the clinical situation. More importantly, elevated RHR is an independent risk factor for cardiovascular events and mortality in patients with and without cardiovascular disease (21-23). Although it has been described that the presence of the metabolic syndrome is associated with an elevated RHR (24), it is unclear what the origin of elevated RHR is. VAT may increase RHR by activating the sympathetic nerve system via high insulin and leptin serum levels. In the present study we investigated the relation between visceral adiposity and RHR in patients with various clinical manifestations of vascular disease.

Methods and procedures

Study design and patients

In this study, data were used from patients enrolled in the SMART study (Second Manifestations of ARTerial disease), an ongoing single centre prospective cohort study carried out at the University Medical Center Utrecht. The SMART study started in September 1996. Patients aged 18-80 years, referred to our institution with clinically manifest atherosclerotic vascular disease (coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm) or with cardiovascular risk factors (hyperlipidemia, diabetes or hypertension) were included. Patients with terminal malignant disease, those not independent in daily activities (Rankin scale >3) and not sufficiently fluent in Dutch were not included.

The aims of the SMART study are to determine (i) risk factors for atherosclerosis, (ii) prevalence of additional vascular disease and (iii) incidence of future cardiovascular events. The Medical Ethics Committee of the UMC Utrecht, the Netherlands, has approved the study and all patients gave written informed consent. Patients were asked to complete a health questionnaire covering medical history, risk factors, smoking habits and medical treatment. A standardized diagnostic protocol was followed consisting of physical examination and laboratory testing in a fasting state. A more detailed description of the design of the study has been published previously (25).

For the present study, patients with manifest vascular disease at study inclusion were selected when they were enrolled in the SMART-cohort from May 2000 to March 2009 (n=3,970), as visceral fat was not measured regularly in the period before May 2000. Patients without a study- electrocardiogram (ECG) (n=64) or without sinus rhythm on the ECG (n=183) were excluded, leaving 3,723 subjects for final analyses (*Figure 1*).

Measurement of RHR

At study inclusion, a 12-lead ECG was obtained after the patient had rested in supine position for 5 minutes. Heart rate was calculated using the digitally stored 12-lead 10-second data, by dividing the number of R-R intervals (number of QRS-complexes minus one) by the time difference between the first and last beat, and the result was converted to beats/minute. This calculation was performed using the Marquette 12SL analysis program (General Electric Healthcare, Hoevelaken, the Netherlands).

Measurement of intra-abdominal and subcutaneous fat

Intra-abdominal fat was estimated anthropometrically by measurement of the waist circumference and ultrasonographically. Waist circumference was measured

halfway between the lower rib and the iliac crest and was taken in standing position. B-mode ultrasound of the abdomen was obtained to measure intra-abdominal and subcutaneous fat and performed by well-trained registered vascular technologists in a certified vascular laboratory. Ultrasonographic measurements were performed in supine position using an ATL HDI 3000 ultrasound device (Philips Medical Systems, Eindhoven, The Netherlands) with a C 4-2 transducer. There was no bowel preparation performed before the ultrasound measurement. Intra-abdominal fat was ultrasonographically measured as the distance between the peritoneum and the lumbar spine using electronic callipers. A strict protocol was used, including the position of the transducer and pressure applied on the transducer. The transducer was placed in a straight line drawn between the left and right midpoints of the lower rib and the iliac crest. Measurements were performed at the end of a quiet expiration, applying minimal pressure without displacement or compression of the abdominal cavity. The distance was measured three times at slightly different positions; the intra-abdominal fat was calculated as the mean value of these three measurements. Previously, the ultrasound protocol for measuring intra-abdominal fat was validated with computed tomography (CT) at our center (26). Ultrasonographic measurements were strongly associated with CT measurements of intra-abdominal fat: Pearson's correlation coefficient was 0.81 ($p<0.001$). Also, an interobserver coefficient of variation of 5.4% was found for ultrasound measurements of intra-abdominal fat, indicating good reproducibility.

For determining subcutaneous fat, the distance between the linea alba and the skin was measured. Abdominal muscles were thus excluded for both intra-abdominal and subcutaneous fat measurements.

Data analyses

Continuous variables are expressed as mean ± SD when normal distributed or as median (interquartile range) in case of skewed distribution. Categorical variables are expressed as numbers (percentage). The baseline characteristics are described per quartile of visceral fat. A p value for trend across the quartiles of visceral fat was calculated with one-way ANOVA. To prevent overrepresentation of male subjects in the higher quartiles of visceral fat, the data from men and women were ranked separately into quartiles and then combined in gender-pooled quartiles resulting in an almost even distribution of gender over quartiles. Potential interaction on the relationship between VAT and RHR was investigated on an additive scale by entering cross-products of visceral fat thickness and possible interaction factors (selected on pathofysiologic grounds: gender, age and the use of β-blockers) in the linear regression analysis. Only gender was a significant interaction term in the relation between VAT and RHR ($p = 0.03$).

Therefore, this relation was analyzed for male and female subjects separately.
The relation between measures of adiposity and RHR was quantified with linear regression analysis. Results are expressed as β-regression coefficients with 95% confidence intervals (95% CI) denoting the change in RHR per quartile measure of adiposity relative to the first (reference) quartile.
Three models were used to estimate this relation. Firstly, a model was made with adjustments for the potential confounding variables age and gender. Secondly, a model was made with additional adjustments for the potential confounding variables current smoking, diagnosis of diabetes mellitus type 2, estimated glomerular filtration rate (eGFR) and the use of medication with effect on the RHR (β-blockers, α-blockers, diuretics and calcium-channel blockers). The main results are based upon this model. Additional adjustment for BMI was conducted in the analyses of the relation between RHR and VAT to explore if the relation was independent of BMI (Model III). The presence of type 2 diabetes mellitus, current smoking, gender and the use of above-mentioned medication were included as categorical variables and age, eGFR and BMI were entered as continuous variables. Finally, an additional, exploratory, analysis was performed including all covariates with a p-value of <0.10 in the baseline table.
To compare the strength of the relations between different measures of adiposity and RHR, an analysis per SD increase in measure of adiposity was conducted. To assess whether the relation between VAT and RHR was different in patients with different manifestations of vascular disease at study inclusion, separate, continuous analyses were conducted stratified for location of vascular disease.
To reduce bias and to improve statistical efficiency, missing values for visceral fat thickness (n=105), subcutaneous fat thickness (n=132), BMI (n=3), waist circumference (n=131), smoking status (n=19) and eGFR (n=26) were completed in the dataset by single regression imputation. Sensitivity analyses excluding the subjects with missing data for the investigated measures of adiposity, instead of imputating these missing data, were conducted.
SPSS version 15.0.1 was used for all analyses.

Results

Baseline characteristics

Baseline characteristics of the study population are presented in sex-pooled quartiles of visceral fat thickness (*Table 1*). Visceral fat thickness ranged from 2.7 to 8.0 cm in quartile 1 (Q1) to 9.4 to 20.6 cm in quartile 4 (Q4). The proportion of male subjects was approximately 74% in all quartiles. In patients within the highest intra-abdominal fat quartile, RHR was 66 ± 13 beats/minute compared to 62 ± 12 beats/minute in patients in the lowest quartile.

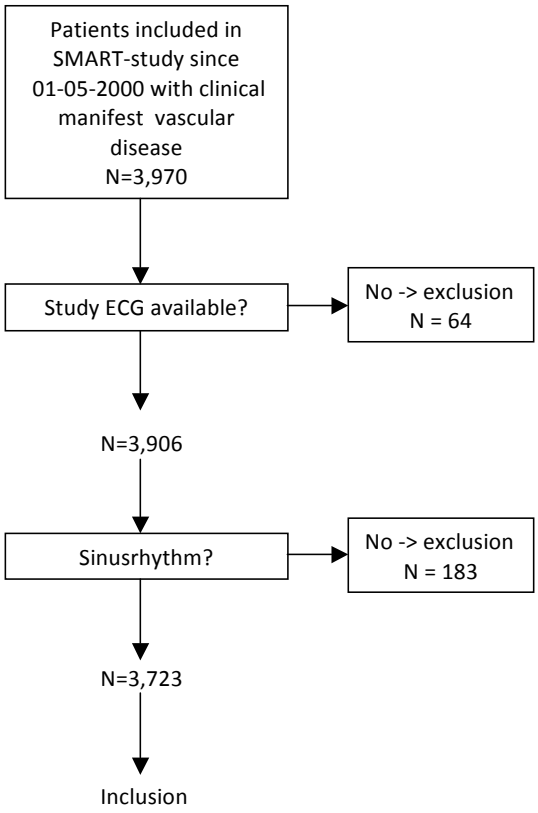


Figure 1. Flow chart.

Intra-abdominal fat and other markers of adiposity in relation to RHR.

High visceral fat thickness was associated with increased RHR in men (Q4 versus Q1, β = 4.36; 95%CI 3.11 to 5.61) adjusted for age, current smoking, eGFR, type 2 diabetes and the use of medication with effect on the RHR (β-blockers, α-blockers, diuretics and calcium-channel blockers) (Model II). All results are based upon this model. This relation was also present in women, although smaller and not statistically significant (Q4 versus Q1, β = 1.48; 95%CI -0.70 to 3.66) (*Figure 2*).

The β-coefficient of 4.36 implies that male patients within the highest quartile of intra-abdominal fat have a mean RHR of 4.36 beats/minute higher than males within the lowest quartile. Analyzed continuously, per increase in 1 SD intra-abdominal fat thickness (1 SD = 2.46 cm for both men and women), RHR increased with 1.80 beats/minute (95%CI 1.35 to 2.25) in men and 0.88 (95%CI 0.11 to 1.66) in women. (*Figure 3*).

Table 1. Baseline characteristics according to quartiles of intra-abdominal fat (n = 3,723).

	Quartile 1 n = 942	Quartile 2 n = 939	Quartile 3 n = 915	Quartile 4 n = 927	P value for trend
Intra-abdominal fat range men (cm) n= 2,724	(3.2 - 8.0) n = 695	(8.1 - 9.4) n = 690	(9.5 - 11.1) n = 655	(11.2 - 20.6) n = 684	
Intra-abdominal fat range women (cm) n= 1,000	(2.7 - 6.1) n = 247	(6.2 - 7.6) n = 249	(7.7 - 9.3) n = 260	(9.4 - 19.0) n = 243	
Intra-abdominal fat (cm)	6.3 ± 1.2	8.3 ± 0.9	9.7 ± 1.0	12.5 ± 1.8	<0.01
Male gender, n (%)	695 (74)	690 (74)	655 (72)	684 (74)	0.77
Age (years)	57.7 ± 11.4	59.1 ± 10.4	60.7 ± 9.8	60.5 ± 9.5	<0.01
Current smoking, n (%)	312 (33)	290 (31)	291 (32)	287 (31)	0.41
BMI (kg/m^2)	24.0 ± 2.9	25.9 ± 2.8	27.8 ± 3.3	30.3 ± 4.0	<0.01
Waist circumference (cm)	86 ± 10	92 ± 9	97 ± 10	105 ± 11	<0.01
Subcutaneous adipose tissue (cm)	2.3 ± 1.2	2.4 ± 1.3	2.4 ± 1.2	2.5 ± 1.6	<0.01
Systolic blood pressure (mmHg)	139 ± 22	140 ± 21	145 ± 21	145 ± 21	<0.01
Diastolic blood pressure (mmHg)	81 ± 12	82 ± 11	84 ± 11	84 ± 12	<0.01
Resting heart rate (beats/minute)	62 ± 12	63 ± 12	65 ± 12	66 ± 13	<0.01
Glucose (mmol/l)	5.5 (5.1 - 6.0)	5.7 (5.3 - 6.2)	5.9 (5.4 - 6.6)	6.2 (5.7 - 7.3)	<0.01
Triglycerides (mmol/l)	1.08 (0.80 - 1.46)	1.30 (1.00 - 1.80)	1.50 (1.08 - 2.09)	1.71 (1.26 - 2.45)	<0.01
HDL-cholesterol (mmol/l)	1.37 ± 0.43	1.26 ± 0.37	1.22 ± 0.36	1.15 ± 0.34	<0.01
LDL-cholesterol (mmol/l)	2.75 ± 0.96	2.79 ± 0.98	2.78 ± 1.03	2.83 ± 0.99	0.13
HOMA IR	1.77 (1.20 - 2.56)	2.24 (1.52 - 3.24)	2.77 (1.87 - 4.34)	3.86 (2.76 - 5.92)	<0.01
eGFR (ml/min/1.73m^2)	79 ± 17	77 ± 18	76 ± 19	75 ± 19	<0.01
HsCRP (mg/l)	1.3 (0.6 - 3.1)	1.6 (0.8 - 3.4)	1.9 (1.0 - 4.0)	2.6 (1.4 - 5.3)	0.01
Thyroid stimulating hormone (mU/L)	1.60 (1.19 - 2.30)	1.60 (1.10 - 2.30)	1.62 (1.10 - 2.50)	1.70 (1.20 - 2.50)	0.28
Diabetes mellitus type 1, n (%)	6 (1)	9 (1)	8 (1)	8 (1)	0.65
Diabetes mellitus type 2, n (%)	82 (9)	112 (12)	181 (20)	248 (27)	<0.01

Table 1. Baseline characteristics according to quartiles of intra-abdominal fat (n = 3,723).

	Quartile 1 n = 942	Quartile 2 n = 939	Quartile 3 n = 915	Quartile 4 n = 927	P value for trend
Inclusion diagnosis					
Coronary artery disease, n (%)	454 (48)	373 (50)	482 (53)	481 (52)	0.07
Cerebrovascular disease, n (%)	177 (19)	160 (17)	157 (17)	133 (14)	0.02
Peripheral arterial disease, n (%)	128 (14)	140 (15)	122 (13)	145 (16)	0.37
Abdominal aortic aneurysm, n (%)	33 (4)	42 (5)	41 (5)	46 (5)	0.14
Medication use					
Platelet aggregation inhibitors, n (%)	716 (76)	702 (75)	724 (79)	700 (76)	0.64
Lipid-lowering medication, n (%)	629 (67)	652 (69)	664 (73)	668 (72)	<0.01
Glucose-lowering medication, n (%)	52 (6)	76 (8)	118 (13)	166 (18)	<0.01
Use of insulin, n (%)	22 (2)	23 (2)	45 (5)	67 (7)	<0.01
Oral anticoagulation, n (%)	83 (9)	90 (10)	77 (8)	97 (11)	0.37
β-blocker, n (%)	448 (48)	506 (54)	511 (56)	549 (59)	<0.01
Diuretic, n (%)	136 (14)	158 (17)	197 (22)	251 (27)	<0.01
ACE-i/ARB, n (%)	301 (32)	309 (33)	386 (42)	416 (45)	<0.01
Calcium-channel blocker, n (%)	146 (16)	180 (19)	197 (22)	222 (24)	<0.01
α-blocker, n (%)	6 (1)	6 (1)	12 (1)	14 (2)	0.03

Data are presented as mean ± SD, median (interquartile range) or numbers (percentage). P-values for trends across the quartiles of visceral fat were calculated with one-way ANOVA tests. ACE-i = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, eGRF = estimated glomular filtration rate, HDL= high-density lipoprotein, HOMA IR = homeostasis model assessment of insulin resistance, hsCRP= high-sensitivity C-reactive protein, LDL= low-density lipoprotein.

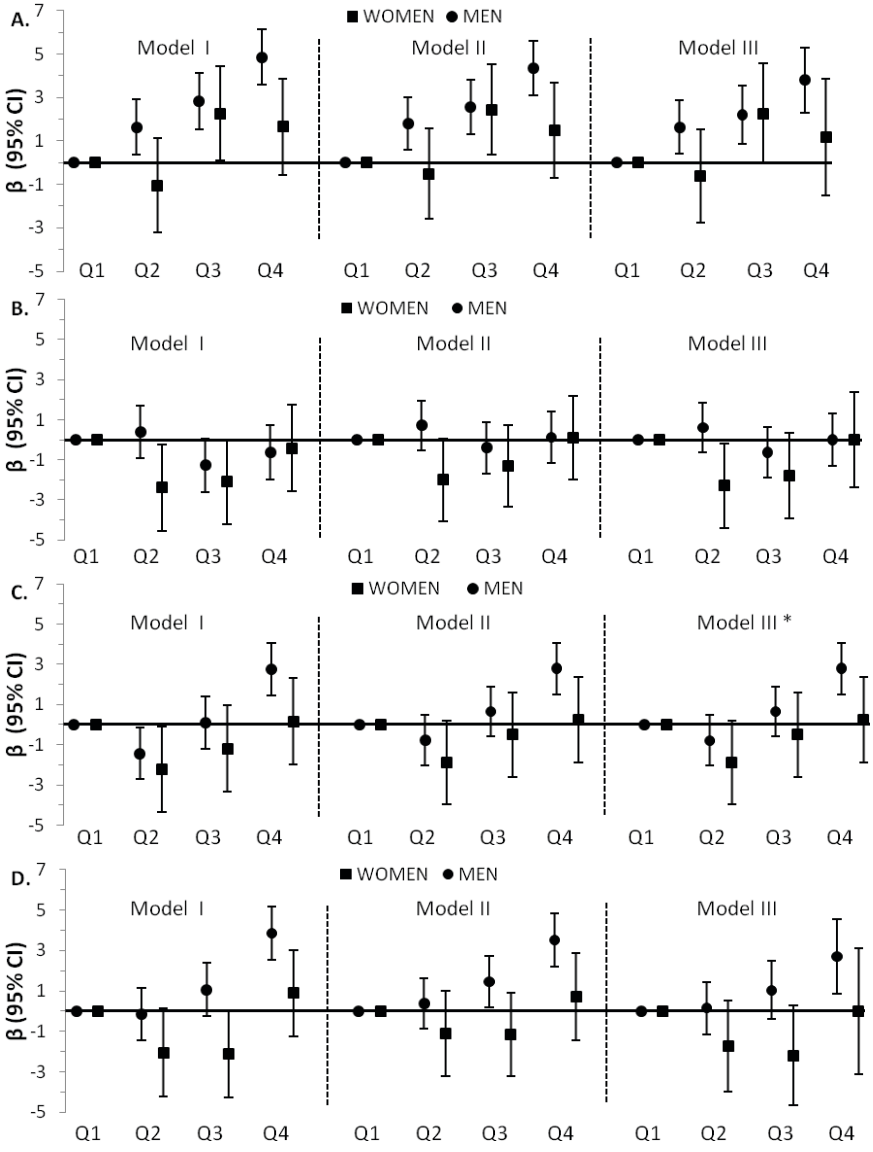


Figure 2. Intra-abdominal fat, subcutaneous fat, BMI and waist circumference in relation to resting heart rate.
The difference in resting heart rate per quartile (Q) of adiposity measure (A) visceral fat, (B) subcutaneous fat, (C) BMI, and (D) waist circumference is expressed relative to quartile 1 (reference). Data are expressed as β- regression coefficients (β) with 95% confidence interval (95% CI). Model I: adjusted for age; Model II: Model I additionally adjusted for current smoking, use of β-blockers, use of α-blockers, use of calcium-channel blockers, use of diuretics, estimated glomerular filtration rate, type 2 diabetes mellitus; Model III: Model II additionally adjusted for BMI. * Data are not adjusted for BMI, as BMI is the determinant. Data in model II and III are therefore equal.

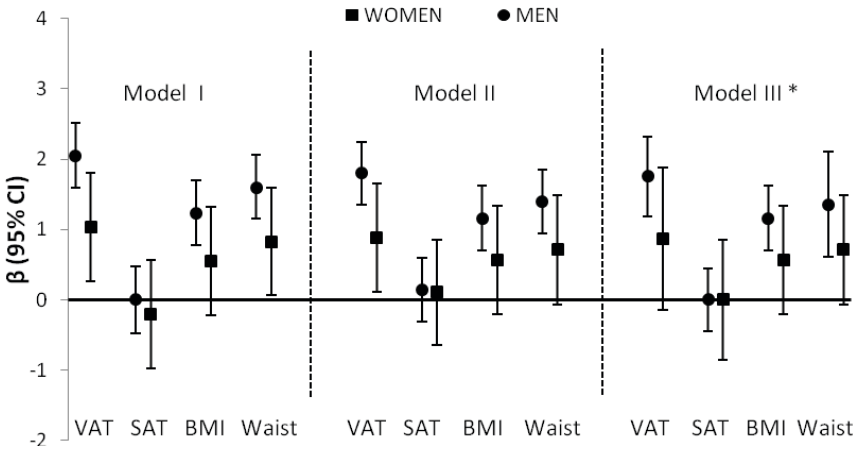


Figure 3. Intra-abdominal fat, subcutaneous fat, BMI and waist circumference in relation to resting heart rate, expressed per SD increase.
The increase in resting heart rate per SD increase in adiposity measure is shown. Data are expressed as β-regression coefficients (β) with 95% confidence interval (95% CI). VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; waist, waist circumference. Model I: adjusted for age; Model II: Model I additionally adjusted for current smoking, use of β-blockers, use of α-blockers, use of calcium-channel blockers, use of diuretics, estimated glomerular filtration rate, type 2 diabetes mellitus; Model III: Model II additionally adjusted for BMI. * Data for BMI are not adjusted for BMI, as BMI is the determinant. Data in model II and model III are therefore equal (for BMI only). Men 1 SD: VAT= 2.46cm, SAT= 1.26cm, BMI= 3.66kg/m², waist= 10.57cm. Women 1 SD: VAT= 2.46cm, SAT= 1.37cm, BMI= 4.88kg/m², waist= 13.07cm.

In men, there was a significant association between both waist circumference and BMI with RHR after adjustment for age (Q4 versus Q1, β = 3.51; 95%CI 2.21 to 4.81 and β = 2.80; 95%CI 1.51 to 4.08 respectively) while these relations were smaller and not significant in women (Q4 versus Q1, β = 0.71; 95%CI -1.44 to 2.85 and β = 0.24; 95%CI -1.90 to -2.37 respectively). Subcutaneous adipose tissue was not related with RHR (Q4 versus Q1, β = 0.13; 95%CI -1.16 to 1.41 in men and β = 0.11; 95%CI -1.99 to 2.21 in women). Continuous analyses showed that an increase in one SD in waist circumference or BMI was significantly associated with an increased RHR in men (β = 1.40; 95%CI 0.95 to 1.85 for waist and β = 1.16; 95%CI 0.70 to 1.62 for BMI) but these relations were smaller and not significant in women (β = 0.71; 95%CI -0.07 to 1.49 for waist and β = 0.57; 95%CI -0.20 to 1.34 for BMI). An increase in one SD of subcutaneous fat was not related to an increase in RHR (β = 0.14; 95%CI -0.31 to 0.59 in men and β = 0.11; 95%CI -0.64 to 0.86 in women). The increase of 1 SD in VAT/SAT ratio resulted in an increase in RHR of β = 0.41; 95%CI -0.04 to 0.86 in men and of β = 0.53; 95%CI -0.20 to 1.26 in women.
After additional adjustment for BMI as a marker of general adiposity, the relation between VAT and RHR decreased only a little in women (Q4 versus Q1, β = 1.19;

95%CI -1.50 to 3.88) and remained statistically significant in men (Q4 versus Q1, β = 3.79; 95%CI 2.29 to 5.29). After exploratory adjustment for all variables in the baseline table with a p-value <0.10, the point estimates were attenuated only slightly.

Separate analysis with exclusion of all patients with thyroid-stimulating hormone levels outside the normal range did not change the age-adjusted relation between intra-abdominal fat and RHR in men and women. Sensitivity analyses with exclusion of the subjects with missing data for the investigated measures of adiposity did not change the results markedly.

Influence of the location of vascular disease on the relation between adiposity and RHR.

The relation between measures of adiposity and RHR appears least strong in patients with peripheral arterial disease or aortic abdominal aneurysm compared with patients with coronary artery disease or cerebrovascular disease (*Figure 4*, only model II is shown).

