PHYSICAL ACTIVITY, CARDIORESPIRATORY FITNESS, AND ABDOMINAL OBESITY IN RELATION TO CARDIOVASCULAR DISEASE RISK – EPIDEMIOLOGICAL STUDIES

Elin Ekblom Bak

Stockholm 2013
Supervisors

Main supervisor
Mai-Lis Hellénius, M.D., Ph.D., Professor
Department of Medicine
Karolinska Institutet, Stockholm, Sweden

Co-supervisor
Björn Ekblom, M.D., Ph.D., Professor emeritus
Åstrand Laboratory of Work Physiology
The Swedish School of Sport and Health Sciences, Stockholm, Sweden

Faculty Opponent

David Dunstan, Ph.D., Professor
The University of Western Australia, School of Sports Science, Exercise and Health and
Baker IDI Heart and Diabetes Institute, Melbourne, Australia

Examination Board

Eva Nylander, M.D., Ph.D., Professor
Department of Medical and Health Sciences
Linköpings University, Linköping, Sweden

Ylva Trolle Lagerros, M.D., Ph.D., Associate Professor
Department of Medicine
Karolinska Institutet, Stockholm, Sweden

Jan Henriksson, M.D., Ph.D., Professor
Department of Physiology and Pharmacology
Karolinska Institutet, Stockholm, Sweden

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet. Printed by Larserics Digital Print AB

© Elin Ekblom Bak, 2013
“The sated day is never first.
The best day is a day of thirst.

Yes, there is goal and meaning in our path
but it's the journey that is the labor's worth.”

Karin Boye
ABSTRACT

Although Sweden saw a decline in death rates related to cardiovascular disease (CVD) between 1987 and 2011, it is still the most common cause of death for both women and men. Lifestyle-related factors such as inadequate physical activity (PA), poor cardiorespiratory fitness (CF), and excess body fat are all recognised as important predictors of CVD morbidity and mortality. More recently, studies have highlighted the possible detrimental effects of prolonged sitting, which mainly substitutes for daily non-exercise PA (NEPA). Conversely, more preferable levels of these lifestyle factors are associated with lower CVD risk and increased life expectancy. Despite the extensive research performed within this field, there is still no consensus.

The main objective of this thesis was therefore to examine the interrelationship between different levels of PA, CF, and abdominal adiposity and their association with CVD risk factors, CVD morbidity, and longevity in population-based samples of Swedish men and women of different ages. A second objective was to develop a new and more precise method for estimation of CF in a mixed, healthy, population.

The main findings were:

- In a cross-sectional population based random sample of Swedish men (n=781) and women (n=890) aged 20 to 65 years were CF and abdominal obesity each independently and strongly beneficially associated with individual CVD risk factors, as well as to a clustered CVD risk factor profile. For the clustered risk, each unit of fitness (ml·kg⁻¹·min⁻¹) was associated with a 5% decrease in risk and each unit of waist circumference (cm) with a 5% increase in risk. This was seen in women as well as men, younger as well as older people, and daily smokers as well as non-smokers; however, there were some differences within the subgroups.

- In the same population, higher levels of self-reported PA and CF, but mainly the latter, were independently associated in a beneficial way with both individual and clustered CVD risk factors. Furthermore, a notable interaction of excess clustered CVD risk was shown for being insufficiently physically active according to general guidelines in combination with not being fit.

- In a representative cohort of 60-year-old men (n=2039) and women (n=2193) in Stockholm County, a generally active daily life was associated with beneficial metabolic health at baseline and an approximately 30% lower risk for a first-time cardiovascular event and all-cause mortality, respectively, after 12.5 years. These relationships were independent of regular exercise.

- A new submaximal cycle ergometer test for estimation of maximal oxygen uptake was developed. The test is simple, low-risk, and easily administered, and does not require laboratory equipment or expertise. In a mixed population (in terms of age, activity status, and gender), the test showed a significantly increased precision compared with one of the most commonly used submaximal exercise tests today.

In conclusion, these results indicate that in clinical practice it is important to evaluate both PA and CF as well as abdominal obesity status. Regarding PA, it is important to highlight the separate beneficial associations of a daily active life including NEPA on the one hand, and intentional regular exercise on the other.
LIST OF PUBLICATIONS

This thesis is based on the four papers listed below, which will be referred to throughout the text by their Roman numerals.


CONTENTS

1 Background........................................................................................................................................ 8
  1.1 Cardiovascular disease.................................................................................................................. 8
  1.2 Atherosclerosis ............................................................................................................................ 8
  1.3 The concept of risk and risk factors ............................................................................................... 9
  1.4 Cardiovascular risk factors ........................................................................................................... 10
  1.5 Traditional risk factors and novel biomarkers ............................................................................... 10
      1.5.1 Hypertension ......................................................................................................................... 10
      1.5.2 Abnormal lipids .................................................................................................................... 10
      1.5.3 Hyperglycaemia and hyperinsulinaemia ............................................................................... 11
      1.5.4 Novel biomarkers ............................................................................................................... 11
      1.5.5 Clustering of metabolic and vascular risk factors ............................................................... 11
  1.6 Lifestyle-related and other factors ............................................................................................... 12
      1.6.1 Physical activity .................................................................................................................... 12
      1.6.2 Sedentary behaviour and non-exercise physical activity ..................................................... 14
      1.6.3 Cardiorespiratory fitness ...................................................................................................... 17
      1.6.4 Overweight and obesity ........................................................................................................ 19
  1.7 Relation between physical activity, cardiopulmonary fitness, and obesity20
      1.7.1 Physical activity versus cardiopulmonary fitness ............................................................... 20
      1.7.2 Physical activity/Cardiorespiratory fitness versus obesity .............................................. 21
  1.8 Diet .................................................................................................................................................. 21
  1.9 The interrelationship between cardiovascular risk factors ....................................................... 21

2 Objectives ........................................................................................................................................... 23

3 Materials and Methods ..................................................................................................................... 24
  3.1 Papers I and II ............................................................................................................................. 24
      3.1.1 Study population .................................................................................................................... 24
      3.1.2 Data collection and measurements ....................................................................................... 25
      3.1.3 Statistical considerations in Papers I and II ....................................................................... 27
  3.2 Paper III ........................................................................................................................................ 28
      3.2.1 Study population .................................................................................................................... 28
      3.2.2 Data collection and measurements ....................................................................................... 28
      3.2.3 Model construction .............................................................................................................. 29
      3.2.4 Statistical considerations in Paper III ................................................................................... 30
  3.3 Paper IV ........................................................................................................................................ 31
      3.3.1 Study population .................................................................................................................... 31
      3.3.2 Data collection and measurements ....................................................................................... 31
      3.3.3 Individual CVD risk factors and the metabolic syndrome .................................................. 32
      3.3.4 CVD event and mortality surveillance .................................................................................. 32
      3.3.5 Statistical considerations in Paper IV ................................................................................... 33
  3.4 Ethical considerations and informed consent ............................................................................. 33

4 Results ................................................................................................................................................ 34
  4.1 Papers I and II ............................................................................................................................. 34
      4.1.1 Characteristics of the study population .................................................................................. 34
      4.1.2 Cardiorespiratory fitness, waist circumference, and CVD risk ........................................ 34
      4.1.3 Physical activity, cardiopulmonary fitness, and CVD risk .................................................. 37
  4.2 Paper III ........................................................................................................................................ 39
      4.2.1 Characteristics of the study population .................................................................................. 39
4.2.2 Final regression model for the new test ................................ 39
4.2.3 Measured versus estimated VO2max .................................... 40
4.2.4 Reliability of the new test ................................................... 41
4.3 Paper IV .................................................................................. 41
4.3.1 Cross-sectional analysis ....................................................... 41
4.3.2 Prospective analysis ............................................................ 43
5 Discussion .................................................................................. 45
5.1 Cardiorespiratory fitness and waist circumference ....................... 45
  5.1.1 Previous knowledge ............................................................ 45
  5.1.2 Cardiorespiratory fitness and waist circumference on CVD risk in relation to age, gender, and smoking habits ......................... 46
  5.1.3 Low cardiorespiratory fitness and body fat often in combination 46
  5.1.4 Possible mechanisms .......................................................... 47
5.2 Physical activity and cardiorespiratory fitness .............................. 50
  5.2.1 Excess risk of interaction ................................................... 50
  5.2.2 Previous knowledge ............................................................ 51
  5.2.3 Is there a true separate effect of physical activity and cardiorespiratory fitness? ........................................................ 51
5.3 Non-exercise physical activity and sedentary behaviour ................ 54
  5.3.1 Previous knowledge ............................................................ 54
  5.3.2 Separate behaviours ........................................................... 55
  5.3.3 Potential mechanisms .......................................................... 55
  5.3.4 Distribution of the activity and breaks in prolonged sitting 57
  5.3.5 The balance between non-exercise physical activity and sitting 58
5.4 A new submaximal method for estimation of cardiorespiratory fitness 58
  5.4.1 Improved precision due to use of ΔHR ................................. 60
  5.4.2 Is it possible to increase the precision even further? ............... 60
  5.4.3 Impact of medicament use on estimated VO2max ................... 61
6 Methodological considerations .................................................. 62
  6.1 Study design and external validity ......................................... 62
  6.2 Sample size ........................................................................... 62
7 Limitations and strengths ............................................................ 63
  7.1 Papers I and II ..................................................................... 63
  7.2 Paper III .............................................................................. 63
  7.3 Paper IV ............................................................................... 64
8 Concluding discussion and clinical implications ......................... 65
  8.1 The unique importance of sedentary behaviour .......................... 65
  8.2 How should we handle different factors in epidemiological analyses? 66
  8.3 Lifestyle factors in risk score prediction ................................ 67
  8.4 Evaluation and counseling in clinical practice ......................... 68
    8.4.1 Obstacles 1 and 2 ............................................................ 68
    8.4.2 Obstacle 3 ....................................................................... 69
    8.4.3 Obstacles 4 and 5 ............................................................. 70
9 Future perspectives ...................................................................... 71
10 Sammanfattning på svenska ..................................................... 72
11 Conclusions ............................................................................. 74
12 Acknowledgements .................................................................... 75
13 References ................................................................................. 77
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>APO</td>
<td>Apolipoprotein</td>
</tr>
<tr>
<td>BAT</td>
<td>Brown adiposity tissue</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>bpm</td>
<td>Beats per minute</td>
</tr>
<tr>
<td>CF</td>
<td>Cardiorespiratory fitness</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variance</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>EE</td>
<td>Energy expenditure</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat free mass</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LIPA</td>
<td>Light-intensity physical activity</td>
</tr>
<tr>
<td>MET</td>
<td>Metabolic equivalent</td>
</tr>
<tr>
<td>MVPA</td>
<td>Moderate-to-vigorous physical activity</td>
</tr>
<tr>
<td>NEPA</td>
<td>Non-exercise physical activity</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PA</td>
<td>Physical activity</td>
</tr>
<tr>
<td>PO</td>
<td>Power output</td>
</tr>
<tr>
<td>RPE</td>
<td>Rating of perceived exertion</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
</tr>
<tr>
<td>SB</td>
<td>Sedentary behaviour</td>
</tr>
<tr>
<td>VAT</td>
<td>Visceral adipose tissue</td>
</tr>
<tr>
<td>VO₂ max</td>
<td>Maximal oxygen uptake</td>
</tr>
<tr>
<td>WC</td>
<td>Waist circumference</td>
</tr>
</tbody>
</table>
PREFACE

When I was four years old I started to play soccer in the club of my hometown, Älta IF. Twenty-eight years later, after a long career that among other things has included ten years of playing soccer in one of the best leagues in the world, playing professionally in Spain, and representing the national team, I am back playing on my home ground in Älta. My passions for the sport remains – hate to lose, love to win. Why is this relevant in this context? I will tell you: I see so many similarities between succeeding in sports and surviving in the research community; and I have already been able to take advantage of lessons learned during my career as a soccer player as I enter this new chapter in my life.

Passion – Without genuine passion, it is impossible to succeed in either of these worlds. Doing it for the money or the fame will mean the journey is neither joyful nor long-lasting.

Stamina – Keep on trying, working, and sweating no matter what. Hard work is the only way, and it always pays off in the end; whether in the lab or on the pitch.

Handling setbacks – Losing a game, being criticised, or having an article rejected; it is all about minimising the slump, learning from the setback, and growing even stronger.

Enjoy success – To really embrace the feeling of winning a game or having an article accepted; this is the pay-off for all your hard work, and the driving force to keep on doing that hard work.

Surviving a man’s world – Soccer and science are each still a man’s world, but only for the sake of old traditions. Nowadays, many women know these worlds as well as the men. It is essential to believe in yourself and in the knowledge you possess.

So while I am a senior in the soccer team, I am a junior in the research team – and I feel just like that little four-year-old girl tying the shoelaces of her first soccer boots, eager and well prepared to start a journey that I hope will last for life.
1 BACKGROUND

Primary aging is the gradual and inevitable deterioration of cellular structure and function, independent of disease and environment [1]. It is the main determinant of the maximum life span of a species, defined as the maximum length of time that one or more individuals have been observed to survive between birth and death. Conversely, secondary aging is caused by diseases, poor health practices, and other environmental factors, which means that these changes are not inevitable [1]. Secondary aging does not alter the maximum human life span, but rather the human life expectancy. The steady increase in human life expectancy over the past century has been due to successful preventive strategies against secondary aging which have aimed to maintain health and capacity. From the 17th to the late 19th century, starvation and infectious diseases were among the main underlying causes of death. Due to improvements in public health and living conditions, people today live longer, and rather experience diseases related to age and to an unhealthy lifestyle, such as cancer, cardiovascular disease (CVD), and other metabolic disorders.

Before the 20th century, CVD was rather rare. William Osler, a famous Canadian cardiologist, reported in a series of lectures given in 1897 at the Johns Hopkins Hospital that during 7 years of practice he only witnessed 4 cases of angina pectoris [2]. A decade later, in 1910, Osler gave a famous speech at the Royal College of Physicians of London, highlighting the increased prevalence of CVD after having seen an additional 208 cases [3]. In 2010, CVD accounted for 25% of the estimated 52 million deaths globally, making it the leading cause of death [4].

1.1 CARDIOVASCULAR DISEASE

CVD includes disorders of the heart and blood vessels. In Sweden, although there was a 60% decline in CVD-related death rates between 1987 and 2011, it is still the most common cause of death for both women (39%) and men (38%) [5]. The most common types of CVD include coronary heart disease (CHD) and cerebrovascular disease. These are related to the narrowing of the blood vessels supplying the heart muscle and brain, respectively, with blood and oxygen. In general, this narrowing results from atherosclerotic plaques made of cholesterol and fats building up in the endothelium of the arteries.

1.2 ATHEROSCLEROSIS

Atherosclerosis is a general term for thickening and hardening in medium and large-sized arteries. This is a normal process of aging that can begin in early life, with impairment of endothelial function as a primary result, followed by gradual remodelling of the arterial wall. It was previously considered as being due to abnormalities in lipid metabolism, but recent findings have shown that low-grade vascular inflammation plays a central role in mediating all stages of the disease; from initiation, through progression, and finally to detrimental thrombotic complications [6]. The atherosclerotic process is initiated by damage to the endothelium and perturbations in endothelial function. This creates an imbalance between the proatherogenic and anti-atherogenic mechanisms in the endothelium; by enhancing the expression of certain leukocyte adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) on the one hand, and reducing the local production of the vasodilator and anti-inflammatory endothelium-derived nitric oxide on the other [7]. The increased adhesion and endothelial dysfunction then trigger an inflammatory cascade including recruitment of inflammatory cells (monocytes and T-cells), with a subsequent release of various cytokines (e.g. IL-1β, TNF-α, IL-6, MCP-1) and loss of functional integrity of the
endothelial surface. The endothelium then becomes more permeable to substances and cells from the circulating blood, and hence more susceptible to, for example, recruitment of lipids into the atherosclerotic plaque. The earliest visible lesion of atherosclerosis is the fatty streak, which consists of aggregated lipid-loaded macrophages, turned into foam cells and oxidised low-density-lipoprotein (LDL). With time, the fatty streak develops into a fibrous plaque and an established atherosclerosis [8].

Atherosclerosis is a gradual process of plaque accumulation. These plaques are separated into two broad categories: stable and unstable plaques. The stable plaques have an intact and thick fibrous cap and are rich in extracellular matrix and smooth muscle cells; they tend to be asymptomatic. The unstable plaques are often more rapidly growing plaques that are prone to rupture. These are characterised by a large necrotic core and a thin fibrous cap containing an abundance of inflammatory cells. A myocardial infarction is usually an acute event occurring when a plaque suddenly ruptures. As an instant response to the disruption, there is a rapid accumulation of clotting factors at the rupture site and a rapid blockage of blood flow to the affected part of the myocardium or brain, which in turn becomes ischemic and often necrotic.

1.3 THE CONCEPT OF RISK AND RISK FACTORS

The concept of risk is the probability that an event will occur. In epidemiology, it is usually used to express the probability that a particular outcome will occur following a particular exposure. A risk factor is generally a type of exposure, behaviour, or characteristic that increases the risk of disease, which by temporal sequence directly increases the probability of disease occurring and, if absent or removed, reduces the probability [9]. A risk factor is a part of the causal chain or exposes the individual to the causal chain, but once the disease occurs, removal of a risk factor may not always result in cure of the disease. A risk marker or a biomarker (the latter term being used for factors circulating in the blood) is an exposure or attribute that is associated with increased probability of disease but is not a causal factor. An intermediate factor is a factor in the causal path between exposure and disease, and normally not a risk factor in itself.

Most diseases have multiple causal mechanisms. In line with the causal pie model proposed by Rothman in 1976 [10], the contribution to disease of an individual risk factor can be seen as one piece of a pie. After all the pieces of a pie fall into place, the pie is complete (a sufficient cause) and disease occurs. A disease may have more than one sufficient cause, with each sufficient cause being composed of several component causes that may or may not overlap. This explains why some people develop a disease, and others do not, despite being exposed to the same risk factor. Disease prevention can therefore be at least partially accomplished by blocking any single component of a sufficient cause, which averts disease through that pathway.

Risk factors also tend to interact with each other biologically. When two or more factors are present at the same time, they may yield a combined effect that is stronger (synergistic) or weaker (antagonistic) than could be expected from simply adding the effects exerted separately by these factors.

Another issue to consider is the relative importance of different risk factors. The prevalence and effect of risk factors vary, which means that in reality, two or more risk factors might not be considered equally important. Attributable risk, or population attributable risk, is often used to compare different risk factors. The
former indicates the number of cases of a disease that can be attributed to the risk factor, while the latter further depends on the prevalence of the risk factor in the population under study, and the strength of its association with the disease.

1.4 CARDIOVASCULAR RISK FACTORS
The aetiology of CVD is complex and multifactorial. Endothelial dysfunction, which is the main initiator of the low-grade inflammation in the vascular wall, is the result of a combined action of local and systemic factors interfering with the dysfunctional endothelial cells. Important local factors are disturbed blood flow and absence of normal laminar shear stress due to naturally present bifurcations, branching points, and curvatures in the arterial tree, but also in response to unfavourable serum lipid profiles [7]. The most important systemic factors may be divided into modifiable and non-modifiable factors. Physical inactivity, smoking, and systemic disorders such as dyslipidemia and high blood pressure are examples of the former, while age, gender, and genetic predisposition are examples of the latter. Both local and non-modifiable risk factors are natural conditions involved in the development of atherosclerosis, but modifiable risk factors are highly correlated to lifestyle, and an unhealthy lifestyle has been shown to accelerate and aggravate the atherosclerotic process. Results published in 2004 from the multinational INTERHEART study showed that nine modifiable risk factors closely linked to the modern western lifestyle (abnormal lipids, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, fruit and vegetable intake, and physical inactivity) could explain 90% of the myocardial infarctions worldwide, in both sexes and at all ages in all regions studied [11].

In recent decades, a link has been established between several risk factors/conditions and increased CVD risk. However, interactions between these risk factors, as well as their partial intermediating effects, make it difficult to clearly define the independent importance of each. A first step is to try to separate them into groups with regard to where in the causal chain of disease development they primarily belong. This thesis uses three main groups: traditional metabolic and vascular risk factors, lifestyle-related risk factors, and other risk factors. A fourth possible group is the novel biomarkers which have been highlighted recently. Although these are not actual risk factors, they ought to be included in this discussion as they are markers for conditions closely linked to the origin of atherosclerosis or the actual atherosclerotic process. How different risk factors could be accounted for and the adequacy of evaluating them together will be discussed in the conclusion of this thesis.

1.5 TRADITIONAL RISK FACTORS AND NOVEL BIOMARKERS

1.5.1 Hypertension
A large number of observational studies have demonstrated a continuous relationship between both systolic and diastolic blood pressure and cardiovascular morbidity and mortality [12]. This association is partly explained by the pathophysiological link between hypertension and inflammation (i.a. through angiotensin II). Endothelial injury and vascular cell proliferation induced by increased pressure are other effects that exacerbate the atherosclerotic process [13].

1.5.2 Abnormal lipids
Abnormal levels of blood lipids and their lipoprotein carriers play a central role in the development of CVD. Elevated serum levels of total cholesterol and its main carrier, low-density lipoprotein (LDL), have the strongest causal evidence with atherosclerosis [14]. Cholesterol is a key component in the atherosclerotic plaque,
while LDL is likely to start the lipid deposition in the endothelium by moving from the blood into the vessel wall. Both in serum and in the vessel wall, LDL is oxidised through actions of free radicals or leukocyte activity and becomes a key player in the development of foam cells. When accumulated over time, these foam cells are significant for the evolution of the atherosclerotic plaque. Elevated plasma apolipoprotein (APO) B is also a strong risk marker, as it represents the total atherogenic particle number. Moreover, high-density lipoprotein (HDL) and its principal apolipoprotein carrier, APO A1, have apparent cardioprotective effects and functions, with multiple mechanisms identified such as reverse cholesterol transport from foam cells to liver, and anti-inflammatory, anti-oxidative, and anti-thrombogenic effects as well as regression of atherosclerotic plaques [14]. However, the causal relationship between low HDL and increased risk of CVD is unclear, and it is rather suggested that low HDL levels are a secondary phenomenon occurring alongside high triglyceride levels [15], which in turn have been shown to have a close association with the disease [16].

1.5.3 Hyperglycaemia and hyperinsulinaemia
Diabetes is a metabolic disease resulting from defects of insulin secretion and/or insulin action, and a prominent risk factor for CVD [17]. The hyperglycaemia characterising both type 1 and type 2 diabetes plays a central pathophysiological role in the atherosclerotic process. Hyperglycaemia may, for example, cause protein glycosylation and accumulation of advanced glycation end products (AGEs), a decrease in endothelium-derived NO availability, and an increase in oxidative stress; it affects vascular function mainly through overproduction of reactive oxygen species (ROS), and increases the endothelial expression of various adhesion molecules, which all results in endothelial dysfunction and vascular inflammation. Hyperinsulinaemia, which in most instances occurs as a reflection of insulin resistance, is another important cardiovascular risk factor. Insulin is involved in the process of atherosclerosis via stimulation of smooth muscle cell proliferation and enhancement of lipoprotein metabolism in arterial tissue [18].

1.5.4 Novel biomarkers
Assessment of the traditional metabolic and vascular risk factors plays a key role in disease detection and prognosis. However, along with recent advances in genetics and vascular cell biology, novel circulating biomarkers have been given attention for their possible ability to add information to the prediction of future disease. This is a fast-growing research area, and new biomarkers are suggested continuously. Some biomarkers have created a large interest due to their potential mechanistic involvement in the atherosclerotic process. Examples include C-reactive protein (CRP), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α), all associated with inflammation; fibrinogen and plasminogen activator inhibitor 1 (PAI-1), associated with haemostasis and thrombosis; and creatine kinase-MB (CKMB) and troponin, associated with myocardial damage [19]. However, the utility of these biomarkers is debated. Studies evaluating the additional predictive power of several new biomarkers beyond the traditional risk factors suggested that the biomarkers only added marginal information [20, 21]. Hence, the majority of these biomarkers should probably only be considered as risk markers and not as risk factors.

