Beyond the Established Risk Factors of Myocardial Infarction

*Lifestyle Factors and Novel Biomarkers*

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People are usually more convinced by reasons they discovered themselves than by those found by others.

Blaise Pascal
1623-1662
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ABSTRACT

Age, male sex, hypertension, smoking, diabetes, dyslipidaemia, and obesity are considered as established risk factors for cardiovascular diseases. Several of these established cardiovascular risk factors are strongly influenced by lifestyle. Novel biomarkers from different mechanistic pathways have been associated with cardiovascular risk, but their clinical utility is still uncertain.

The overall objective of the thesis was to evaluate the associations between certain lifestyle factors (physical activity and snuff use), biomarkers reflecting the haemostatic and the inflammatory systems and risk of a future first-ever myocardial infarction.

A prospective incident nested case-control study design was used with a total of 651 cases of myocardial infarction and 2238 matched controls from the population-based Northern Sweden Health and Disease Study.

The effects of commuting activity, occupational and leisure time physical activity on risk of myocardial infarction were studied. A clearly increased risk of myocardial infarction was found for car commuting compared to active commuting (walking, cycling or going by bus). High versus low leisure time physical activity was associated with decreased risk of myocardial infarction. Low occupational physical activity was associated with risk of myocardial infarction in men.

The risk of myocardial infarction or sudden cardiac death was studied in male snuff users compared to non-tobacco users. No increased risk was found for myocardial infarction or sudden cardiac death among snuff users without a previous history of smoking. However, for sudden cardiac death the study did not have statistical power to detect small differences in risk.

Plasma levels of haemostatic markers have previously shown to be associated with risk of myocardial infarction, but as haemostatic markers are also acute-phase reactants, it is not clear if their association with myocardial infarction is independent of inflammatory markers. In the present study, the haemostatic markers D-dimer, von Willebrand factor (VWF), tissue plasminogen activator (t-PA), and tissue plasminogen activator/plasminogen activator inhibitor-1 complex (t-PA/PAI-1 complex) were associated with risk of myocardial infarction after adjustment for established risk factors and the inflammatory markers C-reactive protein (CRP) and interleukin 6 (IL-6). Furthermore, the addition of eight haemostatic and inflammatory markers could improve the predictive ability for future myocardial infarction beyond that of a model utilizing only established risk factors.

Established risk factors and novel biomarkers were explored as potential mediators of the reduced risk of myocardial infarction related to active commuting. A combination of established risk factors, haemostatic and inflammatory markers appeared to explain a substantial proportion (40%) of the difference in risk for myocardial infarction between active commuters and car commuters. IL-6, t-PA, t-PA/PAI-1 complex, apo B/apo A-1 ratio, and BMI seemed to be the largest potential mediators when tested individually.
In conclusion, regular physical activity such as active commuting is associated with reduced risk of a first-ever myocardial infarction. This effect could in part be mediated through a beneficial influence on haemostasis and inflammation, as well as a positive impact on established risk factors. Several haemostatic markers are associated with risk of myocardial infarction independent of established risk factors and inflammatory markers. The combination of haemostatic and inflammatory markers may enhance predictive ability beyond established risk factors. Our findings do not support the hypothesis that snuff use increases the risk of myocardial infarction.

Keywords: lifestyle factors, physical activity, commuting activity, leisure time physical activity, occupational physical activity, snuff use, haemostatic markers, inflammatory markers, myocardial infarction, epidemiology, primary prevention.
Ålder, manligt kön, högt blodtryck, rökning, diabetes, höga blodfetter och övervikt anses vara etablerade riskfaktorer för hjärt-kärlsjukdomar. Flera av dessa riskfaktorer påverkas i hög grad av livsstil. Vissa biomarkörer i blodet har visat sig ha ett samband med ökad risk för hjärt-kärlsjukdom, men det är fortfarande oklart på vilket sätt de kan användas i klinisk vardag. Det övergripande syftet med denna avhandling var att studera sambanden mellan livsstilsfaktorerna fysisk aktivitet och snusning, markörer från kroppens system för hemostas (blodlevring) respektive inflammation och risken att insjukna i en första hjärtinfarkt.

I studien ingick 651 personer som drabbades av hjärtinfarkt. Samtliga var undersökta och hade lämnat blodprover innan de insjuknade. Dessa jämfördes med 2238 friska personer.


Risken att drabbas av hjärtinfarkt eller plötslig hjärtdöd studerades hos snusare som aldrig varit rökare. Dessa snusare hade ej ökad risk för hjärtinfarkt eller plötslig hjärtdöd jämfört med tobaksfria. Analysen avseende plötslig hjärtdöd är dock osäker på grund av att få personer ingick.

Blodnivåer av hemostasmarkörer har tidigare visat sig vara kopplade till ökad risk för hjärtinfarkt, men då hemostasmarkörerna samvarierar med inflammation är det oklart om sambandet med hjärtinfarkt är oberoende av inflammationsmarkörer. I denna studie var hemostasmarkörerna D-dimer, von Willebrand faktor (VWF), vävnads-plasminogenaktivator (t-PA) och komplexet av vävnads-plasminogenaktivator och plasminogenaktivator-hämmare-1 (t-PA/PAI-1 komplex) kopplade till ökad hjärtinfarktrisk efter kontroll för etablerade riskfaktorer och markörer för inflammation, C-reaktivt protein (CRP) och interleukin 6 (IL-6). När vi lade till åtta markörer för hemostas och inflammation förbättrades förmågan att förutsäga vem som kom att drabbas av hjärtinfarkt jämfört med en modell med enbart de etablerade riskfaktorerna.