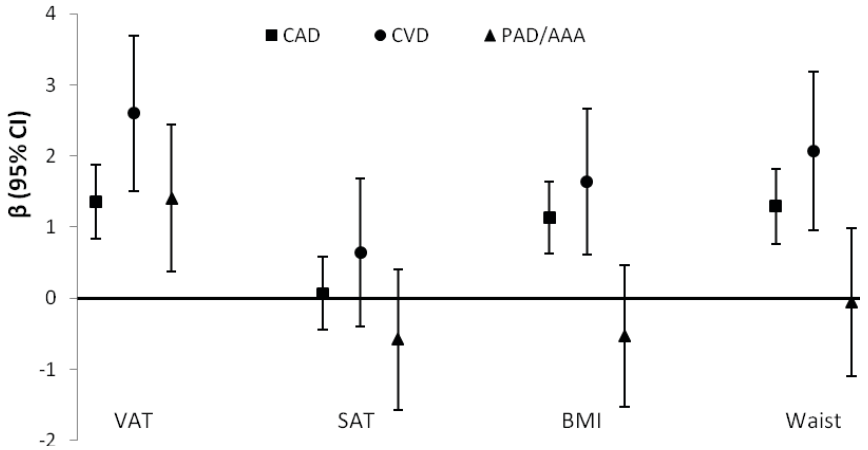


Figure 4. Intra-abdominal fat, subcutaneous fat, BMI and waist circumference in relation to resting heart rate, stratified by location of vascular disease.

The increase in resting heart rate per SD increase in adiposity measure is shown, stratified for location of vascular disease. Data are expressed as β-regression coefficients (β) with 95% confidence interval (95% CI). Data are adjusted for age, gender, current smoking, use of β-blockers, use of α-blockers, use of calcium-channel blockers, use of diuretics, estimated glomerular filtration rate, type 2 diabetes mellitus (Model II in text). AAA, aortic abdominal aneurysm; CAD, coronary artery disease; CVD, cerebrovascular disease; PAD, peripheral arterial disease; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; waist, waist circumference. CAD: 1 SD VAT = 2.49cm, 1 SD SAT = 1.24cm, 1 SD BMI = 3.71kg/m², 1 SD waist = 11.26cm. CVD: 1 SD VAT = 2.55cm, 1 SD SAT = 1.19cm, 1 SD BMI = 3.89kg/m², 1 SD waist = 11.88cm. PAD/AAA: 1 SD VAT = 2.52cm, 1 SD SAT = 1.62cm, 1 SD BMI = 4.02kg/m², 1 SD waist = 11.78cm.

However, this difference was not statistically significant (p for interaction in the relation between visceral fat and RHR 0.58 for CVD, 0.94 for CAD and 0.27 for PAD and AAA). The relation between the other measures of adiposity and RHR was significantly lower for patients with PAD or AAA compared to patients with CAD or CVD (all p-values <0.05). Patients with different locations of vascular disease have different baseline characteristics, for example current smoking (23, 34 and 54% for CAD, CVD and PAD/AAA respectively) or β-blocker use (80, 25 and 26% for CAD, CVD and PAD/AAA respectively).

Discussion

In this cross-sectional study in patients with manifest vascular disease, we observed a positive relation between ultrasonographically measured intra-abdominal fat thickness and RHR. In men, this association was also demonstrated for waist circumference and BMI. Subcutaneous fat thickness did not influence RHR. The relation between visceral fat and RHR was stronger in men than in women and did not differ between patients with different locations of vascular disease. Adjusting for BMI led only to a small decrease in the relation between VAT and RHR, indicating that it is specifically the amount of intra-abdominal fat and not total body fat that is associated with heart rate.

A recent study in patients without cardiovascular disease showed that subjects in the highest quintile of RHR (HR>80 beats/minute) had an odds ratio of 4.2 for men and 3.6 for women of having the metabolic syndrome (24). However, as one of the major determinants of the metabolic syndrome is an increased waist circumference, a measure of intra-abdominal fat, the association between metabolic syndrome and RHR may be a reflection of the underlying relation between visceral fat and RHR, as we describe in the present study. Another recent study found no significant differences in RHR between adolescents with high versus low intra-abdominal fat measured by CT scanning (27). That study was conducted in a considerably smaller, younger and healthier population compared to the study population in the present study, which could explain the fact that no association was found.

Although the present study is a cross-sectional study and causality of the observed relations can only be suggested, a causal pathophysiological explanation for the relation between VAT and RHR can be hypothesized. Increased abdominal fat is associated with elevated plasma concentrations of insulin and leptin (7). The infusion of insulin in humans has shown to increase MSNA and serum noradrenalin levels, both measures of SNA (8). In rats, insulin has been shown to directly act on the insulin receptors in the central nervous system, for example in the hypothalamus (9;10). Similar evidence is available for leptin, although obtained from animal models. In rats, leptin infusion leads to an increase in

mean arterial pressure and heart rate, both measures of SNA (13). Furthermore, a leptin receptor (OB-R) has been identified in mice, in the hypothalamus and other parts of the central nervous system (14). As RHR is a marker of sympathetic activity (20), it can be argued that the high leptin and insulin plasma levels found in visceral obesity stimulate the sympathetic nerve system via receptors in the central nervous system, resulting in an increase in RHR. This obesity-induced SNA seems to be independent of the presence or absence of the sleep apnea syndrome (28). On the other hand, there are also reports describing that both an acute and a chronic increased SNA can precede the development of insulin resistance (19;29). Furthermore, RHR was found to be an independent predictor of body weight gain in young adults with hypertension, which was more pronounced in men than women (30). Whether increased SNA is the cause or the effect of high insulin levels is therefore unknown. Explanations for the different relation between VAT and RHR in male and female patients in the current study could be the larger amount of visceral fat in men than in women (*Table 1*) with possible corresponding effect on the adipose tissue (dys)function.

In line with our findings is that increased sympathetic nerve activity, measured by MSNA, has been associated with abdominal obesity, measured by waist-hip ratio (6), or CT-measured visceral fat mass (31). Both studies were conducted in small populations without cardiovascular disease, and do not present a quantitative relation. Although heart rate is under major influence of the sympathetic nerve system, and correlates with other measures of sympathetic nerve activation such as MSNA (r=0.38) and plasma norepinephrine (r=0.32) (20), there are marked regional differences in SNA and the increased SNA seen in many cardiovascular diseases is organ specific rather than generalized (16). Although certainly not the perfect marker of general sympathetic activity, heart rate probably is the most clinically relevant one, as an increase in heart rate has a strong and independent relation with adverse cardiovascular events and death in both the general population and in patients with cardiovascular disease (21-23). Furthermore, decreasing RHR could become a therapeutic goal, as selectively decreasing RHR with a new pharmacologic agent, ivabradine, has shown to reduce death and hospitalisation in patients with heart failure (32). Finally, RHR is a noninvasive, nonexpensive and easily obtainable parameter, in contrast to MSNA and serum norepinephrine levels.

Although the present study is a cross-sectional analysis of the relation between measures of adiposity and RHR, these results can be placed in clinical perspective using data from prospective studies. An increase in RHR of 10 beats/minute in a large population of patients with stable coronary disease was associated with an increased risk on major cardiovascular events of 8% during a median follow-up of 4.9 years (33). Thus, the increase in RHR in the highest quartiles

of visceral fat thickness in the present study of around 4 beats/minute in men and 1.5 beats/minute in women compared to the lowest quartile, corresponds to an increased estimated risk on major cardiovascular events of around 3.5% for men and 1.5% for women over a 5-year period. In patients with hypertension and electrocardiographic left ventricular hypertrophy an increase in RHR of 10 beats/minute was associated with an increased risk of 16% on cardiovascular mortality during a 5-year follow up (22). Men and women in the highest quartiles of visceral fat thickness in the present study would have an increased risk on cardiovascular mortality of 7% and 3%, respectively compared to the lowest quartiles. These rough estimations give an impression of the possible clinical relevance of our findings, but should be confirmed by prospective studies.

A strength of this study is the large sample size of a clinically relevant and well-characterized group of patients with vascular diseases. Furthermore, abdominal fat compartments and RHR were accurately measured with ultrasonography and ECG, respectively. Assessment of several clinical and laboratory parameters allowed for identification of possible confounding factors. The major limitation of this study is the cross-sectional design, not allowing definitive conclusions about causality of the relation between intra-abdominal fat and RHR. Inherent to etiologic research, the described relation between measures of adiposity and RHR in the present study could be influenced by residual confounding. For example, we were not able to adjust for cardiorespiratory fitness and daily energy expenditure. Cardiorespiratory fitness is related to heart rate (34), and individuals with high VAT accumulation have a low cardiorespiratory fitness, irrespective of BMI (35). However, as we adjusted for other potential confounding variables related to daily energy expenditure and cardiorespiratory fitness (such as age, gender, current smoking and BMI), we have at least partly accounted for the potential confounding effect of cardiorespiratory fitness on the results. Furthermore, as RHR is susceptible to environmental influences, multiple ECG-recordings or continuous recording via 24-hour Holter ECG may increase the accuracy of the RHR. However, the standardized ECG-recordings in our study were conducted with the patient resting in supine position and in a quiet environment, which limits variability.

In conclusion, increased intra-abdominal fat thickness is associated with a higher RHR in patients with manifest vascular disease. Waist circumference and BMI are only significantly associated with RHR in men, while subcutaneous fat is not associated with RHR in men and women. The relation between intra-abdominal fat and RHR is present in male and female subjects and in patients with different locations of vascular disease, and even persists after adjusting for BMI, suggesting that intra-abdominal fat is the major fat compartment responsible for increased RHR in this population.

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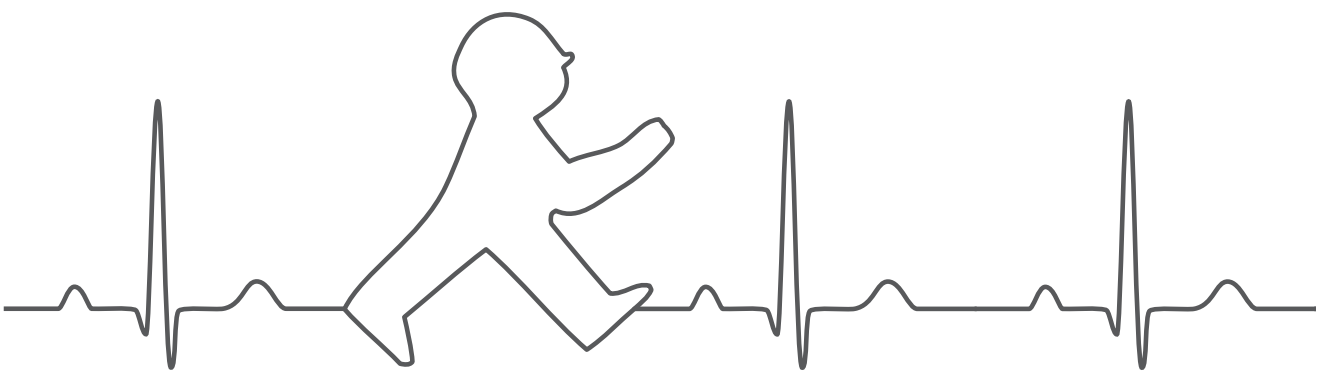

Chapter 6

The risk of resting heart rate on vascular events and mortality in vascular patients

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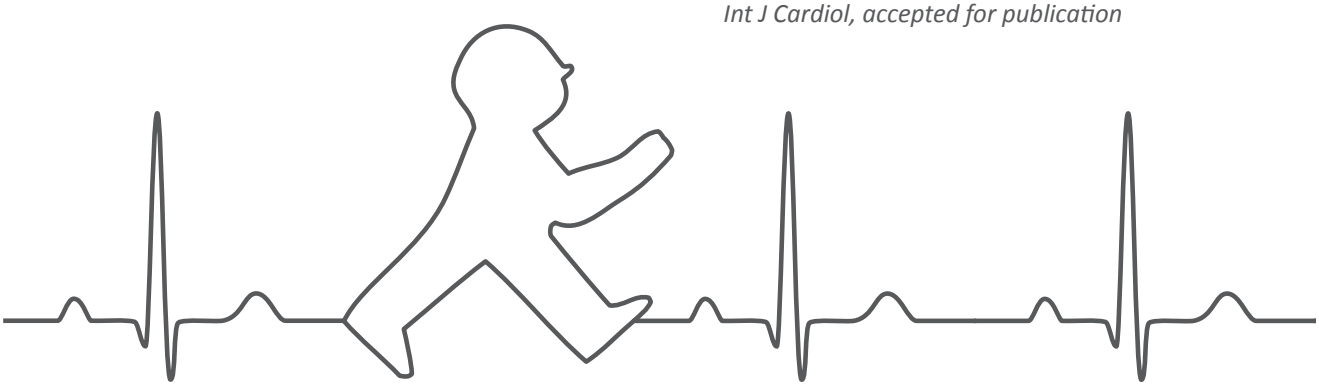

Abstract

Background

Resting heart rate (RHR) reflects sympathetic nerve activity and is independently related to the occurrence of cardiovascular events and death in healthy subjects, patients with coronary artery disease (CAD) and patients with cardiovascular risk factors. We investigated and compared the risk of RHR on the occurrence of cardiovascular events and death in patients with CAD, cerebrovascular disease (CVD), peripheral arterial disease (PAD) or abdominal aortic aneurysm (AAA).

Methods

Data were used from a prospective cohort study of 4272 patients with manifest vascular disease: CAD (n=2244), CVD (n=930), PAD (n=823) or AAA (n=275). RHR was obtained at baseline from an electrocardiogram. The median follow-up time was 4.4 (interquartile range 2.1-7.4) years. The relation between RHR and the occurrence of cardiovascular events and death was estimated by Cox proportional hazard analyses.

Results

Each increase in RHR of 10 beats/minute was related to an increased risk for all-cause mortality (hazard ratio (HR) 1.14; 95% confidence interval (CI) 1.07-1.21) and vascular mortality (HR 1.15; 95%CI 1.06-1.25), but not for myocardial infarction (HR 1.03; 95%CI 0.94-1.14) or ischemic stroke (HR 1.05; 95%CI 0.92-1.20). The relation between an increased RHR and increased risk for all-cause mortality was present irrespective of beta-blocker use and irrespective of the location of vascular disease: CAD (HR 1.23; 95%CI 1.05-1.44), CVD (HR 1.18; 95%CI 1.05-1.33) and PAD/AAA (HR 1.10; 95%CI 1.01-1.20).

Conclusions

Elevated RHR is associated with increased risk for mortality but not for myocardial infarction or stroke in patients with manifest vascular diseases irrespective of location of vascular disease.

Introduction

Sympathetic nerve activity (SNA) is increased in patients with heart failure, myocardial infarction or cerebrovascular disease, and also in patients with diabetes mellitus, hypertension, obesity or metabolic syndrome (1;2). Resting heart rate (RHR) reflects sympathetic tone, and correlates with other measures of SNA such as muscle sympathetic nerve activity (MSNA) and serum noradrenalin levels (3). Furthermore, elevated resting heart rate can also be a reflection of severe disease, such as heart failure. Finally, RHR is an independent risk factor for cardiovascular events and mortality in patients with coronary artery disease (4;5), in patients with diabetes mellitus (6) or hypertension (7) and in healthy subjects (8-13).

Proposed direct adverse effects of an increased RHR on cardiac and vascular function and morphology include endothelial dysfunction, direct stimulation of atherosclerogenesis and atherosclerotic plaque rupture, increased susceptibility for ventricular arrhythmias and a negative influence on the balance between myocardial oxygen demand and supply (14;15). Moreover, in patients with a recent myocardial infarction, heart rate reduction using beta blockers or non-dihydropyridine calcium channel blockers reduces the risk for all-cause- and cardiac mortality and recurrence of non-fatal myocardial infarction, although these agents not only reduce heart rate but also reduce blood pressure (16). Recently, ivabradine, a pure heart-rate-lowering agent without other known cardiovascular effects, has shown to reduce the risk for cardiovascular events and death in patients with coronary artery disease or chronic heart failure who were already on beta blocker treatment, indicating a pathophysiologic role of elevated RHR in the occurrence of cardiovascular events and death (17-19).

The independent relation between elevated RHR and cardiovascular events and death and the beneficial effects of reducing heart rate are based on studies in patients with coronary artery disease. As RHR is a parameter reflecting heart function, and adverse effects of RHR include an increased susceptibility to cardiac events such as ventricular arrhythmias, the adverse pathophysiologic effects of increased RHR could be predominantly present in patients with coronary artery disease. However, as elevated SNA is also present in patients with vascular diseases at other locations and other adverse effects of RHR such as atherosclerotic plaque rupture and endothelial dysfunction are systemic, we hypothesize that the detrimental effects of an elevated RHR could affect patients with clinical manifest vascular disease irrespective of the locations of vascular disease.

Therefore, in this study we investigated the relation between RHR and cardiovascular events and mortality in patients with coronary artery disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm.

Materials and methods

Study design and patients

In this study, data were used from patients enrolled in the SMART study (Second Manifestations of ARTerial disease), an ongoing single centre prospective cohort study carried out at the University Medical Center Utrecht since 1996. Patients aged 18 to 80 years, referred to our institution with clinically manifest atherosclerotic vascular disease (coronary artery disease (CAD), cerebrovascular disease (CVD), peripheral arterial disease (PAD) or abdominal aortic aneurysm (AAA)) were included. Patients with terminal malignant disease, those not independent in daily activities (Rankin scale >3) and not sufficiently fluent in Dutch were not included.

The aims of the SMART study are to determine (i) risk factors for atherosclerosis, (ii) prevalence of additional vascular disease and (iii) incidence of future cardiovascular events. The study complies with the Declaration of Helsinki; the Medical Ethics Committee of the UMC Utrecht, the Netherlands, has approved the study and all patients gave written informed consent. Patients were asked to complete a health questionnaire covering medical history, risk factors, smoking habits and medical treatment. A standardized diagnostic protocol was followed consisting of physical examination and laboratory testing in a fasting state. A more detailed description of the design of the study has been published previously (20).

For the present study, data were used from patients (n=4636) enrolled in the SMART-cohort from September 1996 to March 2009 with a recent diagnosis of CAD, CVD, PAD or AAA. CAD was defined as either a diagnosis of angina pectoris, myocardial infarction, cardiac arrest or coronary revascularisation (coronary bypass surgery or coronary angioplasty). Patients with CVD had a transient ischemic attack, cerebral infarction, amaurosis fugax, retinal infarction or carotid surgery. PAD included patients with symptomatic and documented obstruction of distal arteries of the leg or vascular surgery of the leg (percutaneous transluminal angioplasty, bypass or amputation). Patients with AAA had a supra- or infrarenal aneurysm of the aorta (distal aortic anteroposterior diameter ≥3 cm, measured with ultrasonography) or abdominal aortic aneurysm surgery.

Patients without a study-electrocardiogram (ECG) (n=149) or without sinus rhythm on ECG (n=215) were excluded, leaving a total of 4272 patients for analyses: CAD (n=2244), CVD (n=930), PAD (n=823) or AAA (n=275) (*Figure 1*).

Measurement of resting heart rate

At study inclusion, a 12-lead ECG was obtained with the patient resting in supine position. Resting heart rate (RHR) was calculated using the digitally stored 12-lead 10-second data, by dividing the number of R-R intervals (number of QRS-complexes minus one) by the time difference between the first and last beat, and

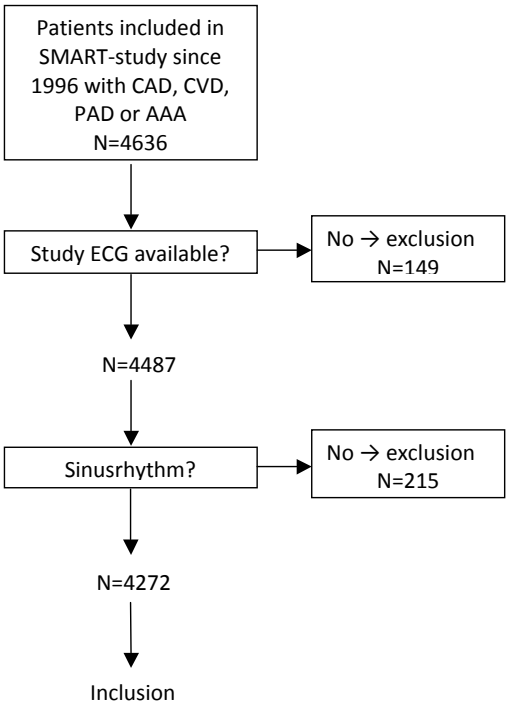

Figure 1. Study flow chart.
CAD: coronary artery disease; CVD: cerebrovascular disease; PAD: peripheral arterial disease; AAA: abdominal aortic aneurysm.

the result was converted to beats per minute. This calculation was performed using the Marquette 12SL analysis program (General Electric Healthcare, Hoevelaken, the Netherlands).

Follow-up

Patients were biannually asked to complete a questionnaire on hospitalisations and outpatient clinic visits. Outcomes of interest for this study were first occurrence of myocardial infarction, ischemic stroke, vascular death, a composite of vascular events (all vascular events) and all-cause mortality. Definitions of events are shown in *Table 1*. When a possible event was reported, hospital discharge letters and results of relevant laboratory and radiology examinations were collected. Death was reported by relatives of the participant, the general practitioner or the vascular specialist. Based on the information from the questionnaire and/or the family, all events were audited by three members of the SMART study

Endpoint Committee, comprising physicians from different departments. If a patient had multiple events, the first recorded event was used in the analyses. Follow-up duration (years) was defined as the period between study inclusion and first cardiovascular event or death from any cause, date of loss to follow-up or the preselected date of 1 March 2009. Due to migration or discontinuation of the study, 123 (2.9%) patients were lost to follow-up.

Table 1. Definitions of vascular events and all-cause mortality.

Ischemic stroke	Relevant clinical features causing an increase in impairment of at least one grade on the modified Rankin scale, without signs of haemorrhage on repeat brain imaging.
Myocardial Infarction	At least two of the following criteria: (I) chest pain for at least 20 minutes, not disappearing after administration of nitrates; (II) ST-elevation > 1 mm in two following leads or a left bundle branch block on the electrocardiogram; (III) Creatinine kinase (CK) elevation of at least two times the normal value of CK and a myocardial band-fraction > 5% of the total CK. Sudden death: unexpected cardiac death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence.
Vascular Mortality	Death from ischemic or haemorrhagic stroke, myocardial infarction, congestive heart failure, or rupture of abdominal aortic aneurysm. Vascular death from other causes
Composite vascular endpoint	A composite of ischemic or haemorrhagic stroke, myocardial infarction, retinal infarction and vascular mortality.
All-cause mortality	Death from any cause

Data analyses

Continuous variables are expressed as mean ± standard deviation (SD) when normal distributed or as median (interquartile range (IQR)) in case of skewed distribution. Categorical variables are expressed as numbers (percentage). Single imputation methods were used to reduce missing covariate data for smoking status (n=17; 0.4%), BMI (n=6; 0.1%), systolic blood pressure (n=4; 0.1%) and eGFR (n=24; 0.6%), since this reduces the chance for bias compared to complete case analysis.

The relation between RHR and the occurrence of cardiovascular events or death was quantified with Cox proportional hazard analysis. Results are expressed as hazard ratios (HR) with 95% confidence intervals (95%CI). Patients were censored if they were lost to follow-up. The proportional hazard assumption was confirmed by

testing the correlations between scaled Schoenfeld residuals for RHR and time. No significant non-proportionality ($p<0.05$) was observed.