1.5.5 Clustering of metabolic and vascular risk factors
CVD risk factors tend to cluster and interact multiplicatively. In line with the causal pie model described above, the presence of a single risk factor will yield a lower probability for disease in comparison to multiple metabolic abnormalities in an
individual. Various risk prediction scores (for example the Framingham formula or SCORE) have therefore been developed to estimate absolute CVD risk via multivariable assessment of known risk factors. The metabolic syndrome (MetS) is another assessment to evaluate clustering of metabolic risk factors, proven to be a strong risk for CVD morbidity and mortality [22].

1.6 LIFESTYLE-RELATED AND OTHER FACTORS

Several lifestyle-related factors have been shown to have a close, independent association with CVD morbidity and mortality. Moreover, clustering of healthy lifestyle-related factors is associated with a major risk reduction for incident CVD [23, 24]. As these factors are related to the individual’s chosen lifestyle, they are modifiable. The negative consequences of an unhealthy lifestyle have been shown to be potentially reversible, thus reducing the complication of atherosclerosis [25]. Decline in risk factors has been related to a remarkable decline in acute myocardial infarctions [26, 27]. Other established risk factors such as age, gender, ethnicity, and genetic predisposition are not modifiable, but their influence on disease risk can be mitigated by a healthy lifestyle.

1.6.1 Physical activity

PA is defined as any bodily movement produced by skeletal muscles that results in energy expenditure (EE) [28]. EE consists of three components: basal metabolic rate (equal to 1 metabolic equivalent, 1 MET, ≈ 3.5 ml O₂·kg⁻¹·min⁻¹), the thermic effect of food, and EE due to PA. Of these, PA is the major modifiable variable for regulation of total EE in an individual. PA is a behaviour and an action that can further be divided into seven different modes: automatic/unaware movements, activity embedded in daily life, travelling to/from places, occupational activity, hobbies, exercise, and sports training [29]. This classification is mainly based on the context, the type of PA, and how the PA takes place. A further classification may be made into different arenas during the day: domestic activity, leisure-time activity, occupational activity, and commuting activity. The reasons and individual motivations for undertaking PA vary between the different arenas. While domestic and occupational PA are incorporated in daily tasks with purposes other than obtaining PA itself, daily commuting (walking/cycling instead of taking the car) and leisure-time PA are more often planned and executed from personal choice. Exercise and sports training in particular are highly dependent on personal preferences; these types of PA are defined as repetitive bodily movement that is planned, structured, and performed to improve or maintain physical fitness [30], to improve health status, or for other social reasons [31]. The effects of PA are also dependent on the three components intensity, frequency, and duration. The intensity of PA is often expressed in METs as multiples of the basal metabolic rate. Light-intensity PA (LIPA) is considered to range from 1.5 to 3.0 METs, moderate PA to range from 3.0 to 6.0 METs, and vigorous PA as > 6.0 METs. The two latter categories are often grouped together as moderate-to-vigorous PA (MVPA). As well as the difference in EE, substrate use and impact on health and fitness also change with increased intensity. However, when discussing or prescribing PA in clinical practice, rated perceived exertion (RPE) is used rather than METs (for example with use of Borg’s RPE scale [32]).

1.6.1.1 Physical activity, health and longevity

Two and a half millennia ago, the Greek physician Hippocrates postulated that disease is a product of diet and lifestyle factors. He believed that the basis of good health was plenty of daily exercise together with proper diet, good hygiene, and fresh air. At the beginning of the 19th century, several observations were reported on the
relationship between occupational PA and chronic disease and mortality [33]. However, at this point the role of PA was not fully clear, and the associations were often attributed to social and emotional factors.

1.6.1.2 CVD risk and mortality

It was not until the landmark work of Morris and colleagues in 1953 that the first epidemiological investigation into the associations between PA and health outcome was performed [34]. Morris and colleagues observed a large cohort of postal office employees and transport workers on London’s double-decker buses, and found that the more active conductors on the buses had significantly lower incidence of CVD mortality than the sedentary bus drivers (yearly incidence of 1.9 and 2.7 per 1000 person-years, respectively). A similar dose-response relationship was established for the active postal delivery workers versus the sedentary postal clerks. The importance of occupational PA was further established in the early work of Paffenbarger and colleagues in the San Francisco longshoremen study [35], and the importance of leisure-time PA and exercise in the College Alumni Study [36]. In the latter of these, PA was quantified as kilocalories (kcal) expended per minute for self-reported activities such as stair climbing, walking, and different sports, in more than 16 000 male Harvard alumni aged 35-74 years. The participants were subsequently divided into those with low (< 2000 kcal per week) and high (≥ 2000 kcal per week) EE. After a mean of 8 years of follow-up, men with low EE at baseline were at 64% higher risk for a first-time CVD event than their classmates with high EE; the results were consistent regardless of other risk factors such as smoking and BMI. Several basic concepts regarding PA and exercise were identified from the results. First, it seemed that PA of at least moderate intensity and volume was required for health benefits. Secondly, the cardioprotective effects of PA could not be accumulated, meaning that previous PA or exercise in early life was not sufficient, and an individual had to be recently engaged in PA or exercise to gain the benefits of reduced CVD risk. Moreover, for the first time it was suggested that exercise-related health benefits can be gained at a later age in life. The 1976 Nurses’ Health Study was one of the first large prospective epidemiological initiatives to examine the association between lifestyle and health in women. The study showed effects of PA on CVD health similar to those previously found in men [37]. In Sweden, several studies have shown important effects of PA on health. For example, in a cohort of middle-aged men, the risk of CVD as well as all-cause mortality after 25 years of follow up was 50% higher in those reporting low PA compared to high PA at baseline [38]. Men who increased their PA in middle age showed the same reduction in all-cause mortality risk as men with constantly high PA [39].

In summary, today there is overwhelming accumulated epidemiological evidence supporting a graded, strong importance of PA on CVD health and longevity in both young and old men and women [40-43], even with traditional risk factors and genetic factors accounted for [44]. International guidelines as well as Swedish national guidelines subsequently promote 150 minutes of weekly exercise of at least moderate intensity [45]. Fulfilling this criterion, which is hence regarded as the definition of being physically active, is in general associated with a 50% reduction in CVD risk compared to an inactive individual who does not fulfil the criterion [46]. This is a degree of risk similar to traditional risk factors such as hypertension and dyslipidemia [11].
1.6.1.3 Mechanisms of physical activity on disease

There are several mechanisms involved in the reduction of CVD risk and mortality by higher levels of PA and exercise. Primarily, regular exercise has been shown to improve intermediating traditional metabolic and vascular risk factors. Reduction in hypertension in response to aerobic exercise has been shown in both hypertensive and normotensive individuals, as well as in overweight and normal-weight individuals [47], with a general systolic and diastolic reduction of 3.8 mmHg and 2.6 mmHg, respectively. Exercise also improves serum lipid profile; the most consistent effect is on increased HDL, but exercise is also associated with reduction in total cholesterol, LDL, and triglycerides [48]. Further improvements have been shown for the prevention and treatment of overweight and obesity [49], as well as impaired glucose tolerance and insulin resistance [50], type 2 diabetes [51, 52], and the MetS [53, 54]. In a Swedish population-based study, re-examination after 20 years of 1860 healthy men who were aged 50 years at baseline revealed that increased leisure-time PA during the follow-up period was associated with improved glucose, insulin, and lipid metabolism [55]. The authors concluded that this could mediate much of the lower CVD mortality seen in more active men. High PA has also been suggested to reduce the risk of CVD events by modifications of inflammatory and haemostatic factors [56], improvement in endothelial function [57], and blood vessel remodelling [58].

1.6.2 Sedentary behaviour and non-exercise physical activity

More recently, prolonged sitting has been recognised to increase the risk for several common public health diseases and premature mortality, regardless of regular exercise [59-61]. Prolonged sitting is a behaviour related to bodily movement (or in this case absence of movement), but distinct from PA. A sedentary behaviour (SB) is defined as any waking behaviour characterised by an EE ≤ 1.5 METs while in a sitting or reclining posture [62]. It implies the absence of muscular contractions (muscular inactivity) within the large skeletal muscle groups of the body. Muscular inactivity and relatively low EE are characteristic of prolonged sitting, and potential pathogenic mechanisms have been suggested linking these to increased health risks. It seems that the molecular and physiological responses in the human body after too much sitting are not always the same as the responses that follow a bout of additional physical exercise [63].

Prolonged sitting is a behaviour that often goes unnoticed. SB typically occurs in the context of workplace sitting, television viewing, computer and video game use, and car travel. SB for an individual varies considerably during the day, and so just as for PA, it can be classified into the four primary arenas during the day (domestic activity, leisure-time activity, occupational activity, and commuting activity) where it takes place. Although it may seem that sitting or moving is a voluntary choice, there are many different factors that influence whether, when, and how we sit for extended periods during the day. For example, in today’s society, sitting is often non-discretionally incorporated in different tasks, mainly as occupational SB. Social norms, such as sitting versus standing during meetings, are another influencing factor, and the possibility of walking during the busy day is limited by the need for quick transport between different activities.

Historically, being sedentary has often been conceptualised as reflecting the lower end of the PA continuum, and considered equivalent to a lack of sufficient MVPA. In the light of new findings, it is suggested that regular exercise and SB should rather be seen as two distinct behaviours, each with partly independent importance for health and disease risk [62]. SB primarily limits the activity embedded into much of daily
life; this activity is mainly light intensity activity which is not intended to constitute exercise, and is hence referred to as non-exercise physical activity (NEPA). Conversely, SB is poorly correlated with time spent in exercise [64]. One reason for this is that the proportion of time spent doing intentional exercise usually consists of only a fraction of the day, leaving much time for NEPA or sitting. Therefore, the importance of NEPA, as the main substitute for prolonged sitting during the day, is subsequently augmented. Recent studies have shown independent associations, regardless of exercise habits and SB, between NEPA and metabolic health [65, 66], CVD risk [67, 68], and mortality [69, 70]. In Sweden, the research is limited. However, in a case-control study, Wennberg and co-workers observed a reduced risk for myocardial infarction in men, but not women, who performed active commuting compared with car commuting [71].

1.6.2.1 The daily movement pattern
As with considering different components of our diet (e.g. carbohydrates, fat, and protein) as unique nutrients with different importance for bodily function, the different aspects of our daily movement pattern should be independently considered in research and health promotion. Daily movement patterns could be described in terms of three main intensity levels: sitting (1.0 to 1.5 METs), LIPA (1.5 to 3.0 METs), and MVPA (> 3.0 METs). An increment in intensity level will generally lead to an increment in biological stimuli and EE in humans. Alternatively, daily movement patterns could be described in terms of behaviours and intention of the PA performance: SB, NEPA, and exercise. These could also be referred to in intensity levels, as SB is equivalent to sitting, NEPA mainly consists of LIPA, and exercise is mainly performed at moderate-to-vigorous intensity levels. However, the possibility to refer to the context or the intention of the PA performance makes it more communicable to the general population, in which only a few individuals rely on objective measures of intensity levels, but everyone usually knows why and in which context they perform different activities.

Figure 1 describes a daily movement pattern in terms of intensity levels, with measurements made using an objective instrument (an accelerometer). This shows the potential effects and volume of sitting, LIPA, and MVPA [72]. The powerful biological effect of MVPA is well-known; however, the daily volume is low, and a large proportion of Swedish adults lack daily MVPA [73]. On the other hand, the emerging evidence of the importance of prolonged sitting and LIPA, considered as separate behaviours, is a central issue. In recent decades, the balance between time spent in LIPA of daily life and time spent sitting has undoubtedly shifted in favour of the latter, resulting in an “unnaturally” high amount of sitting time in the general population [74, 75].
1.6.2.2 Measurements of physical activity and the daily movement pattern

PA is a complex behaviour with many different dimensions, which makes it difficult to assess. Type, intensity, duration, and frequency are the main components of interest, as well as total PA volume. There is no gold standard for measuring PA. Since PA is a result of bodily movement and increased EE, the most accurate method is to measure EE through the double-labelled water technique [76], or by direct or indirect calorimetry [77]. However, these techniques are time-consuming and expensive, and are not feasible in larger population studies. They also cannot capture variation in PA intensity, duration, and frequency during the measurement period. Other objective methods with documented evidence of validity include heart rate (HR) monitoring, accelerometers, and pedometers [78, 79]. The use of an accelerometer in combination with an inclinometer is one of the most promising methods for monitoring human movement, even in larger epidemiological studies.

Until now, the vast majority of research in PA epidemiology has relied on subjective self-reported data, mainly gathered through questionnaires. The advantages of self-administered questionnaires are that they are cheap, easy to use, and easy to distribute, and can be used to collect data from a high number of individuals in large geographic areas. Questionnaires offer a time-efficient method which does not influence the behaviour that is assessed. There are many different PA questionnaires, which aim in different ways to cover the aspects, arenas, and modes of PA. This diversity in questionnaire construction means it is difficult to make valid comparisons between different studies. One frequently-used technique is to express PA in terms of METs; that is, multiples of the basal metabolic rate. The Compendium of Physical Activity was developed to facilitate the coding of and to promote the comparison of PA expressed in METs across different observational studies [80]. Through the coding scheme provided by this compendium, the data gathered by asking specific questions about type, intensity, frequency, and duration can be recalculated into an estimation of total EE, for example as MET hours per week. More recently, the International Physical Activity Questionnaire (IPAQ) was developed to enable multinational comparisons of activity levels [81].
Nevertheless, researchers in the epidemiology field recognise that the method of self-report questionnaires carries a relatively high degree of error, regardless of which questionnaire or approach is chosen. Common challenges include recall bias, social desirability, and personal reference and interpretation of the question [82]. Although acceptable reliability estimates are reported (generally 0.50–0.70), validation estimates between self-reported PA and objective measures of PA (accelerometry, double-labelled water, etc.) suggest that only about 5–25% of the variance in the objective measures is accounted for by the self-report [82]. High-resolution analyses of PA obtained by self-reported data often result in imprecise classification of PA, which reduces the apparent magnitude of any benefits from PA on the outcome. One common approach is to either use a question with lower resolution or to further divide the collected data into, for example, tertiles (low, moderate, or high PA). However, the challenge is even more apparent as the health interest now shifts from mainly considering the structured exercise of specific intensity, to the daily NEPA and SB. The regularly repeated exercise of a special intensity and duration is easier to recall, while the latter components of the daily movement pattern are of a more sporadic and routine nature, and often not registered by the individual. Also, as SB is a distinct behaviour, it cannot be measured just as absence of exercise, but has to be measured in itself.

1.6.3 Cardiorespiratory fitness

In contrast to PA, physical fitness is a set of attributes possessed by an individual [28, 31]. It is often addressed as either performance-related or health-related fitness. Performance-related fitness refers to different subcomponents for optimal work or sport performance, while health-related fitness refers to the ability to perform daily tasks and to maintain good health. Health-related fitness consists of five main components: morphological, muscular, motor, cardiorespiratory, and metabolic fitness. In this thesis, the focus is on cardiorespiratory fitness (CF), although both morphological (abdominal fat) and metabolic (lipids, glucose, insulin) fitness are considered to some extent. CF is defined as the ability of the circulatory and respiratory systems to deliver oxygen to the working skeletal muscle. Maximal aerobic power is an indicator of the maximal capacity of the system, assessed by measuring the individual’s maximal oxygen uptake (VO$_2$max). The main limiting factor of VO$_2$max is the capacity of the cardiorespiratory system (heart, lungs, and blood) to deliver oxygen to the exercising muscles [83, 84]. VO$_2$max is expressed in absolute terms as litres per minute (L∙min$^{-1}$) or in relative terms, where the absolute value is related to body weight (ml∙kg$^{-1}$∙min$^{-1}$).

The absolute VO$_2$max level is mainly determined by sufficient amount of exercise [85], and thus higher reported levels of PA are associated with higher CF [86]. However, there are considerable individual differences in the response of CF to exercise, suggesting that generic propensity has an important influence [87].

1.6.3.1 Cardiorespiratory fitness as an important, independent predictor of CVD health and longevity

Poor CF is recognised as a strong, independent predictor of CVD and mortality in healthy men and women [88, 89] as well as in individuals with various risk factors [90]. The effect seems to be graded, so even small increments in CF will be associated with a significant risk reduction [91]. The association is suggested to be curvilinear, implying that a small change in CF for an unfit individual is associated with a relatively greater risk reduction than an equivalent change in a fitter individual [92]. The effect of CF may partly be due to improved intermediating risk factors such
as serum lipids, blood pressure, glucose levels, waist circumference [93-95], and the metabolic syndrome [94, 96]. Low CF is also independently associated with arterial wall thickness, presence of carotid plaque [97], and epicardial fat tissue [98]. Interestingly, a recent study linked low CF to circulating microRNAs, which have emerged as early biomarkers of CVD risk [99].

The peak or maximal exercise capacity is either referred to as the absolute or relative VO\(_2\)\(_{\text{max}}\), or expressed in METs (1 MET \(\approx 3.5\) ml\(\cdot\)kg\(^{-1}\)\(\cdot\)min\(^{-1}\)). There is no general agreement of an appropriate CF level for risk reduction, similar to the 150 minutes weekly of MVPA for exercise. However, a commonly used optimal CF level is the one adopted from a landmark study by Blair and co-workers in 1989 among over 10,000 men and 3,000 women [100]: 9 METs (\(\approx 33\) ml\(\cdot\)kg\(^{-1}\)\(\cdot\)min\(^{-1}\)) for women and 10 METs (\(\approx 35\) ml\(\cdot\)kg\(^{-1}\)\(\cdot\)min\(^{-1}\)) for men.

In further data from the ACLS, Blair and co-workers calculated the attributable fraction of CF and different traditional risk factors for all-cause mortality in over 52,000 men and women [101]. The attributable fraction is an estimate of the number of deaths attributable to a risk factor. It depends on the strength of association between the risk factor and the outcome, and also on the prevalence of that particular risk factor in the population. Blair and co-workers concluded that in their population, low CF accounted for about 16% of all deaths in both men and women, which was substantially more than obesity (3%), smoking (8%), high cholesterol (2-4%), diabetes (2-4%), and hypertension in women (7%). For hypertension in men, the attributable fraction was 15%. All attributable fractions were adjusted for age and each other.

**1.6.3.2 Measurement of cardiopulmonary fitness**

Despite this evidence of the importance of CF as an independent predictor, traditional metabolic and vascular risk factors are measured and evaluated considerably more frequently than CF in clinical practice. One reason for this is that actual VO\(_2\)\(_{\text{max}}\) is assessed by indirect calorimetry (by measuring ventilation and concentrations of oxygen and carbon dioxide) during an incremental test on cycle ergometer or treadmill to voluntary exhaustion. This procedure involves a health risk in non-athletic populations, and restricts it to laboratories as it requires special techniques and equipment. Several studies have estimated CF from peak power achieved on a cycle ergometer or total time in a standard treadmill test, which eliminates the requirement for a laboratory but still requires maximal effort. However, there are still several situations where VO\(_2\)\(_{\text{max}}\) should be assessed (as in clinical practice) or needs to be assessed (in large epidemiology studies). In these situations, it is vital to have a reasonable simple, low-risk, easily-administered test which does not require laboratory equipment, but preferably has good validity and reliability and is based on good physiological grounds. Several submaximal tests exist for estimation of VO\(_2\)\(_{\text{max}}\) using the HR response to submaximal work for either regression modelling or extrapolation to supposed maximal levels [102-105]. The estimation is then often based on the assumption of a rather linear relationship between HR and power output (PO) up to maximum, and the fact that VO\(_2\) can be estimated from PO with acceptable precision.

The Åstrand (Å-test) was the first submaximal cycle ergometry test to be developed, and is also the most commonly used [106, 107]. It allows VO\(_2\)\(_{\text{max}}\) to be estimated from a nomogram (after consideration of gender and age) by using the steady-state HR achieved after 6 minutes of constant loading at an individually-chosen work rate.
The test has good validity, showing no mean difference between measured and estimated VO\textsubscript{2}\text{max} on a group level [108]. However, the coefficient of variance (CV), expressing the precision of the test, was reported by the authors to be ±10\% in a younger, well-trained population, and ±15\% in a mixed population (with different age and training status). This has been later confirmed in other studies [105, 108]. For the mixed population, this means that for 95 out of 100 individuals with an actual VO\textsubscript{2}\text{max} of 3.0 L\text{-}min\textsuperscript{-1}, VO\textsubscript{2}\text{max} can be predicted within ±0.9 L\text{-}min\textsuperscript{-1}.

Estimation of VO\textsubscript{2}\text{max} from a submaximal test has a number of limitations. Variation between individuals in moving economy, especially during weight-bearing activities, may cause variation in HR response to a given work rate. Moreover, tests relying on extrapolation to supposed maximal levels are limited by the asymptotic relationship between HR and VO\textsubscript{2} near maximum, and to the variability of age-predicted maximal HR, which has a CV of at least 10\% [109]. In addition, results from tests that rely on only one absolute HR at one work rate may be seriously impacted by the variability of submaximal HR due to internal as well as external factors, such as ambient temperature, nervousness, emotions, and intra-individual variability of basal metabolic rate and VO\textsubscript{2} at a given work rate.

With regard to the methodological limitations incorporated in existing submaximal ergometer tests, there is a potential need for a new test with enhanced precision in estimating VO\textsubscript{2}\text{max} (see Paper III).

### 1.6.4 Overweight and obesity

Overweight and obesity are characterised as abnormal or excessive amounts of adipose tissue accumulation that may impair health. They constitute a complex multifactorial chronic disease that mainly results from a long-term positive energy balance, but is to some extent influenced by genotype. The most accurate methods of assessing body composition and quantifying the body fat and its distribution include dual energy X-ray absorptiometry (DEXA), magnetic resonance imaging (MRI), hydrostatic weighing, and computerised tomography (CT). As these methods are time-consuming and expensive, surrogate measures are often used in clinical practice and large epidemiology studies. The Body Mass Index (BMI) has historically been the most widely accepted measure for defining overweight and obesity, and is still used by several leading national and international institutions, including the World Health Organization (WHO). It is calculated as the body weight in kilograms divided by the square of the height in meters (kg\text{-}m\textsuperscript{-2}). Adults with a BMI between 25.0 and 29.9 kg\text{-}m\textsuperscript{-2} are considered overweight, while those with a BMI \( \geq 30 \) kg\text{-}m\textsuperscript{-2} are regarded as obese. Overweight and obesity have been shown to induce a high risk for metabolic disorders such as type 2 diabetes, the metabolic syndrome, and CVD morbidity and mortality [110, 111]. The most recent reports identify approximately 50\% of Swedish men and women [112], and 68\% of American men and women [113] to be overweight or obese, resulting in a major health impact both in Sweden and globally.

More recently, body fat distribution has been ascribed a greater importance and health impact than total body fat mass per se [114]. The upper type of fat distribution in particular, such as excess intra-abdominal fat mass, has been associated with increased metabolic impairment. BMI is an indicator of general adiposity, and lacks the discriminatory power to differentiate between body fat and lean mass. This means that extra weight from muscle mass on trained individuals will affect BMI. In a review of 40 prospective studies, Romero-Corral and co-workers showed that the
definition of overweight as a BMI ≥ 25 had poor specificity to detect excess body fat, and subsequent low ability to predict CVD as well as total mortality [115]. Waist circumference (WC), waist-to-hip ratio, and sagittal abdominal diameter are often used as surrogate markers of abdominal fat mass, reflecting both the subcutaneous and intra-abdominal fat mass. This thesis uses WC as a proxy for abdominal fat mass. WC is generally measured at the midpoint between the lowest rib and the iliac crest [116]. It has been identified as a better predictor of total intra-abdominal adipose tissue than BMI [116], and is also better at detecting CVD risk factors in both men and women [117]. WC also associates significantly with the risk of incident CVD events [118, 119] and all-cause mortality [111]. The WC thresholds representing overweight are ≥ 80 cm in women and ≥ 94 in men, while obesity is defined as ≥ 88 cm in women and ≥ 102 in men [116, 120].

The biological mechanisms responsible for the association between intra-abdominal fat tissue and cardiometabolic risk are not fully clear. However, abdominal fat tissue is known to be highly biologically active and responsible for a range of metabolic and endocrine functions [121]. For example, intra-abdominal adipocytes are more lipolytically active than their subcutaneous counterparts, and an excess of abdominal fat tissue results in increased production of free fatty acids. These are released into the portal vein and directly delivered to the liver, with a subsequent stimulation of very low density lipoprotein (VLDL) synthesis and secretion as well as a negative influence on hepatic glucose production and insulin sensitivity. Free fatty acids also have adverse metabolic effects on muscle, pancreatic β-cells, and the vasculature. In addition, specific proteins and hormones are released by the abdominal adipocytes, with potential contribution to cardiometabolic risk.