En kombination av etablerade riskfaktorer och markörer för hemostas och inflammation tycks kunna förmedla en betydande del (40%) av skillnaden i risk mellan aktiva arbetspendlare och bilpendlare. Då variablerna testades individuellt, föreföll IL-6, t-PA, t-PA/PAI-1 komplex, blodfettsmarkören apo B/apo A-1 kvot och kroppsmasseindex vara de mest betydelsefulla mediatörerna.

Sammanfattningsvis visar avhandlingen att regelbunden fysisk aktivitet, såsom aktiv arbetspendling är kopplat till minskad risk att insjukna i en första hjärtinfarkt. Denna effekt kunde delvis medieras via positiva effekter på hemostas och inflammation samt
gynnsam inverkan på etablerade riskfaktorer. Flera hemostasmarkörer är kopplade
till risk för hjärtinfarkt oberoende av etablerade riskfaktorer och markörer för
inflammation. En kombination av hemostas- och inflammationsmarkörer skulle
kunna förbättra förmågan att förutsöga risken att insjukna i hjärtinfarkt som tillägg
till de etablerade riskfaktorerna. Resultaten stödjer inte hypotesen att snusning ökar
risken för hjärtinfarkt.
ABBREVIATIONS

apo(a) apolipoprotein (a)
apo A-1 apolipoprotein A-1
apo B apolipoprotein B
AUROC area under the receiver operating characteristic
ROC receiver operating characteristic
BMI body mass index
CHD coronary heart disease
CI confidence interval
CRP C-reactive protein
CV coefficient of variation
CVD cardiovascular disease
ECG electrocardiogram
EDTA ethylenediaminetetraacetic acid
ELISA enzyme-linked immunosorbent assay
GLUT4 glucose transporter isoform 4 protein
hsCRP high-sensitivity C-reactive protein
HDL high-density lipoprotein
IL-1 interleukin 1
IL-6 interleukin 6
IPAQ International Physical Activity Questionnaire
kDa kiloDalton
LDL low-density lipoprotein
Lp(a) lipoprotein (a)
LTPA leisure time physical activity
MET metabolic equivalent
MI myocardial infarction
MONICA Multinational Monitoring of Trends and Determinants in Cardiovascular Disease
NO nitric oxide
NSHDS Northern Sweden Health and Disease Study
OPA occupational physical activity
OR odds ratio
PAI-1 plasminogen activator inhibitor-1
SCD sudden cardiac death
SD standard deviation
RCT randomized controlled trial
RNA ribonucleic acid
t-PA tissue plasminogen activator
TSNAs tobacco-specific N-nitrosamines
TNF-α tumor necrosis factor-alpha
u-PA urokinase plasminogen activator
VIP Västerbotten Intervention Program
VLDL very low density lipoprotein
WHO World Health Organization
VWF von Willebrand factor
This thesis is based on the following papers:


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PREFACE

During my first year of residency I met a 60 year old man who had experienced a myocardial infarction in his forties. He had just undergone a routine physical examination and was found to have untreated hypertension, impaired glucose tolerance, and hyperlipidemia. He was overweight. Fortunately, he decided to quit smoking after his heart attack. Using published cardiovascular guidelines I categorised him as “high risk” and we discussed possible interventions. Despite my attempts to persuade him he refused all medications other than aspirin. At the time he was under a lot of stress and requested a six-month reprieve in order to make necessary lifestyle changes. When I met him later that same year he had lost 8 kg through a combination of daily walks and dietary changes. He seemed less stressed and his blood pressure had normalized without any additional medications. His laboratory tests normalized as well. Now, six years later, he remains physically active and is free from angina pectoris.

In my clinical work I meet many patients with coronary heart disease or an increased risk of getting it. By utilizing published guidelines, I try to estimate the patient’s risk of future events and counsel them in terms of lifestyle modification and pharmacotherapy. This is often difficult work, but at the same time very inspiring.

I hope this thesis sheds some light on lifestyle factors and biomarkers beyond the established risk factors and furthers our understanding of their effect on risk and role in cardiovascular prevention. This work would not have been possible without the guidance from my supervisors, Jan-Håkan, Kurt and Lars, and the great feeling of fellowship at Forskningsenheten at Skellefteå Lasarett.
Cardiovascular disease
Cardiovascular disease (CVD) refers to diseases affecting the heart and the blood vessels. The two most common subgroups of CVD are coronary heart disease (CHD) and stroke. Cardiovascular mortality has decreased in Sweden in men 15-74 years from 352 to 145 deaths per 100,000 from 1987 to 2006 (a reduction of 57%). For women the corresponding mortality rates have decreased from 128 to 61 during the same period (a reduction of 52%). Still cardiovascular disease is the leading cause of death, accounting for 42% of the total mortality during 2006.

Coronary heart disease and myocardial infarction
Coronary heart disease is a narrowing of the blood vessels that supply blood and oxygen to the heart. Among the cardiovascular diseases, CHD is the major cause of death, accounting for 21% of the total mortality for men and 18% of the total mortality for women in Sweden during 2006. A myocardial infarction (MI) is usually the result of thrombus formation in the coronary artery lumen resulting in an acute reduction of blood supply to a portion of the myocardium. This region becomes ischemic and eventually necrotic.