Before conducting the analyses, we tested in the whole study population if the relation between RHR and outcome events was modified by the location of vascular disease (CAD, CVD, PAD or AAA) by entering cross-products of RHR and location of vascular disease in the Cox proportional hazards analysis. Other potential effect modification of the relationship between RHR and outcome events was investigated for factors that may act as effect modifier on pathophysiologic grounds in each group (CAD, CVD, PAD or AAA) separately. There was no significant effect modification on a multiplicative scale.

Hazard ratios on cardiovascular events and death were calculated per quartile RHR with quartile 1 as reference. Secondly, linear regression analyses were performed to estimate the risk on cardiovascular events and death per 10 beats per minute (bpm) increase in RHR. To evaluate if there was reverse causality – patients having an increased or decreased RHR because of severe disease – the analyses were repeated after excluding all patients who died (n=59) or had a vascular event (n=153) in the first year of follow-up. Furthermore, to exclude a large influence of extreme heart frequencies, analyses were repeated after exclusion of patients with a RHR <50 bpm (n=364) or >100 bpm (n=44). Analyses were performed separately for patients with (n=2289) or without (n=1983) beta-blocker use, to investigate the effect of beta-blockers. Finally, analyses were performed after stratifying patients according to the vascular diagnosis, being CAD, CVD, PAD or AAA.

All regression analyses were conducted with a model adjusted for age and gender (model I) and with two additional models to adjust for other potential confounding factors. In model II additional adjustments were made for current smoking, the use of medication with effect on the RHR (beta-blockers, alpha-blockers, diuretics and calcium channel-blockers) and the location of vascular disease (CAD, CVD, PAD or AAA). The study conclusions are based on this model. Finally, in an exploratory model (model III) we additionally adjusted for variables which may be in the causal pathway between RHR and events, being body mass index (BMI), type 2 diabetes mellitus, estimated glomerular filtration rate (eGFR) and systolic blood pressure (SBP). The presence of type 2 diabetes mellitus, current smoking, gender, the vascular diagnosis and the use of medication were included as categorical variables and age, BMI, eGFR and SBP were entered as continuous variables. eGFR was calculated using the modification of diet in renal diseases (MDRD) formula.

SPSS version 15.0.1 was used for all analyses.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology (21).

Results

Baseline characteristics

Baseline characteristics per quartile of RHR are presented in *Table 2*. RHR increased from 51±4 bpm in Quartile (Q) 1 to 82±9 bpm in Q4. The mean age was around 60±10 years in all quartiles, and the percentage males decreased from 83% in Q1 to 72% in Q4.

Follow-up, occurrence of new vascular events and all-cause mortality

The total follow-up time in this study was 20,875 person years, with a median follow-up of 4.4 (2.1-7.4) years. During this follow-up, 513 patients died, 295 from a vascular cause. Furthermore, there were 286 patients who had a fatal or non-fatal myocardial infarction and 135 with a fatal or non-fatal ischemic stroke. The combined vascular endpoint occurred in 530 patients.

Resting heart rate and the occurrence of new vascular events and mortality in all patients

Compared to quartile 1 (reference), patients in Q4 had a 63% higher risk for all-cause mortality (HR 1.63; 95%CI 1.25-2.13), a 78% higher risk for vascular mortality (HR 1.78; 95%CI 1.26-2.53) and a 37% higher risk for the composite vascular endpoint (HR 1.37; 95%CI 1.07-1.76) (*Table 3*). The risk for myocardial infarction (HR 1.20; 95%CI 0.85-1.70) and ischemic stroke (HR 1.14; 95%CI 0.71-1.83) was not statistically significant higher in Q4 compared to Q1. These conclusions are based on model II, but further, exploratory, adjustment in model III did not markedly change the results.

Each increase in RHR with 10 bpm was related to an increase in all cause mortality (HR 1.14; 95%CI 1.07-1.21), in vascular mortality (HR 1.15; 95%CI 1.06-1.25) and in all vascular events (HR 1.08; 95%CI 1.01-1.15) (*Table 4*). Again, the risk for myocardial infarction (HR 1.03; 95%CI 0.94-1.14) and ischemic stroke (HR 1.05; 95%CI 0.92-1.20) was not higher with increasing RHR. There was no evidence of reverse causality, as hazard ratios remained largely unchanged when patients who died (n=59) or had a cardiovascular event (n=153) in the first year of follow-up were excluded from analyses (*Table 4*). After exclusion of patients with a RHR <50 bpm (n=364) or >100 bpm (n=44); the hazard ratios did not markedly change. The increased risk per 10 bpm increase in RHR on all-cause mortality was both present in patients on beta-blockers (n=2289) (HR 1.22; 95%CI 1.09-1.38) and in patients not on beta-blocker treatment, although attenuated, (n=1983) (HR 1.11; 95% CI 1.03-1.20) after adjusting for age, gender, smoking, other blood pressure-lowering medication and location of vascular disease (Model II); the p-value for interaction of beta-blocker use was 0.14.

Table 2. Baseline characteristics of all patients according to quartiles of resting heart rate.

	Quartile 1 (n=1102)	**Quartile 2** (n=1074)	**Quartile 3** (n=1042)	**Quartile 4** (n=1054)
Heart rate (bpm)	51 ± 4	59 ± 2	67 ± 3	82 ± 9
Heart rate range (bpm)	31 - 55	56 - 62	63 - 71	72 - 122
Male gender, n (%)	909 (83)	831 (77)	759 (73)	755 (72)
Age (years)	60.2 ± 9.5	60.0 ± 10.0	60.2 ± 10.1	60.7 ± 10.4
Current smoking, n (%)	314 (29)	333 (31)	379 (36)	446 (42)
BMI (kg/m²)	26.4 ± 3.4	26.7 ± 3.6	27.0 ± 3.9	27.0 ± 4.3
Waist circumference (cm)	95 ± 10	96 ± 11	96 ± 12	97 ± 13
Metabolic syndrome, n (%)	351 (32)	399 (37)	463 (44)	524 (50)
Diabetes mellitus type 2, n (%)	103 (9)	135 (13)	196 (19)	215 (20)
Systolic BP (mmHg)	139 ± 21	142 ± 22	143 ± 22	145 ± 23
Diastolic BP (mmHg)	80 ± 11	82 ± 11	83 ± 11	84 ± 12
Laboratory parameters				
Total cholesterol (mmol/l)	4.79 ± 1.11	4.87 ± 1.12	5.04 ± 1.29	5.11 ± 1.24
Triglycerides (mmol/l)	1.33 (0.98 - 1.86)	1.40 (1.04 - 2.00)	1.50 (1.07 - 2.18)	1.58 (1.12 - 2.28)
HDL-cholesterol (mmol/l)	1.21 ± 0.35	1.21 ± 0.35	1.22 ± 0.38	1.22 ± 0.39
LDL-cholesterol (mmol/l)	2.87 ± 1.00	2.92 ± 0.99	3.02 ± 1.06	3.05 ± 1.08
ApoB (g/l)	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.3	0.9 ± 0.2
Glucose (mmol/l)	5.6 (5.2 - 6.2)	5.7 (5.3 - 6.3)	5.8 (5.4 - 6.6)	5.9 (5.4 - 6.8)
HOMA IR	2.27 (1.47 - 3.47)	2.47 (1.65 - 3.81)	2.62 (1.71 - 4.04)	2.88 (1.80 - 4.56)
eGFR (ml/min/1.73m²)	76 ± 16	76 ± 16	77 ± 17	77 ± 20
HsCRP (mg/l)	1.5 (0.7 - 3.1)	1.6 (0.8 - 3.5)	2.0 (1.0 - 4.2)	2.5 (1.1 - 5.3)
TSH (mU/L)	1.7 (1.2 - 2.4)	1.6 (1.1 - 2.4)	1.6 (1.1 - 2.3)	1.7 (1.1 - 2.4)

Table 2. Continued

	Quartile 1 (n=1102)	**Quartile 2** (n=1074)	**Quartile 3** (n=1042)	**Quartile 4** (n=1054)
Vascular disease at inclusion				
CAD, n (%)	757 (69)	599 (56)	518 (50)	370 (35)
CVD, n (%)	168 (15)	234 (22)	232 (22)	296 (28)
PAD, n (%)	132 (12)	175 (16)	216 (21)	300 (29)
AAA, n (%)	45 (4)	66 (6)	76 (7)	88 (8)
Medication use				
Platelet aggregation inhibitor, n (%)	914 (83)	851 (79)	783 (75)	718 (68)
Oral anticoagulation, n (%)	72 (7)	104 (10)	106 (10)	122 (12)
Lipid-lowering med, n (%)	739 (67)	702 (65)	662 (64)	595 (57)
Oral glucose-lowering med, n (%)	55 (5)	92 (9)	127 (12)	134 (13)
Insulin, n (%)	21 (2)	22 (2)	53 (5)	64 (6)
Beta blocker, n (%)	754 (68)	629 (59)	536 (51)	370 (35)
Diuretic, n (%)	128 (12)	178 (17)	199 (19)	230 (22)
ACE-i/ARB, n (%)	308 (28)	320 (30)	367 (35)	391 (37)
Calcium channel blocker, n (%)	248 (23)	226 (21)	226 (22)	203 (19)

The study population is presented in quartiles according to their resting heart rate. ApoB = apolipoprotein B, HOMA IR= homeostatis model assessment of insulin resistance, HsCRP=high-sensitivity C-reactive protein, TSH = thyroid stimulation hormone, CAD = coronary artery disease, CVD = cerebrovascular disease, PAD = peripheral arterial disease, AAA = aortic abdominal aneurysm, med = medication, ACE-I = angiotensin converting enzyme-inhibitor, ARB = angiotensin receptor blocker.

Table 3. The risk of resting heart rate on vascular events and mortality in patients with vascular disease.

		Q1 (n=1102)		Q2 (n=1074)		Q3 (n=1042)		Q4 (n=1054)	
	Model	# events	HR (95%CI)	# events	HR (95%CI)	# events	HR (95%CI)	# events	HR (95%CI)
Myocardial infarction	I	66	1 (reference)	71	1.16 (0.83 - 1.62)	70	1.13 (0.81 - 1.58)	79	1.28 (0.92 - 1.78)
	II		1 (reference)		1.14 (0.81 - 1.59)		1.06 (0.75 - 1.49)		1.20 (0.85 - 1.70)
	III		1 (reference)		1.13 (0.80 - 1.58)		1.06 (0.75 - 1.50)		1.19 (0.84 - 1.69)
Ischemic stroke	I	32	1 (reference)	29	0.97 (0.59 - 1.60)	28	0.92 (0.55 - 1.52)	46	1.50 (0.95 - 2.36)
	II		1 (reference)		0.83 (0.50 - 1.38)		0.74 (0.44 - 1.24)		1.14 (0.71 - 1.83)
	III		1 (reference)		0.83 (0.50 - 1.38)		0.76 (0.45 - 1.28)		1.13 (0.70 - 1.81)
All vascular events	I	113	1 (reference)	118	1.12 (0.87 - 1.45)	123	1.12 (0.87 - 1.45)	176	1.61 (1.27 - 2.05)
	II		1 (reference)		1.05 (0.81 - 1.36)		0.98 (0.76 - 1.28)		1.37 (1.07 - 1.76)
	III		1 (reference)		1.04 (0.80 - 1.35)		1.00 (0.77 - 1.30)		1.38 (1.07 - 1.78)
Vascular death	I	50	1 (reference)	62	1.36 (0.93 - 1.97)	66	1.32 (0.91 - 1.91)	117	2.26 (1.62 - 3.15)
	II		1 (reference)		1.22 (0.84 - 1.77)		1.07 (0.74 - 1.56)		1.78 (1.26 - 2.53)
	III		1 (reference)		1.20 (0.82 - 1.75)		1.13 (0.78 - 1.65)		1.83 (1.28 - 2.60)
All-cause mortality	I	90	1 (reference)	105	1.28 (0.96 - 1.69)	121	1.37 (1.04 - 1.80)	197	2.15 (1.67 - 2.76)
	II		1 (reference)		1.14 (0.86 - 1.52)		1.11 (0.84 - 1.46)		1.63 (1.25 - 2.13)
	III		1 (reference)		1.16 (0.87 - 1.54)		1.18 (0.89 - 1.56)		1.73 (1.33 - 2.26)

Results are expressed as hazard ratios (95% confidence interval) relative to the risk in quartile 1.
Model I = adjusted for age and gender; model II = additionally adjusted for beta-blockers, calcium-channel-blockers, alpha-blockers, diuretics, current smoking and inclusion diagnosis (CAD, AAA, PAD or CVD); model III = model II with additional adjustment for BMI, eGFR, diabetes mellitus type 2 and systolic blood pressure.
Q = quartile of resting heart rate, HR = hazard ratio, CI = confidence interval, CAD = coronary artery disease, CVD = cerebrovascular disease, PAD = peripheral arterial disease, AAA = aortic abdominal aneurysm.

Table 4. The risk of resting heart rate per 10 bpm on vascular events and mortality in patients with vascular disease.

		All patients (n=4272)		Exclusion of deaths in first year of follow-up (n=4213)		Exclusion of events in first year of follow-up (n=4119)	
	Model	# events	HR (95%CI)	# events	HR (95%CI	# events	HR (95%CI)
Myocardial infarction	I	286	1.06 (0.97 - 1.15)	265	1.06 (0.96 - 1.16)	206	1.12 (1.02 - 1.24)
	II		1.03 (0.94 - 1.14)		1.05 (0.95 - 1.15)		1.08 (0.97 - 1.20)
	III		1.03 (0.94 - 1.13)		1.04 (0.95 - 1.15)		1.07 (0.96 - 1.20)
Ischemic stroke	I	135	1.12 (1.00 - 1.27)	131	1.11 (0.98 - 1.26)	91	1.03 (0.89 - 1.20)
	II		1.05 (0.92 - 1.20)		1.04 (0.91 - 1.19)		0.99 (0.84 - 1.17)
	III		1.05 (0.92 - 1.20)		1.04 (0.91 - 1.19)		0.99 (0.84 - 1.18)
All vascular events	I	530	1.13 (1.06 - 1.20)	489	1.12 (1.05 - 1.20)	395	1.14 (1.06 - 1.22)
	II		1.08 (1.01 - 1.15)		1.08 (1.01 - 1.16)		1.09 (1.01 - 1.17)
	III		1.08 (1.01 - 1.15)		1.08 (1.01 - 1.16)		1.09 (1.01 - 1.18)
Vascular death	I	295	1.22 (1.13 - 1.32)	254	1.22 (1.12 - 1.32)	234	1.24 (1.14 - 1.35)
	II		1.15 (1.06 - 1.25)		1.17 (1.07 - 1.28)		1.18 (1.08 - 1.30)
	III		1.15 (1.06 - 1.25)		1.17 (1.07 - 1.28)		1.18 (1.08 - 1.30)
All-cause mortality	I	513	1.22 (1.15 - 1.29)	454	1.20 (1.13 - 1.28)	424	1.22 (1.14 - 1.30)
	II		1.14 (1.07 - 1.21)		1.13 (1.05 - 1.21)		1.14 (1.06 - 1.22)
	III		1.15 (1.08 - 1.22)		1.14 (1.07 - 1.22)		1.15 (1.07 - 1.23)

Model I = adjusted for age and gender; model II = additionally adjusted for beta-blockers, calcium-channel-blockers, alpha-blockers, diuretics, current smoking and inclusion diagnosis (CAD, AAA, PAD or CVD); model III = model II with additional adjustment for BMI, eGFR, diabetes mellitus type 2 and systolic blood pressure. HR = hazard ratio, CI = confidence interval, CAD = coronary artery disease, CVD = cerebrovascular disease, PAD = peripheral arterial disease, AAA = aortic abdominal aneurysm.

Resting heart rate and the occurrence of new vascular events and mortality according to location of vascular disease

There was no effect modification on a multiplicative scale on the relation between RHR and all-cause mortality (p for interaction 0.07 for CAD, 0.73 for CVD, 0.90 for PAD and 0.90 for AAA) and on the relation between RHR and all vascular events (p for interaction 0.39 for CAD, 0.62 for CVD, 0.67 for PAD and 0.67 for AAA). In patients with CAD, each increase in RHR of 10 bpm was associated with a 23% increase in the risk of all-cause mortality (HR 1.23; 95%CI 1.05-1.44) (*Table 5*). Patients with CVD had a 18% increase in all-cause mortality per 10 bpm increase in RHR (HR 1.18; 95% CI 1.05-1.33), while this was 10% in patients with PAD or AAA (n=1093) (HR 1.10; 95%CI 1.01-1.20). There was no relation between an increase of 10 bpm in RHR and the occurrence of myocardial infarction or stroke in patients with CAD, CVD, PAD or AAA. Again, further, exploratory, adjustment in model III had only minimal influence on the results, and there was no indication of reverse causality in these analyses either.

Discussion

In the present study it is shown that increased resting heart rate is related to increased vascular death and total mortality in patients with CAD, CVD, PAD or AAA. Each increase in RHR of 10 bpm corresponds with an increased risk on all-cause and vascular mortality of around 15% and with an 8% increased risk for all vascular events, irrespective of beta blocker use and location of vascular disease. An increase in RHR was not related to an increased risk on myocardial infarction or ischemic stroke.

The independent relation between an increase in RHR and increased risk for mortality and cardiovascular events has been firmly established in patients with acute or stable CAD and/or heart failure (4;5;22;23). The results of CAD patients in the present study are in line with the results of other studies in patients with suspected or proven CAD (4;5). In the present study, each increase in RHR of 10 bpm was independently associated with a hazard ratio of 1.23 (95%CI 1.05-1.44) for all-cause mortality in the patients with CAD, which is comparable to the HR of 1.40 (95%CI 1.14-1.71) per 20 bpm difference observed in the TNT Trial where patients with a RHR of ≥70 bpm (mean 77±7 bpm) were compared with patients with a RHR <70 bpm (mean 57±7 bpm) (5). In the Coronary Artery Surgery Study (CASS) the increased risk for all-cause mortality per standard deviation (12.4 bpm) increase in RHR was about 10% for all subgroups (4). The CASS study population was a population undergoing coronary arteriography, not necessarily having CAD, which may be an explanation for the lower risk for all-cause mortality. In patients with hypertension and left ventricular hypertrophy, an increase in RHR of 10 bpm was associated with a hazard ratio of 1.25 (95%CI 1.17-1.33) for all-cause

Table 5. The risk of resting heart rate per 10 bpm on vascular events and mortality stratified for various locations of vascular disease.

		CAD (n=2244)		CVD (n=930)		PAD (n=823)		AAA (n=275)	
	Model	# events	HR (95%CI)	# events	HR (95%CI)	# events	HR (95%CI)	# events	HR (95%CI)
Myocardial infarction	I	121	1.02 (0.86 - 1.20)	55	1.06 (0.87 - 1.29)	69	1.02 (0.86 - 1.22)	41	1.04 (0.86 - 1.27)
	II		0.96 (0.81 - 1.15)		1.08 (0.88 - 1.32)		1.02 (0.85 - 1.23)		1.08 (0.88 - 1.33)
	III		0.95 (0.80 - 1.13)		1.07 (0.88 - 1.32)		1.02 (0.85 - 1.23)		1.05 (0.85 - 1.30)
Ischemic stroke	I	23	0.80 (0.53 - 1.19)	77	1.10 (0.93 - 1.30)	24	0.99 (0.73 - 1.35)	11	1.05 (0.71 - 1.54)
	II		0.82 (0.54 - 1.25)		1.11 (0.94 - 1.31)		1.05 (0.77 - 1.43)		1.03 (0.68 - 1.57)
	III		0.84 (0.54 - 1.30)		1.09 (0.92 - 1.29)		1.09 (0.80 - 1.49)		1.06 (0.69 - 1.63)
All vascular events	I	161	1.04 (0.90 - 1.19)	158	1.08 (0.96 - 1.21)	128	1.06 (0.94 - 1.21)	83	1.10 (0.96 - 1.26)
	II		0.99 (0.86 - 1.15)		1.09 (0.97 - 1.23)		1.09 (0.95 - 1.24)		1.14 (0.99 - 1.31)
	III		0.98 (0.84 - 1.14)		1.08 (0.96 - 1.22)		1.11 (0.97 - 1.27)		1.11 (0.96 - 1.28)
Vascular death	I	60	1.27 (1.04 - 1.56)	83	1.11 (0.95 - 1.30)	88	1.14 (0.99 - 1.32)	64	1.10 (0.95 - 1.29)
	II		1.20 (0.97 - 1.50)		1.16 (0.99 - 1.37)		1.13 (0.97 - 1.32)		1.17 (0.99 - 1.38)
	III		1.18 (0.94 - 1.47)		1.15 (0.98 - 1.35)		1.17 (1.00 - 1.36)		1.14 (0.97 - 1.35)
All-cause mortality	I	117	1.31 (1.13 - 1.51)	147	1.16 (1.04 - 1.31)	144	1.11 (0.99 - 1.24)	105	1.08 (0.96 - 1.22)
	II		1.23 (1.05 - 1.44)		1.18 (1.05 - 1.33)		1.10 (0.97 - 1.24)		1.10 (0.97 - 1.25)
	III		1.25 (1.07 - 1.45)		1.19 (1.05 - 1.34)		1.14 (1.01 - 1.28)		1.07 (0.94 - 1.21)

Model I = adjusted for age and gender; model II = additionally adjusted for beta-blockers, calcium-channel-blockers, alpha-blockers, diuretics, current smoking and vascular history (CAD, AAA, PAD or CVD); model III = model II with additional adjustment for BMI, eGFR, diabetes mellitus type 2 and systolic blood pressure.
HR = hazard ratio, CI = confidence interval, CAD = coronary artery disease, CVD = cerebrovascular disease, PAD = peripheral arterial disease, AAA = aortic abdominal aneurysm.

mortality, very similar to the present study as well (7). Most previous studies have focused on mortality, and the relation between RHR and the risk for non-fatal cardiovascular events is less well understood. In line with our results are the findings in the TNT study (CAD-patients) (5) and the 3C study (elderly subjects from the population) (24) that no significant relation between RHR and risk for myocardial infarction and stroke was demonstrated, but positive results have been reported as well (4).

In the present study, we show that the detrimental effects of an increased RHR are not restricted to patients with coronary artery disease only, but are also present in patients with other locations of vascular disease, such as CVD, PAD or AAA. RHR is a functional parameter of the heart and some pathophysiological effects of an elevated RHR, such as increased susceptibility to ventricular arrhythmias are of cardiac origin. However, atherosclerosis is a systemic disease and a large proportion of patients with atherosclerotic disease at other locations in the vascular tree also have (subclinical) coronary artery disease as well (25). Secondly, other pathophysiologic effects of an increased RHR, such as endothelial dysfunction and atherosclerotic plaque rupture, are not restricted to the heart but are present in the entire arterial circulation. Thirdly, elevated RHR is a measure of increased sympathetic nerve activity which is shown to be present in patients with different locations of cardiovascular diseases or risk factors (1;2). Whether the negative effects of an increased RHR on mortality are a reflection of the harmful effects of an increased sympathetic tone or of an increase in heart rate itself cannot be determined definitively from this study. However, in our exploratory analyses (Model III), we adjusted for some determinants of sympathetic activity (eGFR, BMI, type 2 diabetes mellitus) and another measure of present sympathetic tone (systolic blood pressure). Correction for these variables did not lead to substantial differences in the results, suggesting that is it not (only) sympathetic activity, but increased RHR itself that plays an important role in the increased risk of mortality. This could be effectuated by direct adverse effects of an increased RHR which are independent of sympathetic activity, such as increased risk of atherosclerosis and plaque rupture, endothelial dysfunction, increased susceptibility to ventricular arrhythmias and a negative effect on the balance between myocardial oxygen demand and supply (14;15).