1.7 RELATION BETWEEN PHYSICAL ACTIVITY, CARDIORESPIRATORY FITNESS, AND OBESITY

1.7.1 Physical activity versus cardiorespiratory fitness

Although there is an inevitable interrelationship between PA and CF, CF has generally been credited with a stronger relationship to single CVD risk factors [93, 95, 122] as well as to CVD incidence or mortality [89, 123]. In contrast, some claim that increasing levels of PA may protect against metabolic disease even in the absence of improved CF [124]. Along with the recently highlighted importance of NEPA of daily life to reduce the negative health effects of prolonged sitting, it also becomes evident that metabolic health benefits may be achieved at PA intensities that will not enhance CF. No consensus exists regarding whether there is a true difference between the effects of PA and CF, or if the divergence is due to other explanations such as different measurement methods (PA is often assessed by self-report, while CF is assessed through objective stress tests), influence from genetic endowment, or just the fact that PA is seen as an action or behaviour while CF is seen as the result of this action.

Plowman [125] stresses the importance of weighing the relative significance of PA and CF and determining whether it is necessary to achieve higher levels of CF, or whether simply participating in PA is sufficient for health benefits. Ignorance of the independent contribution of each of these variables could lead to misclassifications. Inactive individuals with a strong genetic endowment that allows them to score well on CF tests may get a false sense of security; and, conversely, highly active individuals with low cardiovascular adaptation to exercise may experience a sense of discouragement and false worry when tested as unfit.
1.7.2 Physical activity/Cardiorespiratory fitness versus obesity

Low CF and inactivity as well as excess body fat often occur in combination, which subsequently poses the question of the independent importance of the two variables for CVD risk. Some have suggested that high PA/CF creates a protective effect which diminishes the obesity-related risk for CVD and all-cause mortality [126-129]. Others claim that PA/CF may not completely eliminate the negative effects [130, 131] or that they might be considered as two independent risk contributors [132, 133]. Regarding CVD risk factors, less is known, but the results available are equivocal [124, 134-136]. A review by Fogelholm and co-workers reported that the health risk for hard endpoints, such as disease and mortality, seems to be attenuated to a large extent with higher levels of PA/CF in both obese and normal-weight individuals [137]. Conversely, for CVD risk factors the influence of obesity seems to be more important, regardless of PA/CF.

Due to the divergence in previous results, there is no consensus regarding the interrelationship between these variables and the question of whether one is more important than the other. These divergent results can be partly explained by the complex multifactorial aetiology of CVD, but also by a large variation between studies in measurements of or method used for assessing PA, CF, and fatness. For example, studies using self-reported PA suffer from rather low validity, and hence CF is often used as a surrogate measure even though it is also determined by other factors such as age and genes. In turn, CF may be estimated by treadmill time, or by direct or indirect measurement of VO$_2$max. Excess body fat is often assessed by the crude estimate of BMI, but also by the more valid WC, waist to hip ratio, and % body fat. Variation of the distribution of these exposure measurements within different cohorts and using different endpoints are other important aspects that may explain the divergent results. Furthermore, there is a great lack of research into the role of prolonged sitting for the interrelationship between PA, CF, and abdominal obesity.

1.8 DIET

Both total energy intake and the qualitative aspects of diet (nutrients, patterns, etc.) have an important independent impact on disease development. For example, a high intake of saturated fats of animal origin is generally considered unfavourable, while vegetable-based diets containing mainly unsaturated fats (such as the Mediterranean diet) are seen as protective [138, 139]. Similar comparisons have been made between diets with low versus high proteins and carbohydrates [140, 141]. In recent years, high consumption of soda, sweets, and fast food with high levels of sugar and empty carbohydrates and low levels of nutrients has probably played an important role in the dramatic increase of overweight and obesity worldwide. Food culture and habits vary considerably between different global regions, and the variation is partly reflected by the distribution of disease burden. There is also an important interaction between PA, SB, and diet [142, 143]. This thesis does not consider diet as a primary factor. However, as it is important to take diet into account, attempts have been made to adjust for dietary habits in the full analysis.

1.9 THE INTERRELATIONSHIP BETWEEN CARDIOVASCULAR RISK FACTORS

As noted above, a variety of factors are associated with the development and manifestation of CVD: traditional metabolic and vascular risk factors, lifestyle-related factors, and other factors. The interrelationships between these are complex, with direct, indirect, and intermediating effects. There are also interactions between factors, and reversed relationships and reversed causality are also possible.
Despite the extensive research performed within this field, there is no consensus. One reason for this is that the pattern of risk factors has changed over time, with a subsequent impact on disease burden. In Sweden, abdominal obesity and prolonged sitting are examples of lifestyle-related risk factors which have increased over the last 20-30 years in both men and women, while smoking and total cholesterol have decreased significantly during the same time span [27, 144, 145]. It is also important to consider that lifestyle habits and patterns of risk factors considerably between communities [146], countries, and cultures. Therefore, it is essential that epidemiological studies are performed and conclusions are drawn using data from the population to which the results will be applied.

The methodology used and the specificity of the measurements have also evolved over time. Examples of this include the need to measure the distribution of body fat (as WC or waist-to-hip ratio) rather than total amount of body fat (BMI), or the call to evaluate all aspects of the daily movement pattern. Further, if results are to be extrapolated to the general population, analyses must be performed in random population-based samples. In Sweden, we have a strong history of qualitative registration in different contexts. For a general overview, the Swedish Population and Address Registry (SPAR) includes all people registered as Swedish citizens. This enables epidemiological studies to be based on randomly drawn samples from the general population.
2 OBJECTIVES
The main objective of this thesis was to examine the interrelationship between different levels of PA, CF, and abdominal adiposity and their association with CVD risk factors, CVD morbidity, and longevity in Swedish men and women of different ages. A second objective was to develop a new and more precise method for estimation of CF in a mixed healthy population.

The specific objectives of each paper were:

I. To examine the relationships between CF and abdominal obesity and individual dichotomised CVD risk factors, as well as a clustered CVD risk factor profile, in a cross-sectional random sample of Swedish men and women aged 20-65 years. In addition, to study the impact of gender, age, and smoking habits on these relationships.

II. To examine the interrelationship between different levels of PA and CF and individual dichotomised CVD risk factors, as well as a clustered CVD risk factor profile, in a cross-sectional random sample of Swedish men and women aged 20-65 years.

III. To create and evaluate a new submaximal cycle ergometry test to be used in a mixed, healthy population for estimation of VO$_2$max. The test should meet the criteria of being simple, low-risk, time-effective, and easily administered; having good validity and reliability; avoiding the need for laboratory equipment; and being based on good physiological grounds.

IV. To examine the importance of NEPA for metabolic health at baseline and the risk of a first-time CVD event and total mortality after 12.5 years in a population-based sample of 60-year-old men and women.
3 MATERIALS AND METHODS

3.1 PAPERS I AND II

3.1.1 Study population
The study population in Papers I and II was compiled from two cross-sectional random sample studies on Swedish women and men aged 20 to 65 years, LIV 90 [147] and LIV 2000 [73]. LIV 90 aimed to study PA, physical fitness, lifestyle, and living conditions in a representative sample of the adult Swedish population in 1990/1991. LIV 2000 had the same study aim in 2000/2001, but also aimed to study the trends over 10 years. The participants were randomly drawn from the Swedish Population and Address Registry (SPAR), which includes all people registered as Swedish citizens.

In LIV 90, a total of 2400 participants were drawn from eight defined geographical representative regions of Sweden (150 men and 150 women from each region). The study finally included 2203 participants, of which 1879 (85%) answered a questionnaire regarding lifestyle and living conditions and 1180 fulfilled measurements of anthropometrics and physical performance. In LIV 2000, a total of 2000 participants were drawn from four defined geographical representative regions of Sweden (250 men and 250 women from each region). The study finally included 1357 participants, of which 1065 (79%) answered the questionnaire and 491 fulfilled the testing. A more detailed description of dropout and participation rates is presented in Figure 2 below.

Figure 2. Dropout and participation rates in the LIV 90 and LIV 2000 studies.
The division of geographical regions was altered between the two studies due to administrative reasons, and so the studies did not use exactly the same regions. However, the reason for the random sampling was to get a cohort of men and women which was representative of the Swedish population of these ages. We therefore compared both the LIV 90 and LIV 2000 samples with gender and age matched cohorts (25-34 years, 35-44 years, 45-54 years, 55-64 years) from the nationwide Swedish Survey of Living Conditions (ULF) [112] from 1990 and 2000, respectively, for several important variables: mean height, mean weight, prevalence of overweight (BMI > 25), daily smoking, some protracted disease conditions, and high educational level (≥ university). Using these data, 95% confidence intervals (CI) for the mean values/percentages from the LIV 90 and LIV 2000 studies were compared with the national data mean values/percentages. In LIV 90, older women had higher prevalence of overweight and high educational level, and lower prevalence of protracted disease conditions, compared with older women in the national register data. For participants in the LIV 2000 study, younger and middle-aged women were more likely to be highly educated and the oldest women to be less overweight. Regarding the men in LIV 2000, the youngest and oldest ones were more likely to be overweight and less likely to smoke daily, whilst middle-aged men were higher educated than men in the national data. Otherwise there were no differences, and we concluded that the samples were a good representation of men and women of these ages at each specific time.

Comparing the two cross-sectional samples revealed no significant differences in PA levels either in men or in women [73, 108]. For CF, there was no change in women, but men showed a decline over the 10 years. For cardiometabolic factors, there was a significant decline in total cholesterol level and both systolic and diastolic BP. Most of the decrease in total cholesterol levels is probably attributable to change in diet [26]. WC was significantly higher in men in LIV 2000 compared to LIV 90, with no corresponding change in women. To allow compilation of the two samples, original study participation was adjusted for in the analysis.

3.1.2 Data collection and measurements
All participants were sent an extensive questionnaire covering PA, lifestyle, and living conditions as well as an invitation to visit a nearby test centre to undergo physical examination and laboratory testing. The test centres were locally distributed within each geographical region, and staffed by trained medical personnel. To ensure that all tests were performed in a standardised way, all personnel underwent a 1-day training course where all theoretical and practical parts were discussed and performed. Also, as the study was running, the personnel at each test centre had frequent contact with the investigators.

3.1.2.1 Leisure-time physical activity
Leisure-time PA was determined from the questionnaire responses. There were some differences in the PA questions between LIV 90 and LIV 2000. Table 1 presents the two questions and the scoring system used to compile these into three PA levels (low, medium, and high PA). These levels were derived according to the general guidelines on PA for health promotion and risk prevention.
Table 1. Questions used in LIV 90 and LIV 2000 to assess PA (top), and the classification used in this thesis to determine PA level (bottom).

The low PA group was considered as physically inactive or active at very low levels, a state associated with many health risks. Participants with high PA were regarded as regular exercisers and sufficiently active to achieve many health benefits. The medium PA group comprised the remaining participants not included in the low or high PA groups. To validate the compilation of the two questions and test the concordance between them, we conducted a separate validation study. A total of 199 men and women answered both questions, and a significant association was found between the questions divided into the three PA categories (γ = 0.86; P < 0.001).

3.1.2.2 Other self-reported variables
For the multi adjusted analysis in Papers I and II, confounding variables were derived from the questionnaire responses. Educational level was classified into ≤ 3-4 years of secondary school or ≥ university. Smoking habits were classified as current daily smoker or non-smoker.

3.1.2.3 Cardiorespiratory fitness
CF was estimated from the Åstrand-Rhyming submaximal cycle ergometer test [148] and assessed as VO\textsubscript{2max} (ml·kg\textsuperscript{-1}·min\textsuperscript{-1}). Participants with cardiovascular disorders, or who were on medication for cardiovascular disorders, were not tested. Before testing, all participants were requested to refrain from smoking and vigorous activity the day before and from heavy meals during the hours before the test. The participants cycled on a calibrated mechanically braked cycle ergometer (Monark, Varberg, Sweden) at an individually chosen submaximal work rate for 6 minutes to achieve a steady state HR within the interval 120-170 beats per minute. The pedal rate was 50 rpm. HR was recorded during the last minute using telemetry (Polar Electro OY, Kempele, Finland). VO\textsubscript{2max} was estimated from a nomogram using the steady state HR, gender, and age.

3.1.2.4 Anthropometrics and blood sample
Body weight and height were assessed with the participants wearing light clothes, standing on a calibrated standard scale and a standard stadiometer, to the nearest 0.1 kg and 0.1 cm, respectively. WC was determined in a standing position to the nearest 0.5 cm with a tape measure midway between the lower rib margin and the iliac crest. Systolic and diastolic BP were measured in the right arm in a lying position after 10 minutes of rest, using standard auscultatory method, and calculated as the mean value of two determinations. A venous blood sample was drawn after more than 4 hours of fasting. All blood samples were analysed at the same laboratory in Stockholm,
Sweden. Conventional enzymatic methods [149] were used to determine total cholesterol, HDL cholesterol, and triglycerides and turbidity immunoassay methods [149] were used to determine APO A1 and APO B with an Abbott Aeroset analyser (Abbott Laboratories, Abbott Park, IL, USA). LDL cholesterol was subsequently calculated with the Friedewald equation [150].

3.1.2.5 CVD risk assessment

We chose to assess dichotomised risk factors rather than continuous variables, as we considered it more important to evaluate how the exposures coincide with unhealthy levels of the risk factors, rather than how they correlate. We therefore used conventional cut-off points derived from large epidemiological studies on the relationship between these risk factors and CVD risk: WC (in Paper II) ≥ 80 cm for women and ≥ 94 cm for men; systolic BP ≥ 140 mmHg; diastolic BP ≥ 90 mmHg; triglycerides > 1.7 mmol/l; total cholesterol ≥ 5.0 mmol/l; APO A1 < 1.25 g/l for women and < 1.15 g/l for men; HDL cholesterol < 1.2 mmol/l for women and < 1.0 mmol/l for men; APO B ≥ 1.2 g/l; and LDL cholesterol ≥ 3.0 mmol/l. APO A1 and APO B were uniquely measured in LIV 90 and HDL and LDL in LIV 2000 to assess lipoprotein levels. To enable a full-sample analysis, the dichotomised variables of APO A1 and HDL as well as APO B and HDL were merged into single risk factors referred to as “anti-atherogenic lipid profile” and “atherogenic lipid profile”, respectively. Moreover, a total risk factor variable was derived and further dichotomised into having three or more risk factors compared to fewer. This is line with conventional definitions regarding clustering of risk factors and the association with increased CVD risk [151].

3.1.3 Statistical considerations in Papers I and II

The continuous descriptive characteristics for the study population in Papers I and II are summarised as means with standard deviations. Two software packages were used in the statistical analyses: SPSS (version 15.0) and CIA (Confidence Interval Analysis).

3.1.3.1 Paper I

Partial correlations derived from linear regression models were assessed to determine the associations between CF and WC as continuous variables with the total number of risk factors. The variable of total number of risk factors was logarithmically transformed owing to its skewed distribution. Logistic regression models were used to evaluate how each dichotomised individual risk factor was associated with continuous levels of CF and WC, as well as how the dichotomised total risk factor variable (three or more risk factors compared with fewer) was associated with gender-specific tertiles of CF and WC (all as odds ratios [OR] with 95% CI). In the latter analysis, the sample was further divided into subgroups that were evaluated one at a time: men vs. women; ≤ 43 years vs. > 43 years (median age of the total sample); and non-smoker vs. low daily smoker (irregular or one to two cigarettes/day) vs. heavy daily smoker (three or more cigarettes/day).

3.1.3.2 Paper II

To enable comparison of the association between CF and PA, gender- and age-specific quartiles of CF were derived; CF quartile 1 was considered as low CF, quartiles 2 and 3 as medium CF, and quartile 4 as high CF, according to the same general guidelines used for assignment of PA levels (see Table 1). Logistic regression models were used to assess OR and 95% CI associated with PA and CF levels, respectively, for each individual risk factor. The three PA and CF levels were cross-
tabulated to study the combined effects of PA and CF on clustering of CVD risk. To test for possible interaction between PA and CF for the individual risk factors as well as the clustered CVD risk factor profile, an additive model of interaction (mean deviation from the sum of the OR for each exposure respectively) was used to calculate the relative excess risk of interaction (RERI) [152, 153]. For two dichotomised exposure variables A and B, the following equation was then used: RERI = RR (AB) – RR (A) – RR (B) + 1. The RERI 95% CI was estimated using modified regression models [154]. There was evidence of an excess risk due to interaction if the RERI 95% CI excluded zero.

3.2 PAPER III
The study described in Paper III aimed to create and evaluate a submaximal cycle ergometer test to increase the precision of VO\textsubscript{2}max estimation by submaximal testing. The hypothesis for the new test was based on the fact that HR response to a fixed increase in work rate will be steeper in individuals with lower VO\textsubscript{2}max compared with individuals with higher VO\textsubscript{2}max. By using the delta HR (ΔHR) response between a lower, standard work rate and an individually-chosen higher work rate, rather than absolute HR response to one work rate, VO\textsubscript{2}max may be estimated with greater precision in mixed populations by diminishing some of the sources of error mentioned in the background section of this thesis. One previous study made a similar attempt, but the study population was limited to young males and the pedalling rate was 90 rpm, and so the method was not appropriate for a mixed population [155].

3.2.1 Study population
After public announcement in the nearby region of Stockholm, Sweden, a total of 143 participants (65 men and 78 women) were included in the study. The sample was composed of a mixed population with regard to sex, age (21 to 65 years), and self-reported activity status (inactive to highly active).

3.2.2 Data collection and measurements
The participants visited the laboratory on two occasions at an interval of one week, on the same weekday and at the same time, to perform submaximal and maximal testing. A subsample (n = 49) visited the laboratory only once, and without measuring actual VO\textsubscript{2} during the submaximal test. However, analysis showed no significant difference in age (men), height, weight, actual VO\textsubscript{2}max, and HR\textsubscript{max} between this subsample and the rest of the study population. All participants were healthy and free from medication that could influence the relationship between HR and VO\textsubscript{2}. All participants were requested to refrain from smoking and vigorous activity the day before and from heavy meals three hours prior to testing. All tests were performed in laboratory environment with a normal ambient climate. HR was monitored during the tests with a Polar Electro monitor (Kempele, Finland). VO\textsubscript{2} was measured by a computerised metabolic system (Jaeger Oxycon Pro, Hoechberg, Germany) breath-by-breath with a flowmeter connected to a face mask. Before each test, ambient temperature, humidity, and barometric pressure were measured, and gas analysers and an inspiratory flowmeter were calibrated. High-precision gases (15.00 ± 0.01% O\textsubscript{2} and 6.00 ± 0.01% CO\textsubscript{2}, Air Liquid, Kungsängen, Sweden) and ambient indoor air were used for gas analyser calibration.

3.2.2.1 Submaximal test
The seat and handlebars of the cycle ergometer were individually adjusted using standard procedures. Next, the participants were introduced to the Borg RPE scale
which they would use to rate their perceived exertion during the submaximal test and after the maximal test. The participants then cycled on a calibrated, mechanically braked cycle ergometer (Monark, model 828E, Varberg, Stockholm) at three submaximal work rates for 4 minutes each, with no rest in between the work rates. Four minutes was sufficient to reach steady state HR on the lower rate, and as the work was continuous, steady state was also reached on the higher rates within 4 minutes. The pedalling rate was 60 rpm. Submaximal cycling on a standard rate with a resistance of 0.5 kilopond (kp) preceded two individually chosen higher work rates. The first higher work rate was chosen by the test personnel to obtain a RPE of 12-13 on the Borg scale, and the second to obtain a RPE of 14-15. HR and VO\textsubscript{2} were recorded as the mean value during the last minute at each work rate. Tests resulting in RPE above 16 were excluded due to suspicion of effects of anaerobic energy production on HR and mechanical efficiency.

3.2.2.2 Maximal test
A maximal treadmill test was used to determine VO\textsubscript{2max}. After a short period of rest followed by a 5-min warm up, the participant started off at a speed and incline level corresponding to approximately 60–65% of maximal capacity. Direct measurements of VO\textsubscript{2} were conducted with increasing speed and incline every minute until voluntary exhaustion. The criteria for acceptance of the VO\textsubscript{2max} value achieved were respiratory quotient greater than 1.10, “levelling off” of VO\textsubscript{2} despite an increase in work rate, an RPE above 16, a work time above 6 minutes, and supported by a HR during the maximal effort within ± 15 beats per minute from age-predicted maximum HR. VO\textsubscript{2max} and HR\textsubscript{max} were calculated as the mean values during the highest 30 seconds registered.

A maximal test was performed on both occasions, but due to the criteria mentioned above, not all participants had two acceptable measurements. We studied the variation between the first and second VO\textsubscript{2max} achieved in 59 participants with two acceptable measurements. Mean values did not differ between the first and second occasion (VO\textsubscript{2max} was 3.49 L ∙ min\textsuperscript{-1} and 3.50 L ∙ min\textsuperscript{-1}, respectively, paired t-test P = 0.341). The CV was 2.70%, which is regarded as small and in concordance with internal laboratory measurements and other previous reports \cite{156}. Therefore, the first accepted VO\textsubscript{2max} value was used.

3.2.3 Model construction
\(\Delta\text{HR} \) was calculated as the difference between the HR for the higher individually chosen work rate and the HR for standard work rate. To standardise \(\Delta\text{HR} \) and make it comparable between individuals cycling at different final work rates, it had to be related to the increased VO\textsubscript{2} demand (\(\Delta\text{VO}_{2} \)). The VO\textsubscript{2} cost of submaximal constant work rate cycle ergometry at the different work rates was measured in the study. However, as the main aim of this new predictive test was that it should be easily administered with no need for laboratory equipment, VO\textsubscript{2} will not normally be measured in actual test situations. Therefore, it was preferable to use the delta work rate (delta power output, \(\Delta\text{PO} \)) as a proxy for \(\Delta\text{VO}_2 \). PO at each work rate was calculated as the work rate from the cycle ergometer, multiplied by 1.08 to correct for frictional losses at the chain and drive train \cite{157, 158}. This added load must be considered when comparing VO\textsubscript{2} and HR between work rates on the Monark ergometer, or between the mechanically braked Monark and electronically braked ergometers. \(\Delta\text{PO} \) was calculated as the difference between the higher work rate and the standard work rate, and the individually measured \(\Delta\text{VO}_2 \) was plotted against \(\Delta\text{PO} \). There was no significant increase in delta efficiency for the different work rates.
above standard rate (as ΔVO₂ per ΔPO) either for men (p=0.399) or for women (p=0.801). This justified the use of ΔPO as a proxy for ΔVO₂. Therefore, ΔPO factors were developed for different final work rates pedalling at 60 rpm. As there was a similar increase in VO₂ with increased work rate for men and women, no gender-specific ΔPO factors were needed. For more details and tables with directly measured and calculated VO₂ cost for the different work rates, and the ΔPO factors to be used, see Paper III [159].

Further, a ΔHR/ΔPO score was calculated for each participant from the results of the submaximal test obtained at the first visit to the laboratory. However, the submaximal test on this visit was not valid for 15 participants due to reported RPE > 16 (n=4), illness (n=4), failure to follow the standardised procedure before the test (n=3), and abnormal ambient climate during the test (n=4). Hence, the data obtained at the second occasion was used. Linear regression analysis determined the suitability of using the ΔHR/ΔPO score to predict VO₂max. Further partial correlation analysis identified age and sex to be possible confounders. Hence all these variables were entered into a multiple linear regression model with VO₂max as dependent variable. A forward selection method was used to add the independent variables in order of significance.

For comparative purposes, VO₂max was also estimated with the Åstrand submaximal test (Å-test), applying the work rate and HR of the higher work rate to the Åstrand nomogram with associated age-correction factors [106]. The Å-test was originally based on a pedalling rate of 50 rpm. However, Jessup and colleagues showed no difference in predicted VO₂max calculated from the Å-test at 50 and 80 rpm [160], and negligible differences in mechanical efficiency between 50 and 60 rpm have also been reported [161, 162]. In line with the Å-test prerequisites, a submaximal pulse rate within the range of 120 to 170 bpm was accepted. However, for some of the older participants with a low maximal HR, a pulse rate of ≥ 110 bpm was accepted as they reported a RPE of 14-15 on the Borg scale even at these pulse rates. For twelve participants, VO₂max could not be estimated with the Å-test, as their HR during the submaximal test was too low (< 110 or 120 bpm).