Atherosclerosis
The atherosclerotic process is recognized as the main culprit of coronary heart disease. During the last decades our view of the pathogenesis of atherosclerosis has changed dramatically. What used to be described as “a plumbing problem” in which lipid deposits formed on the surface of arteries and proliferated until they restricted and eventually blocked the blood supply to the tissues, is now considered as a chronic inflammatory process. We view arteries as highly organized organs comprised of living cells, not as inanimate conduits.

The formation of an atherosclerotic plaque is a complex process with many interconnected phases. Initially, lipids such as LDL-particles (low-density lipoproteins) accumulate and are modified (usually oxidized) inside the arterial wall. The oxidative stress leads to recruitment of macrophages and lymphocytes into the vascular intima by expression of adhesion molecules on the endothelial surface. The macrophages and lymphocytes are then activated, leading to accumulation of so-called foam-cells, which are macrophages filled with lipid droplets. Smooth muscle cells differentiate, proliferate and migrate from the media into the intima layer of the arterial wall.

Atherothrombosis
Rupture of atherosclerotic plaque in a coronary artery is the most common mechanistic event initiating MI (see Figure 1). Inflammation plays a critical role in plaque destabilization e.g. by the secretion of so-called matrix metalloproteases from activated macrophages. These proteolytic enzymes weaken the protective fibrous cap of the plaque and eventually induce its rupture. The damaged vessel wall activates platelets, primary haemostasis, and plasma coagulation, secondary haemostasis, leading to thrombus formation. The activation of platelets is induced when
subendothelial tissues such as collagen are exposed to the circulating blood. The platelets adhere to the subendothelial tissue and aggregate. The complex coagulation cascade leads to the formation of fibrin, which is polymerised to form a mesh in conjunction with platelets. The fibrin mesh is stabilized by cross-links in the presence of factor XIII.

**Figure 1.** Rupture of the fibrous cap on an atherosclerotic plaque (arrow). Adapted from Pasterkamp et al^7^ with permission from the publisher.

The main function of the fibrinolytic system is to counterbalance coagulation by prevention or removal of thrombus formation. The key enzyme in the fibrinolytic system is plasmin. Plasmin degrades fibrin into soluble fibrin degradation products, including D-dimer, by cutting the fibrin mesh at various sites. Plasmin is converted from its inactive proenzyme, plasminogen, by plasminogen activators such as tissue plasminogen activator (t-PA). Active t-PA can be inactivated by plasminogen activator inhibitor-1 (PAI-1). The balance between t-PA and PAI-1 regulates the fibrinolytic capacity in the blood vessels.

**Risk and risk factor**

The term *risk* is usually defined as the probability that an event will occur, for example, that an individual will become ill or die, within a period of time^8^. There is no general agreement on the definition of the term *risk factor*. *A Dictionary of Epidemiology* by Last, emphasizes a risk factor’s relation to a disease of major significance:

> An aspect of personal behaviour or lifestyle, an environmental exposure, or an inborn or inherited characteristic which on the basis of epidemiological evidence is known to be associated with health-related condition(s) considered important to prevent^8^.

The term *risk factor* has several related terms like *risk marker*, an attribute or event that is associated with increased probability of disease, but is not necessarily a causal factor, and *determinant*, an attribute or event that increases the probability of
occurrence of disease or other specified outcome. A modifiable risk factor is a determinant that can be modified by intervention, thereby reducing the probability of disease. Another definition of the term risk factor, proposed by Beck, accentuates the causal relationship of a risk factor to a disease process:

An environmental, behavioral, or biologic factor confirmed by temporal sequence, usually in longitudinal studies, which if present directly increases the probability of a disease occurring, and if absent or removed reduces the probability. Risk factors are part of the causal chain, or expose the host to the causal chain. Once disease occurs, removal of a risk factor may not result in a cure.

In a classic essay from 1965, Sir Austin Bradford Hill suggests nine perspectives of an association that researchers should consider before deciding that the most likely interpretation infers causation: strength, consistency, specificity, temporality, biologic gradient, plausibility, coherence, experimental evidence, and analogy. These perspectives have often been viewed as strict causal criteria. Hill himself was ambivalent about their utility and emphasized that he did not believe that we can establish hard rules of evidence that must be obeyed before cause and effect can be accepted. Still, the principles set forth by Hill form the basis of evaluation of causality.

The gold standard for establishing a causal relation is the randomized controlled trial (RCT) in which the study participants are randomly assigned to either a “treatment” group or a “control” group and followed over time. If the two groups show different outcomes (assuming the study is well designed and corroborated by other well-designed studies), then the researchers have demonstrated that changing the risk factor changes the outcome, i.e. that the risk factor is causal. However, to study the effects of a lifestyle factor such as physical activity on morbidity and mortality, a RCT is seldom an option. It would not be ethical and probably impossible to randomly assign participants to a given level of physical activity that must be maintained for a long time. Similarly, it is difficult to assign a control group prolonged inactivity. Generally, the evidence for causality of lifestyle factors needs to be based on convincing associations in observational studies. In observational studies (mainly cohort and case-control studies) the treatment and control group are usually self-selected, potentially introducing a number of possible biases. A large number of well-designed observational studies in different settings are therefore needed to establish causality. Since this thesis is based on observational studies it is not possible to prove causality for the studied variables.

Generally, research in risk factors tends to have two separate main objectives; to enhance our understanding of the pathogenesis of diseases by studying risk factors as possible etiological links, or to improve our ability to predict risk by seeking risk factors for predictive models. In this thesis, etiological as well as predictive research approaches are employed.