The results from the present study have possible implications for the clinical situation, as resting heart rate is a non-invasive, non-expensive and easily obtainable parameter, and therefore easily applicable in clinical practice. As elevated RHR identifies CAD, CVD, PAD or AAA patients at increased risk for mortality, RHR can be considered in risk estimation models to predict future cardiovascular events (26). The European Society of Cardiology and the European Society of Hypertension (ESC/ESH) guidelines for the management of hypertension

already suggest to include elevated heart rate when assessing the cardiovascular risk profile of an individual (27). More importantly, reducing elevated RHR is strongly related to a reduction in all-cause mortality in patients with coronary artery disease (16). In a meta-analysis of 25 randomized clinical trials with beta blockers (n=21) and calcium channel blockers (n=4), each reduction in RHR of 10 bpm is estimated to reduce the relative risk of all-cause mortality by about 20% (16), with the remark that this not likely to be attributed to heart rate reduction alone, as these therapies also reduce blood pressure. However, pure heart rate reduction with ivabradine, which does not affect blood pressure, has recently been shown to improve clinical outcome in patients with heart failure (17) by decreasing the heart rate (19), as well as in a subgroup of patients with stable coronary disease, left ventricular dysfunction and a baseline heart rate of ≥70 bpm (18). Another promising treatment modality reducing RHR is chronic vagus nerve stimulation, which has recently been evaluated in a phase II study showing improvement in left ventricular function and quality of life in patients with heart failure (28). The results of the present study suggest that not only patients with CAD, but also patients with CVD, PAD or AAA may benefit from therapies aiming to reduce heart rate.

Strengths of this study include the prospective cohort design and the large sample size of a clinically relevant and well-characterized group of patients with vascular diseases. Furthermore, RHR was accurately and consistently measured with ECG and clinical endpoints were thoroughly assessed. Assessment of several clinical and laboratory parameters allowed for identification of possible confounding factors. Finally, we excluded the possibility of reverse causality in the relation between RHR and mortality.

Study limitations need to be considered. As heart rate is susceptible to environmental influences, multiple recordings, or 24-hour registration with Holter-ECG could have increased the accuracy and may account for variation during the day. However, measurement of RHR in the present study was very well standardized, resembles the way RHR is measured in clinical practice and is easy to measure at low costs. Moreover, clinical and ambulatory (24-hour) heart rate are highly correlated (r=0.60) and have similar associations with all-cause- and cardiovascular mortality (29). Furthermore, because this is an observational cohort study, theoretically, the relation between RHR and mortality could be subject to residual confounding. We were for example not able to adjust for physical activity, exercise capacity or for left ventricular function. However, as we adjusted for other potential confounding variables closely related to physical activity or exercise capacity (such as age, gender, smoking and BMI), we assume the influence of residual confounding on our results to be minimal. As we stratified for location of vascular disease at study inclusion and further adjusted

for location for vascular disease in the past medical history, the results are adjusted for a history of myocardial infarction, making the residual confounding effect of left ventricular function probably very small. Therefore, we consider the relation between RHR and mortality to be causal.

In conclusion, an increase in RHR is associated with increased risk for vascular mortality, all-cause mortality and a composite endpoint of vascular events in patients with clinical manifest vascular diseases, irrespective of beta blocker use and the location of vascular disease. Similar as patients with CAD, patients with clinical manifest vascular diseases at other locations may also benefit from heart rate reduction therapy, although this needs first to be investigated in prospective clinical trials.

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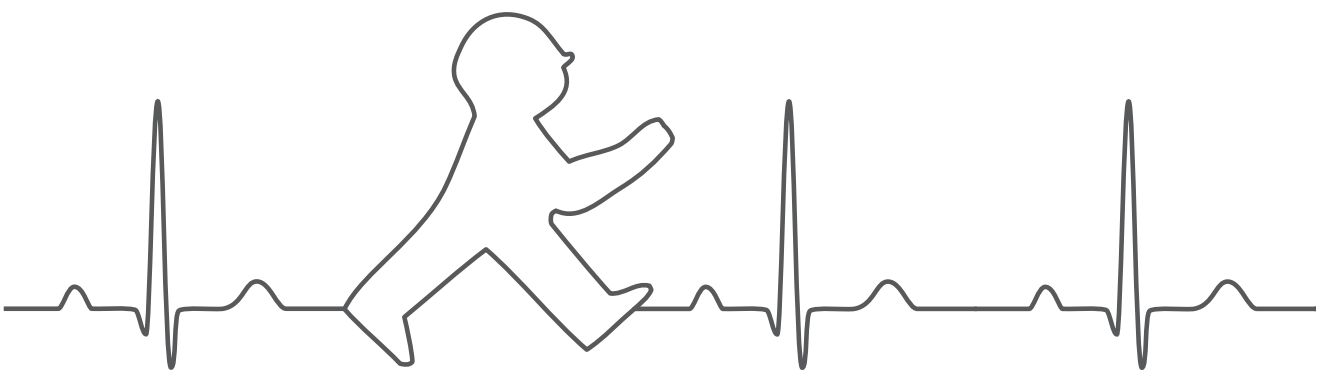

Chapter 7

The risk of elevated resting heart rate on the development of type 2 diabetes in patients with clinically manifest vascular diseases

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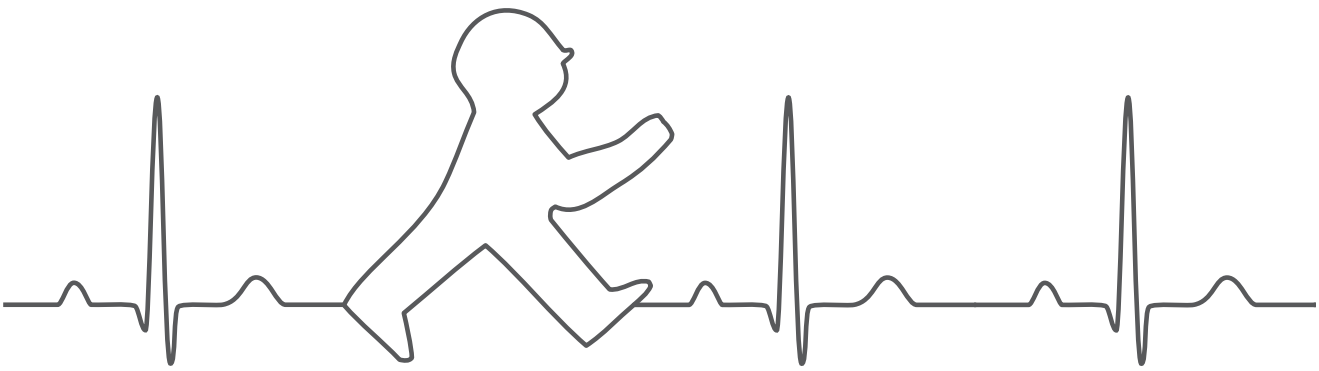

Abstract

Objective

Sympathetic nerve activation is causally related to insulin resistance as both a cause and a consequence. Resting heart rate (RHR) reflects sympathetic nerve activity. We investigated the effect of RHR on the incidence of type 2 diabetes mellitus (T2DM) in patients with clinically manifest vascular diseases.

Design

Data were used from the second manifestations of arterial disease (SMART) study: a prospective cohort study of patients with clinically manifest vascular diseases (n=3646).

Methods

RHR was obtained using an electrocardiogram. Patients were followed up for incident type 2 diabetes (n=289) during a median period of 5.5 (interquartile range 3.2-8.4) years. The relation between RHR and incident T2DM was estimated by Cox proportional hazard analysis. As age was an effect modifier (p=0.048), analyses were stratified for age.

Results

Patients in quartile 4 (Q4) of RHR had a 65% increased risk for T2DM compared with those in Q1 (reference; hazard ratio (HR), 1.65; 95% confidence interval (95% CI) 1.15-2.36) adjusted for age, gender, smoking, estimated glomerular filtration rate, systolic blood pressure, location of vascular disease, and antihypertensive medication. Every 10 beats per minute (bpm) increase in RHR increased the risk for T2DM with 10% (HR, 1.10; 95% CI 1.00-1.21) in the total population. This risk was particularly high in subjects aged 55-63 years (per 10 bpm: HR, 1.22; 95% CI 1.04-1.43) and was independent of the location of vascular disease and beta-blocker use.

Conclusions

Increased RHR, an indicator of sympathetic nerve activity, is associated with an increased risk for T2DM in patients with manifest vascular diseases, particularly in middle-aged patients.

Introduction

Insulin resistance and elevated sympathetic nerve activity are closely related, but the precise pathophysiologic relation is complex and only partially understood. Visceral obesity is associated with a more pronounced sympathetic nerve activity than subcutaneous obesity (1), probably to a large extent caused by elevated serum levels of insulin and leptin (2). Administration of insulin in humans increases muscle sympathetic nerve activity, the reference standard of measuring sympathetic nerve activity (3), by a direct effect on insulin-receptors in the central nervous system (4;5). Plasma leptin levels are independently associated with resting heart rate (RHR) in healthy male subjects (6). Furthermore, the infusion of leptin in rats increases the heart rate, through leptin-receptors in the central nervous system (7;8).

The causal relation between sympathetic nerve activity and insulin resistance could also be the other way around. Acute reflex activation of the sympathetic nerve system is reported to induce acute insulin resistance in humans (9). Moreover, chronically increased sympathetic nerve activity can precede the development of insulin resistance and obesity (10;11). RHR reflects sympathetic tone, and correlates with muscle sympathetic nerve activity and noradrenalin serum levels (12). An increased RHR identifies subjects at higher risk for developing cardiovascular disease and mortality (13;14). Furthermore, an increased RHR at baseline is independently associated with an increased risk for developing type 2 diabetes (T2DM) in healthy populations (15-19) and in subjects with obesity or impaired glucose tolerance (20), but this has not been shown in patients with vascular diseases.

Identifying subjects at high risk for developing T2DM is important, as diabetes is a strong risk factor for cardiovascular morbidity and mortality (21;22) and patients with diabetes have a reduced quality of life (23). Furthermore, the risk for T2DM can be reduced through lifestyle interventions and medication (24). Patients with clinically manifest vascular diseases are at particularly high risk of developing new vascular events and T2DM given shared pathophysiological pathways (‘common soil’ hypothesis) of diabetes and atherosclerosis such as insulin resistance and low-grade inflammation (25). As increased sympathetic nerve activity, reflected by an elevated RHR, is associated with increased risk for vascular diseases and mortality on the one hand (13;26), and diabetes on the other hand (15-20), increased sympathetic tone may also be part of the shared pathophysiology in the development of vascular diseases and T2DM. Patients with vascular diseases who have a particular high risk for the development of diabetes are an ideal group for preventive measures, given their high risk and the fact that they are already receiving medication and lifestyle advices, in contrast to the general population. Therefore, in this study we investigated the relation between RHR and incidence of T2DM during follow-up in a cohort of patients with clinically manifest vascular diseases.

Subject and methods

Study design and patients

In this study, data were used from patients enrolled in the second manifestations of arterial disease (SMART) study, an ongoing single-centre prospective cohort study carried out at the University Medical Center Utrecht. This study started in September 1996. Patients aged 18-80 years, referred to our institution with clinically manifest atherosclerotic vascular diseases were included. Patients with terminal malignant disease, those not independent in daily activities (Rankin scale >3) and not sufficiently fluent in Dutch were not included.

The aims of the SMART study were to determine (i) risk factors for atherosclerosis, (ii) prevalence of additional vascular disease and (iii) incidence of future cardiovascular events and type 2 diabetes. The study complies with the Declaration of Helsinki Principles; the Medical Ethics Committee of the UMC Utrecht, the Netherlands, approved the study and all patients gave written informed consent. Patients were asked to complete a health questionnaire covering medical history, risk factors, smoking habits and medical treatment. A standardized diagnostic protocol consisting of physical examination and laboratory testing in a fasting state was followed. A more detailed description of the design of the study has been published previously (27).

For this study, data were used from patients (n=5280) enrolled in the SMART cohort from September 1996 to March 2010 with a recent diagnosis or history of coronary artery disease (CAD), cerebrovascular disease (CVD), peripheral arterial disease (PAD) or abdominal aortic aneurysm (AAA).

Patients who had died (n=480) or were lost to follow up (n=91) before the assessment of incident T2DM started in 2006 (see Follow up and assessment of type 2 diabetes) were excluded (*Figure 1*). Patients with diabetes mellitus (type 1 or 2) at study inclusion (n=809), defined as a referral diagnosis of diabetes, self-reported diabetes (use of glucose-lowering agents) or a known history of diabetes, were excluded. To make sure that all patients with diabetes at baseline were excluded, subjects without a history of diabetes, but with the combination of a fasting plasma glucose level ≥7.0 mmol/l at baseline and receiving treatment with glucose-lowering agents within 1 year after baseline were considered as having diabetes at baseline and were excluded (n=50). Furthermore, patients without a study-electrocardiogram (ECG) (n=85) or without sinus rhythm on the ECG (n=169) were excluded, leaving a total of 3646 patients for analyses (*Figure 1*).

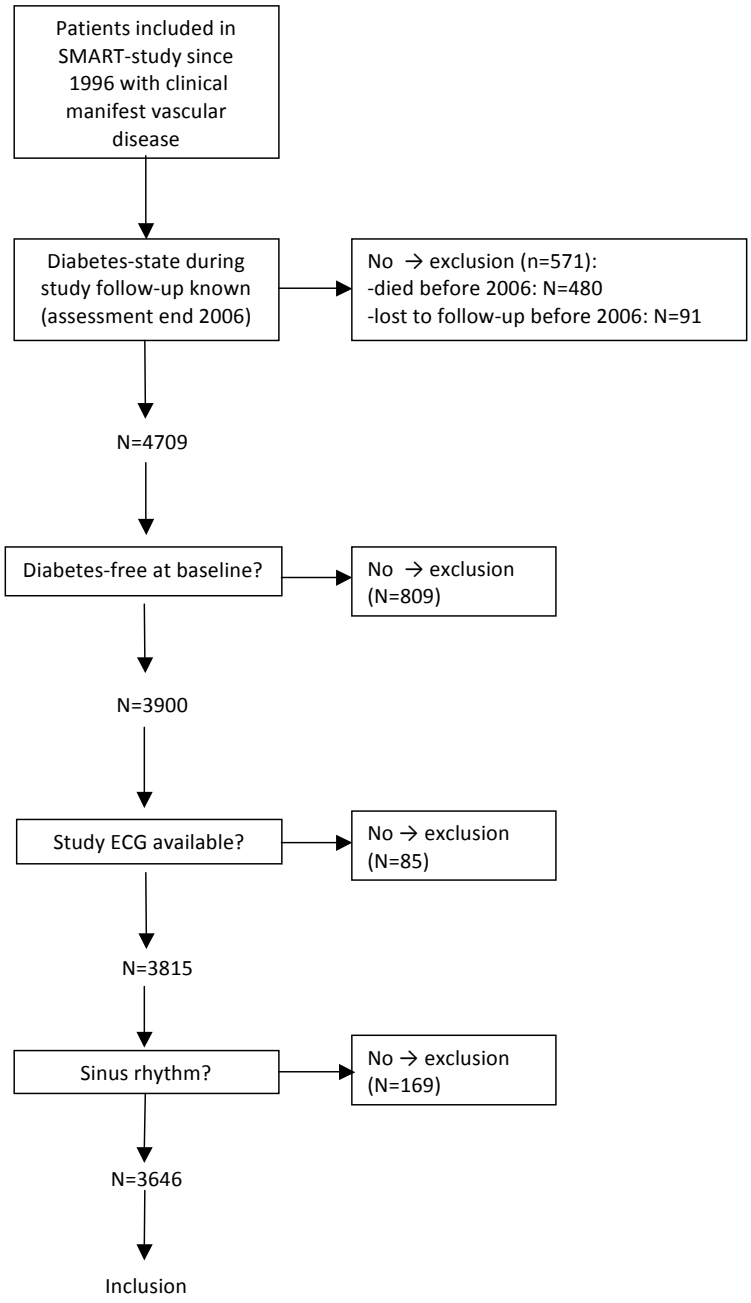

Figure 1. Flow chart.

Measurement of RHR

At study inclusion, a 12-lead ECG was obtained after the patient had rested in supine position for 5 minutes. RHR was calculated using the digitally stored 12-lead 10-second data, by dividing the number of R-R intervals (number of QRS-complexes minus one) by the time difference between the first and last beat, and the result was converted to beats per minute (bpm). This calculation was performed using the Marquette 12SL analysis program (General Electric Healthcare, Hoevelaken, the Netherlands).

Follow-up and assessment of type 2 diabetes

The main outcome of interest for this study was incident T2DM. All participants that had been included until June 2006 without diabetes at baseline received a questionnaire in the period between June and December 2006 to assess the incidence of T2DM after study inclusion. After 2006, all patients were biannually asked to complete this questionnaire. Patients were asked whether they had diabetes and if 'yes', patients received a supplementary questionnaire regarding date of diagnosis, initial treatment (diet, oral medication or insulin), current treatment and family history of diabetes. If the answers were incomplete or unclear, patients and/or their general practitioner were called on phone for further information. To validate the diagnosis of diabetes, two independent physicians audited and classified all diabetes cases. Furthermore, cross-validation with the hospital diagnosis registry revealed that none of the patients who reported not to have diabetes, had a physician's diagnosis of diabetes.

Follow-up duration (years) was defined as the period between study inclusion and date of diagnosis of T2DM, date of death, date of loss to follow-up, or the preselected date of 1 March 2010. From 1996 until 1 March 2010, a total of 72 (2%) patients were lost to follow-up.

Data analyses

Continuous variables are expressed as mean ± SD when normally distributed or as median (interquartile range (IQR)) in case of skewed distribution. Categorical variables are expressed as numbers (percentage). Single imputation methods were used to reduce missing covariate data for smoking status (n=15; 0.4%), body mass index (BMI; n=5; 0.1%), systolic blood pressure (SBP; n=5; 0.1%) and estimated glomerular filtration rate (eGFR, calculated using the Modification of Diet in Renal Disease formula; n=18; 0.5%), as this method reduces the chance for bias compared to complete case analysis.

The incidence rate of T2DM was calculated as the number of new T2DM patients divided by total amount of person-years during the study follow-up. Corresponding 95% confidence intervals (95% CIs) were calculated using Fisher's exact test. The

relation between RHR and incident type 2 diabetes was quantified with Cox proportional hazard analysis. Results are expressed as hazard ratios (HR) with 95% CI. Patients were censored if they died or were lost to follow-up. The proportional hazard assumption was confirmed by testing the correlations between scaled Schoenfeld residuals for RHR and time. No significant nonproportionality ($p<0.05$) was observed.

Hazard ratios on incident T2DM were calculated per quartile RHR, using patients in quartile 1 (Q1) as reference category. Second, linear regression analyses were performed to estimate the risk on incident T2DM per 10 bpm increase in RHR, for the whole study population and after stratification for age in tertiles (<55, 55-63 and >63 years). Third, to explore if associations between RHR and incident T2DM were different in patients with different locations of vascular diseases, analyses were performed per 10 bpm increase in RHR after stratifying patients according to the vascular diagnosis at study inclusion, being CAD, CVD, PAD or AAA. If study inclusion in the SMART study was not due to a recent vascular diagnosis, the first vascular diagnosis in their medical history was used to classify them as CAD, CVD, PAD or AAA.

All regression analyses were conducted with a crude model (I) and three models to adjust for potential confounding factors. In model II, we adjusted for age and gender and in model III, additional adjustments were carried out for current smoking, SBP, eGFR, the use of medication with possible effect on the RHR and on incident T2DM (beta-blockers, diuretics, calcium channel-blockers, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers) and the location of vascular disease at study inclusion (CAD, CVD, PAD or AAA). The study conclusions are based on this fully adjusted model. Finally, in an exploratory model (IV) we additionally adjusted for variables which may be in the causal pathway between RHR and incident T2DM, being BMI and fasting glucose. Current smoking, gender, the vascular diagnosis and the use of medication were included as categorical variables and age, eGFR, SBP, BMI and fasting glucose were entered as continuous variables.

Analyses were repeated after exclusion of patients with baseline fasting glucose values ≥7.0 mmol/l (n=212) and after exclusion of patients with extreme heart rates (≤50 (n=436) or ≥100 bpm (n=35)), to avoid a large effect of patients with impaired fasting glucose or extreme heart rates. Additional exploratory correction for thyroid-stimulating hormone (TSH), another possible factor in the causal pathway between RHR and incident T2DM, was also carried out. Finally, besides correcting for beta-blocker use, analyses were conducted in patients on beta-blocker (n=1934) and in patients not on beta-blocker (n=1712) treatment separately, as beta-blockers influence both the RHR and the risk for diabetes. SPSS version 15.0.1 was used for all analyses.

Results

Baseline characteristics

Baseline characteristics per quartile of RHR are presented in *Table 1*. The average RHR increased from 50±4 bpm in Q1 to 79±9 bpm in Q4. The mean age of the study population was 59±10 years and the percentage male participants decreased from 81 in Q1 to 66 in Q4. Over the quartiles with increasing RHR, slight increases were observed in fasting serum glucose levels (Q1, 5.7±0.7 - Q4, 5.8±0.8 mmol/l) and BMI (Q1, 26.2±3.2 - Q4, 26.8±4.2 kg/m^2).

Follow-up and incident type 2 diabetes

The total follow-up time in this study was 21535 person years, with a median follow-up of 5.5 (IQR 3.2-8.4) years. During this follow-up, 289 patients were diagnosed with incident T2DM and 188 patients died. The unadjusted incidence rate (new cases of T2DM per 1000 person-years of follow-up) increased across quartiles of RHR (from 9.6 (95% CI, 7.3-12.5) in Q1 to 16.0 (95% CI, 13.0-19.6) in Q4; *Figure 2A*) and with increasing age (from 11.9 (95% CI, 9.7-14.5) for subjects <55 years to 14.2 (95% CI, 11.6-17.2) for subjects >63 years (*Figure 2B*).

RHR and incident T2DM

Subjects in the highest quartile of RHR (Q4) had a 65% higher risk of incident T2DM compared with those in the reference Q1 (HR 1.65; 95% CI, 1.15-2.36) based on the fully adjusted model (III; *Figure 3*). Every 10 bpm increase in RHR was related to a 10% increase in incident T2DM (HR 1.10; 95% CI, 1.00-1.21) in the total population. The point estimates in this paragraph should be interpreted with caution as there is effect modification by age.

Effect modification of age on the relation between RHR and incident T2DM

Before conducting the analyses, we investigated potential effect modification on a multiplicative scale of the relationship between RHR and incident T2DM for factors that may act as effect modifier on pathophysiological grounds, by entering cross-products of RHR and the possible effect modifier in the Cox proportional hazard model. Gender was not a significant effect modifier (p=0.38), nor was beta-blocker use (p=0.16). Only age appeared to modify the relation between RHR and incident T2DM significantly (p for interaction=0.048).

Stratification for age in three groups (tertiles) showed that in subjects between 55 and 63, every 10 bpm increase in RHR increased the risk of incident T2DM with 22% (HR, 1.22; 95% CI, 1.04-1.43), while there was no increased risk of T2DM with increasing RHR in patients <55 years (HR, 1.08; 95% CI, 0.91-1.30) and patients >63 years (HR, 1.00; 95% CI, 0.84-1.18; *Table 2*). Further exploratory analyses in smaller age groups (quintiles) confirmed the finding that the relation between increased RHR and incident T2DM is absent in the youngest and oldest groups of patients, and is present in middle-aged patients (between 51 and 68 years in this analysis).

Table 1. Baseline characteristics according to quartiles of resting heart rate (n=3646).