### 3.2.4 Statistical considerations in Paper III

Continuous descriptive characteristics were summarised as means with standard deviations. A multiple linear regression with forward selection was used to include independent variables in the VO₂max estimation model in order of significance (probability of F=0.05 for entry, and 0.10 for removal). The final model was checked for equal variance. Pearson’s coefficient of correlation (r) with corresponding 95% CI was used to evaluate the association between estimated and observed VO₂max. CV was calculated as the ratio between the standard deviation of the difference between estimated and measured VO₂max, and the mean measured VO₂max. For the reliability analysis, paired sample t-tests were used to compare the absolute HR and ΔHR for the first and second occasion at the standard and higher work rate, respectively. Correlation analysis was used to analyse the influence of maximum HR, deviation from age-predicted maximum HR (220 bpm - age), and VO₂max for the difference between measured and estimated VO₂max. A repeated measures ANOVA was used to test the distribution in difference from mean VO₂ for the standard rate and the first and second higher work rates. Statistical significance was set at p < 0.05 for all analyses. The statistical analyses were conducted using version 19.0 of the SPSS software package (SPSS Inc., Chicago, Illinois, USA) and version 12.2.1.0 of MedCalc.
3.3  PAPER IV

3.3.1 Study population
The study population in Paper IV consisted of a cohort of men and women who had attended a health screening study in 1997–1999. Every third man and woman born between 1 July 1937 and 31 June 1938 and living in Stockholm County, Sweden, was invited to participate. In total, 5460 individuals were invited, and 4232 individuals (2039 men, 2193 women; 78%) agreed to participate.

3.3.2 Data collection and measurements
The participants were invited to visit the laboratory after overnight fasting to undergo physical examination and laboratory tests, and to complete an extensive self-administered questionnaire covering PA habits, medical history, lifestyle, and living conditions.

3.3.2.1 Non-exercise physical activity and regular exercise
A NEPA index was derived from the questionnaire responses at baseline. Participants were asked to report how frequently ("never", "occasionally", or "frequently or regularly") during the last 12-month period they performed 24 different activities typical for older adults of the Swedish and Scandinavian culture (see Appendix 1, Paper IV). Five of these activities predominantly promoted NEPA of daily living: "Performing home repairs", "Cutting the lawn, hedge, etc.", "Car maintenance", "Taking bicycle rides, skiing, ice-skating, going hunting or fishing", and "Gathering mushrooms or berries". These activities mainly elucidated the context in which PA was performed (as part of daily life), and did not refer to a specific intensity span. Regarding the other 19 activities, 12 could not clearly be defined as promoting daily activity or not, four predominantly promoted sitting, two were mainly intentional exercise, and one was an exclusive activity not available to all study participants. For construction of the NEPA index, reporting "never" was equal to one point, "occasionally" to two points, and "frequently or regularly" to three points, thus resulting in a possible range of 5 to 15 points. A reliability analysis revealed moderate internal consistency of the five single items (Cronbach's α = 0.67). A total of 71 participants had an internal missing observation for one of the five NEPA activities; these values were replaced by the estimated gender-specific series mean to obtain a full score and inclusion in the analysis. The score was subsequently divided into tertiles; low, moderate and high levels of NEPA. Since some of the NEPA activities were more common in men than women, sex-specific tertiles were used to ensure that the NEPA index analyses elucidated differences in NEPA patterns and not gender differences (cut-off points were ≤ 8, 9-10, > 10 points in women and ≤10, 11-12, > 12 points in men).

To determine exercise habits, the participants were asked to report their leisure-time PA level during the past year as either 1. “Sedentary” (light-intensity activity <2 hours/week); 2. “Light-intensity PA” (≥ 2 hours/week); 3. “Regular moderate-intensity PA” (at least 30 minutes, 1-2 times a week) or 4. “Regular high-intensity PA” (at least 30 minutes, ≥ 3 times a week) (see Appendix 2, Paper IV). In line with current guidelines for health promotion and risk prevention recommending regular exercise (defined as PA of at least a moderate intensity level), these were further dichotomised into regular exercise of at least moderate intensity (3 or 4 above) or not (1 or 2 above).
### 3.3.2.2 Other self-reported variables

For the multi adjusted analysis in Paper IV, lifestyle-related factors with the potential to confound the analysis were dichotomised from the questionnaire data: marital status (married/living together vs. not), education level (university degree vs. not), current smoking (yes vs. no), dietary intake of vegetables (high intake: one portion daily/almost daily vs. low intake: occasionally/never), general wellbeing (very/quiet good vs. not), and living conditions (apartment vs. house/townhouse). Regarding alcohol, consuming 4-6 bottles of strong beer, 2-3 bottles of wine, or 0.35–0.75 L spirits per week was considered a high intake, while less than this was considered a low intake. Self-rated financial status was based on a seven-point scale ranging from 0 (very bad) to 7 (excellent); scoring 1-4 was considered bad and 5-7 good. Heredity of high blood pressure, dyslipidemia, diabetes mellitus, or CVD was determined as self-reported presence of each individual condition, respectively, in the participant’s mother or father.

### 3.3.2.3 Anthropometrics and blood sample

WC was measured with the participant in a standing position, using a tape measure midway between the lower rib margin and the iliac crest. Systolic and diastolic BP were measured twice with an automatic device (HEM 71, Omron Healthcare, IL, USA) after 5 min of rest in a sitting position, and the mean of the measurements was calculated. A venous blood sample was drawn from an antecubital vein after overnight fasting, and all blood samples were analysed continuously. Serum total cholesterol and triglycerides were analysed using enzymatic methods (Bayer Diagnostics, Tarrytown, NY, USA). Serum HDL was measured after isolation of LDL and VLDL (Boehringer Mannheim GmbH, Germany), and LDL was calculated with the Friedewald equation. Serum glucose was measured with an enzymatic colorimetric test (Bayer Diagnostics), serum insulin levels with the ELISA technique (Boehringer Mannheim), and plasma fibrinogen with a functional spectrophotometric test (Boehringer Mannheim).

### 3.3.3 Individual CVD risk factors and the metabolic syndrome

Nine individual dichotomised risk factors were defined using conventional cut-off points for CVD risk: high WC (≥ 88 cm in women, ≥ 102 cm in men), systolic BP (≥ 130 mmHg), diastolic BP (≥ 85 mmHg), LDL (> 3.0 mmol·l⁻¹), triglycerides (≥ 1.7 mmol·l⁻¹), insulin (75th percentile: ≥ 11.6 mU·l⁻¹ in women, ≥ 13.0 mU·l⁻¹ in men), glucose (75th percentile: ≥ 5.6 mmol·l⁻¹), fibrinogen (75th percentile: ≥ 3.5 g·l⁻¹), and low HDL (< 1.3 mmol·l⁻¹ in women, < 1.0 mmol·l⁻¹ in men). Dichotomised risk factors were used in order to evaluate how the exposure coincided with and predicted the presence of an ‘unhealthy’ level of risk factors.

The metabolic syndrome was defined using criteria proposed by the American Heart Association and the National Heart, Lung, and Blood Institute [163], as a clustering of three or more of the following five individual risk factors: high WC, high triglycerides, low HDL, high systolic or diastolic BP, and high triglycerides.

### 3.3.4 CVD event and mortality surveillance

All participants were followed from the date of completion of baseline questionnaire and physical examination until the date of their death or until December 31, 2010. Incident cases of first-time CVD event (fatal or non-fatal myocardial infarction, angina pectoris, or ischemic stroke) and death from any cause were ascertained through regular examinations of the national cause of death registry and the national
in-hospital registry. We could guarantee registration of first CVD events only, as care was taken to exclude participants with a history of CVD in the analysis.

3.3.5 **Statistical considerations in Paper IV**
Logistic regression models were used to assess the OR and 95% CI associated with higher tertiles of NEPA for each individual risk factor as well as for prevalence of metabolic syndrome at baseline. For the prospective analyses, Cox regression models were used to assess the hazard ratio (HR) and 95% CI between higher NEPA tertiles and the risk of a CVD event and mortality from any cause, respectively. Both the baseline and prospective analyses were tested for confounding by sex, marital status, education level, current smoking, regular exercise, dietary intake of vegetables, alcohol intake, self-rated financial status, living conditions, and heredity. To identify possible confounding, univariate models were used for each different outcome. The outcomes (the individual risk factors and metabolic syndrome at baseline, and CVD event and mortality from any cause after follow-up) were included one by one as the dependent variable, and each confounder was included together with the NEPA variable as independent variables. Confounders were regarded as significant and introduced into the main analysis if the 95% CI for the OR or HR did not include 1. However, any that did not remain significant (under the same criterion) after the inclusion of the other significant confounders in the main analysis were then excluded. As the cross-sectional outcomes of this study are commonly present in older adults, and the incidences of the prospective outcomes are rather high, even small significant changes in OR or HR are regarded as clinically meaningful. Kaplan-Meier survival curves were plotted to examine differences in cumulative survival across the cross-tabulated variable of NEPA level and regular exercise. The statistical analyses were conducted using version 21.0 of the SPSS software package (SPSS Inc., Chicago, Illinois, USA).

3.4 **ETHICAL CONSIDERATIONS AND INFORMED CONSENT**
The local ethics committee approved all studies. All participants were fully informed about the details of the studies, and provided written informed consent.
4 RESULTS

4.1 PAPERS I AND II

4.1.1 Characteristics of the study population
A total of 1180 participants (53% women) were included from LIV 90, and 491 (55% women) from LIV 2000. As shown in Table 2, men and women were evenly distributed with regard to age, and BMI and WC indicated prevalence of overweight in both sexes. Mean values are presented for the individual risk factors (top) as well as the proportion above defined cut-off levels (bottom).

Table 2. Characteristics of the study population in papers I and II in relation to sex.

<table>
<thead>
<tr>
<th></th>
<th>Women (n=860)</th>
<th>Men (n=781)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.9 (41.1–42.7)</td>
<td>41.5 (40.6–42.3)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.7 (165.3–166.0)</td>
<td>179.1 (178.6–179.5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.1 (65.4–66.8)</td>
<td>80.5 (79.6–81.3)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.1 (23.9–24.3)</td>
<td>25.1 (24.8–25.3)</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>79.6 (79.0–80.2)</td>
<td>90.9 (90.2–91.7)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>122.8 (121.8–123.9)</td>
<td>128.8 (127.8–129.9)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75.9 (75.2–76.5)</td>
<td>79.2 (78.6–79.9)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.01 (0.97–1.06)</td>
<td>1.37 (1.29–1.45)</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.4 (5.3–5.4)</td>
<td>5.4 (5.4–5.5)</td>
</tr>
<tr>
<td>Antiatherogenic lipid profile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APO A1 (g/l)</td>
<td>1.35 (1.34–1.37)</td>
<td>1.20 (1.18–1.21)</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.60 (1.55–1.64)</td>
<td>1.30 (1.26–1.35)</td>
</tr>
<tr>
<td>Atherogenic lipid profile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APO B (g/l)</td>
<td>1.04 (1.01–1.07)</td>
<td>1.11 (1.08–1.14)</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.14 (3.01–3.27)</td>
<td>3.44 (3.31–3.57)</td>
</tr>
<tr>
<td>PA level (%)</td>
<td>16</td>
<td>23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Daily smokers (%)</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2max&lt;/sub&gt; (ml/kg per min)</td>
<td>34.0 (33.4–34.6)</td>
<td>35.2 (34.5–35.9)</td>
</tr>
<tr>
<td>Proportion above defined cut-off levels&lt;sup&gt;b&lt;/sup&gt; (%)</td>
<td>40</td>
<td>34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Waist circumference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Systolic BP</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Diastolic BP</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Antiatherogenic lipid profile</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Atherogenic lipid profile</td>
<td>34</td>
</tr>
</tbody>
</table>

Continuous characteristics are shown as means (95% CI).
<sup>a</sup>Proportion difference vs. women after 95% CI evaluation.
<sup>b</sup>For cut-off levels, see method section.

4.1.2 Cardiorespiratory fitness, waist circumference, and CVD risk

4.1.2.1 Associations with individual CVD risk factors
As shown in Table 3, both CF and WC were significantly associated with each individual risk factor after adjustment for gender, age, and study (Model 1). After further adjustment for CF and WC, respectively (Model 2), the associations were modified but still significant. Furthermore, significant correlations were revealed between the total number of risk factors and levels of CF (r=−0.28; p < 0.001) and WC (r=0.29; p < 0.001) after adjustment for gender, age, and study. After additional
adjustment for each other, both CF (r=-0.19; p < 0.001) and WC (r=0.21; p < 0.001) were modified to a similar extent.

Table 3. Associations (expressed as OR with 95% CI) for each individual dichotomised risk factor with increasing levels of CF or WC.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Fitness</th>
<th>Waist circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.95 (0.93-0.96)</td>
<td>0.96 (0.94-0.98)</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.94 (0.92-0.96)</td>
<td>0.96 (0.94-0.98)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.93 (0.91-0.95)</td>
<td>0.95 (0.94-0.97)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.97 (0.95-0.98)</td>
<td>0.97 (0.96-0.99)</td>
</tr>
<tr>
<td>Anti-atherogenic lipid profile</td>
<td>0.96 (0.95-0.98)</td>
<td>0.98 (0.96-0.99)</td>
</tr>
<tr>
<td>Atherogenic lipid profile</td>
<td>0.96 (0.95-0.97)</td>
<td>0.97 (0.96-0.99)</td>
</tr>
</tbody>
</table>

Model 1, adjusted for gender, age, and study; Model 2, additionally adjusted for WC or CF, respectively.

4.1.2.2 Associations with clustered CVD risk factor profile
Each higher tertile of CF was associated with lower risk, and each higher tertile of WC was associated with higher risk compared with reference levels after adjustment for gender, age, and study (Table 4). Additional adjustment for educational level, smoking habits, and PA modified the associations only slightly (Model 2). Further adjustments for CF and WC as continuous variables (Model 3) modified the risk somewhat; however, all higher tertiles still remained significantly different from reference level. The fully adjusted model (Model 3) revealed that each unit of CF (ml·min⁻¹·kg⁻¹) was associated with a 5% lower clustered risk and each unit of WC (cm) was associated with a 5% higher clustered risk.

Table 4. OR (95% CI) for having three or more risk factors compared with fewer in relation to different levels of CF and WC.

<table>
<thead>
<tr>
<th>Fitness</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 1</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>0.49 (0.37–0.64)</td>
<td>0.52 (0.39–0.70)</td>
<td>0.68 (0.51–0.92)</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>0.23 (0.16–0.34)</td>
<td>0.27 (0.19–0.40)</td>
<td>0.46 (0.30–0.68)</td>
</tr>
<tr>
<td>Per mL</td>
<td>0.92 (0.91–0.94)</td>
<td>0.93 (0.91–0.95)</td>
<td>0.95 (0.93–0.97)</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Tertile 1</td>
<td>2.38 (1.68–3.37)</td>
<td>2.30 (1.62–3.28)</td>
<td>1.98 (1.38–2.84)</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>5.68 (4.03–8.01)</td>
<td>5.26 (3.71–7.46)</td>
<td>3.75 (2.59–5.43)</td>
</tr>
<tr>
<td>Per cm</td>
<td>1.07 (1.05–1.08)</td>
<td>1.06 (1.05–1.08)</td>
<td>1.05 (1.03–1.06)</td>
</tr>
</tbody>
</table>

Model 1, adjusted for gender, age, and study; Model 2, additionally adjusted for educational level, smoking habits, and PA; Model 3, additionally adjusted for WC or CF (as continuous variables). aCut-off points for CF: women <29.0; 29.0–37.5; >37.5, men <30.0; 30.0–38.6; >38.6 ml·min⁻¹·kg⁻¹. bCut-off points for WC: women <74.0; 74.0–83.0; >83.0, men <86.0, 86.0–95.0; >95.0 cm.

As shown in Figure 3, leaner tertiles were associated with lower clustered risk in both fit and unfit participants. Fitter tertiles were associated with lower risk to some extent in fat and moderately fat participants, but mainly in lean participants. However, being lean and having a high level of CF was associated with a more than 10-fold lower risk compared to being fat and unfit.
**Figure 3.** OR (95% CI) for having three or more risk factors compared with fewer in association with cross-tabulation of tertiles of WC and CF. 1st, 2nd and 3rd tertiles of CF are referred to as unfit, medium (CF) and fit. 1st, 2nd and 3rd tertiles of WC are referred to as fat, medium (fatness) and lean. Adjusted for gender, age, study, educational level, smoking habits and PA. 95% CI fat–medium (CF) (0.44–1.00); fat–fit (0.31–1.23); medium (fatness)–unfit (0.33–0.77); medium (fatness)–medium (CF) (0.20–0.48); medium (fatness)–fit (0.17–0.47); lean–unfit (0.17–0.58); lean–medium (CF) (0.12–0.32); lean–fit (0.04–0.14).

### 4.1.2.3 Cardiorespiratory fitness, waist circumference, and CVD risk in relation to gender, age, and smoking

The clustered risk associated with low CF was similar in men and women; however, higher levels of CF were more strongly associated with reduced CVD risk in women (Table 5). Older participants (>43 years) had a 2.5 times higher risk compared with younger (≤43 years) participants in the lowest CF tertile; that is, for the same absolute low level of CF. However, each higher CF tertile was associated with reduced the risk with similar relative power in younger and older adults. The risk for a fit but old participant was comparable to the risk for a young but unfit participant. Daily smokers (three or more cigarettes/day) had a 1.6-fold higher risk in the low CF tertile compared with non-smokers. Higher levels of CF coincided with lower risk in non-smokers as well as daily smokers, but being a highly fit smoker did not significantly reduce the risk compared to that of an unfit non-smoker.

With regard to WC, men in any WC tertile had a two- to three-fold greater risk than their female counterparts. Young and old participants had similar risk in the slimmest group; however, the risk was more pronounced in the older participants with each higher tertile. Being a daily smoker was associated with a 2.1 times higher risk in the slimmest tertile compared with their non-smoking counterparts, but with no differences in the second or third tertile.
Table 5. OR (95% CI) for having three or more risk factors compared with fewer in relation to CF and WC tertiles, respectively, in women and men (top), in younger and older people (middle), and in non-smokers and daily smokers (bottom).

All analyses were adjusted for (when not being evaluated) gender, age, study, educational level, smoking habits, PA, and WC or CF (as continuous variables).

4.1.3 Physical activity, cardiorespiratory fitness, and CVD risk

4.1.3.1 Associations with individual CVD risk factors
Regardless of CF, high self-reported PA was significantly associated with lower triglycerides and an atherogenic lipid profile (Table 6). Regardless of PA, higher CF levels were associated in a dose-response manner with significantly lower OR for all individual risk factors.

Table 6. OR (95% CI) for different levels of PA and CF, respectively, in relation to reference (low PA and low CF).

Adjustment made for sex, age, educational level, daily smoking, and level of fitness or physical activity. Cut-off points for low and high fitness were 31.8 and 46.0 ml∙min⁻¹∙kg⁻¹ in women aged 20–34; 28.3 and 39.4 ml∙min⁻¹∙kg⁻¹ in women aged 35–49; 22.3 and 32.5 ml∙min⁻¹∙kg⁻¹ in women aged 50–65; 36.1 and 47.5 ml∙min⁻¹∙kg⁻¹ in men aged 20–34; 27.9 and 38.4 ml∙min⁻¹∙kg⁻¹ in men aged 35–49; and 24.5 and 33.5 ml∙min⁻¹∙kg⁻¹ in men aged 50–65.
4.1.3.2 **Associations with clustered CVD risk factor profile**

Figure 4 shows that among unfit participants (low CF), those reporting high PA had a 50% lower clustered CVD risk compared with those reporting low PA. Among fit (high CF) participants, too, a high PA level was associated with a reduced risk. Conversely, regardless of PA level, each higher CF level was gradually correlated with significantly lower OR by half or more. Accordingly, reporting high PA and testing as fit was associated with a more than 10-fold lower OR than being inactive and unfit.

**Figure 4.** OR (95% CI) of having three or more risk factors compared with fewer for the combined effect of PA and CF. Adjustments were made for sex, age, study, educational level, and daily smoking. 95% CI: low CF–medium PA (0.37–1.14); low CF–high PA (0.25–0.99); medium CF–low PA (0.17–0.53); medium CF–medium PA (0.19–0.55); medium CF–high PA (0.13–0.39); high CF–low PA (0.06–0.36); high CF–medium PA (0.05–0.19); high CF–high PA (0.04–0.16).

4.1.3.3 **Excess risk of interaction for neither reporting high physical activity nor being fit**

To further evaluate the importance of being regularly physically active on a moderate intensity level (high PA) and tested as fit, we examined the relative excess risk of interaction (RERI) for neither reporting high PA nor being fit. Significant excess risk of interaction was seen for high WC (RERI = 1.45; 95% CI 0.02–2.88), high triglycerides (RERI = 1.48; 95% CI 0.07–2.90), and the clustered CVD risk (RERI = 1.56; 95% CI 0.26–2.86; adjusting for gender, age, study, educational level, and daily smoking). No significant interactions were seen for the other individual risk factors (data not shown). The proportion attributable to interaction (AP) was 0.25 (95% CI 0.02 to 0.48) for high WC, 0.33 (0.02 to 0.63), for high triglycerides, and 0.30 (0.07 to 0.54) for clustered CVD risk profile.
4.2 PAPER III

4.2.1 Characteristics of the study population

Table 7. Characteristics of the study population in Paper III, in relation to sex.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Men (n = 65)</th>
<th>Women (n = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>43.3</td>
<td>39.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180.2</td>
<td>167.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.0</td>
<td>63.0</td>
</tr>
<tr>
<td>VO(_{2}\max) (L/min)</td>
<td>3.83</td>
<td>2.73</td>
</tr>
<tr>
<td>VO(_{2}\max) (ml/kg/min)</td>
<td>48.8</td>
<td>43.7</td>
</tr>
<tr>
<td>HR(_{max}) (beats/min)</td>
<td>182</td>
<td>183</td>
</tr>
<tr>
<td>RPE on the higher work rate</td>
<td>14.1</td>
<td>14.5</td>
</tr>
<tr>
<td>Reported activity status*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactive/low (%)</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Moderate (%)</td>
<td>32</td>
<td>39</td>
</tr>
<tr>
<td>High (%)</td>
<td>44</td>
<td>45</td>
</tr>
</tbody>
</table>

Continuous characteristics are given as mean and standard deviation.

*Proportion of each sex selecting the given category.

RPE, rating of perceived exertion.

4.2.2 Final regression model for the new test

The final regression model included ∆HR/∆PO score for the second higher individually determined work rate, along with sex and age, to give the best estimate for VO\(_{2}\max\):

\[ \text{VO}_{2}\max = 4.98196 - 2.88618 \times \frac{\text{∆HR/∆PO}}{\text{Sex}} - 0.01712 \times \text{Age} \]

where male = 1 and female = 0 for sex. The adjusted \( R^2 \) was 0.82 for the final model. Sex-specific models did not show any improvement over this model for either the explained variance or the precision.

The mean estimated VO\(_{2}\max\) was 3.23 L ∙ min\(^{-1}\) (SD 0.65) and 3.19 L ∙ min\(^{-1}\) (SD 0.74) for the new test (the Ekblom-Bak test or EB-test; VO\(_{2}\max_{\text{EB-test}}\), and the Å-test (VO\(_{2}\max_{\text{Å-test}}\)), respectively. Mean measured VO\(_{2}\max\) (VO\(_{2}\max_{\text{measured}}\)) was 3.23 L ∙ min\(^{-1}\) (0.72). The mean difference between VO\(_{2}\max_{\text{measured}}\) and VO\(_{2}\max_{\text{Å-test}}\) was -0.018 L ∙ min\(^{-1}\) (95% CI -0.119 to 0.083) for the total sample, though underestimating men (0.37 L ∙ min\(^{-1}\), 95% CI 0.23 to 0.51) and overestimating women (-0.30 L ∙ min\(^{-1}\), 95% CI -0.40 to -0.19). As VO\(_{2}\max_{\text{measured}}\) was the dependent variable for estimation of VO\(_{2}\max_{\text{EB-test}}\), mean VO\(_{2}\max_{\text{EB-test}}\) and VO\(_{2}\max_{\text{measured}}\) were the same and hence mean difference calculations are not relevant.
4.2.3 Measured versus estimated VO$_{2\text{max}}$

Figure 5 shows the estimated VO$_{2\text{max}}$EB-test and VO$_{2\text{max}}$Å-test, respectively, plotted against VO$_{2\text{max}}$measured.