The cardiovascular risk factor concept
In April 1945, US president Franklin D. Roosevelt died from a cerebral haemorrhage. For a long time he had suffered from severe hypertension with blood pressure measurements as high as 260/150 mmHg. He smoked twenty cigarettes a day and
had symptoms of angina pectoris and congestive heart failure. At this time, CVD had become the leading cause of death in the US, yet very little was known about the general causes of coronary heart disease and cerebrovascular disease. Consequently, even the president's severe hypertension was overlooked by his doctors. Roosevelt's death seems to have served as a wake-up call, and in 1948 the National Heart, Lung, and Blood Institute started a health research project in the town of Framingham, Massachusetts. The researchers recruited over five thousand men and women and began the first round of physical examinations and lifestyle interviews that would later be analyzed for common patterns related to CVD development. The Russian pathologist, Nicolai Anitschkow, demonstrated as early as 1913 that simply feeding rabbits purified cholesterol dissolved in sunflower oil induced vascular lesions closely resembling those of human atherosclerosis, both grossly and microscopically. These findings were more or less ignored for over thirty years. For the researchers of the Framingham Heart Study, hypercholesterolemia became one of the main suspects. Over the years a number of characteristics such as hypercholesterolemia, hypertension, smoking, obesity, and diabetes were found to be associated with CVD in the Framingham Heart Study. Even if the risk factor concept originally was developed by the insurance industry early in the previous century, the actual term risk factor was coined by Dr William Kannel, one of the pioneers of the Framingham Heart Study. The risk factor concept has had profound implications for the understanding and the management of cardiovascular disease.

Since the pioneering work of the Framingham study, many prospective population-based and clinical studies have identified a number of independent cardiovascular risk factors which are considered “established” (also called “traditional”, “conventional” or “classical”) including increasing age, male sex, smoking, hypertension, dyslipidaemia, diabetes, and obesity. These risk factors are considered to be part of a causal chain leading to CHD and have shown to be useful for prediction of risk. Several of the established risk factors have therefore been integrated in risk scores which provide quantitative prediction of an individual’s future risk of cardiovascular disease. The assessment, treatment, and monitoring of these established risk factors are major emphases in clinical practice. The list of established cardiovascular risk factors sometimes includes physical inactivity, even though physical inactivity has not been included in any of the more common risk scores.

Beyond the established cardiovascular risk factors
In addition to the established cardiovascular risk factors, a large number of clinical, biochemical and genetic markers have shown significant associations with CVD events in previous studies. At least 246 candidate risk markers for CHD were suggested already in 1981. However, only a few of these associations have shown to be reproducible in other studies and independent of established cardiovascular risk factors. There is also an increasing interest in the effect and understanding of the lifestyle factors, which are considered the main underlying causes of the established risk factors; physical inactivity, poor dietary habits, smoking, and excessive alcohol consumption. The interest in lifestyle factors was further heightened in 2004 when the Canadian-led INTERHEART study was published. This global study identified nine modifiable risk factors (smoking, lipids, hypertension, diabetes, obesity, diet, physical activity, alcohol consumption, and psychosocial factors) that account for over 90% of the risk of myocardial infarction. The INTERHEART investigators found these risk factors universal, occurring in almost every geographic region and
racial/ethnic group worldwide, and are consistent in men and women. The lifestyle factors are, in turn, strongly influenced by a number of psychosocial factors such as depression, anxiety and social support, and socioeconomic factors like education, occupational status and income. A simplified presentation of the relations between cardiovascular disease, established and novel risk factors, lifestyle factors, socioeconomic and psychosocial factors are described in Figure 2. This thesis places focus on two of the lifestyle factors; physical activity and snuff use (a Swedish form of smokeless tobacco) and their association with MI.

Figure 2. A simplified schematic presentation of the relations between cardiovascular disease, established and novel risk factors, lifestyle factors, socioeconomic and psychosocial factors.

Physical activity
Physical activity, exercise and physical fitness are related terms which describe different concepts. The following definitions have been suggested:

Physical activity is defined as any bodily movement produced by skeletal muscles that result in energy expenditure. Exercise is physical activity that is planned, structured, and repetitive, and done to improve or maintain physical fitness. Physical fitness is defined as a set of attributes (such as aerobic endurance, muscular endurance, muscular strength, flexibility and body composition) that people have or achieve, which relate to the ability to perform physical activity. Cardiorespiratory fitness is a health-related component of physical fitness that relates to the ability of the circulatory and respiratory systems to supply fuel during sustained physical activity and to eliminate fatigue products after supplying fuel.
Physical activity can be described in different dimensions, commonly activity type, frequency (the number of events of physical activity during a specific time period), duration (time of participation of a single bout of physical activity), intensity (the physiological effort associated with participating in a special type of physical activity) and context. The two principal contexts in previous research have been occupational physical activity, which is activity associated with the performance of a job, usually within the time frame of an 8-hour day, and leisure time physical activity, which is a broad descriptor of activities performed during free-time, based on personal interests or needs. The time trends of occupational and leisure time physical activity in northern Sweden from 1990 to 1999 was investigated using the cross-sectional surveys in the Northern Sweden MONICA study. Sedentary working conditions increased in men but not in women. This trend was especially pronounced among highly educated men in which nearly 50% reported sedentary working conditions in 1999. In women, sedentary working conditions were more stable at about 20% during the study period. No clear trends were seen in leisure time physical activity. Approximately 80% of men and women reported at least two hours per week of light to moderate leisure time physical activity, such as walking or bicycling.