	Quartile 1 (n=889)	Quartile 2 (n=965)	Quartile 3 (n=838)	Quartile 4 (n=954)
RHR, mean (bpm)	50 ± 4	58 ± 2	65 ± 3	79 ± 9
RHR, range (bpm)	31 - 54	55 - 61	62 - 69	70 - 122
Male gender, n (%)	723 (81)	711 (74)	578 (69)	628 (66)
Age (years)	58.9 ± 9.9	59.0 ± 10.3	58.2 ± 10.6	58.7 ± 10.8
Current smoking, n (%)	246 (28)	289 (30)	313 (37)	395 (41)
Body mass index (kg/m²)	26.2 ± 3.2	26.7 ± 3.7	26.7 ± 3.9	26.8 ± 4.2
Waist circumference (cm)	94 ± 10	95 ± 11	94 ± 12	95 ± 13
Systolic BP (mmHg)	138 ± 22	140 ± 22	142 ± 22	144 ± 21
Diastolic BP (mmHg)	80 ± 11	82 ± 11	84 ± 12	85 ± 12
Laboratory parameters				
Total cholesterol (mmol/l)	4.82 ± 1.14	4.89 ± 1.14	5.09 ± 1.30	5.19 ± 1.28
Triglycerides (mmol/l)	1.29 (0.96 - 1.80)	1.37 (1.00 - 1.97)	1.43 (1.04 - 2.04)	1.49 (1.09 - 2.14)
HDL-cholesterol (mmol/l)	1.24 ± 0.36	1.25 ± 0.37	1.27 ± 0.40	1.27 ± 0.40
LDL-cholesterol (mmol/l)	2.89 ± 1.01	2.90 ± 1.00	3.05 ± 1.05	3.09 ± 1.07
ApoB (g/l)	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.3
Glucose (mmol/l)	5.7 ± 0.7	5.7 ± 0.7	5.8 ± 0.9	5.8 ± 0.8
eGFR (ml/min/1.73m²)	76 ± 16	76 ± 16	78 ± 17	77 ± 19
HsCRP (mg/l)	1.5 (0.7 - 3.1)	1.6 (0.7 - 3.2)	1.9 (1.0 - 4.0)	2.5 (1.1 - 4.9)
TSH (mU/L)	1.70 (1.20 - 2.40)	1.60 (1.10 - 2.30)	1.60 (1.10 - 2.30)	1.70 (1.20 - 2.50)
Vascular disease at inclusion, n (%)				
CAD	605 (68)	565 (59)	401 (48)	344 (36)
CVD	160 (18)	224 (23)	239 (29)	309 (32)
PAD	96 (11)	131 (14)	144 (17)	226 (24)
AAA	28 (3)	45 (5)	54 (6)	75 (8)

Table 1. Continued.

	Quartile 1 (n=889)	Quartile 2 (n=965)	Quartile 3 (n=838)	Quartile 4 (n=954)
Medication use, n (%)				
Platelet aggregation inhib	721 (81)	769 (80)	616 (74)	624 (65)
Oral anticoagulation	58 (7)	79 (8)	74 (9)	106 (11)
Lipid-lowering med	596 (67)	639 (66)	519 (62)	559 (59)
BP-lowering med	705 (79)	722 (75)	578 (69)	611 (64)
Beta-blocker	604 (68)	580 (60)	403 (48)	347 (36)
Diuretic	92 (10)	154 (16)	135 (16)	195 (20)
ACE-i/ARB	251 (28)	294 (31)	271 (32)	336 (35)
Calcium channel blocker	188 (21)	204 (21)	157 (19)	166 (17)

The study population is presented in quartiles according to their resting heart rate. BP, blood pressure; bpm, beats per minute; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ApoB, apolipoprotein B; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; TSH, thyroid-stimulating hormone; CAD, coronary artery disease; CVD, cerebrovascular disease; PAD, peripheral arterial disease; AAA, abdominal aortic aneurysm; inhib, inhibitors; med, medication; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; RHR, resting heart rate.

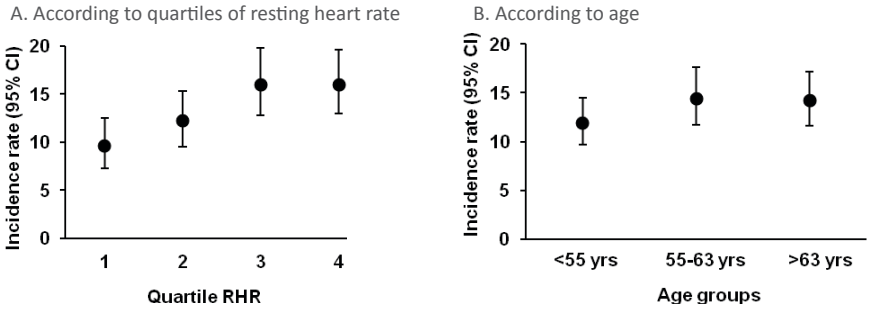

Figure 2. Incidence rates for T2DM in patients with clinically manifest vascular diseases.
Incidence rates are expressed as cases per 1000 person-years of study follow-up. 95% CI, 95% confidence intervals; RHR, resting heart rate; yrs, years of age.

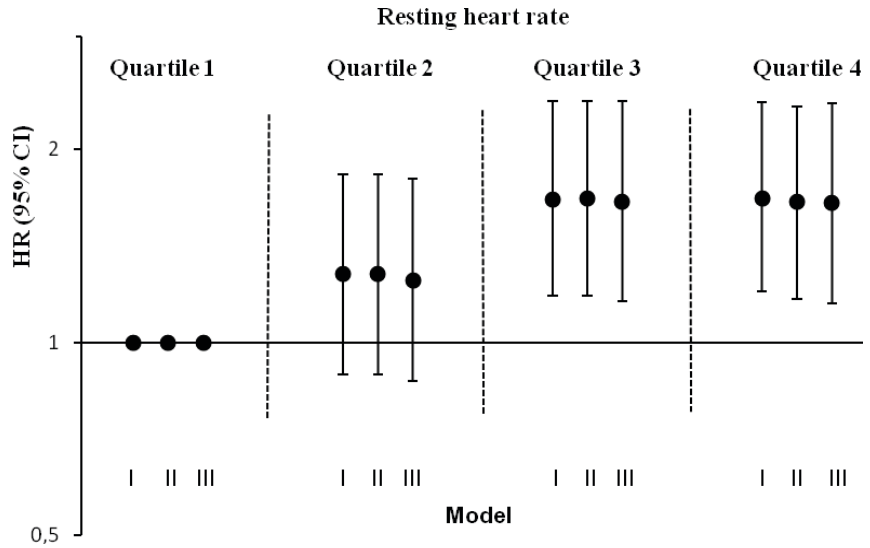

Figure 3. The risk of increasing resting heart rate on incident T2DM in patients with clinically manifest vascular diseases. Results are expressed as hazard ratios (HR) with 95% confidence intervals (95% CI) per quartile of resting heart rate, relative to quartile 1 (reference). Model I: crude; model II: age and gender, model III: age, gender, beta-blocker use, diuretic use, calcium blocker use, use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, current smoking,estimated glomerular filtration rate, systolic blood pressure, location of vascular disease (coronary artery disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm).

Exploratory analyses

In an exploratory analysis (model IV), the relation between RHR and incident T2DM was attenuated to a large extent by adding BMI and fasting serum glucose to the fully adjusted model, indicating that BMI and serum glucose levels are part of the causal pathway between sympathetic nerve activity measured with RHR and incident T2DM (*Table 2*).

Table 2. The risk of resting heart rate per 10 bpm on incident type 2 diabetes in patients with clinically manifest vascular diseases, after stratification in three age groups.

		Age groups		
		1	2	3
n		1255	1127	1264
Age (median; range)		49 (19-54)	59 (55-63)	69 (64-80)
RHR (mean ± SD; range)		63±12 (31-117)	63±12 (39-114)	63±13 (36-122)
No. of events		94	94	101
Model HR (95% CI)	I	1.13 (0.95 - 1.33)	1.32 (1.14 - 1.53)	0.94 (0.80 - 1.10)
	II	1.13 (0.96 - 1.34)	1.31 (1.13 - 1.51)	0.92 (0.79 - 1.08)
	III	1.08 (0.91 - 1.30)	1.22 (1.04 - 1.43)	1.00 (0.84 - 1.18)
	IV	1.01 (0.83 - 1.23)	1.03 (0.86 - 1.22)	0.92 (0.76 - 1.10)

Results are expressed as hazard ratios (HR) with 95% confidence intervals (95% CI) per 10 bpm increase in resting heart rate.
Model I: crude; model II: age and gender; model III: age, gender, beta-blocker use, diuretic use, calcium blocker use, use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, current smoking, estimated glomerular filtration rate, systolic blood pressure, and inclusion diagnosis (coronary artery disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm); model IV (exploratory): model III and body mass index and fasting glucose.

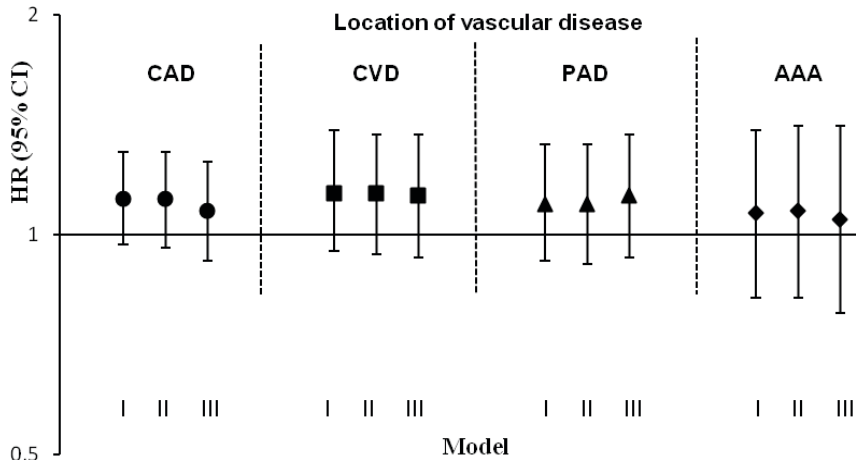

Figure 4. The risk of resting heart rate per 10 bpm on incident T2DM in patients with clinically manifest vascular diseases, stratified for the location of vascular disease.
Results are expressed as hazard ratios (HR) with 95% confidence intervals (95% CI) per 10 bpm increase in resting heart rate for patients with different locations of vascular diseases. CAD, coronary artery disease; CVD, cerebrovascular disease; PAD, peripheral arterial disease; AAA, aortic abdominal aneurysm; Model I: crude; model II: age and gender; model III: age, gender, beta-blocker use, diuretic use, calcium-blocker use, use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, current smoking, estimated glomerular filtration rate, systolic blood pressure, location of vascular disease (CAD, CVD, PAD or AAA).

Furthermore, in separate analyses after exclusion of patients with a baseline fasting plasma glucose level ≥7.0 mmol/l or after exclusion of patients with a RHR ≤50 or ≥100 bpm, the results were not essentially different. Adjustment for levels of TSH did not change the results either. Each 10 bpm increase in RHR was associated with a 19% higher risk for incident T2DM in patients on beta-blocker treatment (HR, 1.19; 95% CI, 1.04-1.37) and only with a 3% higher risk in patients without beta-blocker use (HR, 1.03; 95% CI, 0.90-1.18); however, this was not statistically significant (p for interaction = 0.16).

RHR and incident T2DM according to location of vascular disease

The relation between RHR and incident T2DM was not significantly different between patients with different locations of vascular disease (p for interaction 0.72 for CAD, 0.69 for CVD, 0.82 for PAD and 0.84 for AAA); every 10 bpm increase in RHR was associated with a 13% higher risk for T2DM in patients with CVD and PAD (HR, 1.13; 95% CI, 0.93-1.37 and HR, 1.13; 95% CI, 0.93-1.37 respectively), an 8% higher risk in patients with CAD (HR, 1.08; 95% CI, 0.92-1.26) and a 5% higher risk in patients with AAA (HR, 1.05; 95% CI, 0.78-1.41) (*Figure 4*).

Discussion

In this study it is shown that an increased RHR is associated with an increased risk for developing T2DM in patients with clinically manifest vascular diseases, particularly in middle-aged (55-63 years) patients, independently of the location of vascular disease or beta-blocker use. These results are in accordance with previous findings in healthy subjects and in subjects with increased BMI or impaired glucose tolerance (15-20). As patients with clinically manifest vascular diseases have an increased sympathetic nerve activity (26), reflected by an increased RHR, increased sympathetic tone can be considered to be part of shared pathophysiological pathways for developing diabetes and atherosclerotic vascular diseases, next to insulin resistance and low-grade inflammation as proposed in the 'common soil' hypothesis (25).

The overall 10% risk of developing T2DM (HR, 1.10; 95% CI, 1.00-1.21) for every 10 bpm increase in RHR in the present study fits within the range of risks in other study populations (15-20). The reported risks in various populations are remarkably similar although population characteristics vary widely. Also, the method for recording RHR varied across studies including ECG, heart rate meter or pulse palpation.

In this study it is shown that age is an important effect modifier in the relation between RHR and incident type T2DM. The risk for incident type T2DM is particularly high in middle-aged patients (age range 55-63 years), while there is no clear relation in younger and older patients. Main analysis were performed

in tertiles of age. This was an arbitrary decision balancing statistical power and contrast between patient groups. The results were confirmed in exploratory analyses in smaller age groups (quintiles). In the other studies there was no statistically significant interaction of age on the relation between RHR and incident diabetes (15;16;18), or at least this was not reported (17;19;20). In a study conducted in healthy Chinese women, stratified analyses for age revealed that the effect of RHR was larger in women <55 years of age compared to women >55 years of age, although this was not a statistically significant difference (18). Furthermore, the study conducted in the youngest population (mean age 44±7 years) reports the highest risks of developing diabetes per SD increase in RHR: HR 1.37 (95% CI, 1.29-1.45) for men and HR 1.46 (95% CI, 1.31-1.62) for women (19) compared to the HR ranging from 1.10 to 1.27 in the other populations (mean age ranging from 48 to 54 years) (15;16;18;20). Although drawing a firm conclusion on the basis of these data is not appropriate, an elevated RHR seems to be associated with a higher the risk on incident T2DM in younger subjects or subjects of middle age than in older subjects.

Different pathophysiological explanations for the relation between RHR and incident T2DM may be considered. RHR is an indicator of sympathetic activity (12) and an increased sympathetic nerve system induces both acute and chronic insulin resistance (9-11). The major organs involved in insulin secretion, glucose production and glucose metabolism, including the pancreas, liver and skeletal muscle, are innervated by autonomic nerves (28-30). Stimulation of β-adrenergic receptors causes a reduction in the insulin-stimulated uptake of glucose (31) an increase in the proportion of insulin-resistant fast-twitch muscle fibers in rats (32), and most importantly, vasoconstriction leading to a decreased skeletal muscle blood flow, with impaired glucose uptake into the muscle cells as a result (33).

Both insulin resistance and autonomic dysfunction are the result of, and can be influenced by, physical inactivity and obesity, which are established risk factors for the development of T2DM (34;35) and are also known to influence autonomic function (36;37). Obesity and fasting serum glucose levels are very likely to be part of the causal pathway between RHR and incident T2DM, as shown in the present study by the attenuation of the results after adding BMI and fasting serum glucose levels to the regression model. However, obesity and elevated fasting glucose levels could have been the result of autonomic dysfunction (elevated RHR) during patients' life, or could have been caused by the high RHR. Obesity is a cause of insulin resistance, which in turn leads to increased sympathetic nerve activity, and to increased glucose levels progressing to overt T2DM. However, cause and effect could also be the other way around. Increased sympathetic nerve activity, as measured by RHR, could also lead to higher BMI and elevated plasma glucose and insulin levels with progression to T2DM. Which of these two explanations

is true cannot be concluded from our study. As discussed before, sympathetic nerve activation can be both the cause and the consequence of insulin resistance. The recent finding that reducing sympathetic nerve activity, by renal sympathetic denervation (38), causes improvements in glucose metabolism and insulin sensitivity further underlines the pathophysiological role of the sympathetic nerve system in the development of insulin resistance.

The overall association between RHR and incident T2DM in the present study is found despite a high prevalence of beta-blocker use (53%). It seems that the predictive role of RHR in patients on beta-blocker treatment is greater (HR, 1.19; 95% CI, 1.04-1.37) than in those without beta-blocker use (HR, 1.03; 95% CI, 0.90-1.18), although this was not statistically significant. This is not surprising considering the fact that beta-blockers favor insulin resistance and increase the risk of developing diabetes (39).

The results from the present study are in line with previous results obtained in the general population (15-20), but are of potential greater clinical significance because of the potential therapeutic implications. RHR is a noninvasive, nonexpensive and easily obtainable parameter, and therefore easily applicable in clinical practice, in contrast to other measurements that reflect sympathetic nerve activity such as MSNA or plasma noradrenalin levels. Prevention of diabetes in patients with vascular diseases is important, as patients with diabetes have an increased risk for (cardiovascular) morbidity and mortality (21;22) and a reduced quality of life (23). Reducing the risk of T2DM is feasible with lifestyle interventions, such as increasing physical activity and reducing body weight, and with medical treatment (24;40).

Strengths of this study include the prospective cohort design and the large sample size of a clinically relevant and well-characterized group of patients with various locations of clinically manifest vascular diseases. Furthermore, RHR was accurately and consistently measured with ECG and the incidence of T2DM was thoroughly assessed. Assessment of several clinical and laboratory parameters allowed for identification of possible confounding factors.

Study limitations need to be considered. As we excluded subjects who died or were lost to follow-up before the assessment of diabetes started in 2006, and diabetes is associated with a higher mortality, this could have affected the results of this study toward the null hypothesis. This effect is possibly of greatest influence in the older age group, as older persons are at the highest risk of death, which could be an explanation for the absence of a relation between RHR and incident diabetes in this group. Unfortunately, serum insulin levels were not routinely measured in our study, so we could not adjust our exploratory model for insulin resistance expressed as homeostatis model assessment of insulin resistance (HOMA). However, by adjusting for BMI and fasting serum glucose, the

role of insulin resistance is explored for an important part, as both parameters are strong determinants of HOMA. Finally, physical activity and cardiorespiratory fitness, factors in the causal pathway of RHR and incident T2DM were not assessed in this study. In the exploratory analyses, we adjusted for other factors in the causal pathway between RHR and incident diabetes (BMI and fasting serum glucose levels). As not only BMI and fasting serum glucose levels, but also variables such as age, gender and smoking are closely related to physical activity and cardiorespiratory fitness, we believe the residual confounding effect in the exploratory analysis of physical activity and cardiorespiratory fitness to be limited. In conclusion, increased RHR, an indicator of increased sympathetic nerve activity, is associated with an increased risk for incident T2DM in patients with clinically manifest vascular diseases. This risk was particularly elevated in middle-aged patients and was irrespective of the location of vascular disease and of the use of beta-blockers.

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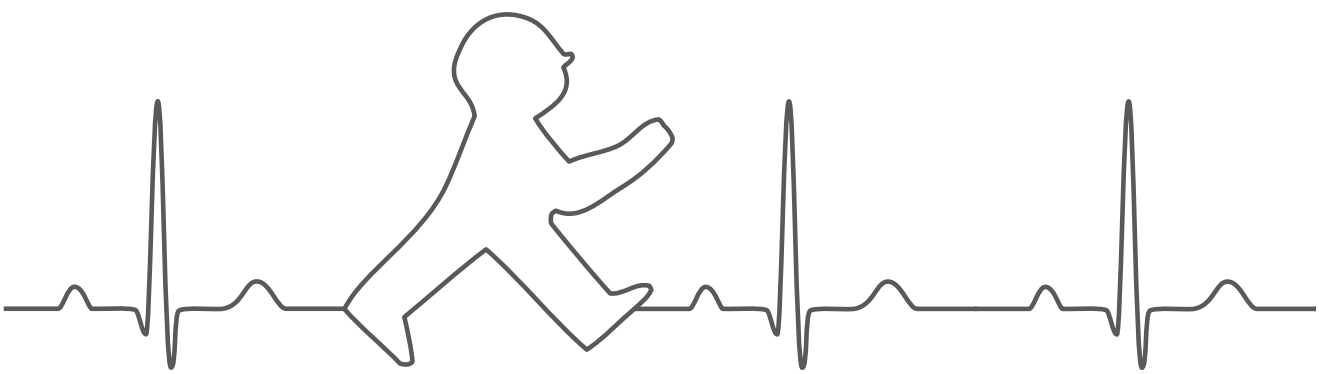
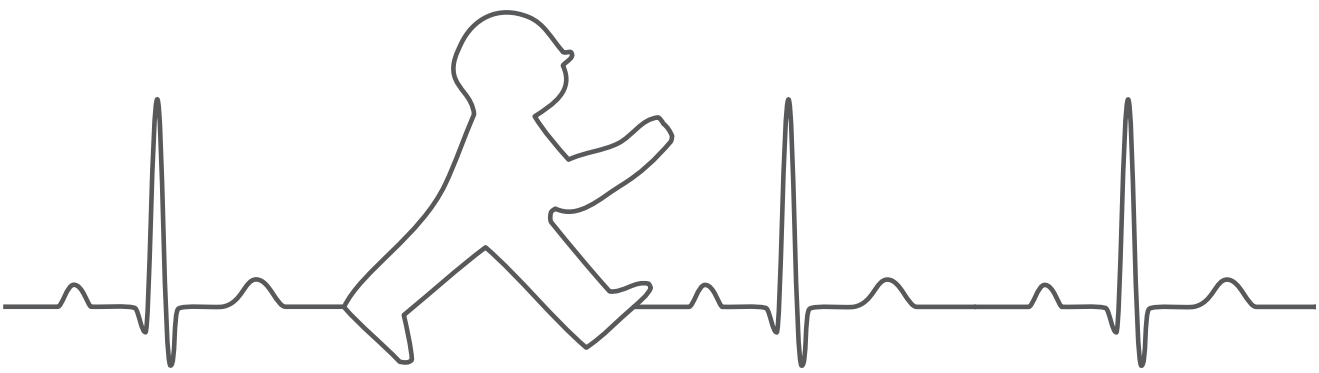


Chapter 8

General discussion



Part one. Physical exercise

The clinical relevance of prolonged daily walking

For the vast majority of patients who are at risk for, or already have, clinically manifest vascular disease, increasing the amount of physical exercise is one of the lifestyle advices that is repeatedly given in clinical practice. Numerous studies have shown that physical exercise has beneficial effects on virtually all aspects of cardiovascular disease. The large majority of these studies are conducted under controlled circumstances, such as using treadmills, with exercise programs designed to control exactly for exercise duration and intensity. Although many people in the Western society indeed exercise, at fairly low frequency (e.g. once a week) in fitness centres under controlled circumstances, an even larger part of daily physical exercise is conducted outdoors. For example, walking is, together with gardening, the major component of leisure time physical activity (1). Furthermore, the number of people conducting specific exercise types in a natural environment is increasing; for example Nordic Walking is increasingly popular (2). It is therefore important that more studies are conducted to investigate the health effects of various exercise types conducted in real life by many people.

Since it is estimated that millions of people walk parts of old pilgrimage routes, and this number is increasing (3), walking part of a pilgrimage route is one such example of exercise in a natural environment that is conducted by many people, justifying scientific research to its health effects.

Walking part of an ancient pilgrimage route to Santiago de Compostela in Spain consisting of 280 km in 12 days, resulted in a sustained weight loss of around 2 kg, but no other beneficial metabolic or vascular effects persisting for at least 2 months after the pilgrimage (**chapter 2**) (4). However, during the pilgrimage, the most remarkable finding was a large steady decrease in LDL-cholesterol (LDL-c), reaching a maximal decrease on day 10 of 1.1±0.7 mmol/l (-29%) compared to study baseline LDL-c. Changes of this size in LDL-c are comparable to LDL-c reduction with statins and have not been reported before as a result of physical exercise. We can only speculate about the possible mechanisms behind this decrease in LDL-c, which is to our opinion most likely due to an increased uptake and use of cholesterol for cellular mechanism and repair due to muscle damage after intense exercise (5).