Further internal validation revealed a standard deviation (SD) of 0.30 L·min$^{-1}$ (0.33 L·min$^{-1}$ for men and 0.28 L·min$^{-1}$ for women) for the difference between VO$_{2\text{max}}$measured and estimated VO$_{2\text{max}}$EB-test. The corresponding coefficient of variation (CV) for the EB-test was 9.3% for the total population, 8.5% for men, and 10.3% for women. For the difference between VO$_{2\text{max}}$measured and VO$_{2\text{max}}$Å-test, the SD was 0.59 L·min$^{-1}$ (0.51 L·min$^{-1}$ for men and 0.47 L·min$^{-1}$ for women). The CV was 18.1% for the total population; 13.3% for men and 17.0% for women. We also estimated VO$_{2\text{max}}$ using the Å-test with the maximal HR factor [106], but the precision was still not as good as for the EB-test, with the mean difference between measured and estimated VO$_{2\text{max}}$Å-test maximal HR factor being -0.13 L·min$^{-1}$ (SD 0.47), with a CV of 14.6%. Additionally, evaluating the estimated VO$_{2\text{max}}$ in relative terms (ml·kg$^{-1}$·min$^{-1}$) did not alter the CV for either the EB-test (9.4% for the total population, 8.4% for men, and 10.4% for women) or the Å-test (18.9% for the total population, 14.0% for men, and 17.3% for women).

Differences between VO$_{2\text{max}}$measured and VO$_{2\text{max}}$EB-test did not depend on maximum HR ($r=0.01$, 95% CI -0.16 to 0.17) or deviation from age-predicted maximum HR ($r=0.15$, 95% CI -0.16 to 0.17), but did depend on increased VO$_{2\text{max}}$ ($r=0.42$, 95% CI 0.28 to 0.55). The corresponding differences between VO$_{2\text{max}}$measured and VO$_{2\text{max}}$Å-test depended on maximum HR ($r=0.38$, 95% CI 0.23 to 0.52), deviation from age-predicted maximum HR ($r=0.60$, 95% CI 0.48 to 0.70), and increased VO$_{2\text{max}}$ ($r=0.37$, 95% CI 0.22 to 0.52).

In the method section of this thesis, the use of ΔPO as a proxy for increased VO$_2$ demand above standard rate was justified by the non-significant increased ΔVO$_2$ per ΔPO at higher work rates. To strengthen this further, we analysed the 94 participants that had actual VO$_2$ measured during the submaximal test, and constructed two models including the same variables as the original model presented in the result section, using ΔHR/ΔPO score and ΔHR/ΔVO$_2$ score, respectively. The ΔHR/ΔVO$_2$ model showed the same explained variance and SEE (adjusted $R^2 = 0.78$, SEE = 0.330 L·min$^{-1}$) as the ΔHR/ΔPO model (adjusted $R^2 = 0.78$, SEE = 0.332 L·min$^{-1}$).
4.2.4 Reliability of the new test
Table 8 shows the test-retest reliability over one week.

<table>
<thead>
<tr>
<th>Variable</th>
<th>First occasion</th>
<th>Second occasion</th>
<th>Mean diff (95% CI)</th>
<th>Correlation, r (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR standard load (bpm)</td>
<td>84.3 (11.8)</td>
<td>81.6 (10.8)</td>
<td>2.8 (0.7–4.8)</td>
<td>0.76 (0.62–0.85)</td>
</tr>
<tr>
<td>HR higher load (bpm)</td>
<td>135.9 (15.0)</td>
<td>132.0 (14.8)</td>
<td>3.8 (2.2–5.5)</td>
<td>0.91 (0.85–0.95)</td>
</tr>
<tr>
<td>ΔHR</td>
<td>51.5 (12.8)</td>
<td>50.5 (12.2)</td>
<td>1.1 (–0.6–2.7)</td>
<td>0.88 (0.80–0.92)</td>
</tr>
<tr>
<td>Estimated VO(_2)max</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-test (L/min)</td>
<td>3.14 (0.67)</td>
<td>3.16 (0.69)</td>
<td>–0.02 (–0.07–0.03)</td>
<td>0.96 (0.93–0.98)</td>
</tr>
<tr>
<td>Å-test (L/min)</td>
<td>3.17 (0.80)</td>
<td>3.40 (0.92)</td>
<td>–0.23 (–0.31–0.15)</td>
<td>0.95 (0.91–0.97)</td>
</tr>
</tbody>
</table>

A total of 57 participants had a valid submaximal test at the same workload on both the first and second occasion. The absolute HR at both standard and higher work rate was significantly higher for the first occasion compared with the second occasion. However, mean ΔHR for the first occasion and mean ΔHR for the second occasion were highly correlated with no mean difference. The reliability analysis also revealed a high agreement between estimated VO\(_2\)max\(_{EB}\)-test at the first and second occasions, with no mean difference in estimated VO\(_2\)max between these two occasions. For the Å-test, there was a strong agreement between estimated VO\(_2\)max between the two occasions, but with a significantly higher mean estimated VO\(_2\)max for the second occasion. The test-retest CV was 6.2% for the EB-test and 9.8% for the Å-test.

4.3 PAPER IV
After exclusion of 205 participants with reported myocardial infarction (n=110), heart failure (n=53), and stroke (n=60), and 66 participants with missing data on two or more NEPA activities, 1816 men and 2023 women remained to be included in the analysis.

4.3.1 Cross-sectional analysis
Table 9 shows the characteristics of the study population and different lifestyle variables by NEPA tertiles. In both women and men, higher levels of NEPA were generally associated with more favourable lifestyle profiles. Cross-tabulation analysis revealed a low association between the NEPA tertiles and the dichotomised exercise variable (γ=0.33 for women and γ=0.30 for men). Concerning the individual risk factors, high reported NEPA level was, regardless of regular exercise and other confounding factors, significantly associated with a more preferable profile of WC, HDL cholesterol, and triglycerides in both women and men, and also with insulin, glucose, and fibrinogen in men (Table 10).
Table 9. Characteristics of the study population (top) and commonly recognised favourable lifestyle factors in relation to sex-specific tertiles of NEPA (bottom).

<table>
<thead>
<tr>
<th>NEPA tertiles</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (n = 886)</td>
<td>Moderate (n = 624)</td>
<td>High (n = 513)</td>
</tr>
<tr>
<td>High education</td>
<td>22%</td>
<td>29% a</td>
</tr>
<tr>
<td>Non smoking</td>
<td>74%</td>
<td>78% a</td>
</tr>
<tr>
<td>Regular exercise</td>
<td>19%</td>
<td>30% a</td>
</tr>
<tr>
<td>High intake of vegetables</td>
<td>64%</td>
<td>71% a</td>
</tr>
<tr>
<td>Good perceived general well-being</td>
<td>69%</td>
<td>77% a</td>
</tr>
<tr>
<td>Low intake of alcohol</td>
<td>91%</td>
<td>92%</td>
</tr>
<tr>
<td>Good self-rated financial status</td>
<td>67%</td>
<td>76% a</td>
</tr>
</tbody>
</table>

Continuous characteristics are given as mean and standard deviation.

a Proportion difference vs. Low
b Proportion difference vs. Moderate

Table 10. Odds ratio (95% CI) for different NEPA levels in relation to being at risk for each dichotomised risk factor.

<table>
<thead>
<tr>
<th>Dichotomised risk factors</th>
<th>Women</th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Waist circumference</td>
<td>1</td>
<td>0.90</td>
<td>0.73</td>
<td>1</td>
<td>0.92</td>
<td>0.70</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>1</td>
<td>0.96</td>
<td>1.01</td>
<td>1</td>
<td>1.05</td>
<td>0.90</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>1</td>
<td>0.93</td>
<td>0.94</td>
<td>1</td>
<td>1.09</td>
<td>1.01</td>
</tr>
<tr>
<td>S-HDL-C</td>
<td>1</td>
<td>0.90</td>
<td>0.72</td>
<td>1</td>
<td>0.74</td>
<td>0.65</td>
</tr>
<tr>
<td>S-LDL-C</td>
<td>1</td>
<td>1.27</td>
<td>1.26</td>
<td>1</td>
<td>1.01</td>
<td>1.18</td>
</tr>
<tr>
<td>S-TC</td>
<td>1</td>
<td>1.15</td>
<td>1.03</td>
<td>1</td>
<td>1.27</td>
<td>1.26</td>
</tr>
<tr>
<td>S-Triglycerides</td>
<td>1</td>
<td>0.81</td>
<td>0.68</td>
<td>1</td>
<td>0.77</td>
<td>0.64</td>
</tr>
<tr>
<td>S-Insulin</td>
<td>1</td>
<td>0.97</td>
<td>0.86</td>
<td>1</td>
<td>0.88</td>
<td>0.75</td>
</tr>
<tr>
<td>S-Glucose</td>
<td>1</td>
<td>0.98</td>
<td>0.98</td>
<td>1</td>
<td>0.91</td>
<td>0.72</td>
</tr>
<tr>
<td>P-Fibrinogen</td>
<td>1</td>
<td>0.81</td>
<td>0.78</td>
<td>1</td>
<td>0.65</td>
<td>0.70</td>
</tr>
</tbody>
</table>

BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol. Adjusted for marital status, education level, smoking habits, regular exercise, dietary intake of vegetables, alcohol intake, self-rated financial status, living conditions, and heredity (high blood pressure, dyslipidemia, and diabetes mellitus, respectively).
Regarding the metabolic syndrome, Figure 6 shows the interaction effect between higher levels of NEPA and regular exercise, with the reference group set as low NEPA and no regular exercise. Participants with moderate or high NEPA levels but no regular exercise showed lower ORs than the reference group. Those exercising but with low NEPA showed a lower OR than for the reference group, though this was not significantly different from that of the non-exercisers with higher levels of NEPA. Exercisers with high NEPA levels had the lowest OR.

![Figure 6](image-url)

**Figure 6.** Odds ratios for metabolic syndrome at baseline in relation to tertiles of NEPA and exercise. 95% CIs were 0.65 – 0.98 for non-exercise and moderate NEPA, 0.58 – 0.95 for non-exercise and high NEPA, 0.50 – 0.89 for exercise and low NEPA, 0.56 – 0.97 for exercise and moderate NEPA, and 0.28 – 0.52 for exercise and high NEPA. The analysis was adjusted for sex, marital status, education level, smoking habits, dietary intake of vegetables, alcohol intake, self-rated financial status, and living conditions. The dashed line represents OR=1.

### 4.3.2 Prospective analysis

During the 12.5 years of follow-up, 476 participants experienced a fatal or non-fatal first-time CVD event and 383 deaths were registered from all causes. Figure 7 shows the adjusted hazard ratio (HR) for higher and lower levels of NEPA at baseline in relation to first-time CVD event (Figure 7A) and all-cause mortality (Figure 7B). High NEPA level was associated with a 27% lower HR for CVD event compared with low NEPA, and with a 30% lower HR for all-cause mortality. In further sensitivity analyses, we excluded cases and deaths, respectively, occurring in the first, second, or third year of follow-up, with no significant change in the results.
Figure 7A and B. Hazard ratio for higher levels of NEPA compared with low levels for a first CVD event (Fig 7A) and all-cause mortality (Fig 7B). For CVD event, 95% CIs were 0.69 - 1.07 for moderate NEPA and 0.57 – 0.94 for high NEPA. For all-cause mortality, 95% CIs were 0.67 – 1.08 for moderate NEPA and 0.53 – 0.93 for high NEPA. All analyses were adjusted for sex, marital status, education level, smoking habits, regular exercise, dietary intake of vegetables, alcohol intake, self-rated financial status, and living conditions. CVD event analysis was also adjusted for family history of CVD events. The dashed line represents OR=1.

The cumulative survival across the cross-tabulated variable of NEPA level (low vs. moderate/high) and regular exercise is presented in Figure 8. There was a significant difference in survival probability across the different levels of exercise and NEPA (log-rank $\chi^2=20.81$, df = 3, $p<0.0001$), with the lowest probability seen for those reporting no regular exercise and low NEPA.

Figure 8. Kaplan-Meier survival curves for all-cause mortality across the cross-tabulated variable of NEPA level (low vs. moderate/high) and regular exercise. Log-rank $\chi^2 = 20.81$, df = 3, $p<0.0001$. 
5 DISCUSSION

The main aim of this thesis was to examine the interrelationship between different levels of PA, CF, and abdominal adiposity and their association with CVD risk factors, CVD morbidity, and longevity in Swedish men and women of different ages. A second aim was to develop a new and more precise method for estimation of CF in a mixed, healthy population.

5.1 CARDIORESPIRATORY FITNESS AND WAIST CIRCUMFERENCE

The present results suggest that higher CF and lower WC are independently associated, to a similar extent, with a lower CVD risk in this population. More specifically, for the individual risk factors, each unit of higher CF (ml·min⁻¹·kg⁻¹) was associated with a decreased risk ranging from 2% to 5%, independent of WC. Each unit of higher WC (cm) was associated with an increased risk ranging from 2% to 5%, independent of CF. For the clustered CVD risk factor profile, each unit of CF was associated with a 5% decrease in risk and each unit of WC with a 5% increase in risk. This was maintained in men as well as women, younger as well as older people, and daily smokers as well as non-smokers; however, some differences were seen within the subgroups.

As shown in Figure 3, unfit participants benefited from a threefold lower CVD risk if they were lean rather than fat. Conversely, participants who were fat but more fit also benefited from a lower CVD risk than their unfit counterparts. The non-significance of the association seen for fat but fit participants may be due to the lower number of participants (n=54) in this subgroup compared with the other subgroups. Further, similar trends were also seen in more preferable subgroups: leaner tertiles were associated with lower risk in already fit participants, and higher CF level induced lower risk in already lean participants. This strengthens the previous findings of an independent contribution of CF and abdominal obesity to CVD risk, while not singling out either variable as more important than the other. It rather implies that unfit and/or fat participants should be encouraged to become more fit and/or reduce their abdominal obesity.

5.1.1 Previous knowledge

There is extensive previous research on the independent effects of CF and overweight/obesity on CVD and mortality. Some studies have suggested that higher levels of PA or CF offset the risk of premature death associated with overweight and obesity in adults [126-128]. Others claim that PA/CF may not completely eliminate the negative effects [130, 131] or that they might be considered as two independent risk contributors [132, 133]. Regarding CVD risk factors, previous research has mainly used BMI as a measure of obesity [164, 165]. Studies evaluating abdominal obesity are few, and the results are equivocal. Christou and co-workers showed in a sample of 135 healthy men aged 20 to 79 years that WC was strongly and consistently associated with metabolic and haemodynamic risk factors (the same as those evaluated in this thesis) after adjustment for CF, but there was no significant association with CF after adjustment for WC [134]. Further, prospective data on 393 healthy middle-aged men and women showed that both change in PA energy expenditure (PAEE) and WC after 5.6 years were associated with change in risk factor profile; however, the magnitude of WC change was two to three times stronger than for PAEE [124]. Conversely, Lee and co-workers reported that high levels of CF were associated with a substantial reduction in metabolic risk for a given level of WC [166]. The results in this thesis are in concordance with previous cross-sectional data,
which suggests a similar independent importance of CF and WC for risk factors [167]. Borodulin and co-workers examined the association of CF estimated from a non-exercise test and waist-to-hip ratio on CVD risk factors in 3820 adults (53% women) aged 20 to 64 years from the FINRISK 2002 Study [168]. They concluded that a good CF and a low waist-to-hip ratio were each independently associated with a better risk factor profile (total cholesterol, HDL, triglycerides, systolic BP, and diastolic BT). In addition, a recent prospective study examined the change in CF and % body fat on the development of CVD risk factors during a 6-year follow-up in 3185 healthy adults [136], and concluded that both maintaining and/or improving CF and preventing fat gain is important to reduce the risk of developing CVD risk factors.

5.1.2 Cardiorespiratory fitness and waist circumference on CVD risk in relation to age, gender, and smoking habits

Several important findings are shown in Table 5 in the result section. Firstly, male gender seemed to be disadvantageous for the independent associations of CF and abdominal obesity with the clustered CVD risk factor profile. This has previously been shown for all-cause mortality and CVD [89, 111, 133]. Jensen and co-workers found a stronger hazard ratio reduction for acute coronary syndrome with higher levels of PA in women compared to men [169]. However, for CVD risk factors, reports on gender differences are limited, with mainly gender-specific rather than comparative analysis [124, 168].

Secondly, even though older adults (>43 years) had 2.5 times higher clustered risk in the lowest CF tertile compared with younger adults (≤ 43 years), the relative risk reduction with higher CF was similar in younger and older participants. This may illuminate the increased absolute risk that goes along with ageing, but conversely, it also suggests that PA resulting in higher CF influences risk with a similar relative impact in adults regardless of age. This is consistent with a recent study on PA and telomere length (a marker for biological ageing), which showed that younger and older participants active on a comparably high level did not differ in telomere length [170].

A third important finding shown in Table 5 is that neither a higher CF level nor a lean body reduces the hazard of smoking. Lean smokers were associated with a two-fold higher risk compared with their non-smoking counterparts. A fit smoker did not have a significantly reduced risk of CVD compared to an unfit non-smoker. These findings agree with previous observations that daily smokers benefit from higher PA levels, but that activity cannot diminish the risk associated with smoking per se to the same levels as that of non-smokers [171]. Lee and co-workers studied the combined effect of three lifestyle factors (being moderate or highly fit, having a waist girth of < 94 cm, and not smoking) on morbidity and mortality in a large cohort of middle-aged and older individuals followed prospectively for 14.7 years [172]. The results indicated a dose–response relationship between increasing number of favourable risk factors (0 to all 3) and lower risk of CHD event, CVD mortality, and all-cause mortality. Hence, fatness, low CF, and smoking seem to affect CVD risk and mortality in different ways. Therefore, aside from increment in CF and reduction of WC, cessation is the only option for further risk reduction in smokers.

5.1.3 Low cardiorespiratory fitness and body fat often in combination

An important issue to consider is that low CF and excess body fat often occur in combination, and that some of the favourable associations of high CF may be
attributable to leanness and vice versa. Tables 3 and 4 in the results section illustrate the importance of taking both variables, CF and WC, into consideration. Significant modification of risk associations was revealed for the individual CVD risk factors as well as for the clustered CVD risk factor profile after adjustment for CF and WC, respectively. These reduced risk associations indicate that both variables may act as intermediate factors in the causal chain from the other factor to the risk factors. However, as significant risk associations remained for both variables after adjustment for each other, it seems that both variables also have an effect which is mediated through other mechanisms. Inclusion of lifestyle factors (Table 4, Model 2) only contributed a slight modification. This may be because the self-reported questionnaire responses were weaker and less precise than the objectively obtained measurements of CF and WC [173]. Not including appropriate measurements of both CF and fatness in future analyses may cause loss of important information and misinterpretation of the results.

5.1.4 Possible mechanisms
One central explanation of the strong cross-sectional relationship between PA/CF and obesity is their reciprocal relationship; PA makes weight control easier as it increases EE and may have a beneficial effect on appetite control and food intake [174, 175], while unsuccessful weight control may reduce the possibility of engaging in PA. However, the role of PA in weight reduction and weight gain prevention without dietary restrictions in long-term interventions is debatable, as studies have shown only modest effects [176]. As PA is the major modifiable variable for regulation of the output part in the energy balance equation, this uncertainty in the role of PA in weight control is probably due to other circumstances. Thomas and co-workers reviewed the area, and concluded that the small magnitude of weight loss observed from the majority of the exercise interventions was primarily due to too low doses of prescribed exercise EE and a simultaneous increase in caloric intake [177]. This conclusion elucidates the difficulty of incorporating a sufficient amount of PA in daily life, and simultaneously of avoiding compensating the increase in EE by an increase in caloric intake. To further illustrate this, Church and co-workers concluded that jobs requiring higher EE had decreased in U.S. private industry over the last five decades, resulting in an average decrease of 130 kcal in daily occupation-related EE [178]. Subsequent energy balance modelling showed that this EE reduction could account for a significant proportion of the increase in mean body weight in the U.S. during recent decades. Hence, with sufficient levels of PA and with diet intake kept constant, PA has an important beneficial effect on weight status [179].

5.1.4.1 Brown adipose tissue
Approximately 80% of the variation in daily EE can be explained by fat free mass (FFM) [180]. More active individuals will preserve or increase their FFM, with a subsequent impact on their total daily EE. However, there is a significant unexplained variability in EE between individuals with similar FFM. One possible source of this unexplained variation between individuals beyond variation in FFM could be intra-individual variation in basal metabolic rate due to variation in mitochondrial oxygen affinity (p50_mito) [158]. Another is the recently highlighted thermogenic brown adipose tissue (BAT). BAT was initially considered to only play a significant role in newborn babies as a heat generator for preserving core body temperature, with significant reduction in volume and function with age. More recent studies have established the presence of fully functional BAT in adult humans, mainly located in the upper chest and neck region [181, 182]. BAT possesses large numbers of mitochondria, and BAT substrate metabolism is estimated to consist of approximately
90% free fatty acids and 10% glucose [183], released as heat. BAT is responsible for adaptive thermogenesis in response to exposure to cold temperatures (non-shivering thermogenesis) and high energy intake, but also to be activated by the sympathetic nervous system through norepinephrine-stimulation of beta-3 adrenergic receptor (β3-AR) [184]. However, while this receptor is identified as a major receptor in rodents, its role in human BAT is not fully clear. The presence of BAT in the population is reported within a wide range, from 5% up to 90%, and is more frequently seen in younger individuals and non-smokers [181]. The age gradient could to some extent work in parallel with the tendency to gain weight during aging. Women tend to have higher quantities of BAT, but the significance of this is unclear as women do not have higher EE than men (standardised for FFM). The difference in BAT between lean and obese individuals is debated. Some studies have found similar BAT volumes between obese and lean individuals, but significantly lower BAT activity in the obese in response to similar stimuli [181, 182]. This under-stimulated BAT in obese people might go some way towards explaining why they have higher body weights.

Although the development and regulation of BAT in humans is still largely unclear, Boström and co-workers recently reported the discovery of the myokine irisin. Irisin is suggested to stimulate the browning of white fat and to drive an increment in EE with no changes in PA movement or food intake [185]. Irisin itself is secreted from the skeletal muscle in response to muscular contraction, and is known to be significantly associated with anthropometric, metabolic, and hormonal parameters [186]. Interestingly, Swick and co-workers reported that in 17 post-menopausal overweight and obese women, irisin level correlated highly with EE among women with EE greater than predicted by FFM [187]. Although the contribution of irisin as a single, significantly important exercise-modulated protein for BAT development and function has been challenged [188], and much uncertainty still remains over the contribution of BAT metabolism to total body EE in humans, BAT has been suggested to serve as a potential target for treatment and prevention of obesity and other metabolic disorders [184]. One thought is that BAT may provide a further possibility to enhance EE as a result of PA; apart from EE due to actual PA performance or as a result of increased muscle mass, the muscular contraction would result in a release of irisin, with a subsequent impact on BAT development and EE through heat production.