During the last few decades there has been an increased public health interest in commuting activity, defined by the different modes of transportation to work or school. The World Health Organization (WHO) definition of active commuting includes walking and bicycling as single transportation modes, as well as in combination with other modes (e.g. walking to catch a bus or train).

Assessment of physical activity
Physical activity is a complex behaviour and difficult to measure. Over 30 different methods have been employed and no single instrument fulfills the criteria of being highly valid, reliable, and practical, while at the same time not affecting behaviour. The methods that are very precise tend to be impractical on a population basis.

The level of physical activity can be measured by subjective and objective methods. Subjective methods include self-reporting such as questionnaires, diaries and interviews. Objective methods of physical activity level are usually made by the use of equipment, both for direct assessment (pedometers and accelerometers) or indirect assessment (heart rate monitors). Combinations of these techniques have also been developed.

In large-scale studies the level of physical activity is usually measured by questionnaire, which is relatively cheap and easily applicable when compared to other methodologies. However, questionnaires are limited in validity and reliability since they rely on self-reports. Different factors such as social desirability and seasonal variation in physical activity may lead to under- and overestimation. Moreover, a tendency to overestimate the activity level in questionnaires has been related to certain personal characteristics including age, cultural factors and body mass index. In most studies, uniquely developed questionnaires have been used which makes international comparisons more complicated. To enable cross-national monitoring of physical activity levels, the development of the International Physical Activity Questionnaire (IPAQ) started in 1998 and is still in progress.

Physical activity results in increased energy expenditure which can be estimated and used as a surrogate for physical activity. Energy expenditure can be measured by...
Background

direct methods such as the doubly-labelled water method and calorimetry. Indirect methods such as indirect calorimetry, which considers oxygen uptake and carbon dioxide production can also be used for measurements of energy expenditure. These methods are usually used in validation studies or experimental studies.

In some studies, information on activity type, intensity, and duration are collected and converted into an approximation of the activity-related energy expenditure using a coding scheme. The most commonly used unit is the metabolic equivalent (MET), which is defined as the ratio of work metabolic rate to a standard resting metabolic rate. However, standard compendia have been criticized, particularly for overestimating the intensity of activity in middle-aged and older people, since they are based on data for young adults.

Physical activity and cardiovascular disease

A large number of observational studies in different settings have shown that regular physical activity is associated with decreased risks of CVD morbidity and mortality. The association has shown a clear dose-response pattern with continuously lower risk with higher levels of regular physical activity. However, industrialization, urbanization, and motorized transport have reduced physical activity and more than 60% of the world’s population are not sufficiently active. Physical inactivity has grown to become a major concern for public health. Globally, physical inactivity is believed to account for 22% of coronary heart disease. Successful promotion of physical activity has a potential to save 2 million premature deaths and a loss of 19 million disability-adjusted life years in the world every year.

Over the last decades, evidence for substantial health benefits from physical activity, even at a moderate intensity, has been assembled. The current recommendation (from the American College of Sports Medicine and American Heart Association) suggests moderate-intensity aerobic (endurance) physical activity for a minimum of 30 minutes on five days each week or vigorous-intensity aerobic physical activity for a minimum of 20 minutes on three days each week to promote and maintain health.

Most of the early studies investigating the effects of physical activity on CVD and other health outcomes were conducted among men, partly because of the higher absolute risk in men. The pattern of physical activity may vary between women and men which can have an impact on the validity of assessment tools. However, the inverse relationship between physical activity and CVD has now been clearly demonstrated in women as well as in men.

Both cardiorespiratory fitness and physical activity have shown to be associated with a reduced risk of CVD morbidity and mortality. In addition, being physically fit seems to protect against the influence of detrimental factors such as smoking and obesity. The shape of the dose-response curve across activity and fitness groups has been compared. While the gradient across fitness groups shows a curvilinear gradient with a steep slope at low levels of fitness, the gradient across activity groups is more consistent. As yet, it has not been possible to judge whether physical activity or cardiorespiratory fitness is more important to health.

The inverse relation between physical activity and CVD has been shown predominantly in studies of leisure time physical activity. Results from
studies on the association between occupational physical activity and CVD have been more inconsistent and the separate effect of commuting activity on CVD risk has not been widely studied.

Mechanisms
Epidemiological studies suggest that physically active individuals have a 30-50% lower risk of CHD than do sedentary persons. Regular physical activity has effects on several mechanisms which may contribute to CHD risk reduction. These may be classified in terms of cardiovascular effects, metabolic effects, lifestyle effects and adverse effects.

Cardiovascular effects
Regular physical activity decreases the resting heart rate probably by altering autonomic function and increasing heart stroke volume. Reduced sympathetic activity may also favourably impact blood pressure. In a meta-analysis involving 72 trials of 4 to 52 weeks of dynamic aerobic endurance exercise, the average reduction of systolic and diastolic blood pressure was 3.0/2.4 mmHg and the effect was more pronounced in the 30 hypertensive study groups (-6.9/-4.9). The plasma levels of noradrenaline were 29% lower in the fit state compared with untrained values, indicating a beneficial effect on blood pressure by a decrease in activity of the autonomic nervous system.