Furthermore, the male and female subjects walking this pilgrimage with higher speed showed a larger increase in HDL-c, but a smaller decrease in LDL-c and total cholesterol than the participants walking with lower speed, and a higher walking speed was independently related to increases in HDL-c and LDL-c (**chapter 3**). Again, we did not investigate the underlying mechanism, but we speculate that consumption of cholesterol, both from HDL and LDL particles, for cellular metabolism and repair due to muscle damage, leads to less increase in HDL-c and more decrease in LDL-c with longer walking at lower walking speed compared to walking the same distance in a shorter period at higher speed.

As the large decrease in LDL-c during the pilgrimage was only present during the period of prolonged daily exercise, and walking daily for about 5-6 hours is almost impossible to apply in normal daily life, the results of these studies are not directly of impact for daily clinical practice. An advice to walk a daily distance of about 25 kilometres or to exert a comparable amount of other exercise with the objective to lower LDL-c is not likely to be followed by many patients, and whether we should advice people to walk with higher speed to increase HDL-c more, or with lower speed to decrease LDL-c more, remains unsure. However, both evaluations of the Santiago pilgrimage reveal that serum lipid levels may change notably during prolonged exercise, and that the exercise intensity or duration might be of specific interest. These are both new findings. As few previous studies have focussed on lipid changes during periods of prolonged exercise or on the influence of exercise intensity, the results presented in **chapters 2 and 3** of this thesis can be seen as hypothesis generating. LDL-c can be seen as a pool, from which cholesterol is used as a source of fuel, just like triglycerides, or for cellular mechanisms and repair, when needed.

The role of physical activity in lifestyle modification programs for treatment of the metabolic syndrome

The metabolic syndrome is increasingly prevalent, currently affecting at least a quarter of the US general population (6;7) and about 50% of patients with clinically manifest vascular disease (8). Patients with the metabolic syndrome have a 3-8 times increased risk for the development of type 2 diabetes mellitus (9;10), and a 2-3 times increased risk for the development of cardiovascular disease (11-13), and also a 2-3 times increased risk for premature all-cause and cardiovascular mortality (13). Treatment of the metabolic syndrome starts with lifestyle modification, consisting of the combination of changing dietary habits and increasing physical activity, as recommended by leading health organisations (14-16). This approach has been shown to lead to improvements in multiple metabolic risk factors and even resolution of the metabolic syndrome (17-20), and the combination of diet and exercise is more effective than either approach alone (21). Despite the favourable results in these studies, the unsatisfactory reality in clinical practice is, that in spite of repeatedly given lifestyle recommendations, the majority of patients with the metabolic syndrome remain poorly controlled regarding treatment targets of HbA1C, LDL-c and blood pressure and the adherence to diet and physical activity recommendations is low (22-24).

The difficulty to achieve results with lifestyle modification in the clinical practice setting, even when there is intensive follow-up contact, is confirmed by the results of a 1-year lifestyle modification program for overweight patients with the metabolic syndrome (**chapter 4**). Decreases in bodyweight, BMI, waist circumference and insulin resistance after 1 year were seen, but they were predominantly achieved during the first 6 months of the program, when

contact with the study nurse was most intense, and attenuated to a large extent in the follow-up year after the lifestyle modification program had stopped. Furthermore, although it was a major goal of the program, patients did hardly increase the amount of daily physical activity during the lifestyle modification program. Our population consisted of patients with the metabolic syndrome, and a large percentage also had diabetes (42%) or a history of vascular disease (25%), which is a possible explanation for the limited results. These patients are often restricted by their underlying disease or medical treatment to lose weight (insulin, sulfonylurea derivatives) or to increase physical exercise (fear of hypoglycaemia, symptoms of cardiovascular disease or beta-blocker use). Any weight loss in this population achieved by lifestyle modification can be considered as a valuable result, although maintenance of the weight loss achieved is another problem. Therefore, and also regarding the fact that only dietary advices were given instead of an hypocaloric diet, we consider the average body weight loss of 2.5% after 1 year reached in this lifestyle modification program, to be successful.

Both the amount of baseline physical activity and the change in physical activity during the study were related to beneficial changes in parameters of bodyweight and insulin resistance. Physically active patients are more likely to benefit from a lifestyle modification program, although the magnitude of the relation between baseline physical activity and improvements in measures of bodyweight and insulin resistance was small. Furthermore, patients who increased their level of daily physical activity during the study had larger decreases in measures of bodyweight and insulin resistance, but only very large increases in physical activity were related with clinically meaningful improvements. Therefore we conclude that lifestyle modification is of value for any patient with the metabolic syndrome, regardless of their baseline physical activity level, and that the effect of increasing physical activity on bodyweight loss is limited.

Despite the small effects of the physical exercise on measures of bodyweight and insulin resistance in this lifestyle modification program, we are still convinced that this is the best treatment as a first step. Physical exercise has important beneficial effects on cardiovascular risk factors besides weight loss or insulin resistance, as discussed before. Furthermore, pharmacological options to induce weight loss, such as rimonabant or sibutramine are also causing only modest weight loss and are associated with serious adverse events (25;26). Bariatric surgery is highly effective in achieving and maintaining weight loss, but is a major invasive strategy associated with peri-operative mortality and morbidity, and therefore not suitable as first step (27). Therefore, physical exercise remains a cornerstone in the first step treatment of the metabolic syndrome.

Part two. Resting heart rate

The relation between obesity/insulin resistance and sympathetic nerve activation: cause or consequence?

The relation between obesity and insulin resistance on the one hand and sympathetic nerve activation on the other hand is complex, and only partially understood. Obesity and insulin resistance have been considered to be causal factors for increased sympathetic activation, as especially visceral obesity is related to elevated serum levels of leptin and insulin (28), and administration of either hormone leads to an increase in sympathetic nerve activation (29;30), through activation of insulin- and leptin-receptors in the central nervous system (31-33). However, more recently, sympathetic nerve activation has also been discovered as a possible causal factor in the development of obesity and insulin resistance. Acute sympathetic nerve activation induces insulin resistance (34) and chronically increased sympathetic nerve activity is reported to precede the development of insulin resistance and obesity (35;36). Furthermore, resting heart rate, a marker of sympathetic activity (37), is independently associated with an increased risk for developing type 2 diabetes mellitus (38-43). Finally, the recent finding that reducing sympathetic nerve activity, by renal sympathetic denervation, causes improvements in glucose metabolism and insulin sensitivity (44), further underlines the pathophysiological role of the sympathetic nerve system in the development of insulin resistance. Therefore, obesity and insulin resistance can be considered as both a cause and a consequence of increased sympathetic nerve activity.

In the present thesis, arguments are presented for obesity/insulin resistance as both a cause and a consequence of sympathetic nerve activation. The cross-sectional relation between visceral adipose tissue, subcutaneous adipose tissue, body mass index and waist circumference on the one hand and resting heart rate as a marker of sympathetic nerve activity on the other hand shows that typically ultrasonographically measured visceral adipose tissue thickness is independently related to increased resting heart rate in men and, to a smaller extent, in women (**chapter 5**) (45). Waist circumference and body mass index, parameters of obesity reflecting visceral adipose tissue thickness also, but less specifically, were less strongly related to resting heart rate, while subcutaneous adipose tissue, not reflecting visceral adipose tissue mass, was not related with resting heart rate. Even after additional adjustment for body mass index, the relation between visceral adipose tissue and resting heart rate remained present, indicating that it is specifically the amount of visceral fat and not total body fat that is associated with resting heart rate. Although causality of the relation between visceral adipose tissue and resting heart rate in this cross-sectional study can only be suggested, the pathophysiological pathway from visceral adipose tissue leading

to elevated serum insulin and leptin levels, which both cause sympathetic nerve activation leading to an increase in resting heart rate, is well documented. Theoretically, it cannot be excluded that increased visceral adipose tissue is the result from elevated resting heart rate in this cross-sectional study, but there is no pathophysiological explanation for a possible causal effect of resting heart rate on specifically visceral adipose tissue, and not on for example, subcutaneous adipose tissue.

However, instead of a cause, obesity/insulin resistance can also be seen as a consequence of sympathetic nerve activation. The independent relation between increased resting heart rate and increased risk for the development of type 2 diabetes mellitus during follow-up in a prospective cohort study of patients with clinically manifest vascular disease supports this view (**chapter 7**) (46). The causal pathway of this relation is formed by obesity and elevated fasting serum glucose, as is made plausible in **chapter 7** as well: adding BMI and fasting serum glucose to the regression model attenuates the results to a large extent. The precise sequence of the pathophysiological pathway cannot be determined for sure, one possibility is that obesity causes insulin resistance, leading to increased sympathetic nerve activation and elevated resting heart rate, and at the same time to increased glucose levels progressing to overt type 2 diabetes. However, increased sympathetic nerve activity, measured by resting heart rate, could also lead to obesity and insulin resistance with higher serum glucose levels, and progression to type 2 diabetes.

From previous evidence in combination with the results from this thesis, we conclude that obesity/insulin resistance is causally related to sympathetic nerve activation as both a cause and a consequence. Visceral adipose tissue is at the basis of leptin- and insulin-mediated sympathetic nerve activation, but increased sympathetic nerve activation measured by resting heart rate causes insulin resistance and overt diabetes as well.

The place in daily clinical practice for routine measurement of resting heart rate

In the 1940s, high resting heart rate and elevated blood pressure were both shown to predict the occurrence of future cardiovascular diseases independently (47;48). Since then, education about hypertension as a cardiovascular risk factor and therapeutic lowering of blood pressure have become priorities in the management of cardiovascular disease. In contrast, resting heart rate as a cardiovascular risk factor has failed to make the step to clinical practice until very recently. Nowadays, a high resting heart rate is considered to be an important predictor of premature all-cause and cardiovascular mortality in patients with cardiovascular disease such as ischemic coronary artery disease or heart failure, irrespective of the presence of hypertension (49;50). Moreover, heart rate

reduction using beta blockers or calcium channel blockers has been shown to be associated with improved outcome in patients with coronary artery disease or heart failure (51;52). However, as these agents also have other cardiovascular actions besides heart rate reduction, such as lowering of blood pressure, resting heart rate has failed to become a formal target of therapy. Recently, pure heart rate reduction using ivabradine, which does not affect blood pressure, has shown to improve clinical outcome in patients with heart failure, as well as in a subgroup of patients with stable coronary artery disease, left ventricular dysfunction and increased resting heart rate (53-55). Another promising treatment modality reducing resting heart rate is chronic vagus nerve stimulation, which has been evaluated in a phase II study showing improvement in left ventricular function and quality of life in patients with heart failure (56). These recent findings underline a pathophysiologic role of elevated resting heart rate in the occurrence of cardiovascular events and death.

Although an elevated resting heart rate has also been shown to be an independent risk factor for premature mortality in the population (57-62), it is unlikely that this will lead to studies investigating the beneficial effects of heart rate reduction in healthy subjects. This could be different for patients with vascular diseases at other locations than the heart, as these patients are at high risk for new cardiovascular events and mortality, and are already receiving other medication to lower their cardiovascular risk. Indeed, elevated resting heart rate is not only independently related to all-cause and vascular mortality in patients with coronary artery disease, but also in patients with cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm (**chapter 6**). Moreover, there was no statistically significant difference in the relation between resting heart rate and all-cause and vascular mortality between patients with various locations of vascular disease, despite a large sample size. These findings were obtained after adjustment for multiple covariates, irrespective of beta blocker use and not due to reverse causality. Furthermore, the multiple-adjusted hazard ratio per 10 bpm increase in resting heart rate on all-cause mortality in patients with coronary artery disease were comparable to what has been reported in other studies in such patients.

Besides being related to an increased risk for mortality, an increased resting heart rate in patients with vascular diseases is also related to an elevated risk for the development of type 2 diabetes, predominantly in patients at middle-age (**chapter 7**). Again, these results were independent of beta blocker use and valid for patients with coronary artery disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm.

Resting heart rate is a non-invasive, non-expensive and easily obtainable parameter, which is already routinely reported on an electrocardiogram and

during electronic blood pressure measurements; both standard examinations in the management of patients with vascular diseases. We have shown that elevated resting heart rate identifies patients with vascular disease who are at increased risk of adverse outcomes, as an increase in resting heart rate of 10 bpm is independently related to an increase in all-cause mortality of 14% (**chapter 6**), in vascular mortality of 15% (**chapter 6**) and in incident type 2 diabetes of 10% (**chapter 7**). Therefore, we suggest that measurement and evaluation of resting heart rate should become routine practice in the management of patients with clinically manifest vascular disease, irrespective of the location, to obtain information about the risk for adverse outcome. The European Society of Cardiology and the European Society of Hypertension (ESC/ESH) guidelines for the management of hypertension already suggest to include elevated resting heart rate when assessing the cardiovascular risk profile of an individual, and resting heart rate is also considered in a risk estimation model to predict future cardiovascular events (63;64).

Heart rate reduction is currently perceived as a potential target for therapy in patients with coronary artery disease or heart failure. We argue that not only patients with coronary artery disease, but also patients with cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm may benefit from therapies aiming to reduce heart rate (**chapter 6**). This is pathophysiologically plausible, as a large proportion of the patients with clinically manifest vascular diseases at other locations than the heart have (subclinical) coronary artery disease as well (65) and proposed adverse effects of an increased heart rate include systemic effects such as endothelial dysfunction and atherosclerotic plaque rupture. Therefore we suggest to include patients with clinically manifest vascular diseases, irrespective of the location, in studies investigating heart rate reduction therapy, for example with ivabradine. Treatment of elevated resting heart rate has the potential to become a new target of treatment in patients with clinically manifest vascular disease, irrespective of the location.

In conclusion, studies presented in this thesis showed that:

- walking a pilgrimage covering 280 km in 12 days immediately lowers triglycerides, body weight and most prominently, LDL-c, and increases HDL-c; however, apart from the weight loss, these changes were short-lived, and there was no effect on vascular function
- walking a pilgrimage at higher walking speed was related to a more pronounced increase in HDL-c, but also to a less decrease in LDL-c, compared to walking at lower walking speed
- both baseline physical activity and an increase in physical activity during a lifestyle modification program were related to decreases in weight, waist circumference, body mass index and insulin resistance in overweight patients with the metabolic syndrome
- increased visceral adipose tissue is associated with a higher resting heart rate in patients with clinically manifest vascular disease, even after adjusting for body mass index
- increased resting heart rate is associated with increased risk for vascular mortality, all-cause mortality and vascular events in patients with clinically manifest vascular disease, irrespective of the location of vascular disease
- increased resting heart rate, a reflection of increased sympathetic nerve activity, is associated with an increased risk for incident type 2 diabetes mellitus in patients with clinically manifest vascular disease, in particular in patients at middle-age

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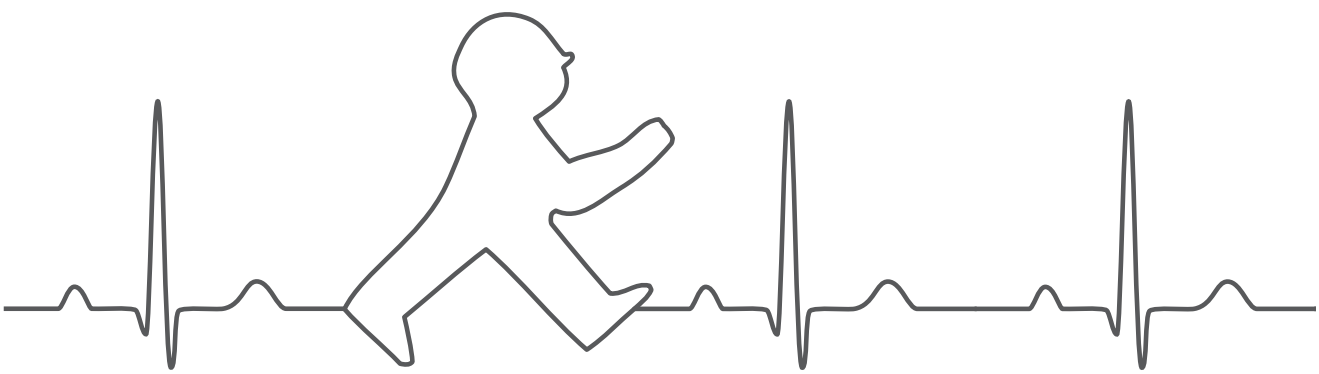

Chapter 9

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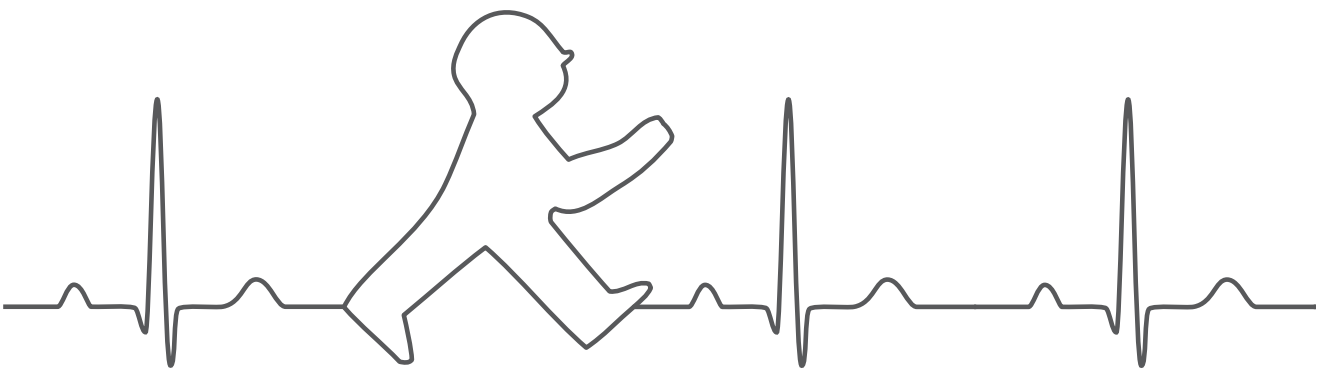


Since the early years of the 20st century, atherosclerotic cardiovascular disease (CVD) has been the leading cause of mortality in the United States every year but 1918. Moreover, globally, low- and middle income countries contribute about 80% of all CVD-related deaths, and tripling of ischemic hart disease- and stroke mortality is expected in the next two decades in these countries.

Physical exercise has beneficial effects on virtually all aspects of cardiovascular disease, including improvements in almost all classical cardiovascular risk factors. Therefore, increasing physical activity is widely advocated in guidelines as a first step, together with other lifestyle measures, in the treatment and prevention of cardiovascular disease.

Among mammals, the total number of heart beats per lifetime is remarkably constant, despite wide variations in body size and resting heart rate (RHR). This implies that the RHR is strongly related to life expectancy. Also in humans, RHR is independently related to mortality, an increase in RHR of 10 beats per minute is associated with around 10-30% increased risk for premature all-cause mortality. Direct adverse events of an increased RHR on cardiac and vascular function and morphology include endothelial dysfunction, direct stimulation of atherogenesis and atherosclerotic plaque rupture, increased susceptibility for ventricular arrhythmias and a negative influence on the balance between myocardial oxygen demand and supply. Heart rate reduction therapy, using beta-blockers or non-dihydropiridine calcium channel blockers, has shown to reduce the risk for all-cause and cardiac mortality with about 30% per 10 beats per minute heart rate reduction in patients with a recent myocardial infarction.

The main aim of this thesis is twofold: to determine the effects of physical exercise and lifestyle changes on cardiovascular risk factors (**Part 1**) and to determine the relation between resting heart rate and obesity, diabetes risk and vascular risk in patients with clinically manifest vascular disease (**Part 2**).

Part 1 Physical exercise, lifestyle changes and vascular risk factors

Most studies investigating the effects of physical exercise focus on exercise under artificial conditions in training centres, controlling exercise intensity and duration. However, walking is one of the major components of leisure time physical activity, often conducted in a natural environment. For example, it is estimated that yearly over five million persons walk a pilgrimage in various parts of the world, and this number is increasing.

In **chapter 2** the effects of a 12-day pilgrimage to Santiago de Compostela in Spain on vascular function and cardiovascular risk factors are reported. Twenty-nine healthy male and female subjects between 40 and 70 years were included in the intervention group, while 29 age- and gender-matched subjects formed the control group who stayed at home. The intervention consisted of walking the last 280 km of the ancient pilgrim route to Santiago de Compostela. Measures of endothelial function, vascular stiffness, autonomic function and cardiovascular risk factors were measured 2 months and 2 weeks before the pilgrimage, and 2 weeks and 2 months afterwards. During the pilgrimage, cardiovascular risk factors were measured every other day. All subjects completed the pilgrimage.

The mean daily walking distance during the pilgrimage was 23.42±0.80 km taking 5.39±0.36 hours/day. During the pilgrimage, there was a remarkable steady decrease in LDL-cholesterol (LDL-c) of 0.6±0.6 mmol/l (-17%) from start to finish, as well as a decrease in weight of 1.4±1.8 kg (-2%), in waist circumference of 1.8±2.9 cm (-2%) and in triglycerides of -0.39±0.58 mmol/l (-30%), while HDL-cholesterol (HDL-c) increased 0.20±0.30 mmol/l (+15%). Maximum decreases in LDL-c during the pilgrimage were seen on day 10 (-0.75±0.70 mmol/l; -21%; $p<0.01$). After the end of the pilgrimage, all changes came back to baseline, except for a 2.0 kg (95% confidence interval (CI) -3.2--0.8; $p<0.01$) weight loss, which persisted for two months in the intervention group compared to the control group. There was no change in vascular function in the intervention group compared to the controls. We conclude that walking a pilgrimage of 280 km in 12 days did not result in beneficial metabolic or vascular effects persisting for 2 months, except for a sustained weight loss of around 2 kg. However, the large steady decrease in LDL-c during the pilgrimage is comparable to LDL-c reduction with statins and has not been reported before as a result of physical exercise. We can only speculate about the possible mechanisms behind this decrease in LDL-c, which is to our opinion most likely due to an increased uptake and use of cholesterol for cellular metabolism and repair due to muscle damage after intense exercise.

Guidelines recommend a minimum weekly physical activity equal to 150 minutes of brisk walking, however, it is not specified at what intensity this exercise should be preferable conducted; brisk walking or shorter periods of exercise at a higher intensity are considered equally effective. Knowledge about the effects of exercise

intensity, specifically walking speed, on cardiovascular risk factors is limited.
In **chapter 3**, we report the relation between walking speed and changes in cardiovascular risk factors in participants of the 12-day pilgrimage to Santiago de Compostela. During the pilgrimage described above, walking speed was calculated from the recorded walking distance, walking time and measured steps with a pedometer. Changes in measured cardiovascular risk factors during the pilgrimage were compared between gender-pooled groups with higher and lower walking speed. Secondly, the relation between walking speed and changes in cardiovascular risk factors was quantified using a mixed linear effects model. In the higher walking speed (4.6±0.2 km/h) group HDL-c increased more than in the lower walking speed (4.1±0.2 km/h) group (difference in change between the groups: 0.20; 95%CI -0.02-0.42), while LDL-c and total cholesterol decreased more in the lower walking speed group (differences in changes between the groups LDL-c: -0.50; 95%CI -0.88--0.12 mmol/l and total cholesterol: -0.75; 95%CI -1.19--0.31 mmol/l). A 1 km/h higher walking speed was related to an increase in HDL-c (0.24; 95%CI 0.12-0.30 mmol/l), LDL-c (0.18; 95%CI -0.16-0.42 mmol/l) and total cholesterol (0.36; 95%CI 0.12-0.60 mmol/l), adjusted for age, gender, smoking, BMI and heart rate, during the whole walking tour. These changes were also independent of changes in body weight during the pilgrimage. There was no relation between walking speed and changes in weight, waist circumference, blood pressure, triglycerides or glucose.
Again, we did not investigate the underlying mechanism, but we speculate that consumption of cholesterol, both from HDL en LDL particles, for cellular metabolism and repair due to muscle damage, leads to less increase in HDL-c and more decrease in LDL-c with longer walking at lower speed compared to walking the same distance in a shorter period at higher speed.
The metabolic syndrome is considered a constellation of metabolic abnormalities including abdominal obesity, insulin resistance, atherogenic dyslipidemia, elevated blood pressure, a prothrombotic profile and systemic, low-grade inflammation. Patients with the metabolic syndrome are at increased risk for type 2 diabetes mellitus, vascular disease and premature mortality. The prevalence of the metabolic syndrome has been increasing over the past decades and is now estimated to affect at least a quarter of the US population and about 50% of patients with clinically manifest vascular disease in the Netherlands. Increasing physical activity and weight reduction are two important lifestyle changes that are widely advocated in guidelines as a first step in the treatment and prevention of the metabolic syndrome.
In **chapter 4** we investigate the relation between baseline daily physical activity or changes in daily physical activity and changes in bodyweight and insulin resistance during a lifestyle modification program in patients with the metabolic

syndrome. Overweight (BMI>25 kg/m2) patients with the metabolic syndrome (n=76) willing to change their lifestyle were included in a 1-year nurse-led lifestyle modification program with the goals to increase physical exercise and change dietary habits. During the program, patients were counselled on healthy dietary habits according to guidelines, but without describing a specific diet or a low-calorie diet. The exercise recommendation of a daily minimum of 30 minutes of moderate-intensity physical activity was given, according to guidelines. Patients visited the study-nurse monthly during the first 6 months and every other month during the second 6 months. After 1 year the intervention stopped and patients returned to regular care. Two years after the start of the intervention program an evaluation visit was scheduled.