### 5.1.4.2 Regional fat distribution

Altering of regional adipose tissue may be another important mechanism in the health-protective effect of CF. Visceral adipose tissue (VAT) is regarded as a more harmful fat depot, while subcutaneous adipose tissue (SAT), both in the abdomen and in the thighs, may be a protective fat depot [189]. Amati and co-workers studied the interrelationship between abdominal VAT and thigh SAT and their association with insulin resistance [190]. Their data showed that excess abdominal VAT was associated with insulin resistance across a range of middle-aged to older men and women, while higher levels of thigh SAT was favourably associated with insulin sensitivity. VAT has also been shown to be lower in fit than unfit men of a given BMI [191], even in those regarded as fat [192]. In the latter study, the authors reported that the ratio between abdominal VAT and SAT was approximately 0.5 in slim-and-fit individuals as well as their fat-and-fit counterparts. However, for slim-and-unfit and fat-and-unfit individuals the ratio was closer to 1.0. This implies that higher CF/exercise has an important beneficial effect on preferable fat storage, regardless of WC. It also strengthens the importance of evaluating both WC and CF in clinical practice, as WC alone cannot distinguish between visceral and
subcutaneous abdominal depots. Available evidence also suggests that exercise and higher CF levels may be associated with lower VAT/reduction in VAT regardless of weight/weight loss in obese adults and the general population [193]. Moreover, Lee and co-workers showed that a more beneficial CVD risk profile (with lower triglycerides and higher HDL) was seen in fit men of a given waist girth or amount of visceral fat [166].

5.1.4.3 Healthy obese – the fat but fit concept
The metabolic profile is generally better in normal-weight adults. However, a report from the National Health and Nutrition Examination Study (NHANES) of adults in the U.S revealed that approximately 25% of the normal-weight adults were metabolically abnormal (with clustering of metabolic risk factors) and just above 30% of the obese adults were metabolically healthy [194]. The metabolically healthy individuals in the report, regardless of fatness, were more likely to be physically active. This has previously been highlighted and conceptualised as the “fat but fit” phenomenon; that is, the fact that obese but fit individuals have a lower risk of type 2 diabetes, CVD, and mortality than lean but unfit individuals [126]. For example, Farrell and co-workers found that in 11 335 women followed for 12.3 years, high CF was associated with lower mortality with each category of all adiposity measures evaluated (BMI, WC, waist-to-height ratio, waist-to-hip ratio, and % body fat), and that rates of all-cause mortality among fit-and-overweight/obese women were not significantly different from mortality rates among fit-and-normal-weight women [129].

The fat but fit phenomenon might be especially relevant for ectopic fatness. Ectopic fat is the accumulation of lipids in non-adipose tissue such as the liver and the skeletal muscle. This occurs when the adipocytes or associated fat storages are unable to expand further or have become insulin resistant, which limits the individual’s capacity to store more fat and results in an excess of lipids in the blood flow. As exercise-induced improvements in glucose metabolism and insulin sensitivity have been reported regardless of fatness and in the absence of weight loss [193], fat but fit individuals are less likely to be exposed to this overflow of lipids, and have rather the capacity to store the excess of fat in the more insulin-sensitive subcutaneous adipose tissue [195]. Stefan and co-workers found that obese individuals who were insulin sensitive had significantly lower levels of VAT compared to obese individuals who were insulin resistant [196]. Subsequently, cross-sectional as well as prospective studies have found that CF or CF change is associated with a better lipoprotein profile independently of fatness or change in fatness [136, 166, 197]. Moreover, with new, more advanced techniques, it is now possible to not only analyse the “traditional” lipid profile, but also to examine lipoprotein subfraction particle concentration, the size of these, and their composition. Halverstadt and co-workers showed that 24 weeks of moderate endurance exercise training in 100 middle-aged men and women (3 times per week, progressing from 20 to 40 minutes of moderate intensity) induced favourable changes in VO₂max. However, most importantly, there were improvements in lipids and lipoprotein concentrations, increment of HDL particle size, and decrement in the concentration of small LDL particles; all regardless of diet and baseline or change in body fat [198]. Metabolically healthy obese people may also have a lean tissue which is able to maintain or alter the fat oxidation along with fluctuations in triglycerides levels.

Other suggested important mechanisms in the fat but fit concept are the improvements in vascular function and reductions of inflammatory markers seen with
higher PA or CF independent of fatness. Lee and co-workers showed that incident hypertension was lower in those who maintained or increased their CF during a 6-year follow-up, adjusted for change in fatness [136]. Hamer showed in a review of 40 observational studies that two thirds of the studies reported an inverse relationship between inflammatory factors (including fibrinogen, cytokines, and CRP) and CF after adjustment for fatness [199]. However, Hamer also concluded that the present literature has not yet provided consistent causal evidence to support the independent association between CF and inflammation.

### 5.1.4.4 Independent mechanisms of fatness
Although several potential health hazards of obesity may be ameliorated by higher CF or exercise levels, they are not totally eliminated. Important associations of fatness and traditional risk factors as well as novel inflammatory biomarkers still remain to some extent independently of CF [136, 199]. One of the central mechanisms is the low-grade inflammation induced by excess adipose tissue, especially the ectopic fat depots. With increased obesity, the adipocytes enlarge and increase the recruitment of macrophages and promote inflammation with a high expression of pro-inflammatory cytokines. One interesting aspect is that the role of fatness in relation to CF and inflammatory pathways seems to be especially important in women [199]. The impact of sex hormones and an observed stronger association between adiposity and low-grade systemic inflammation in women are two possible mechanistic explanations. Obesity-induced hypertension is another central link, which is due to factors such as an increase in sympathetic nervous system activity, renal sodium retention, and systemic vascular resistance [200]. Insulin resistance, oxidative stress, elevated fatty acids, and atherogenic dyslipemia are other suggested mechanisms linking obesity to adverse metabolic health [195].

### 5.2 PHYSICAL ACTIVITY AND CARDIORESPIRATORY FITNESS
The present results suggest that higher PA and higher CF (but mainly the latter) are independently associated with lower risk of individual risk factors as well as clustered CVD risk factor profile. Regardless of CF, high in comparison to low PA level was associated with lower triglycerides and a beneficial atherogenic lipid profile. For the clustered CVD risk factor profile, unfit individuals reporting high PA had a 50% lower OR compared with unfit individuals reporting low PA. Among fit participants, higher PA level was also associated with a favourable risk factor profile. Conversely, regardless of PA, both moderate and high CF were associated with lower OR for all individual risk factors, in comparison to low CF. The distinct pattern was also reflected for the clustered CVD risk factor profile, with a higher CF level producing a significantly lower OR in all PA groups.

#### 5.2.1 Excess risk of interaction
We also found a significant relative excess risk due to interaction between neither reporting high PA (i.e. not fulfilling national guideline recommendations) nor being tested as fit, for high WC and high triglycerides, but most interestingly, for the clustered CVD risk factor profile. As far as we are aware, this has not been reported previously. More relevantly, we also calculated the proportion attributable to interaction, and concluded that 25% of the cases of high WC, 33% of those with high triglycerides, and 30% of those with clustered CVD risk profile were attributable to the interaction between the two adverse exposures. These findings add a new important implication to consider, and further illustrate the importance of evaluating both PA and CF.
5.2.2 Previous knowledge

This is, to our knowledge, the first study assessing risk estimations of PA and CF for CVD risk factors in a random general population sample. The independent importance of CF for individual risk factors has previously been stressed in cross-sectional studies [93, 122, 201]. Prospective data has suggested that to reduce CVD risk factors, an increase in aerobic power appears to be more important than just becoming more active [95]. Although previous findings of the independent importance of PA in the above mentioned studies were few or inconsistent, two recent prospective studies have shown, in agreement with the present study, improvements in risk factors after prolonged low-intensity training [202] and increase in PAEE from baseline [124], even in the absence of improved CF. In the latter study, Ekelund and co-workers found that in a population-based sample of middle-aged men and women followed prospectively for 5.6 years, increasing levels of PA were associated with significant improvements in triglycerides, insulin sensitivity, glucose tolerance, and clustered metabolic risk, regardless of changes in CF, adiposity, or baseline PA.

For the clustered CVD risk, similar independent associations with CF as demonstrated in the present thesis have previously been established for risk factor profile [122], the metabolic syndrome [96], CHD risk [203] and all-cause mortality [89, 204]. Conversely, being very fit has been shown to provide no protection against ischemic heart disease in sedentary men [205]. The present findings of separate associations between high self-reported PA and lower CVD risk in both fit and unfit participants have previously mainly been reported in unfit participants [96, 206]. Laaksonen and co-workers [96] found a reduced number of incident cases of the metabolic syndrome in unfit middle-aged Finnish men with higher PA levels, and only minor effects of PA in fit participants. Franks and co-workers [206] reported a strong inverse relationship between PA and the metabolic syndrome in middle-aged British men and women, and this relationship was much steeper in unfit participants. This additional effect of PA suggests that even slightly increased PA levels which do not result in increased CF are associated with lower CVD risk. It also illuminates the importance of simultaneously evaluating both PA and CF to maximise the predictive power of a health evaluation and minimise misclassifications of disease risk. In the present study, 15% of women and 14% of men reported being inactive (low PA), but tested as highly fit (quartile 4), while 16% of women and 15% of men reported being highly active (high PA), but tested as unfit (quartile 1). The sizeable discrepancy from the logical congruence between PA and CF in this general population sample further highlights this notable and important issue. Unless adjustments are made for PA level, unfit individuals will be told that they are at high risk of disease, and advised to increase their activity level. This could lead to an unnecessary sense of worry in those individuals who are sufficiently physically active but due to low CF response to activity or subclinical medical conditions are still regarded as unfit.

5.2.3 Is there a true separate effect of physical activity and cardiorespiratory fitness?

According to the present results as well as most previous studies in this area, PA and CF seem to have somewhat separate associations with CVD risk and longevity, with a much stronger association for CF. However, the question of whether true separate effects of PA and CF really exist, or whether CF should just be considered as a more reliable surrogate for recent level of PA, is a complicated one which depends on many factors. Primarily, one must consider that PA and CF have evident differences in characteristics and area of influence. PA is a self-imposed action which results in increased EE and has effects on various bodily functions and systems. CF refers to
the capacity of the circulatory and respiratory systems to meet the demands of the active bodily tissue; it reflects the actual status of these systems, but is largely dependent on recent PA habits.

5.2.3.1 Methods of measurement
Another potential explanation is the divergent methods used to measure the components. When PA is measured in a weak and imprecise way through subjectively conducted self-report questionnaire responses, more false negative associations with health outcomes can be expected compared with more standardised and objective measurements of CF, such as those obtained with stress tests [173]. The inherent misclassification of PA by questionnaire responses hence reduces the apparent benefits from PA. This is a central reason why, for example, increasing tertiles or quartiles of CF have a more distinct influence on health and longevity compared with analysis of PA classified into corresponding groups. Low correlations are also often observed between PA and CF, with \( r \) ranging from 0.02 to 0.44 in a review of 75 studies [207]; similarly, in this study, we found correlations of \( r=0.24 \) in women and \( r=0.28 \) in men. A study using more standardised, ongoing recording data of PA showed stronger correlations between PA and CF \( (r=0.66-0.83) \) [208]. In the abovementioned study by Ekelund and co-workers that uniquely showed an evidential independent effect of PA on both individual and clustered risk factors regardless of CF or fatness [124], PA was measured objectively by the flex-heart rate method. In addition, Celis-Morales and co-workers compared objectively measured accelerometer-derived PA and self-reported PA (via IPAQ) and their relationship with conventional metabolic and vascular risk factors [209]. Their data suggested that using IPAQ to estimate PA may result in failure to detect real relationships with several of the risk factors (which were apparent when PA was assessed by accelerometer) or to underestimate the strength of those relationships. These differences in results clearly illustrate the methodological importance of using more valid methods for measuring PA.

5.2.3.2 Genetics and intrinsic prerequisites
PA, CF, and health outcomes are all influenced by genetic endowment. There is great variation in the heritable contribution, suggested to be at least 25-35% for all three variables [125]. Improvements in CF are linked to inheritance of CF phenotypes [210] as well as cardiovascular pathology [211]. For example, studies by Bouchard and co-workers have shown that an increase in PA to the same volume, but adjusted for individual tolerance level, produced an increase in \( \text{VO}_2\text{max} \) and changes in risk factors [87]. However, the adaptation varied widely, with a strong familial aggregation, and genetics accounting for 25-40% of the change, regardless of initial level of \( \text{VO}_2\text{max} \).

The idea of CF being a separate entity with an important effect on metabolic health, rather than just a surrogate measure of PA habits, has been further tested using specially bred rats. In 2005, Wisløff and co-workers presented their original report using the 11\(^{th}\) generation of rats selectively bred for being either high (HCR) or low (LCR) capacity runners [212]. LCR had the capacity to run for on average 191 meters (14.3 minutes) until exhaustion, while the HCR averaged 853 meters (41.6 minutes); a difference of 347% in running capacity. The main findings were that the LCR had higher blood pressure, visceral adiposity, fasting glucose, insulin, triglycerides, and free fatty acids. Conversely, the HCR had better endothelial function, and higher levels of skeletal muscle oxidative enzymes and proteins known to be essential for mitochondrial function (such as PGC-1\(\alpha\) and PPAR-\(\gamma\)). As these rats were not
allowed to exercise, the differences were largely dependent on individual intrinsic physiological variance attained from the selective breeding. The authors suggested that the impairment of mitochondrial function may be a vital link between lower CF and metabolic and cardiovascular disease. In a recent report from 2013, the same research group investigated the effects after 8 weeks of treadmill training in the 15th generation of the HCR and LCR rats [213]. Before exercise training, the HCR and LCR had similar aerobic exercise capacity. However, after the 8 weeks of training, the LCR had not increased their capacity while the HCR improved theirs by 54%. Subsequently, the authors found that the aerobic-non-responding LCR had a prominent metabolic dysfunction (insulin resistance, inflammation, and increased adiposity). Interestingly, in contrast to the earlier findings, the trained LCR had a normal increase in mitochondrial capacity with exercise. The results hence indicated that impaired mitochondrial function was not responsible for either the non-response in aerobic capacity or the metabolic dysfunction. Rather, the authors found that LCR had impaired exercise-induced angiogenesis in skeletal muscle, and suggested that “the supply side of aerobic energy transfer is limiting to exercise capacity, rather than the demand side”. The conclusion from these studies on bred rats is that there is variation in intrinsic physiological factors which may determine the oxidative capacity of the skeletal muscle and CF level, and may impact metabolic health regardless of PA level.

5.2.3.3 **Skeletal muscle fibre types**

Although care should be taken in applying results from rat models in humans, these models provide important hints on where to search for possible mechanisms explaining the differences between fit and unfit humans [214]. One interesting example of this is the oxidative capacity of the skeletal muscle, which is closely related to the individual variation in type I (high oxidative capacity) and type II (high glycolytic capacity) fibres. Individuals with a high prevalence of type I fibres are considered to be better suited for endurance activities, while individuals with predominantly type II fibres are selected for more power-orientated activities. Previous studies have shown that high presence of type II fibres is closely linked to weight gain, CHD risk factors, increased left ventricular mass, and low CF level [215, 216]. Interestingly, type I fibres accounted for approximately 7% of the total fibres in the LCR rats discussed above, compared to more than 20% in the HCR rats. This has led to a hypothesis that the low-fitness category of individuals is composed of two distinct types [214]. On one hand, there are individuals who have low fitness due to a physically inactive lifestyle, but otherwise have a normal metabolic healthy muscle composition which has no further impact on their health risk. On the other hand, there are low-fitness individuals with disadvantageously constituted skeletal muscles, affecting both their metabolism and their behaviour. These individuals may be selected for an inactive lifestyle due to a low oxidative capacity and a sense of discouragement when they fail to benefit from PA. This leads to an exaggerated metabolic risk from being both inactive and with an unfavourable intrinsic muscle profile. Encouragingly, Chomistek and co-workers showed in a large prospective cohort of middle-aged women that genes associated with physical fitness did not modify the inverse relationship between PA and coronary heart disease [217]. Their results hence suggest that genetic predisposition which influences baseline CF or the capability to improve CF from exercise training did not prevent PA from producing benefits on CVD risk.

In summary, there seems to be some evidence that genetic variance may have an important impact on both CF and PA. However, the genetic component should be
seen as a natural element that continues to be a factor throughout life, and does not reduce the importance of CF as a predictor for health or PA as a part of a healthy lifestyle [125]. In the future, it is important to define the mechanisms responsible for the high risk associated with the low-fitness phenotype, to be able to identify pre-determined low-fitness individuals and design well-suited training programs to maximise the effect on these individuals’ physical capacity and health [214]. Another important issue to consider here is the recently-highlighted separate effect of NEPA and avoidance of SB (see below). These aspects of the daily movement pattern have shown important effects on metabolic and vascular health as well as longevity, but correspond to such light intensity levels that for most people they will have no effect on CF level. Hence, PA and CF should be seen as two partly different important measures that each contain important aspects; they cannot substitute for each other, and hence they may differ in their effect on disease risk.

5.3 NON-EXERCISE PHYSICAL ACTIVITY AND SEDENTARY BEHAVIOUR

A 12.5-year follow-up in a sample of 60-year-old Swedish men and women revealed that a generally active daily life, regardless of regular exercise habits, reduced the risk of a first-time CVD event by 27% and that of all-cause mortality of 30%, in comparison to low daily activity. Sensitivity analysis revealed that the results remained unchanged after exclusion of cases and deaths, respectively, occurring in the first three years, minimising potential reverse causality issues. At baseline, the association with metabolic syndrome was significantly lower for those reporting higher NEPA levels in both the non-exercising and the regularly exercising group. High NEPA was also associated with a more preferable profile of WC, HDL, and triglycerides in both sexes, and insulin, glucose, and fibrinogen in men.

5.3.1 Previous knowledge

Previous cross-sectional studies have revealed associations between NEPA (or LIPA) and the same metabolic risk factors as in the present thesis. Healy and co-workers monitored the daily activity pattern with objective accelerometer measurements in a sample of Australian men and women (n=153, mean age 53.3 years). They found that independent of time spent in MVPA, there were significant beneficial associations between time spent in LIPA and WC, clustered metabolic risk [65] and 2-h plasma glucose [218], as well as borderline associations with triglycerides and HDL [65]. Moreover, Camhi and co-workers found that in 1371 men and women (mean age 47.1 years), independently of MVPA, greater time spent in lifestyle activity was associated with lower OR for elevated triglycerides, low HDL, elevated WC, the metabolic syndrome, and diabetes [219]. In addition, Sisson and co-workers found that in 1446 men and women (mean age 47.5 years), as steps taken per day increased, the OR was significantly lower for high WC, low HDL, high triglycerides, and the metabolic syndrome [66]. Further, two experimental studies indicated adverse metabolic health effects after reducing NEPA in both exercising and non-exercising young men and women [220, 221]. Interestingly, neither the present thesis nor the above mentioned studies found any associations between NEPA/LIPA and systolic or diastolic blood pressure. This might reflect that while NEPA/LIPA has important metabolic effects, a higher intensity might be needed to have effect on blood pressure. Another interesting finding was the beneficial association between high NEPA and fibrinogen in the present thesis (significant association in men, and borderline in women). As a higher fibrinogen level has been shown to have a moderately strong association with CVD risk and all-cause mortality [222], these findings may be an important link to the
prospective results of lower risk of CVD events and all-cause mortality in the high NEPA group.

The prospective results of this study are in line with previous research in older adults [67, 69]. A meta-analytic review including eight studies found an integrated cardiovascular risk reduction of 11% associated with active commuting (walking and cycling) compared to non-active commuting (mainly by car) [68]. Further, a meta-analysis found that the all-cause mortality risk was 36% lower for the highest level of PA of daily living compared with the lowest [70]. The authors of a systemic review concluded that the largest benefit was found from moving from no activity to low levels of activity [223]. As strong inverse associations (r=-0.96) are reported between LIPA and sedentary time [65], the present prospective results are comparable to studies examining the effects of prolonged sitting. For example, in a recent systematic review including 794 577 participants, Wilmot and co-workers found that the greatest sedentary time compared with the lowest was associated with a 147% increase in cardiovascular event RR, and a 49% increase in that of all-cause mortality [224]. In the light of a recent report in the Lancet which revealed high sitting time in older adults especially, the present results seem particularly relevant in that they show a connection between a higher level of NEPA and a lower risk for CVD event and mortality [225].

5.3.2 Separate behaviours
The associations between NEPA and cardiovascular health and longevity, regardless of intentional exercise habits, should be highlighted. As it is widely known that regular exercise has a major impact on health, our findings have high clinical significance. Epidemiological studies have implied that in today's society it is not only possible, but very common, to exercise regularly yet be highly sedentary during the day, an "active couch potato" [226]. The present results also show a rather low association between NEPA and exercise. Finni and co-workers studied how time in SB and NEPA varied between days with and without intentional exercise by measuring EMG activity in the quadriceps and hamstring muscle [227]. They found that a day including exercise did not significantly alter the time distribution between SB and NEPA, compared to a day without exercise.

5.3.3 Potential mechanisms
Potential mechanisms to explain the observed independent importance of NEPA are largely interchangeable with the reversed proposed mechanisms of prolonged sitting.

5.3.3.1 Energy expenditure
One important mechanism is linked to EE; prolonged sitting results in low EE close to the basal metabolic rate, while standing up and engaging in NEPA multiplies it [228]. Therefore, variation in NEPA is suggested to play a significant role in the maintenance of energy balance and in regulating body fat storage. Levine and co-workers found different NEPA patterns during the day between lean and obese individuals [229]. The latter spent on average 2 hours more sitting compared with their lean counterparts, who instead performed light-intensity NEPA such as standing and walking. The authors concluded that if the obese individuals adopted the NEPA behaviour of their lean counterparts, they could have expended an additional 350 kcal per day. Moreover, comparisons of different daily movement patterns have shown that the daily energy expended in activity for standing or ambulatory workers might be double the energy expended in seated workers [63]. The cumulative number of thousands of daily muscle contractions during NEPA, compared to the inactive
muscle while sitting, simply produces a much greater energy demand. As referred to previously in the discussion section of this thesis, the importance of daily NEPA for long-term energy balance has also been shown on a greater scale in middle-aged adults in the U.S [178]. Moreover, a study among healthy, normally active men revealed that a reduction in daily steps taken from an average 10 501 to 1344 over 2 weeks resulted in a significant increase in intra-abdominal fat and impairment of other important metabolic markers (with habitual dietary intake kept constant) [230]. Even if the model in that study reflects deconditioning effects rather than the situation among inactive, highly sedentary adults over a longer period, it still provides valuable insights.

WC is considered to be a reliable surrogate for the highly hormonally active visceral adipose tissue, which as described earlier in this thesis, has been linked to several pathological conditions. The baseline association between lower levels of NEPA, an adverse lipid profile, and the metabolic syndrome may partly be explained by the intermediating effect of higher amounts of visceral fat mass. This is strengthened by the recently-introduced hypothesis of “the diseasome of physical inactivity”, where physical inactivity is suggested to play an important, independent role for accumulation of visceral fat, followed by activation of a network of systematic inflammatory pathways, and subsequent promotion of conditions such as insulin resistance and atherosclerosis [231].

However, it is reasonable to assume that some of the negative metabolic associations with low levels of NEPA might be due to energy surplus, as energy surplus has for example been shown to reduce insulin action [232]. Stephens and co-workers showed that whole-body insulin action was significantly reduced following one day of sitting in energy surplus compared to one day of standing in energy balance [221]. When the same individuals were re-tested for one day of sitting, but with energy intake reduced to match expenditure, the effect was reduced but not prevented. The authors concluded that the negative metabolic effects related to prolonged sitting do not depend solely on energy surplus, and as the effect was only present for whole-body insulin action and not for hepatic insulin action, the metabolic effects are most likely manifested and mediated in the skeletal muscle.

5.3.3.2 The skeletal muscle and the myokine concept
Another potential mechanism linked to the skeletal muscle is the concept of myokines [233]. The skeletal muscle, just like the adipose tissue, should be considered as an endocrine organ; and cytokines and other peptides produced and released by the contracting muscle fibres (which have paracrine as well as endocrine effects in the body) should be classified as myokines. Lack of muscular contractions as a consequence of sitting still will undermine the endocrine function of the skeletal muscle, and cause malfunction of several organs and tissues of the body. However, activation of the skeletal muscle per se, and not necessarily the intensity of the activity, will ensure sustained endocrine function. Several potential myokines have been proposed [233], for example lipoprotein lipase (important for fat metabolism and linked to CVD risk), IL-6 (with central anti-inflammatory effects), contraction-induced GLUT-4 translocation in skeletal muscle, and IL-15 (which may have a role in the muscle-fat cross-talk via modulation of the visceral fat mass).