Endothelial-derived nitric oxide (NO) is a major mediator of vasorelaxation in coronary and peripheral arteries. Coronary atherosclerosis is associated with reduced NO bioavailability resulting in a paradoxical vasoconstriction of atherosclerotic coronary artery segments in response to acetylcholine, indicative of endothelial dysfunction. Endurance exercise training in patients with CHD has been shown to attenuate ST-segment depression during exercise and decrease perfusion defects on thallium scanning, indicating an increase in myocardial perfusion. Recent studies have shown that this effect is more likely to be explained by an improved endothelial function in the coronary arteries rather than formation of collaterals or regression of coronary artery stenosis.

Strenuous exercise produces a short-lived inflammatory response, whereas both cross-sectional comparisons and longitudinal studies of regular physical activity demonstrate a long-term "anti-inflammatory" effect. It is not clear how regular physical activity reduces inflammation. Physical activity is inversely associated with age, body mass index, smoking and several other factors that are also independently associated with inflammatory markers. However, lower levels of inflammatory markers persist in more active subjects in most studies even after adjustment for these factors. Decreased cytokine production by skeletal muscles, adipose tissue, and endothelial and blood mononuclear cells, as well as improved endothelial function by increased NO bioavailability have been suggested as possible additional mediating mechanisms.

The association between physical activity and haemostasis is more unclear. Scientific data are sparse and sometimes inconsistent. Short-term strenuous exercise has been shown to induce a pro-thrombotic state by increasing platelet counts and enhancing platelet activity. These effects are balanced by an increase in fibrinolytic capacity. For regular physical activity it appears that platelet activity is decreased. A majority of cross-sectional studies and a few smaller intervention studies have shown an
inverse association between regular physical activity and several haemostatic markers. The effect of regular physical activity on haemostatic markers has been suggested to be most pronounced among patients with CHD who exhibit an impaired balance between pro-thrombotic and fibrinolytic parameters. Conflicting results have been published showing similar effects in men with and without manifest cardiovascular disease.

**Metabolic effects**

Regular physical activity has a clear beneficial effect on glucose homeostasis and insulin sensitivity. This effect has been shown in insulin resistant as well as normal populations. Interventions with exercise or exercise and diet have been found to markedly reduce the risk of type II diabetes in patients with impaired glucose tolerance. Repeated physical activity results in a persistent increase of skeletal muscle glucose uptake via an insulin-independent mechanism that bypasses the typical insulin signalling defects associated with insulin resistance. The enhanced glucose uptake related to physical activity has been attributed to the increased expression and/or activity of glucose transporter isoform 4 protein, GLUT4, but recent research has suggested additional mechanisms such as increased oxidative capacity by exercise-induced expression of proteins involved in mitochondrial biogenesis.

Several studies have shown that individuals who are physically active have favourable blood lipid profiles compared to non-active counterparts. Regular physical activity increases HDL-cholesterol levels and decreases triglyceride levels, mainly by an increased lipoprotein lipase activity. Lipoprotein lipase is an important enzyme in lipid metabolism that hydrolyses triglycerides in chylomicrons and very low density lipoproteins (VLDL), thereby providing fatty acids for tissue utilisation, but also surface components needed to form the mature HDL particle. Physical activity induces a transient rise in lipoprotein lipase messenger RNA followed by a rise in lipoprotein lipase plasma mass concentration. The exact mechanism for this induction of lipoprotein lipase gene expression is not clear but may result from dynamic changes in serum catecholamines, plasma insulin, or events intrinsic to muscle contraction. The beneficial effect on blood lipid profile has been shown for aerobic exercise, whereas lipid changes are usually not found following resistance exercise. The difference in overall caloric expenditure between aerobic and resistance exercise have been suggested as an explanation for the absence of this effect.

Randomized controlled trials have shown significant weight loss and reduction in central obesity in sedentary overweight individuals even for rather modest amounts of exercise. Regular physical activity appears to be particularly efficient in maintaining weight after weight-loss. The minimal level may be as low as about 10 km of walking (or other equivalent caloric expenditure) a week. Even if weight loss is not achieved, regular physical activity may reduce the hazards related to obesity. In a cohort study of over 21,000 American men, obese but fit men had lower all-cause and CVD mortality than did men who were unfit and lean. Substantial CHD risk reduction was also seen for the obese but physically active women in the American Nurses’ Health Study.

**Lifestyle effects**

Other health behaviours may be influenced by the adherence to a physically active lifestyle. Previous cross-sectional studies have shown that higher levels of regular
physical activity are associated with a healthier lifestyle in general. Physically active
individuals tend to have healthier dietary habits with greater consumption of fruit
and vegetables and are more likely to eat breakfast regularly. Physically active
individuals are also less likely to smoke. In addition, regular physical activity
may affect psychological well-being and the ability to endure psychosocial stress.

Adverse effects
Several studies have shown that heavy physical exertion may trigger the onset of
myocardial infarction particularly in untrained individuals. Available data
suggest that short-term vigorous exercise in sedentary individuals can lead to
increased platelet activity and platelet hyperreactivity, whereas regular, long-term
exercise may abolish or reduce this response. The relative risk for myocardial
infarction was as high as 107 during physical exertion compared to at rest in men who
were sedentary in a retrospective study with case-crossover design. In men who
exercised at least five times a week the relative risk for myocardial infarction was 2.4
during physical exertion compared to at rest.