During the study, patients hardly changed their physical activity, but after 1 year the mean body weight was 2.3 kg lower (95%CI -3.8--0.8). A 1 metabolic equivalent (MET) higher baseline daily physical activity level was related to changes in weight (-0.69 kg; 95%CI -1.77-0.40), waist circumference (-0.51 cm; 95%CI -1.65-0.63), BMI (-0.18 kg/m2; 95%CI -0.53-0.17) and HOMA-IR (-0.78; 95%CI -1.62-0.07) per 3 months during the intervention. A 1 MET increase in physical activity during 1 year was related to changes in weight (-2.3 kg; 95%CI -3.9--0.7), waist circumference (-2.8 cm; 95%CI -4.4--1.1), BMI (-0.80 kg/m2; 95%CI -1.32--0.28) and insulin resistance (-0.34; 95%CI -1.39-0.71) per 3 months, adjusted for age, gender, smoking, history of vascular disease and baseline level of physical activity. As any weight loss in this population achieved with lifestyle modification can be considered as a valuable result, and also regarding the fact that only dietary advices were given instead of a hypocaloric diet, we consider the average body weight loss of 2.3 kg (-2.5%) after 1 year reached in this lifestyle modification program, to be a successful result. As both the amount of baseline physical activity, and the change in physical activity during the study were related to beneficial changes in parameters of body weight and insulin resistance, although the quantitative effects were small, we conclude that lifestyle modification is of value for any patient with the metabolic syndrome, regardless of their baseline physical activity level, and that the effect of increasing physical activity on body weight loss is limited.

Part 2 Resting heart rate and vascular disease

Obesity, and in particular abdominal obesity, is an independent risk factor for the development of cardiovascular disease, and strongly related to the development of metabolic complications such as dyslipidemia, insulin resistance and diabetes mellitus. Visceral adipose tissue (VAT) is the predominant adipose tissue compartment producing various proinflammatory cytokines and adipokines, and is considered to play a central role in the development of the metabolic syndrome.

Furthermore, central obesity is associated with more pronounced sympathetic nerve activation than peripheral obesity, possibly mediated by high serum levels of insulin and leptin present in central obesity.

Therefore, in **chapter 5** we investigate the relation between visceral adiposity and RHR, a measure of sympathetic tone, in patients with various clinical manifestations of vascular disease. In 3,723 patients with manifest vascular disease from the Second Manifestations of ARTerial disease (SMART) study, visceral and subcutaneous fat tissue was measured with ultrasonography. RHR was obtained from a electrocardiogram (ECG). The study population was categorized into sex-pooled quartiles (Q) according to their visceral fat thickness, ranging from 2.7-8.0 cm in Q1 (reference) to 9.4-20.6 cm in Q4.

High visceral fat thickness was associated with increased RHR, in men (Q4 versus Q1, β = 3.51; 95%CI 3.11-5.61) and women (β = 1.48; 95%CI -0.70-3.66), after adjusting for age, current smoking, estimated glomerular filtration rate, type 2 diabetes and the use of medication with effect on the RHR (β-blockers, α-blockers, diuretics and calcium-channel blockers). Waist circumference and BMI had a significant relation with RHR in men (β = 3.51; 95%CI 2.21-4.81 and β = 2.80; 95%CI 1.51-4.08 respectively) but these relations were smaller and not statistically significant in women (β = 0.71; 95%CI -1.44-2.85 and β = 0.24; 95%CI -1.90-2.37 respectively). There was no relation between subcutaneous fat and RHR in men and women. The relation between visceral fat and RHR was similar in patients with different locations of vascular disease. Even after additional adjustment for BMI as a marker of general adiposity, the relation between VAT and RHR decreased only a little in men and women, indicating that it is specifically the amount of intra-abdominal fat and not total body fat that is associated with heart rate.

Although this study is a cross-sectional study and causality of the observed relations can only be suggested, a causal pathophysiological explanation is available. Increased intra-abdominal fat is associated with elevated serum insulin and leptin levels, and both hormones are known to increase sympathetic nerve activity, which is reflected by increased RHR. We conclude that increased intra-abdominal fat thickness is associated with a higher RHR in patients with clinically manifest vascular disease, even after adjusting for BMI.

RHR is an independent risk factor for cardiovascular events and mortality in patients with coronary artery disease, in patients with diabetes mellitus or hypertension and in healthy subjects. As elevated sympathetic nerve activation is also present in patients with vascular diseases at other locations than the heart, and some adverse effects of increased RHR such as atherosclerotic plaque rupture and endothelial dysfunction are systemic, we hypothesize that the detrimental effects of an elevated RHR could affect patients with clinical manifest vascular disease irrespective of the location of vascular disease.

In **chapter 6** we investigate the relation between RHR and cardiovascular events and mortality in patients with coronary artery disease (CAD), cerebrovascular disease (CVD), peripheral arterial disease (PAD) or abdominal aortic aneurysm (AAA). Data were used from 4,272 patients with manifest vascular disease from the Second Manifestations of ARTerial disease (SMART) study: CAD (n=2,244), CVD (n=930), PAD (n=823) or AAA (n=75). RHR was obtained at baseline from an ECG. The median follow-up time was 4.4 (interquartile range 2.1-7.4) years. The relation between RHR and the occurrence of cardiovascular events and death was estimated by Cox proportional hazard analysis.

In the total study population, each increase in RHR with 10 beats per minute was related to an increase in all-cause mortality (HR 1.14; 1.07-1.21), in vascular mortality (HR 1.15; 95%CI 1.06-1.25) and in the composite vascular endpoint (HR 1.08; 95%CI 1.01-1.15) after adjustment for age, gender, smoking, blood pressure lowering medication and location of vascular disease. The risk for myocardial infarction (HR 1.03; 95%CI 0.94-1.14) and ischemic stroke (HR 1.05; 95%CI 0.92-1.20) was not higher with increasing RHR. There was no evidence of reverse causality, as hazard ratios remained largely unchanged when patients who died (n=59) or had a cardiovascular event (n=153) in the first year of follow-up were excluded from analyses. There was no effect modification by the location of vascular disease (CAD, CVD, PAD or AAA) on the relation between RHR and all-cause mortality or the combined endpoint of vascular events (p for interaction all >0.05). The increased risk per 10 beats per minute increase in RHR on all-cause mortality was both present in patients on beta-blockers (n=2289, HR 1.22; 95%CI 1.09-1.38) and in patients not on beta-blockers treatment, although attenuated (n=1983, HR 1.11; 95%CI 1.03-1.20), p for interaction of beta-blocker use was 0.14. In an exploratory analysis, we adjusted for some determinants of sympathetic activity (estimated glomerular filtration rate, BMI, type 2 diabetes) and another marker of present sympathetic tone (systolic blood pressure), which did not lead to substantial differences in the results. This suggests that it is not (only) sympathetic activity, but increased RHR itself that plays a detrimental role in the increased risk for mortality.

We suggest that measurement and evaluation of resting heart rate should become routine practice in the management of patients with clinically manifest vascular disease, irrespective of the location, to obtain information about the risk for adverse outcome. Furthermore, not only patients with CAD, but also patients with CVD, PAD or AAA may benefit from heart rate reduction therapy, for example with ivabradine, and should be included in future randomized clinical trials.

Besides being a cause, obesity and insulin resistance could also be a consequence of increased sympathetic nerve activity. And increased RHR, as a marker of increased sympathetic nerve activity, is independently associated with an

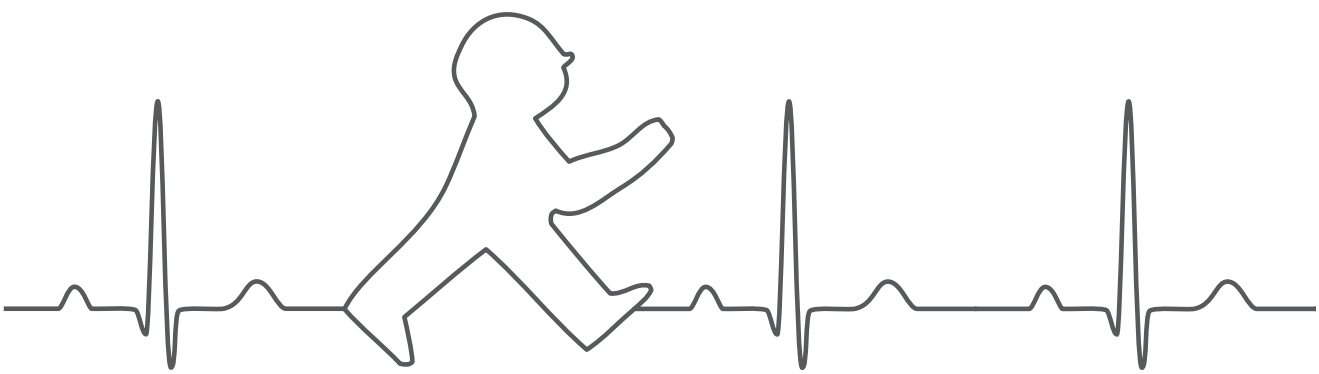
increased risk for developing type 2 diabetes mellitus in healthy populations, and in subjects with obesity or impaired glucose intolerance, but this has not been shown in patients with vascular diseases.

In **chapter 7**, we investigate the relation between RHR and incidence op type 2 diabetes mellitus during follow-up in a cohort of patients with clinically manifest vascular disease. Data were used from 3646 patients with manifest vascular disease from the Second Manifestations of ARTerial disease (SMART) study. RHR at baseline was obtained from an ECG. Patients were followed for incident type 2 diabetes mellitus (n=289) during a median period of 5.5 (interquartile range 3.2-8.4) years. The relation between RHR and incident type 2 diabetes mellitus was estimated by Cox proportional hazard analysis. As age was an effect modifier (p for interaction = 0.048), analyses were stratified for age.

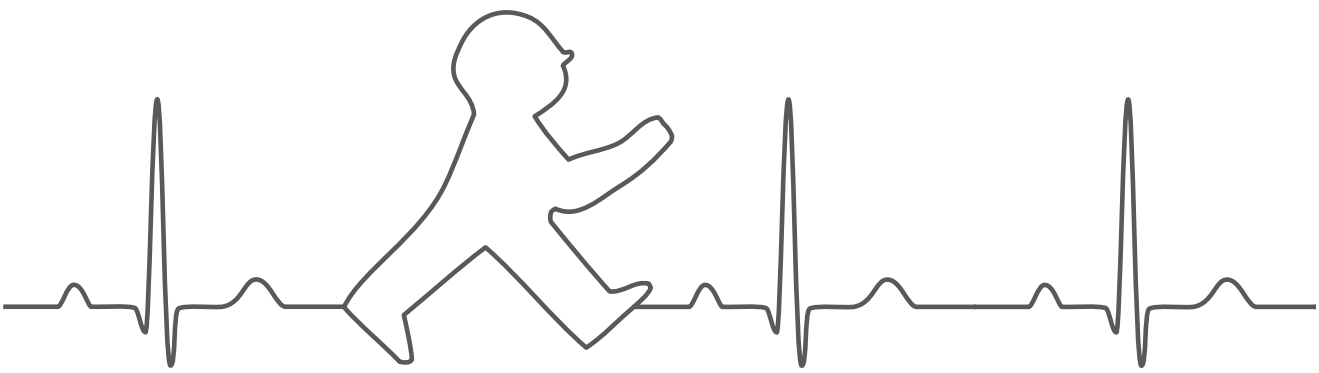
Every 10 beats per minute increase in RHR increased the risk for type 2 diabetes mellitus with 22% (HR 1.22; 95%CI 1.04-1.43) in patients between 55 and 63 years of age, while there was no increased risk of type 2 diabetes mellitus with increasing RHR in patients <55 years (HR 1.08; 95%CI 0.91-1.30) and patients >63 years (HR 1.00; 95%CI 0.84-1.18). Further exploratory analysis in smaller subgroups confirmed the finding that the relation between increased RHR and incident type 2 diabetes mellitus is absent in the youngest and oldest groups of patients, and is present in middle-aged patients (between 51 and 68 years in this analysis). Other exploratory analysis showed that the relation between RHR and incident type 2 diabetes mellitus was attenuated to a large part by adding BMI and fasting serum glucose to the fully adjusted model, indicating that BMI and fasting serum glucose levels are part of the causal pathway between sympathetic nerve activity measured with RHR and incident type 2 diabetes mellitus. The relation between RHR and incident type 2 diabetes mellitus was independent of the location of vascular disease or beta-blocker use.

The results of this study are in line with previous results obtained in the general population, but are of potential greater clinical significance because of the potential therapeutic implications. Prevention of diabetes in patients with vascular diseases is important, as patients with diabetes have an increased risk for (cardiovascular) morbidity and mortality, and a reduced quality of life. Reducing the risk for type 2 diabetes mellitus is feasible with lifestyle interventions such as increasing physical activity and reducing body weight, and with medical treatment.

Finally, in the general discussion in **chapter 8**, the clinical relevance of the main findings of the above studies is discussed.



Chapter 9



Sinds het begin van de 20ste eeuw zijn hart- en vaatziekten ieder jaar de belangrijkste doodsoorzaak geweest in de Verenigde Staten, met als enige uitzondering 1918 (Spaanse griep). Wereldwijd vindt 80 procent van alle sterftegevallen aan hart- en vaatziekten plaats in de landen met een laag- of middeninkomen. In de komende 20 jaar wordt in deze landen zelfs een verdrievoudiging van het aantal sterfgevallen ten gevolge van hartinfarcten en beroertes verwacht. Naast de vele sterfgevallen, leidt deze epidemie aan hart- en vaatziekten ook tot een enorme hoeveelheid ziektegevallen, met grote sociale en economische kosten voor de samenleving tot gevolg.

Bewegen heeft gunstige invloeden op vrijwel alle aspecten van hart- en vaatziekten. Het leidt onder meer tot verbeteringen in het merendeel van de klassieke risicofactoren, waaronder bijvoorbeeld overgewicht, verhoogde bloeddruk, een verhoogd cholesterol, en suikerziekte. Daarom wordt het stimuleren van lichamelijke inspanning veelvuldig in medische richtlijnen genoemd als een belangrijke eerste stap bij de behandeling en preventie van hart- en vaatziekten.

Het is zeer opmerkelijk dat het totaal aantal hartslagen gedurende het leven overeenkomt tussen verschillende zoogdieren, ondanks een grote variatie in lichaamsgrootte en de hoogte van de hartslag in rust. Hieruit volgt dat de hoogte van de hartslag in rust sterk gerelateerd is aan de levensverwachting; een muis met een hoge hartslag leeft veel korter dan een olifant met een hele lage hartslag. Bij mensen blijkt de hoogte van de hartslag in rust eveneens gerelateerd te zijn aan de kans op vroegtijdig overlijden. Iedere tien hartslagen per minuut méér, hangen samen met een 10-30% hoger risico op vroegtijdig overlijden, door welke oorzaak dan ook. Schadelijke gevolgen voor hart en vaten van een verhoogde hartslag in rust zijn: een verhoogde kans op hartritmestoornissen, een verhoogde kans op aderverkalking en het plots losschieten van een stukje kalk uit de slagaderwand, een ongunstigere balans tussen zuurstofaanbod en zuurstofverbruik in het hart, en een verminderde functie van de bloedvatwand. Bij patiënten met een doorgemaakt recent hartinfarct blijkt het verlagen van de hartslag in rust met medicatie de kans op vroegtijdig overlijden ook te verlagen, met ongeveer 30% per bereikte hartslagverlaging van 10 hartslagen per minuut.

Het hoofddoel van dit proefschrift is tweeledig: het bepalen van de effecten van lichamelijke inspanning en leefstijlveranderingen op risicofactoren voor hart- en vaatziekten (**Deel 1**) en het onderzoeken van de relatie tussen de hoogte van de hartslag in rust en overgewicht, het risico op het ontstaan van type 2 suikerziekte en het risico op nieuwe hart- en vaatziekten bij patiënten die al een eerste uiting van hart- en vaatziekten hebben (**Deel 2**).

Deel 1 Lichamelijke inspanning, leefstijlveranderingen en risicofactoren voor hart- en vaatziekten

De meeste onderzoeken naar de gunstige effecten van lichamelijke inspanning zijn uitgevoerd onder gecontroleerde omstandigheden, in laboratoria, waar de hoeveelheid en intensiteit van de inspanning nauwkeurig gecontroleerd is. Wandelen is echter één van de belangrijkste vormen van lichaamsbeweging in het dagelijks leven. Jaarlijks lopen bijvoorbeeld naar schatting meer dan 5 miljoen mensen een deel van een pelgrimstocht op verschillende plaatsen in de wereld, en dit aantal groeit nog steeds.

In **hoofdstuk 2** van dit proefschrift worden de effecten op de functie van bloedvaten en op risicofactoren voor hart- en vaatziekten beschreven van een 12-daagse pelgrimstocht naar Santiago de Compostela in Spanje. Negenentwintig gezonde mannen en vrouwen tussen de 40 en 70 jaar vormden de loopgroep, terwijl 29 mensen van hetzelfde geslacht en vergelijkbare leeftijd de controlegroep vormden. De loopgroep heeft de laatste 280 km afgelegd van de eeuwenoude pelgrimstocht naar Santiago de Compostela. De bloedvatfunctie en verschillende risicofactoren voor hart- en vaatziekten werden 2 maanden en 2 weken voor de start van de tocht, en 2 weken en 2 maanden na het beëindigen van de tocht gemeten. Tijdens de pelgrimstocht werden risicofactoren voor hart- en vaatziekten om de dag gemeten. Alle deelnemers volbrachten de tocht. De gemiddelde dagelijkse loopafstand was ruim 23 kilometer, en dagelijks werd gemiddeld ruim 5½ uur gelopen.

Tijdens de pelgrimstocht bleek het ongunstige LDL-cholesterol zeer sterk te dalen. Het LDL-cholesterol is een belangrijke risicofactor voor hart- en vaatziekten, terwijl het HDL-cholesterol juist beschermend werkt. Dit HDL-cholesterol steeg juist tijdens de pelgrimstocht, terwijl het gewicht en de middelomtrek daalden. Nadat de pelgrimstocht volbracht was, verdwenen de gunstige veranderingen vrij snel, met uitzondering van het gewichtsverlies van 2 kg, wat tot 2 maanden na afloop van de tocht behouden bleef. Het lopen van deze pelgrimstocht had geen enkel effect op de bloedvatfunctie. De meest opmerkelijke bevinding, namelijk de sterke daling in het LDL-cholesterol tijdens de tocht, is in grootte vergelijkbaar met het effect van cholesterolverlagende medicatie. Een dergelijke daling in het LDL-cholesterol is niet eerder beschreven als gevolg van lichamelijke inspanning. Welk mechanisme deze forse daling in het LDL-cholesterol verklaart is onduidelijk. Mogelijk wordt het cholesterol gebruikt als brandstof of als bouwsteen voor het herstellen van schade aan spieren door de intensieve inspanning.

In medische richtlijnen voor de preventie en behandeling van hart- en vaatziekten wordt een wekelijkse minimum hoeveelheid lichamelijke inspanning geadviseerd vergelijkbaar met 150 minuten stevig doorwandelen. Er wordt echter niet vermeld met welke intensiteit deze inspanning bij voorkeur moet worden

verricht; 150 minuten stevig doorwandelen of een kortere periode van meer intensieve inspanning (zoals hardlopen) worden als even effectief beschouwd. De wetenschappelijke kennis over de effecten van de intensiteit van lichamelijke inspanning, en in het bijzonder de wandelsnelheid, op risicofactoren voor hart- en vaatziekten, is beperkt.

In **hoofdstuk 3** beschrijven we de relatie tussen wandelsnelheid en de veranderingen in risicofactoren voor hart- en vaatziekten bij de deelnemers aan de 12-daagse pelgrimstocht naar Santiago de Compostela. Tijdens het lopen werd de wandelsnelheid berekend uit de genoteerde wandelafstand, wandeltijd en het aantal stappen gemeten met een stappenteller. De veranderingen in risicofactoren voor hart- en vaatziekten werden vergeleken tussen de groep mannen en vrouwen met een hogere dan gemiddelde wandelsnelheid, en de groep mannen en vrouwen met een lagere dan gemiddelde wandelsnelheid. Vervolgens werd ook de relatie berekend tussen de wandelsnelheid en de veranderingen in de risicofactoren voor hart- en vaatziekten.

In de groep met de hogere wandelsnelheid steeg het HDL-cholesterol meer dan in de groep met de lagere wandelsnelheid, terwijl het LDL-cholesterol meer daalde in de groep met lagere wandelsnelheid dan in de groep met hogere wandelsnelheid. Sneller wandelen bleek, onafhankelijk van andere factoren zoals veranderingen in lichaamsgewicht tijdens de tocht, samen te hangen met een meer uitgesproken stijging in HDL-cholesterol, en een minder uitgesproken daling in het LDL-cholesterol. Het omgekeerde geldt voor langzaam wandelen. Er was geen relatie tussen de wandelsnelheid en veranderingen in gewicht, middelomtrek, bloeddruk, vrije vetzuren en bloedsuiker. Ook hier blijft het onduidelijk welk mechanisme de relatie tussen wandelsnelheid en de veranderingen in het cholesterolprofiel verklaart. Wij veronderstellen dat langer met een lagere wandelsnelheid lopen meer spierschade veroorzaakt, waarvoor cholesterol uit zowel LDL als HDL deeltjes nodig is, dan dezelfde afstand met een hogere wandelsnelheid lopen in minder tijd.

Bij mensen met het metabool syndroom komen een aantal risicofactoren voor hart- en vaatziekten tegelijk voor, waaronder overgewicht, verminderde gevoeligheid voor insuline (een voorstadium van suikerziekte), verhoogde bloeddruk en een ongunstig cholesterolprofiel. Patiënten met het metabool syndroom hebben een verhoogde kans op het krijgen van suikerziekte, hart- en vaatziekten en vroegtijdig overlijden. Het metabool syndroom komt steeds meer voor, bij tot wel 25% van de inwoners van de Verenigde Staten en tot wel 50% bij Nederlandse patienten met hart- en vaatziekten. Twee belangrijke leefstijlaanpassingen die veelvuldig worden geadviseerd in richtlijnen als een eerste behandelstap voor het metabool syndroom zijn afvallen en lichaamsbeweging.