5.3.3.3 Shear stress and vascular function
Even short periods (30 to 60 minutes) of sitting have been shown to induce different adverse haemodynamic responses, such as turbulent blood flow due to bends within
the arterial tree, blood pooling in the legs, decreased thigh blood flow, and increased blood viscosity [234]. However, one of the most potent detrimental haemodynamic responses is low laminar shear stress, which has been shown to cause elevation of oxidative stress and endothelial dysfunction, both of which promote atherosclerosis. Hence, prolonged bouts of sitting during the day will expose the endothelium to an atherogenic haemodynamic environment. Even though bouts of exercise increase shear stress and thus promote preserved endothelial function, the increase seems to be short-lived. Moreover, in a recent study, Boyle and co-workers found that a transition from high to low levels of activity (average step-reduction of approximately 12500 to 3700 steps per day) in 11 healthy, active men for only 5 days resulted in impaired flow-mediated dilatation in the popliteal artery, decreased diameter of the brachial artery, and an increase in circulating levels of endothelial microparticles; all indicators of deleterious vascular consequences [235]. Hence, daily repeated periods of NEPA seem to be important; this abolishes many of the consequences of prolonged sitting, interrupts the pro-atherogenic haemodynamic responses, and preserves vascular and endothelial function.

5.3.4 Distribution of the activity and breaks in prolonged sitting
The distribution of daily time spent in NEPA or sitting seems to be as relevant as the total time spent. In a cross-sectional analysis of 4757 participants from the NHANES cohort, a higher number of breaks in sedentary time (a transition from sedentary to an active state, measured by accelerometer) were, independently of total sedentary time and exercise, significantly associated with lower WC (on average 4.1 cm when comparing the highest and the lowest quartile) and C-reactive protein [236]. In addition, promising findings from recent experimental trials on the acute negative metabolic effects of prolonged sitting have shown benefits of intermittent LIPA or NEPA, further strengthening the findings in this thesis. In a randomised crossover including 19 overweight/obese adults, the acute effects on postprandial metabolism [142] and gene expression [237] were examined after an initial standardised test drink (75 g glucose, 50 g fat) followed by either A) uninterrupted sitting for 5 hours, B) sitting for 5 hours with 2 min bouts of LIPA walking every 20 min, and C) sitting for 5 hours with 2 min bouts of moderate intensity walking every 20 min. When the prolonged sitting was broken up with walks, irrespectively of walking intensity, the postprandial glucose and insulin levels were lower, and beneficial changes were induced in the expression of a number of genes linked to factors including glucose homeostasis, inflammation, and metabolic risk.

Other interesting approaches have been made to not only studying the importance of breaking up prolonged sitting, but also comparing the mode of activity within these breaks. In a crossover study on eighteen young, healthy participants, Duvivier and co-workers compared three different regimens: prolonged sitting (sitting for 14 h per day), exercise (sitting for 13 h per day and substituting vigorous exercise for the remaining 1 h), and NEPA (substituting 6 h sitting with 4 h walking and 2 h standing) [238]. The prolonged sitting and exercise regimen had similar hours per day of sitting, and the exercise and NEPA regimen had the same EE (for the sitting regimen, the daily EE was approximately 500 kcal lower). Even though the exercise regimen induced an increased EE compared to the sitting regimen, the insulin response to an oral glucose tolerance test in the morning after each regimen was significantly lower for the NEPA regimen compared to both the sitting and the exercise regimen, and no significant improvements comparing the exercise and sitting regimen. The same trends were also seen for triglycerides, non-HDL cholesterol, and Apo B levels. The authors stated that one hour of daily exercise does not seem to be able to compensate
for the negative effects of insulin and plasma lipid levels, if the rest of the day is spent sitting. Rather, the importance of an active day with repeated bouts of NEPA is evident.

Peddie and co-workers came to similar conclusions in their study on seventy healthy adults when comparing three different regimens: (1) sitting for 9 hours, (2) sitting for most of this time but substituting 30 minutes with walking (∼60% of maximal capacity), and (3) breaking up the sitting time by walking (∼45% of maximal capacity) for 1 min and 40 seconds every 30 minutes [239]. The last of these three regimens corresponded to the same total time in activity as the regimen with 30 min of continuous activity. Both glucose and insulin response after intake of three meal replacements during each day of intervention were significantly lower in the regular breaks regimen compared to both the prolonged sitting and the 30 min continuous activity regimen, while no differences were seen between the two latter. Triglyceride response did not differ between the three regimens.

Although the experimental studies described above cover only the short-term effects of breaking up prolonged sitting with NEPA, and long-term research is still needed, these studies provide important insights.

5.3.5 The balance between non-exercise physical activity and sitting
Along with the revolution in technology and restructuring of society in recent decades, there has been a shift in the balance between time spent in NEPA and time spent sitting, in favour of the latter. The result of this is an "unnaturally" high amount of sitting time in the general population [74]. A contrary example is a study among the Old Order Amish, who still live a traditional agricultural lifestyle and maintain a high level of daily movement. The results showed that Amish men and women took on average three times as many steps per day as other adults in the USA [240]. As the human genetic constitution has probably changed little in the past 30,000 years [241], and is therefore not selected for a sedentary lifestyle, it is hardly surprising that the contemporary lifestyle has generated health consequences for the humans of the modern 21st century.

In conclusion, the results in this thesis suggest that a generally active daily life has important beneficial associations with cardiovascular health and longevity in older adults, which seems to be the case regardless of regular exercise habits. As it is widely known that regular exercise has a major impact on health, these results have high clinical relevance. For future health, promoting everyday NEPA might be as important as recommending regular exercise for older adults.

5.4 A NEW SUBMAXIMAL METHOD FOR ESTIMATION OF CARDIORESPIRATORY FITNESS
The Ekblom-Bak test (EB-test) is a new test for estimating VO₂max from submaximal cycle exercise in a mixed population. ΔHR response between a lower standard and an individually chosen higher work rate is used, rather than one absolute HR response to a single work rate as used in most previous similar tests. Internal validation analysis showed a significantly increased precision (a 50% lower CV) for the EB-test compared with one of the most commonly used submaximal exercise tests, the Åstrand test (Å-test). The difference between measured VO₂max and the value estimated by the EB-test showed no influence from maximum HR and deviation from age-predicted maximum HR, but there was a partial influence from
VO₂max level. In addition, test-retest reliability analysis over one week showed that the EB-test can be performed with good precision already on the first test occasion.

The present test was intended to make it feasible for professionals such as clinicians and healthcare workers to evaluate a patient’s CF. Therefore, like the Å-test, the EB-test was created to be simple, low-risk, time-effective, easily administered with no need for laboratory equipment, and applicable in a sex- and age-mixed population. The main difference between the Å-test and the EB-test is the use of ΔHR in the EB-test. The validity correlation coefficient for the EB-test was \( r = 0.91 \), which is significantly higher than the \( r = 0.68 \) seen for the Å-test. The latter was in concordance with Åstrand’s own validation reports of \( r = 0.78 \) [148] and other validation reports of \( r = 0.58–0.76 \) [155, 160, 242-244]. Furthermore, the CV for the EB-test was 9.3%, which means that for two-thirds of the individuals in a mixed population, the difference between measured and estimated VO₂max will be less than ±9.3%. This is a significantly improved precision compared with the Å-test, which had a CV of 18.1% in the present study and ±15% for a mixed sample of individuals in the original report by Åstrand [148]. This means that for 95 out of 100 individuals with an actual VO₂max of 3.0 L·min⁻¹, the EB-test could predict VO₂max within ±0.55 L, while the Å-test could predict it within ±1.06 L. For individuals with an actual VO₂max of 2.0 L·min⁻¹, VO₂max could be predicted within ±0.36 L by the EB-test and within ±0.71 L by the Å-test.

The CV for estimation of VO₂max and for test-retest reliability (presented in the result section) indicates an uncertainty in making fine-tuned statistical comparisons of estimated VO₂max between individuals or over time for an individual. As stated previously, the EB-test is intended to make it feasible in many different situations to estimate and evaluate an individual’s CF; for example, to screen and detect individuals at risk, or to correlate estimated VO₂max with different health outcomes in mixed population studies. For these reasons, we believe that VO₂max estimated by the EB-test will be sufficient, especially as the precision of the estimation is significantly improved compared with the Å-test. We are aware that measuring actual VO₂max by a maximal test would be optimal for comparing individuals as well as detecting change over time within an individual, but this is not feasible in many practical situations in the general population.

Some previous attempts have been made to improve the precision of the Å-test [102, 245] or to find the optimal work rate to use [246, 247]. Like the Å-test, these attempts are mainly based on one HR response to exercise, which may be affected by ambient temperature, nervousness, emotions, the intra-individual variation from age-predicted HRmax, and mechanical efficiency. Only one previous study has used ΔHR response to work rate. Legge and Banister created a nomogram for prediction of VO₂max in young men by plotting ΔHR response (elevation of HR at a higher individually chosen work rate above that reached at zero rate, pedalling at 90 rpm) against percentage of VO₂max [155]. Two separate regression lines were developed for trained and untrained participants. External validation showed a significantly better correlation between VO₂max estimated by the Legge–Banister nomogram and measured VO₂max (\( r = 0.98 \)), compared with the Å-test and measured VO₂max (\( r = 0.80 \)). The nomogram of Legge and Banister has not been used to a great extent, possibly due to the pedalling rate of 90 rpm, the limited population (young men), and the unclear instructions on how to separate trained from untrained participants. Exercise tests exist to determine VO₂max with very low prediction error (CV reduced to 5% or less) [248, 249], but these require special laboratory equipment and
expertise or maximal effort from the participant. They are therefore infeasible to employ in clinical practice or at most public fitness centres.

5.4.1 Improved precision due to use of ΔHR

We believe that the improved precision of the EB-test is mainly due to the use of ΔHR, which reduces the impact of variability in absolute submaximal HR response due to ambient temperature, nervousness, or emotions as well as work efficiency. The reliability analysis showed that although the absolute HR at both standard and higher work rate was significantly higher for the first occasion compared with the second occasion one week later, there was no difference in either ΔHR or estimated VO2max between the two occasions. Higher HR and a lower mechanical efficiency at the first occasion are common features in series testing, and are mainly due to excitement, anxiety, and/or tension among the participants [250]. Submaximal tests relying on absolute HR response to one work rate, like the Å-test, will generate a systematic underestimation of VO2max for these participants. The prerequisites of the Å-test therefore require exclusion of a first test. However, exclusion of a first test and the need for a new test occasion are not feasible in most health evaluation situations. The reliability of the results derived on the first occasion of the EB-test is therefore very important.

Another potential error that may be reduced by the use of ΔHR is the variability in mechanical efficiency. Mechanical efficiency is represented not only by moving economy, but also by intra-individual variation in basal metabolic rate and oxygen consumption at higher work rates, due to variation in mitochondrial oxygen affinity (p50mito) [158]. An effective moving economy and a high p50max would both lead to a substantially lower oxygen cost for a given work rate. Subsequently, this would introduce a variation in absolute HR response between individuals of similar capacity but different mechanical efficiency. This error could be diminished by using ΔHR. To study this, we compared the individual variation from mean VO2 at each work rate. ANOVA from repeated measures revealed the same distribution among participants for the standard rate and the two higher work rates. This means that if an individual shows a low VO2 cost (compared with mean) at the standard rate, it is most probable that they also show a low VO2 cost at both the first and the second higher individually chosen work rate. This analysis strengthens the advantage of using ΔHR. The EB-test also showed no influence from variation in HRmax. The influence of HRmax has previously been particularly hard to account for in submaximal tests extrapolating to supposed maximal levels (e.g. through commonly used formulas like HRmax = 220 bpm - age), as the individual variance in this value from the age-predicted value is more than ±10%. However, it has been proposed that the HR range seems to be fairly stable, as the resting or standard rate HR varies along with HRmax [155]. This suggests that ΔHR is less prone than absolute HR to vary with HRmax, and the influence on estimated VO2max is reduced.

5.4.2 Is it possible to increase the precision even further?

As stated above, the EB-test was intended to be simple, with no need for laboratory equipment or expertise, and to be available as an easy way for clinicians and healthcare workers to evaluate a patient’s CF. Due to the “simple” nature of the EB-test and similar tests such as the Å-test, with measurements taken just by observing work rate and HR during a few minutes of submaximal cycling, I believe that the precision error cannot be reduced much more in mixed population samples. Obviously, there will be random variability errors when performing the test; while measuring the HR response, in the HR response to exercise, as well as in the manual
adjustment of the work rate of the mechanically braked cycle ergometer. However, one possibility could be to increase the number of individuals included in the model for construction of the test. To test if a greater number of individuals could theoretically improve the precision, we randomly divided the 143 individuals included in the present model into three subsamples (n₁=48, n₂=48, n₃=47). In each of the subsamples, we created linear regression models including the same variables as the EB-test model (sex, age, and ΔHR/ΔPO). Using the first subsample, the SEE was 0.326 L·min⁻¹ and the CV was 9.8%. Increasing the number of individuals in the model by adding subsample no 2 (n₁+n₂=98) resulted in a model with SEE=0.306 L·min⁻¹ and CV=9.4%. Including all three subsamples produced the model presented in Paper III (SEE=0.303 L·min⁻¹, CV=9.3%). This implies that even a significantly increased number of individuals with characteristics similar to those in the present model will probably not dramatically reduce the precision error of the EB-test. However, the test could be improved further by increasing the valid range of VO₂max values, as well as by including more individuals to make gender-specific models possible.

5.4.3 Impact of medicament use on estimated VO₂max
As a considerable proportion of the general population is on medication which has either a direct or indirect effect on the heart and circulatory system, it is highly relevant to study the impact of different medications on VO₂max as estimated by the EB-test. Preliminary results from a pilot study examining the effects of inhaled bronchodilators show small differences between estimated VO₂max by the EB-test with vs. without inhalation of bronchodilators; however, the results are yet not published.

In conclusion, although VO₂max has been shown to be an important, independent predictor of CVD health and longevity, it is rarely measured in clinical practice. This is often due to limited time and limited knowledge of exercise testing, but is mainly due to limited feasibility of maximal testing in the general population. We believe that the nature of the EB-test makes it suitable for use in such situations.
6 METHODOLOGICAL CONSIDERATIONS

6.1 STUDY DESIGN AND EXTERNAL VALIDITY
The population in Papers I and II constituted a random population sample which was considered a good representation of men and women aged 20 to 65 years in the whole Swedish population, allowing extrapolation of the results to the general Swedish population between these ages. A similar study design was adopted for the study population in Paper IV, though the source population was men and women living in Stockholm County. These study designs are highly suitable for the aim of the studies in Papers I, II, and IV. For Paper III, the study population was included after public announcement in the nearby region of Stockholm. From a larger original sample of interested individuals, participants were included to create a mixed study population with regard to sex, age, and activity status. As the measurement method included maximal exercise effort from the participants, random sampling was not feasible. This study design may result in a biased study population not representative of the general population. However, as the study was initiated to develop a new method and hence to study mechanisms (HR response in relation to VO₂max level), the random sample design was not as important in this case; it was more important to have a mix of the different prerequisites that we hypothesised would influence the HR – VO₂max relationship (i.e. sex, age, and activity status). While we were successful in creating a study population that was mixed with respect to sex and age, the requirement of a maximal treadmill test made it difficult to recruit participants with low VO₂max (< 2.5 L·min⁻¹), particularly among men. Hence, the study population had slightly higher VO₂max than the general population, and the validity range of the test does not include these extremely unfit men.

Regarding the other end of the validity range, a cohort of well-trained participants (n=16, all men) with high capacity (VO₂max > 4.5 L·min⁻¹) was originally tested. However, in line with previous experience, we discovered that the ΔHR response for these highly trained participants did not follow the same pattern as that for participants with low/normal/normal-high VO₂max (< 4.5 L·min⁻¹). In contrast to the narrower ΔHR range in response to a fixed increase in work rate among participants with lower VO₂max, the ΔHR range in these participants varied randomly. A possible explanation is that the standard work rate was too low, perhaps due to a high parasympathetic drive at lower loads in these highly-trained participants [251]. Inclusion of this specific cohort significantly altered the model and lowered the precision for the test in the low/normal/normal-high VO₂max range. We therefore decided to restrict the new test to be only valid for prediction of VO₂max < 4.5 L·min⁻¹. However, we do not consider this condition to be an overly restrictive one, as the main aim of the new test was to give a valid and precise prediction of VO₂max in the population with low and normal capacity, in which maximal testing is not feasible. Data from a representative cohort of the Swedish population (men and women aged 20–65 years) [73] revealed that only 3% of the cohort had a VO₂max > 4.5 L·min⁻¹. Hence, the EB-test is still valid in the absolute majority of the Swedish population.

6.2 SAMPLE SIZE
The sample sizes for the study populations in Papers I, II, and IV were fairly large, permitting all the main analyses performed in these papers. However, the wide confidence intervals for the OR for single subgroups in cross-tabulation analysis may be due to the low number of participants in these subgroups compared with other subgroups. The study sample in Paper III is extensively discussed in the discussion section above.
7 LIMITATIONS AND STRENGTHS

7.1 PAPERS I AND II

Some limitations in Papers I and II ought to be considered. Different measures of lipoproteins were used in LIV 90 and LIV 2000. APO B reflects the content of LDL but also VLDL, which in high triglyceride situations can be important. However, the cut-off levels for increased risk of APO A1 and HDL, as well as APO B and LDL, respectively, have been shown to correspond [252]. In addition, the dichotomised nature of the variables used in the present study might be preferable, because divergent outliers of APO B were spotted mainly in levels above the cut-off point. Although the cross-sectional design of the study limits the possibility of causality analyses, it may still identify lifestyle patterns associated with increased risk. The compilation of data from two cross-sectional studies 10 years apart should be considered, although adjustment for participation in the original study was made in the analyses. The lack of adjustment for food intake, as well as other important lifestyle factors such as stress, should be noted. A limitation in Paper I was the use of two different questionnaires in the baseline studies regarding PA level. However, the statistical analyses of the compilation make us confident in the validity of this variable. The questions only concerned leisure-time PA, and not work-related PA. For methodological reasons, the PA questions covered only the aspects of frequency (regularly weekly) and intensity, omitting duration (except in LIV90, where only PA > 20 minutes was asked for). Self-reported questions may be the easiest way of measuring PA in larger population samples, but their limited validity and reliability is recognised. Future studies must use more objective methods, such as accelerometry, to better analyse the importance of PA. Finally, in Paper II, WC was used as a risk factor together with the traditional metabolic and vascular risk factors, although I argue that this is not a suitable method (see concluding discussion below).

A strength in the studies of Papers I and II is the large population-based random sample of both men and women within a wide age span; this means that the validity of the results is not limited to a specific cohort, but can be extrapolated to the general population. Another strength might be the assessment of dichotomised risk factors, rather than continuous variables, as we considered it more important to evaluate how the exposure variables are associated with the presence of an “unhealthy” level of risk factors, rather than how they correlate. The inclusion of objectively estimated CF through submaximal testing, rather than subjective estimates through questionnaire responses, enables more valid analysis.

7.2 PAPER III

A limitation of the new submaximal test is that it is yet only valid for men and women within the same VO2max range as the model group (1.56 to 3.73 L·min⁻¹ for women and 2.75 to 4.49 L·min⁻¹ for men). However, the restriction in the upper end was necessary (see the discussion section), and leaves only a small fraction of individuals unable to be tested. Mean VO2max in the model group was somewhat higher than in the general Swedish population [73], but this should not limit the use of the test in the general population, as the VO2max range in the model group embraces a wide capacity range. The new test was developed on a mixed sample with regard to age. Subsequently, the regression coefficients for age are an average approach to the total population sample, and might not reflect the variation in effect of age on VO2max for young and old adults. The same is true for gender, as no gender-specific prediction models were developed due to the narrow range of
VO\textsubscript{2}\text{max} within each gender. This makes a partial contribution to the systematic bias in the estimated VO\textsubscript{2}\text{max} that is seen with increased VO\textsubscript{2}\text{max}.

The strength of this study is that with the new equation, VO\textsubscript{2}\text{max} can be predicted with reduced influence from previously acknowledged sources of error such as HR variability due to other factors than the actual work performed and maximal HR.

7.3 PAPER IV

A limitation of Paper IV was that the cut-offs points for WC, systolic BP, and diastolic BP were not the same as in Papers I and II. In Papers I and II, we used conventional cut-off points for the single risk factors, while in Paper IV the presence of the metabolic syndrome was evaluated (and the risk factors dichotomised) according to the criteria of the American Heart Association and National Heart, Lung, and Blood Institute. A methodological limitation is the use of self-reported data for the daily activities and the NEPA index, the intentional exercise, and the confounding variables. The NEPA index has not yet been validated, and we cannot rule out potential bias which could result in it not reflecting actual NEPA in the population. Therefore, the NEPA activities are not defined as activities within a specific intensity span, but rather elucidating the context of NEPA as part of daily living rather than as intentional exercise. However, the nature of the questions constituting the NEPA score was well-suited for the study population, as these questions covered NEPA activities commonly performed by older adults in Sweden. As it is necessary to consider differences in NEPA activities between different cultures, the present NEPA index should be used with caution in populations of other cultures. An additional limitation of this study includes our inability to rule out possible effects of residual or unmeasured confounding. However, to minimise the potential for reverse causality, we excluded all individuals with reported myocardial infarction, heart failure, and stroke at baseline as well as deaths occurring in the first, second, and third year of follow-up in the prospective analysis. All participants in the study were 60 years old at baseline, and interpretation of the results should be restricted to individuals around this age.

The strengths of the present study are the large and representative cohort of women and men of the relevant age in Stockholm County, the high participation rate, and the long follow-up period. The cohort was thoroughly characterised by a well-defined questionnaire and physical examination, which enabled adjustment for many possibly important confounders. Although the cross-sectional part of the study cannot prove causality for the metabolic factors, the prospective part is a strength. The Swedish national population registers used to ascertain the prospective outcomes are highly valid, and particularly suitable for large-scale population-based epidemiological research [253, 254].
The main results of this thesis were the independent beneficial associations of higher PA, higher CF, and lower abdominal obesity with individual risk factors as well as a clustered CVD risk factor profile in a population-based random sample of 20 to 65-year-old Swedish men and women. In addition, in a representative cohort of 60-year-old men and women in Stockholm County, higher levels of daily activity (NEPA), regardless of exercise habits, were associated with lower metabolic and vascular risk at baseline and a decreased risk of CVD events and all-cause mortality after 12 years. Finally, a new submaximal cycle ergometer test was developed to further improve the precision of VO2max estimation.

The present results emphasise the importance of evaluating different aspects of the daily movement pattern, and not just exercise or aerobic capacity, to enable effective interventions in health care. As the analyses were carried out in population-based samples of Swedish men and women, the results can be extrapolated to the general population of the same age group. The results of Paper IV show the independent importance of an active daily life, and not just the importance of regular exercise, in 60-year-old men and women. The findings are particularly important for older adults, as in comparison to other age groups, individuals in this age group tend to spend a relatively greater portion of their active day performing NEPA due to the fact that they often find it difficult to achieve recommended exercise intensity levels. Along with the demographic shift towards an older population, this is essential not only for individual well-being but also for the national and global burden of disease. The results of Paper II further emphasise the importance of PA per se, regardless of CF level, which may act as encouragement for those individuals who do not reach sufficient intensity levels to increase their CF or individuals who are low or slow responders to exercise in terms of CF. Importantly, Paper I suggests an independent strong association of both CF and abdominal obesity with metabolic and vascular health. Taken together, these results encourage PA at all levels; both daily NEPA for sustained muscular activity and prolonged increase in EE during the day, and also PA on higher intensity levels to improve CF. However, the independent risk associations with smoking in Paper I should be considered. We found that lean smokers were associated with a two-fold higher clustered CVD risk than their non-smoking lean counterparts, and a fit smoker did not have a significantly altered clustered risk compared to an unfit non-smoker. Hence, daily smokers benefit from PA, but PA cannot diminish the risk associated with smoking to the same level as that of non-smokers. Cessation is the only option for further risk reduction in smokers.