Snuff use
Moist snuff (“snus” in Swedish) has been the most common form of smokeless
tobacco in Sweden since the beginning of the 19th century. The tobacco in snuff is
air- and sun-cured and ground. The tobacco is then mixed with water (45-60% of
total content) and sodium chloride (1.5-3.5%) and processed by steam treatment (and
not fermented as in North American smokeless tobacco). After this process, sodium
carbonate (1.2-3.5%), moisturizing agents like glycerol and propylene glycol (1.5-
3.6%), and aroma additives (< 1%) are added. Snuff is sold either loose or portion-
packed and placed behind the upper lip. It is typically kept in the mouth for
approximately 30 minutes before it is discarded. The contents of Swedish snuff
differs from other smokeless tobacco and the following presentation is mainly
focused on different aspects of Swedish snuff.

Nicotine is the most addictive substance in snuff. The nicotine content varies
between different varieties of snuff, usually about 1%. The absorption and blood
levels of nicotine and risk for nicotine addiction are probably equal between
smoking and snuff use. Animal studies have shown that high doses of nicotine,
bioequivalent to smoking two standard cigarettes, may induce cardiac arrhythmias of
simple to severe nature.

Other substances in snuff that may have detrimental effects on health are tobacco-
specific N-nitrosoamines (TSNAs), which are suspected to be carcinogenic. The
content of TSNAs are significantly lower in snuff than in most North American
smokeless tobacco products, probably due to the fact that snuff is heat-treated and
not fermented. Since there may be health hazards associated with TSNAs, its content
in snuff has been greatly reduced during the last decades through efforts made by the
manufacturers. The sodium content in snuff, which is added as a part of an alkaline
buffer to facilitate buccal absorption of nicotine, has also been suspected of causing
health problems, particularly in salt-sensitive individuals. A large number of other
potentially harmful substances are found in snuff such as cadmium, lead, polonium-
210, benzo(a)pyrene, and formaldehyde.
During the last few decades there has been a change in the use of tobacco products in Sweden. A decline in smoking and an increase in the use of snuff have been observed in both sexes (see Figure 3). The prevalence of smoking in northern Sweden is among the lowest in the world119.

Figure 3. Prevalence of tobacco use among men and women, age 25–64 years, in northern Sweden, 1986–2004. Reprint from Stegmayer et al120 with permission from the publisher.

Snuff use and cardiovascular disease
In 1994, Bolinder and co-workers published the results of a study investigating the association between snuff use and risk of cardiovascular mortality in a large Swedish cohort of construction workers121. They found a 40% increased risk of death from cardiovascular diseases among snuff users compared with non-tobacco users. In more recent studies of the same cohort an increased risk of fatal myocardial infarction122 and fatal ischemic stroke123 has been observed. However, no excess risk of cardiovascular diseases have been associated with snuff use in a series of population-based studies124–129.

The finding of an increased risk of fatal myocardial infarction in the construction worker study, together with the sympathico-adrenal activating and possible arrhythmogenic properties of nicotine, has raised the question as whether snuff use may increase the risk of sudden cardiac death. No previous study has investigated the association between snuff use and sudden cardiac death.

Mechanisms
Snuff use has several cardiovascular, metabolic, and lifestyle effects which may impact the risk of cardiovascular disease adversely. Some aspects of snuff use may even have a protective influence on cardiovascular risk.
Cardiovascular effects
The acute effects of snuff use include increased heart rate (10-20 beats/min)\(^\text{118}\) and blood pressure (10-20 mmHg systolic and 2-12 mmHg diastolic)\(^\text{130}\). This is similar to the increase in blood pressure by nicotine replacement therapy. The acute effects of snuff are therefore considered to be mediated mainly through a nicotine-induced activation of the sympathetic nervous system\(^\text{118}\). One study has also shown endothelial dysfunction after administration of snuff in healthy snuff users\(^\text{131}\). Whether regular snuff use causes permanently elevated blood pressure is controversial. Increased blood pressure between periods of snuff use has been observed in large studies of construction workers\(^\text{123, 132}\) and increased mean blood pressure was found in snuff users in a study using ambulatory 24-hour blood pressure monitoring\(^\text{133}\). However, the majority of population-based studies have not detected any adverse effects on blood pressure during periods of non-exposure to tobacco\(^\text{127, 134-138}\). Moreover, no signs of accelerated atherosclerosis has been found in studies using ultrasound assessments of carotid and femoral intima media thickness\(^\text{134, 139}\). Similarly, snuff use does not seem to increase levels of haemostatic and inflammatory markers as seen in tobacco smokers\(^\text{134, 135}\).

Metabolic effects
Several studies have shown no detrimental effects on serum lipids, body mass index, waist-hip ratio, insulin sensitivity, glucose tolerance, or risk for type 2 diabetes associated with the use of snuff\(^\text{134, 135, 140, 141}\). In studies with dose stratification, however, excess risk of type 2 diabetes\(^\text{142}\) and metabolic syndrome\(^\text{137}\) was found for heavy users, suggesting a possible dose-response relationship between snuff use and metabolic determinants.

Lifestyle effects
Regular use of snuff may have an effect on other health behaviours and has been suggested as a potential gateway to smoking, especially in young people. In a study of Swedish boys, an increased risk for taking up smoking was seen among those who had experimented with snuff earlier\(^\text{143}\). The results are difficult to interpret since experimenting with snuff may be an indicator of a drug- and risk-seeking lifestyle, a common behaviour among adolescents who start smoking\(^\text{144}\).

Beneficial effects
Snuff contains a number of substances which may possess antioxidative effects such as carotenoids and phenols\(^\text{116}\), but it is not clear to what extent these compounds can neutralize the effects of nicotine and TSNAs.