In **hoofdstuk 4** beschrijven wij een studie bij mensen met overgewicht en het metabool syndroom die een jaar lang intensief begeleid werden door een gespecialiseerde verpleegkundige met als doel meer lichamelijke inspanning te gaan verrichten en hun voedingsgewoonten te veranderen. Deze patiënten werden een jaar lang, tijdens maandelijkse bezoeken, uitgebreid voorgelicht en begeleid. Ze kregen informatie uit de geldende richtlijnen over gezonde voeding en gezond bewegen, zonder dat ze een specifiek dieetadvies kregen. Tijdens het eenjarige begeleidingsprogramma nam de gemiddelde hoeveelheid lichamelijke inspanning nauwelijks toe. De deelnemers vielen gemiddeld wel 2.3 kg af in dat jaar. Hoe meer lichamelijke inspanning de deelnemers bij de start van het programma verrichtten, hoe meer hun gewicht, middelomtrek en BMI (gewicht/lengte verhouding) daalde en de insulinegevoeligheid steeg. Daarnaast bleek dat hoe groter de toename in lichamelijke inspanning van de deelnemers was tijdens het begeleidingsprogramma, hoe groter de daling in gewicht, middelomtrek en BMI, en hoe groter de stijging in insulinegevoeligheid. De absolute verbeteringen in gewicht, middelomtrek, BMI en insulinegevoeligheid waren echter heel klein. Er moet wel een enorme toename in lichaamsbeweging zijn om grotere verbeteringen te bereiken.
Aangezien ieder gewichtsverlies bij deze patiënten met overgewicht met een hoog risico op hart- en vaatziekten van belang is, zien wij het bereikte gemiddelde gewichtsverlies van 2.3 kg in 1 jaar wel als een succes. Wij concluderen dat een verandering in leefstijl voor iedere patiënt met het metabool syndroom van belang is, ongeacht of ze bij de start meer of minder aan lichaamsbeweging doen, en dat het effect van meer lichaamsbeweging beperkt is wat betreft gewichtsverlies en toename van de insulinegevoeligheid.

Deel 2 Hartslag in rust en ontstaan van hart- en vaatziekten

Overgewicht, en in het bijzonder een toegenomen hoeveelheid buikvet, verhoogt de kans op het ontwikkelen van hart- en vaatziekten, maar ook type 2 suikerziekte. Het buikvet, wat in de buik om de buikorganen ligt, is niet alleen een opslagplek voor overtollige energie in de vorm van vet, maar ook een orgaan wat een enorme hoeveelheid aan hormonen produceert. Deze hormonen zijn van belang bij het ontstaan van de schadelijke gevolgen van overgewicht, zoals hoge bloeddruk, cholesterolafwijkingen en een verminderde gevoeligheid voor insuline en suikerziekte. Twee van die hormonen zijn insuline en leptine. Van deze hormonen is bekend dat in verhoogde concentratie voorkomen bij personen met een verhoogde hoeveelheid buikvet. Insuline en leptine kunnen het onwillekeurig zenuwstelsel activeren, hetgeen onder andere leidt tot een verhoogde hartslag in rust.

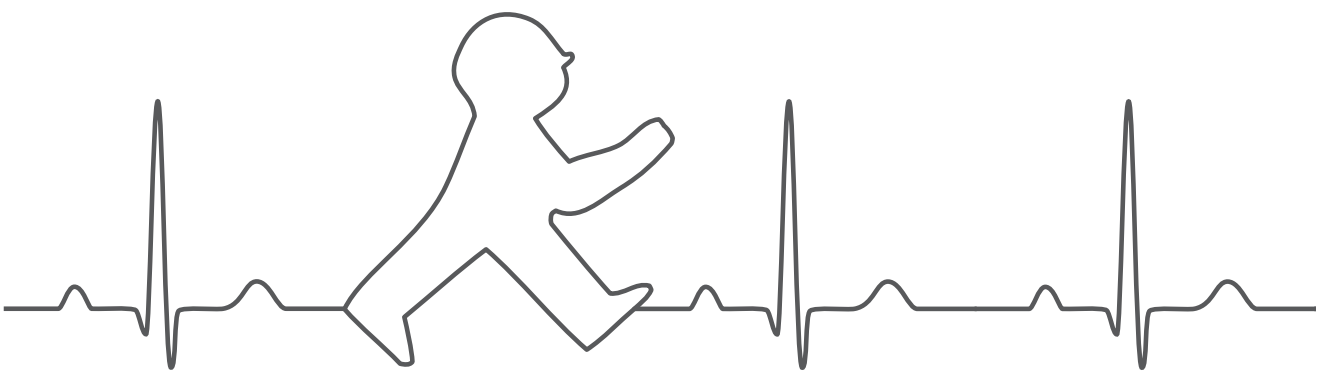
In **hoofdstuk 5**, wordt onderzocht of er een relatie is tussen de hoeveelheid buikvet en de hoogte van de hartslag in rust, bij patiënten met hart- en vaatziekten. Bij 3723 patiënten met hart- en vaatziekten werd met een echo de hoeveelheid buikvet en de hoeveelheid onderhuids vet gemeten. Daarnaast werd een hartfilmpje gemaakt waarvan de hoogte van de hartslag in rust werd afgelezen.
Hoe meer buikvet, hoe hoger de hartslag in rust was. Dit verband was sterker bij mannen dan bij vrouwen. Dezelfde relatie, maar minder sterk, werd gevonden tussen de middelomtrek (ook hiermee wordt de hoeveelheid buikvet gemeten, maar minder nauwkeurig), en BMI (gewicht/lengteverhouding) met de hoogte van de hartslag in rust. De hoeveelheid onderhuids vetweefsel was niet gerelateerd aan de hoogte van de hartslag in rust. De relatie tussen buikvet en de hoogte van de hartslag in rust was zelfs onafhankelijk van het totale lichaamsgewicht. Dit betekent dat het specifiek het buikvet is, en niet het totale lichaamsvet, wat gerelateerd is aan de hartslag in rust. Hoewel het op grond van deze studie niet met 100% zekerheid valt te concluderen, is het wel aannemelijk dat buikvet, door het aanmaken van de hormonen insuline en leptine, het onwillekeurig zenuwstelsel stimuleert. Dit komt tot uiting in een verhoogde hartslag in rust.
Een verhoogde hartslag in rust is een onafhankelijke risicofactor voor het ontstaan van hart- en vaatziekten en voor vroegtijdig overlijden, zoals al is aangetoond bij patiënten met hartziekten, en ook in de gezonde populatie. Het is aannemelijk dat dit ook geldt voor patiënten met vaatziekten op andere plaatsen in het lichaam dan het hart, want sommige schadelijke effecten van een verhoogde hartslag, zoals het ontstaan van aderverkalking en een verminderde functie van de bloedvatwand, blijven niet beperkt tot het hart alleen, maar gelden voor het hele lichaam.
In **hoofdstuk 6** onderzoeken we de relatie tussen de hoogte van de hartslag in rust en het ontstaan van een tweede uiting van hart- en vaatziekten en overlijden bij patiënten die al een eerste uiting van hart- en vaatziekten gehad hebben. Bij patiënten met een doorgemaakt hartinfarct (2244), herseninfarct/beroerte (930), vernauwing in de beenslagaders (823) en een verwijderde lichaamsslagader in de buik (75), werd de hoogte van de hartslag in rust bepaald. Patiënten werden gemiddeld 4½ jaar gevolgd.
In de gehele studiepopulatie werd berekend dat iedere stijging van de hartslag in rust met 10 slagen per minuut gerelateerd was aan een verhoogde kans op overlijden met 14%, en aan een verhoogde kans om te overlijden aan hart- en vaatziekten met 15%. Daarnaast ging iedere stijging van de hartslag in rust met 10 slagen per minuut gepaard met 8% meer kans op het optreden van een nieuwe uiting van hart- en vaatziekten, maar niet aan het opnieuw voorkomen van een hartinfarct of beroerte. Deze bevindingen gelden zowel voor patiënten met een doorgemaakt hartinfarct, een doorgemaakte beroerte, vernauwingen in de beenslagaders of een verwijde lichaamsslagader in de buik.

Op grond van deze resultaten doen wij twee aanbevelingen. Ten eerste stellen wij dat het meten en beoordelen van de hartslag in rust routinematig moet gebeuren bij de behandeling van patiënten met hart- en vaatziekten op welke plek in het lichaam dan ook. De hoogte van de hartslag in rust kan worden gebruikt om het risico op een nieuwe uiting van hart- en vaatziekten en de kans op overlijden in te schatten. Ten tweede adviseren wij dat ook patiënten met hart- en vaatziekten op andere plekken dan het hart aan studies mogen meedoen om te kunnen beoordelen of zij ook, net als hartpatiënten, baat hebben bij medicijnen die de hartslag verlagen.

Naast het feit dat overgewicht en een verminderde gevoeligheid voor insuline oorzaken kunnen zijn van een verhoogde hartslag in rust, zoals beschreven in **hoofdstuk 5**, zijn er ook aanwijzingen dat een verhoogde hartslag in rust juist kan leiden tot type 2 suikerziekte, wat het gevolg kan zijn van een verminderde gevoeligheid voor insuline.

In **hoofdstuk 7** onderzoeken we de relatie tussen de hartslag in rust en de kans op het ontstaan van type 2 suikerziekte bij patiënten met hart- en vaatziekten. Bij 3646 patiënten met hart- en vaatziekten werd de hartslag in rust bepaald. Patiënten werden gedurende gemiddeld 5½ jaar gevolgd. De hoogte van de hartslag in rust was gerelateerd aan de kans op het krijgen van type 2 suikerziekte. Deze relatie was het sterkst in de leeftijdscategorie tussen 55 en 63 jaar. Bij patiënten jonger dan 55, of ouder dan 63 was deze relatie veel zwakker of geheel afwezig. Een verhoogde hartslag in rust kan gebruikt worden om de kans op het ontstaan van type 2 suikerziekte te voorspellen bij mensen met hart- en vaatziekten, en daar naar te handelen. Door middel van aanpassingen in de leefstijl, zoals afvallen of meer lichamelijke inspanning, of door medicatie, kan de kans op suikerziekte bij deze patiëntengroep weer verlaagd worden.

In de algemene discussie in **hoofstuk 8** bespreken wij de waarde van de belangrijkste bevindingen in dit proefschrift voor de dagelijkse klinische praktijk.



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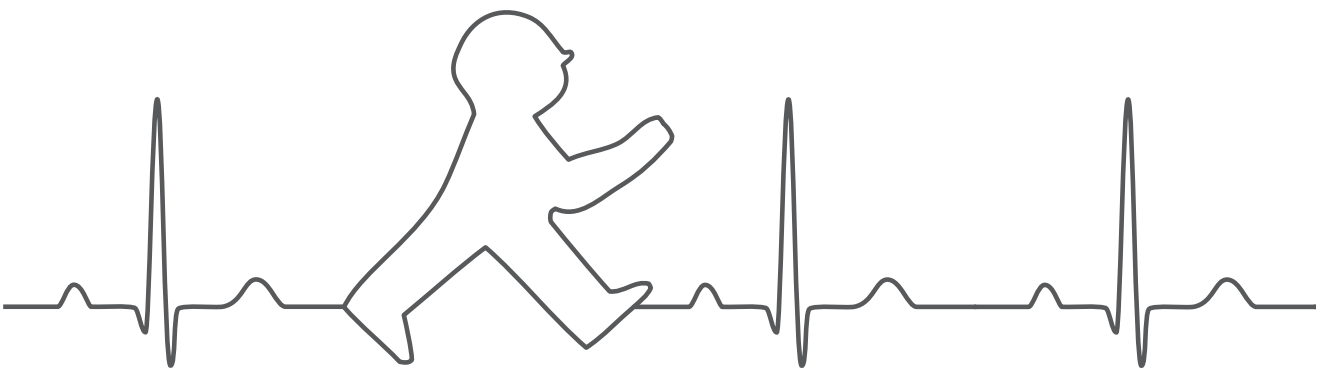

Ik wil iedereen hartelijk bedanken die heeft bijgedragen aan de totstandkoming van dit proefschrift. Een aantal mensen wil ik hieronder in het bijzonder noemen.

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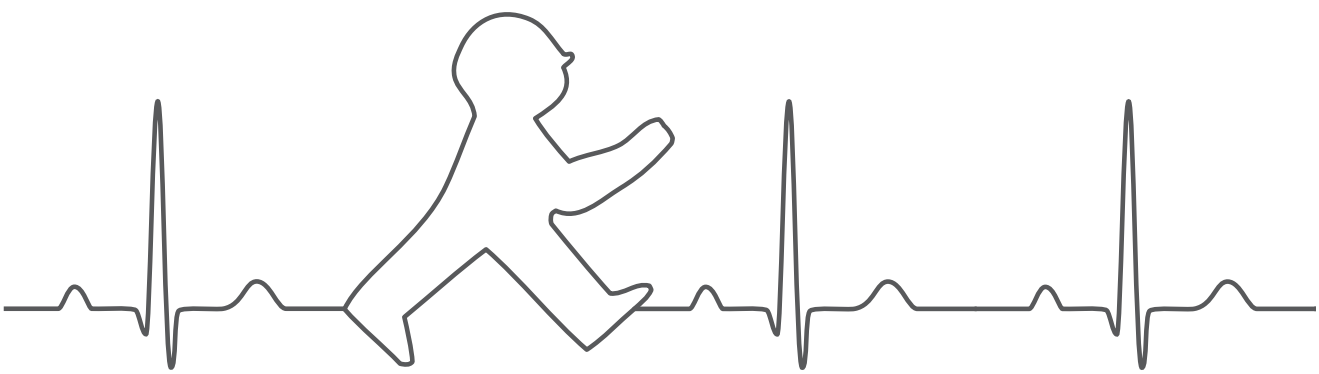
Remy
Mei 2012



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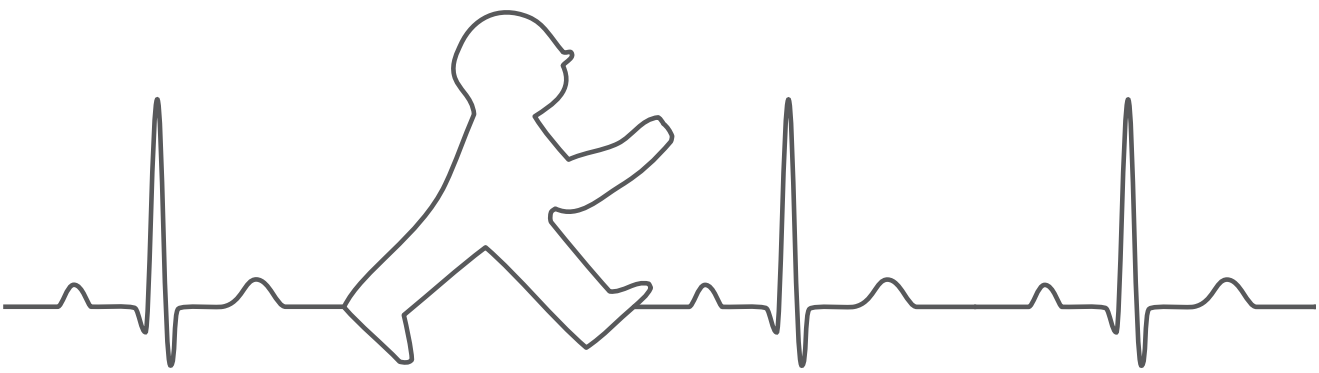
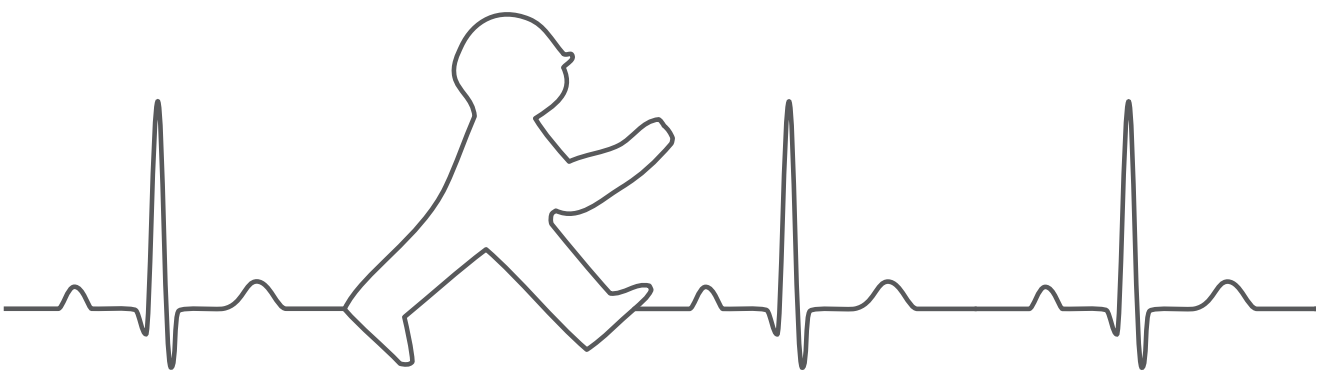


Remy Hubert Henry Bemelmans was born on the 15th of May 1978, in Nijmegen, the Netherlands. After graduating from high school at the “Bernardinus college” in Heerlen in 1996, he studied Medical Biology at the University of Amsterdam. After one year he was able to switch to Medical School at Maastricht University. In October 2003 he obtained his medical degree. From October 2003 until January 2005 he started his clinical career on the Emergency Department of the “St. Annaziekenhuis” in Geldrop. In January 2005 he started his training in Internal Medicine in “Gelre Ziekenhuizen, locatie Apeldoorn”, under supervision of Dr. J.M. Smit and Dr. C.G. Schaar. In this hospital, he started his research career investigating changes in microvascular perfusion during haemodialysis and endotoxemia, at the Department of Intensive Care under supervision of Dr. P.E. Spronk. In May 2007 he continued his training in Internal Medicine at the University Medical Centre Utrecht under the supervision of Prof.dr. E. van der Wall and Prof.dr. D.W. Biesma.

In May 2009 he started the work described in this thesis at the department of Vascular Medicine, University Medical Centre Utrecht, under supervision of Prof. dr. F.L.J. Visseren (department of Vascular Medicine) and Prof.dr. Y. van der Graaf (Julius Centre for Health Sciences and Primary Care).

In November 2011 he continued his training in Internal Medicine at the University Medical Centre Utrecht under supervision of Prof.dr. M.M.E. Schneider and started his specialization in Vascular Medicine under supervision of Prof.dr. F.L.J. Visseren. In April 2014 he will finish his training as Internist with specialization in Vascular Medicine.

Remy Bemelmans is married to Elvira de Vries and they have two children: Aniek (2010) and Jasper (2011).



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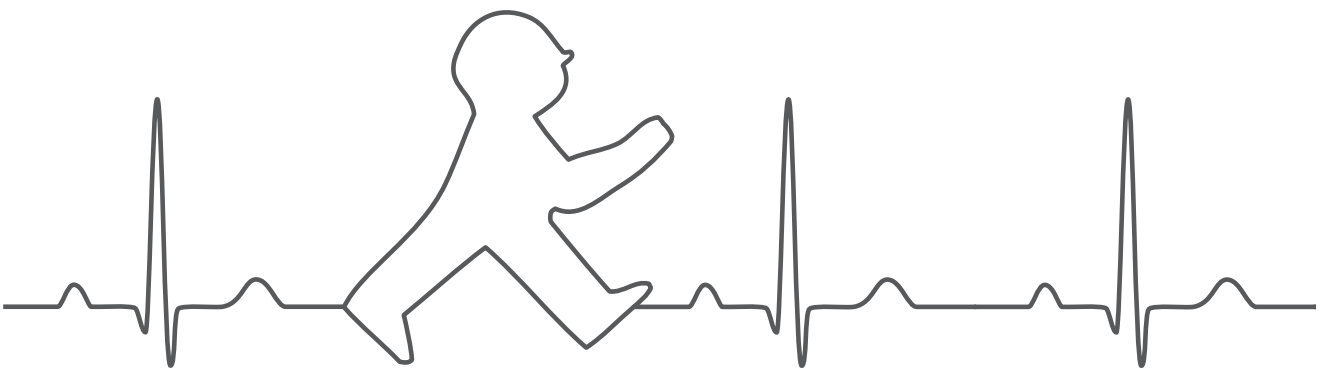

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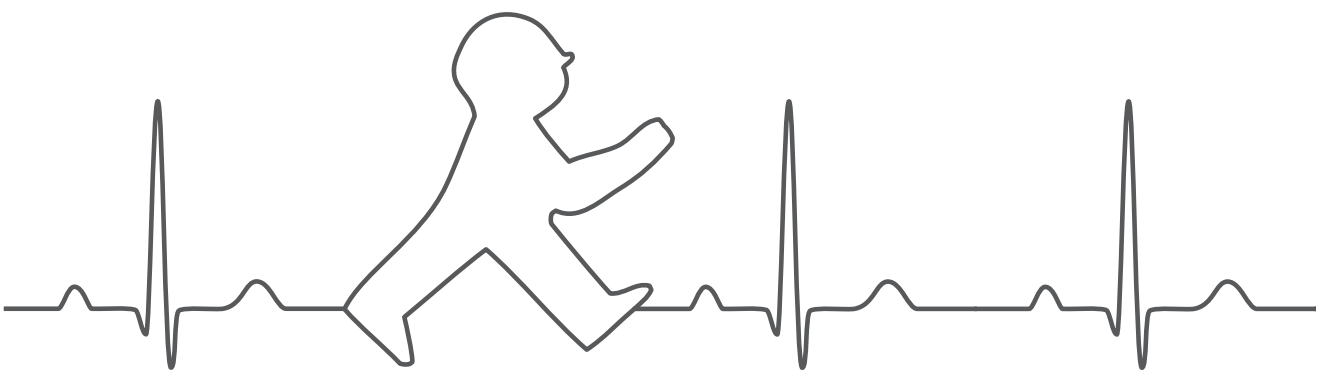

Chapter 9

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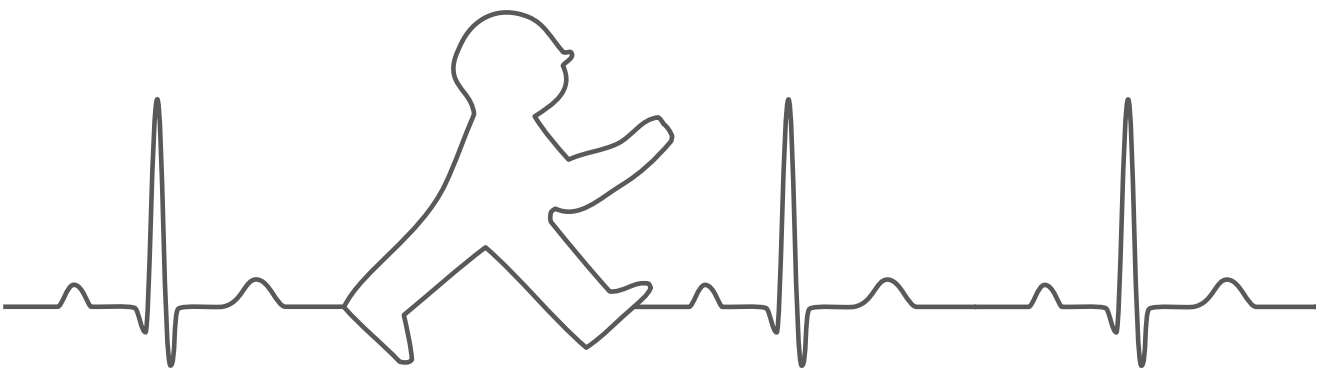
Dankwoord
Curriculum Vitae
List of publications
Appendix



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