8.1 THE UNIQUE IMPORTANCE OF SEDENTARY BEHAVIOUR

Although prolonged sitting was not specifically evaluated in this thesis, NEPA mainly replaces time spent in sedentary behaviour, and hence some of the findings in Papers II and IV could indirectly indicate the independent importance of SB for the relationships studied. Many previous studies of the effect of PA on different health outcomes have failed to evaluate the importance of sedentary time as a unique behaviour with partly separate effects. In analyses of the relationship between PA and health, SB has rather been included in the lower end of the activity continuum and subsequently considered equivalent to a lack of sufficient exercise. There is thus room for speculation on the impact that this lack of separate control for SB (and NEPA) could have. Primarily, I believe that the evidential importance of higher levels of PA for health is probably underestimated. The reference groups in previous
analyses have probably been biased, as they are often defined as the group of individuals who lack regular exercise, and SB level is not taken into account. Another potential impact could be on the relationship between PA/CF and health; the positive effects of PA without an increase in CF might be explained by higher levels of NEPA (substituting sedentary time). It should now be obvious that future analysis must consider all aspects of the daily movement pattern.

8.2 HOW SHOULD WE HANDLE DIFFERENT FACTORS IN EPIDEMIOLOGICAL ANALYSES?

There is an intricate association between traditional metabolic and vascular risk factors, lifestyle-related factors, and other factors in relation to CVD. Figure 9 presents a scheme illustrating this rather complex association.

**Figure 9.** A scheme of the interrelationship between traditional metabolic/vascular, lifestyle-related, and other factors and their association with atherosclerosis and CVD.

The metabolic and vascular risk factors have a direct effect on the underlying causes of the atherosclerotic process and the development of CVD. The lifestyle-related factors affect the traditional risk factors, but also have a direct effect independent of the traditional risk factors. This direct effect may be via unmeasured factors, influence on gene expression, or other as-yet-unknown mechanisms. The lifestyle-related factors are in turn strongly influenced by other modifiable and non-modifiable factors such as age, gender, heredity, and environment. There are also interactions between factors within the same group. For example, CVD morbidity risk for unfit individuals is suggested to be more pronounced in obese individuals compared with lean individuals. Reversed relationships and reversed causality are also possible; the presence of CVD may affect risk factor levels, or prohibit participation in exercise.
programs to increase CF. I believe that it is very important to consider the scheme in Figure 9 and its different levels before performing, analysing, or interpreting any epidemiological data; this is something that is not always done. Rather, previous studies have often included metabolic and vascular risk factors together with lifestyle-related and/or other factors, and tried to isolate the independent importance of each factor for the outcome. With regard to the difference between a risk factor and an intermediate factor, this is not a suitable approach. For example, PA or smoking may affect the risk of CVD development both through the traditional metabolic and vascular risk factors (with the traditional risk factors acting as intermediating factors) and by having a direct effect through new or unknown risk factors which have not been evaluated (for example through a direct effect on endothelial function and inflammation). However, to answer the question of how important different lifestyle factors really are, both the intermediate effect and the unmeasured effect must be evaluated. If the lifestyle factor and a possible intermediating metabolic or vascular risk factor are included together, some of the effect exerted by the lifestyle factor will be accounted for through the risk factor, and the importance of the lifestyle factor will be weakened. One example is the often cited results from the multinational INTERHEART study. Yusuf and co-workers showed that nine modifiable risk factors closely linked to the modern western lifestyle (abnormal lipids, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, fruit and vegetable intake, and physical inactivity) could explain 90% of the myocardial infarctions worldwide, in both sexes and at all ages in all regions studied [11]. In the main results, the OR and population attributable risk (PAR) for each factor is reported after adjustment for the other nine factors. However, all the lifestyle-related factors included among these nine factors (abdominal obesity, smoking, fruit and vegetable intake, psychosocial factors, and physical inactivity) have been shown in previous studies to exert independent effects on the metabolic and vascular risk factors included (blood lipids, hypertension, and diabetes). The importance of the lifestyle factors is therefore probably underestimated in this analysis. For example, in the crude analysis only adjusting for age, sex, and smoking, the OR and PAR were 2.24 and 33.7% for abdominal obesity, and 0.72 and 25.5% for exercise. After adjustment for all other factors, the OR and PAR were 1.62 and 20.1% for abdominal obesity, and 0.86 and 12.2% for exercise. The latter figures were reported as the main results in the abstract of the paper.

Against the background of the example described above, I believe it is vital to analyse which factors should be included from the different groups presented in Figure 9. It is also important to analyse which factors from the same group should be included to avoid confounding issues, in which the effect seen via some lifestyle factor is actually confounded through another.

8.3 LIFESTYLE FACTORS IN RISK SCORE PREDICTION
Another potential area to discuss is the inclusion of lifestyle factors in different risk scores for prediction of future risk of CVD events. Despite convincing evidence of the significance of PA, CF, and abdominal obesity for CVD risk, none of these factors is considered in the existing risk scores [255-257]. In line with the discussion above, one reason for not including these lifestyle factors is that they influence the factors already included in existing risk scores. However, as pointed out above, they also exert effects on CVD risk independent of the clinical conditions included in existing risk scores, by influencing other mechanisms important for the atherosclerotic disease process such as mean lipoprotein size [197], inflammatory factors [199], endothelial function [258, 259], and insulin sensitivity [260].
Appropriate inclusion of valid measurements of PA, CF, and abdominal obesity in the risk evaluation process would therefore enhance the precision of predicting future CVD events, as has been strongly suggested in a review study [119].

8.4 EVALUATION AND COUNSELING IN CLINICAL PRACTICE

Despite the growing evidence of the importance of evaluating PA, SB, CF, and abdominal obesity in clinical practice, these are still rarely measured. Traditional risk factors such as blood lipids and blood pressure are evaluated considerably more frequently. What is the reason for this? I see five important obstacles:

1. a lack of knowledge among healthcare professionals about the importance of these variables for health and longevity,
2. a subsequent lack of awareness among healthcare professionals of the importance of measuring all these variables,
3. a lack of valid methods to measure these variables accurately.
4. a lack of clarity regarding whose responsibility it is to measure these variables, and
5. a lack of routines in clinical practice to measure and follow-up these variables.

8.4.1 Obstacles 1 and 2

Regarding the two first obstacles, the results from the present thesis as well as other epidemiological studies are essential to establish solid recommendations and implement evidence-based knowledge in clinical practice. Hence, health care professionals should be recommended to consider PA, CF, and abdominal obesity in health evaluations. Of these lifestyle factors, I would like to further highlight the importance of considering different aspects of PA (or its absence), and describe a good way of doing this. As stated in the background section, the daily movement pattern could either be described in terms of intensity levels (sitting, LIPA, or MVPA) or in terms of behaviour and intention (SB, NEPA, and exercise). These two categorisations could largely be described as equivalent regarding intensity levels; however, the possibility to refer to the context or intention of the PA performance with the latter has a clinically relevant point. It is more communicable to the general population, most of whom do not rely on objective measures of intensity levels but still know why and in which context they perform different activities. This could lead to better communication regarding health-promoting activities, as well as better implementation. The behaviour-based classification is summarised and described below:

- Sedentary behaviour is a behaviour distinct from PA and intentional exercise, with specific determinants and a partly separate effect on disease risk. SB is characterised by muscular inactivity in the large muscle groups of the body and by low EE close to the basal metabolic rate. It has a high potential for detrimental effects because of its large volume during the day in the general population.
- NEPA is PA which is not intended to be exercise, but rather forms part of daily life. It mainly corresponds to light intensity levels. NEPA implies sustained contractions of the skeletal muscles involved in the movement, and increased EE for extended periods during the day. NEPA is mainly replaced
by sitting (and vice versa), and promoting NEPA is hence the most feasible approach to reducing sitting time.

- **Exercise** is PA which is planned, structured, and executed to gain health benefits, to improve/maintain physical fitness, or due to other social factors. It is mainly performed on moderate-to-vigorous intensity levels, with powerful health stimulus and increased EE. However, the daily volume of exercise is low in the general population, with a large number of individuals totally lacking in exercise.

### 8.4.2 Obstacle 3

Regarding obstacle number three, in this thesis I have presented a new test (the EB-test) for estimation of VO₂max from submaximal cycle exercise in a mixed population. The EB-test has a significantly increased precision compared with one of the most commonly used submaximal exercise tests, the Åstrand-test. The new test was intended to make it feasible for professionals such as clinicians and healthcare workers to evaluate a patient’s CF, by being simple, low-risk, time-effective, easily administered with no need for laboratory equipment, and applicable in a sex- and age-mixed population. The use of the cycle ergometer in clinical settings, rather than, for example, treadmill tests, is justified by the fact that mechanical efficiency is fairly constant in mixed populations, even in populations that have never used or even seen a bicycle or cycle ergometer before. Ekblom and Gjessing visited the Eastern Island in the middle of the South Pacific in 1964-1965 to study the aerobic capacity of the native population [261]. The majority of the 178 participants tested (one fifth of the total Eastern Island population) had never used or seen a bicycle before, but their oxygen uptake on submaximal work rates did not differ from corresponding values obtained in the Scandinavian populations.

A mechanically braked Monark cycle ergometer (model 828E) was used to construct the new test. It is important to remember that other types of cycle ergometers may give different work rate responses when adding the same resistance at higher work rates. This new test offers a new possibility to screen CF status in health evaluation situations. To facilitate usage of the EB-test, a manual, protocol, and procedure for estimating VO₂max are freely available from [www.gih.se/ekblombaktest](http://www.gih.se/ekblombaktest).

Until now, evaluation of the daily movement pattern and its individual components has mainly relied on subjective self-reported data, primarily through questionnaire responses. Although the limitations of self-reported data are recognised among epidemiological researchers to contain a relatively high degree of error, I believe that in clinical practice, the validity of the method is secondary; the primary goal is to measure and highlight the behaviour at all. What has become apparent in this thesis and in other research is the importance of separately evaluating all different components of the daily movement pattern, and not just determining whether or not the patient fulfils the national recommendations of regular exercise. However, the most preferable clinical practice would be to integrate the use of self-report and objective measures (e.g. accelerometry) to monitor the daily movement pattern [262]. While an accelerometer produces a valid quantification of the different components of the daily movement pattern and gives feedback to the patient in a pedagogic way, the self-reported data would help to reveal the context of the different behaviours, and thus enable intervention strategies by identifying when and where during the day adverse (e.g. prolonged sitting) or positive behaviours (NEPA or exercise) take place.
8.4.3 Obstacles 4 and 5

Two essential issues for realising measurements of lifestyle variables in clinical practice are clarification of who is responsible for this, and the development of routines to do so. In many countries today, structural problems within primary and secondary health care make this difficult to achieve. Other issues are that although many general practitioners believe it is important to counsel their patients about lifestyle and PA, many are sceptical about their ability to actually help the patient to practice regular PA; and many also experience difficulty finding enough time to actually discuss this with their patients, due to tight schedules [263]. These obstacles to even the discussion of lifestyle and PA in clinical practice further lower the probability of measuring any of the components. In 2011, the Swedish National Board of Health and Welfare presented its National Guidelines for Methods of Preventing Disease [264]. These provide recommendations for methods to prevent disease by supporting patients in their efforts to change four different and unhealthy lifestyle habits. One of these is insufficient PA (the others are tobacco use, hazardous use of alcohol, and unhealthy eating habits). The guidelines stated that “the healthcare system should offer counseling with the adjunct of exercise on prescription or a pedometer, as well as special follow-up, to patients with insufficient PA”. Two evidence-based methods are available to fulfil these recommendations. The first is physical activity on prescription (PAP), which is delivered via a prescription form for PA, similar to an ordinary drug prescription form. The aim of this is both to reduce SB by substituting NEPA, and to increase exercise. The second method is an evidence-based handbook, FYSS (Physical Activity in Prevention and Treatment of Disease), which recapitulates the up-to-date scientific knowledge on the effects of PA on health and gives advice on how to prescribe PA in the prevention and treatment of various diseases and conditions [265]. A recent thesis concluded that individualised PAPs increased PA and decreased sedentary time for at least six months [266]. There also exists an easily accessible and well developed network of local wellness and activity centres which are willing to welcome and guide patients with PAPs. However, despite the availability of national recommendations, evidence-based methods, and a local network, these resources remain underused. I believe that there is a need for a new professional group to co-ordinate this in primary as well as secondary care in Sweden. This group should not consist mainly of already-established professionals, such as doctors or nurses, taking on even more tasks. Instead, the professionals in the new group should have university degrees, evidence-based competence in the importance of PA and lifestyle for health and disease, and wide knowledge in evaluating PA, CF, and other lifestyle-related factors. They should be specialists in behavioural change therapy, allowing them to interview and guide patients who have received PAPs from their physicians, and to follow-up the PAPs appropriately. The Swedish School of Sport and Health Sciences in Stockholm provides education and degrees which match this need, and similar co-ordinators have been tested locally in other cities in Sweden with very promising results.
9 FUTURE PERSPECTIVES

A focus on lifestyle-related factors will be central to further public health improvements and a decrease in CVD prevalence in Sweden. The technology revolution and restructuring of society in recent decades has contributed to the general society becoming more sedentary; hence, an increase in PA and reduction in sitting time will be two essential approaches.

However, future research needs to further clarify the independent role of the different components of the daily movement pattern for cardiovascular health and CVD incidence, and their relationship with CF and abdominal obesity. This must be achieved using more valid methodology for measurement of both exposure (such as accelerometer and posture measure) and outcome (for example by imaging of the coronary arteries). In this way, we will be able to study the whole causal chain: **Lifestyle – Risk factor – Atherosclerosis – Morbidity – Mortality.** Analyses in random population-based samples would be especially valuable, as the results enable extrapolation of the results to the general population. However, analyses within subgroups of the population, for example individuals with manifest disease, on different types of medication, and with functional disabilities, are also highly relevant. Along with these results of future research, the recommendations for PA have to be updated and recommendations for prolonged sitting have to be adopted.

Furthermore, the epidemiological results must be established by intervention studies carried out in the natural environment and not just in laboratory conditions. More extensive research is needed to clarify possible separate mechanisms for prolonged sitting, LIPA/NEPA, and MVPA/exercise, and how they interact with CF and abdominal obesity. To generate the most effective intervention, it is essential to understand the context of the different behaviours and to clarify the internal and external determinants and how they influence the behaviour in these different contexts. Professor Willem van Mechelen once addressed the interaction between humans and their surrounding environment when talking about the increase in inactivity and prolonged sitting in the contemporary society, by posing the question: “Is it an abnormal behaviour in a normal environment, or a normal behaviour in an abnormal environment?” This sums up the essence of focusing on both the individual behaviour change and the surrounding environment.

As well as gathering evidence from future research, sustainable methods and routines must be adopted for evaluating the lifestyle factors. Objective measurement and subjective self-report of the daily movement pattern would be preferable, and the new submaximal test presented in this thesis could be used to assess aerobic capacity. However, we aim to further enhance the validity and precision of the new test and to validate it in different populations, for example in high-risk individuals or patients taking beta blockers, as well as in specific gender and age samples. For the health care sector, an important step would be to establish the new profession suggested above to ensure the usage of evidence-based methods such as PAP and FYSS, to coordinate the work, and to finally to make it successful and long lasting.
10 SAMMANFATTNING PÅ SVENSKA

I ett populationsbaserat, slumpmässigt urval av 1671 män och kvinnor i åldrarna 20 till 65 år från den svenska befolkningen så visade resultaten att;

- Kondition mätt som maximal syreupptagningsförmåga (VO$_{2\text{max}}$) och bukfetma mätt som midjemått var, oberoende av varandra, starkt kopplade till såväl enskilda metabolab och vaskulära riskfaktorer som till samlad kardiovaskulär risk. För den samlade risken, så innebar varje högre ml i VO$_{2\text{max}}$ en 5 % lägre risk, och varje större cm i midjemått 5 % högre risk. Den samlade risken var högre bland otränade, som var över 40 år eller som rökte dagligen. Bukfeta individer hade en högre risk om de var män eller över 40 år. En ytterligare intressant aspekt var att varken bättre kondition eller lägre midjemått kunde reducera risken kopplad till daglig rökning.

- Både hög rapporterad fysisk aktivitet på fritiden och (framförallt) kondition, var oberoende av varandra starkt kopplade till enskilda metabolab och vaskulära riskfaktorer likväl till samlad kardiovaskulär risk. För den samlade risken så innebar varje högre tertil av kondition en lägre risk med 50 % eller mer, oberoende av rapporterad fysisk aktivitet. Ett intressant fynd var att hos en person med låg kondition, men hög rapporterad fysisk aktivitet, var den samlade risken hälften så stor jämfört med en person som hade låg kondition men som också var fysiskt inaktiv. Dessutom accelererade riskökningen hos dem som varken var tillräckligt aktiva enligt gängse rekommendationer eller hade god kondition.

I ett befolkningsbaserat urval av 4232 svenska 60-åring män och kvinnor i Stockholms län visade resultaten att

- En fysiskt aktiv vardag, oberoende av avsiktlig motion och träning, var starkt kopplad till en hälsofammet metabolisk profil samt lägre förekomst av det metabola syndromet vid studiestarten.

- En uppföljning efter 12.5 år, visade att en fysiskt aktiv vardag vid studiestarten, oberoende avsiktlig motion och träning, innebar 27 % lägre risk att drabbas av en hjärt-kärlhänsel samt 30 % lägre risk för förtida död jämfört med en fysiskt inaktiv vardag.

Sammanfattningsvis visar dessa studier att såväl vardaglig aktivitet som motionsvanor, kondition och midjemått har stor betydelse för den kardiovaskulära hälsan hos svenska män och kvinnor i olika åldrar. De visar också att vardagsaktivitet vid 60-års ålder, oberoende av motions och träningsvanor, minskar risken att i framtiden drabbas av en hjärt-kärlsjukdom eller att dö i förtid.

Vidare presenteras ett nytt, icke-maximalt cykeltest för beräkning av VO$_{2\text{max}}$. Testet är baserat på pulsförändringen mellan en lägre, standardiserad och en högre individuellt vald belastning. Resultaten visade att det nya testet hade en signifikant ökad precision jämfört med ett av det i världen mest använda icke-maximala testerna, Åstrands cykeltest. Dessutom undersökte testets reliabilitet genom att två test genomfördes med en veckas mellanrum. Medan pulsen på den lägre, standardiserade belastningen såväl som den högre belastningen var signifikant högre vid första tillfället, så war pulsförändringen liknande och det var ingen skillnad i beräknad VO$_{2\text{max}}$. Vi konkluderade således att det nya testet kan genomföras med god
precision redan vid ett första testtillfälle; något som inte gällt för Åstrandtestet där ett förtest är en grundförutsättning. Det nya testet är enkelt, innebär en låg risk, kräver ingen avancerad laboratorieutrustning eller arbetssfysiolégisk expertis, och är därför passande bland annat i hälsoutvärderingar i klinisk vardag i en frisk blandad population.
11 CONCLUSIONS

In a population-based random sample of Swedish men and women aged 20 to 65 years:

- Cardiorespiratory fitness and abdominal obesity were each independently and strongly associated with individual CVD risk factors, as well as with a clustered CVD risk factor profile. For the clustered risk, each unit of fitness (ml·kg⁻¹·min⁻¹) was associated with a 5% decrease in risk and each unit of waist circumference (cm) with a 5% increase in risk. This was maintained in women as well as men, in younger as well as older people, and in daily smokers as well as non-smokers; however, some differences were seen within the subgroups.

- Higher levels of physical activity and cardiorespiratory fitness, but mainly the latter, were independently associated with both individual and clustered CVD risk factors. Furthermore, a notable interaction of excess clustered CVD risk was shown for neither being sufficiently physically active according to general guidelines nor being fit.

In a population-based sample of 60-year-old men and women:

- A generally physically active daily life, independently of regular physical exercise, was associated with beneficial metabolic health at baseline.

- After 12.5 years of follow-up, a generally active daily life at baseline, independently of regular exercise, was associated with a 27% lower risk for a first-time cardiovascular event and a 30% lower risk for all-cause mortality.

A new submaximal cycle ergometer test for estimation of maximal oxygen uptake was developed. The test is simple, low-risk, and easily administered, and does not require laboratory equipment or expertise. In a mixed (in terms of age, activity status, and gender) healthy population, the test showed a significantly increased precision compared with one of the most commonly used submaximal exercise tests today.
ACKNOWLEDGEMENTS
This thesis is the result of a collaboration between Karolinska Institutet and the Swedish School of Sport and Health Sciences. I am deeply grateful for this collaboration and to the many people around me who in one way or another have contributed to the realisation of these studies and this thesis. Particular thanks go to:

Mai-Lis, my supervisor and role model, for always believing in me, and sharing your deep knowledge and great experience. After talking to you, even the toughest challenges seem easy to manage and the highest mountains seem easy to climb. Without your guidance I would never have come this far. Thank you also for making me believe in Christmas again 😊

Björn, my supervisor and father, for everything. Always honest and straightforward (which I really appreciate!) and with unlimited knowledge and experience. You have taught me what real professionalism is, that everything is possible in life, and that bad knees are not an obstacle!

P-O Åstrand, for being a remarkable person and scientist, and a muse. It is an honour to have known you personally.

, and Örjan (you understand), my brother and close colleague, for being the best biggest big brother one could wish for. Thank you for your admirable skills and knowledge in our research, and for always supporting me in what I am doing.

Maria, my sister-in-law and neuroscientist, for always being positive but at the same time posing those challenging questions regarding my research from another point of view. And for always making me feel organised 😊

Lars-Magnus Engström, for invaluable inspiration and knowledge, and for being one of the first people I got to know within the research world. I am forever grateful for your letting me learn from the LIV studies!

My roomies; Jane, for being my solid English rock and for lightening up even the cloudiest days with your laughter, and Gustav, for showing me what it means to be well-structured and organised — and for reminding me to take a break once in a while!

Matthias Lidin, for always being positive and inspiring me with the true passion you have for the work that you do. Go home LCHF! And Karin Björklund-Jonsson, for your help and assistance throughout my doctoral studies.

Max Vikström, for invaluable assistance in statistics and for being one of the most positive people I have met.

Ulf Bergh, for invaluable discussions regarding exercise physiology. I have learned so much from you!

All my co-workers at “Åstrandlabbet”, especially Frida Björkman and Mikael Flockhart, for your invaluable assistance in the study for Paper III, and Lena Kallings and Mats Börjesson in my research group. To all of you, for always being supportive and encouraging; it makes research simple!

All my present and previous colleagues at GIH.
All the study participants, for taking part in the studies.

All in “Mai-Lis group”, for all the knowledge and support you give me.

Anna-Lena Särström, my external mentor and extra mom, for always being there for me and sharing your love and knowledge.

Sebastian, the love of my life and my best friend, for the unconditional love and support you give, and for all the joy you bring into my life. Words are not enough. By the way, you are holding the space rocket I have been building in your hand!

Mathilda and Emma, my daughters, you make my life complete. I love you so much it hurts!

Lucyna and Anders, my parents-in-law, for all the support you give us.

Ulla, Mom, for absolutely EVERYTHING and for being the best mom I could ever wish for.

Anders, my brother, for being the best youngest big brother ever. You have inspired and taught me so much throughout the years. And Michèle, my sister-in-law, for always sharing your joy and happiness.

All my nieces, Alfred, Mille, Love, Moa, and Wilma, for enriching my life and securing the spirit of the Ekblom and Ferrari families for the future!

Raffe, for being you.

Linda, Milla, Alexandra, Sara, Stefan, and Pjer for always being there for me. I could not manage without you.

All my team mates, coaches, and other people in the clubs I have played for, for giving me all the support and joy on and off the pitch. Without being able to get rid of all my energy running around on the midfield, I do not know what would have happened…! 😊

All those not mentioned here, but who have supported, encouraged, or challenged me throughout the years.

I also owe my deep gratitude to the financial supporters of the studies in this thesis.

- The LIV 90 study was supported by grants from the Folksam Insurance Company. Special thanks to the Swedish National Institute for Public Health and to the Swedish Federation for Company Sports and the Swedish Sport for All Association (Korpen).

- The LIV 2000 study was supported by grants from the Swedish National Institute for Public Health, the Folksam Insurance Company, the Swedish Research Defence Agency, and the Swedish School of Sport and Health Sciences, Stockholm, Sweden. Special thanks to all test personnel at AB Previa.

- The study in Paper IV was supported by grants from the Swedish Order of Freemasons - Grand Swedish Lodge, Stockholm County Council, the Swedish Heart and Lung Foundation, the Swedish Research Council (Longitudinal Research), and the Tornspiran Foundation.
13 REFERENCES


80