Snuff use have also been suggested to be a potential gateway from smoking, particularly from supporters of the “harm reduction strategy” for tobacco control\(^\text{145, 146}\). This public health policy advocates snuff use as a less hazardous alternative to smoking. In a retrospective survey, the smokers who used snuff at the time of their latest cessation attempt increased the probability of abstinence by about 50%\(^\text{147}\). However, 71% of the men who quit smoking did so without using snuff, which indicates that snuff is not a necessary component of smoking cessation on a population level\(^\text{147}\).
Haemostatic and inflammatory biomarkers

A biomarker (or biological marker) can be defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention\textsuperscript{148}.

D-dimer

D-dimer is a fibrin degradation product (see Figure 4)\textsuperscript{149}. The fibrinogen molecule, from which D-dimer originates, consists of two outer D-domains and a central E-domain\textsuperscript{150}. A fibrin mesh is formed when the fibrinogen molecule is activated by thrombin. The fibrin mesh is stabilized by factor XIII, which cross-links the fibrin E-domain to two D-domains\textsuperscript{151}. Plasmin breaks down the fibrin mesh but cannot break down the bonds between an E-domain and two D-domains\textsuperscript{151}. Thus, D-dimer represents the remnant of a fibrin mesh which has been stabilized by factor XIII. In vascular disorders such as acute thromboembolism, D-dimer levels are highly elevated\textsuperscript{152}. In modestly elevated circulating concentrations, however, D-dimer is considered to reflect the extent of cross-linked fibrin turnover in the circulation and minor increases in plasma coagulation\textsuperscript{153}.

von Willebrand factor

The adhesive glycoprotein, von Willebrand factor (VWF), plays a key role in primary haemostasis. It mediates the adhesion of platelets to components of the subendothelial tissue at sites of vascular injury and initiates platelet-to-platelet aggregation\textsuperscript{154}. In addition, it stabilizes factor VIII, a cofactor required for normal thrombin generation\textsuperscript{155}. The mature VWF is composed of identical 2050 amino acid monomers which are disulfide-linked to form a multimeric molecule that ranges from dimers of 500 kDa up to large polymers of over 20000 kDa\textsuperscript{156}. The VWF molecule is expressed by endothelial cells and megakaryocytes only and is to a large extent secreted constitutively\textsuperscript{157}. Secretion of stored VWF can be stimulated by thrombin, fibrin and various other agents\textsuperscript{158}. The plasma concentrations of VWF vary from 50 to 200% of normal and VWF is cleared from the circulation by the liver with a half-life of approximately 12 hours\textsuperscript{156}. While defects of VWF results in a bleeding disorder, von Willebrand disease, elevated VWF levels are considered to reflect an increased risk for thrombosis\textsuperscript{157}.

Tissue plasminogen activator

Plasminogen, tissue plasminogen activator (t-PA), and urokinase plasminogen activator (u-PA) are the main fibrinolytic components\textsuperscript{159}. Both t-PA and u-PA are serine proteases which convert plasminogen into plasmin, which in turn degrades the cross-linked fibrin of a thrombus\textsuperscript{160}. t-PA is the major plasminogen activator in plasma, whereas u-PA binds to a specific cellular u-PA receptor resulting in an enhanced activation of cell-bound plasminogen\textsuperscript{159}. t-PA is expressed and secreted into the circulation by vascular endothelial cells and is normally present at low concentrations in plasma\textsuperscript{161}. However, t-PA stored in a cellular storage pool can be quickly released after stimulation by thrombin and other components of the coagulation process\textsuperscript{162}. The presence of fibrin enhances the activation rate of plasminogen by t-PA\textsuperscript{163}. Circulating t-PA is cleared from plasma by the liver with a half–life of 3–5 minutes\textsuperscript{164}. 
Plasminogen activator inhibitor-1 and t-PA/PAI-1 complex

Plasminogen activator inhibitor-1 (PAI-1) is the principal inhibitor of plasminogen activators. The PAI-1 molecule is a glycoprotein and a member of the serine protease inhibitor superfamily (serpins)\(^{161}\). The concentration is normally low in plasma since the half-life of PAI-1 is short (8-10 min). However, substantial amounts of PAI-1 can be released from activated platelets after vessel trauma. In contrast with t-PA, PAI-1 is synthesized by a variety of cell types, including endothelial cells, macrophages, hepatocytes, and adipocytes. Synthesis is stimulated by several agents, such as thrombin, cytokines, insulin and other hormones\(^{165}\). The released PAI-1 binds to the active site of t-PA and forms a stable 1:1 stochiometric t-PA/PAI-1 complex\(^{165}\). Hence, PAI-1 plays a regulatory role in fibrinolysis by limiting the activation of plasminogen. The PAI-1 molecule is cleared from the circulation mainly by hepatic cells\(^{166}\).

\[\text{Plasminogen} \xrightarrow{\text{Activators: t-PA, u-PA}} \text{Plasmin} \xrightarrow{\text{Fibrin Degradation Products: D-dimer}} \text{Fibrin}\]

**Figure 4.** Schematic representation of the activation of plasminogen.

**Measurements of t-PA, PAI-1, and t-PA/PAI-1 complex**

Active t-PA and PAI-1 are difficult to measure because they are unstable and require specific laboratory procedures to avoid complex formation\(^{167}\). Thus, in epidemiological studies, total t-PA and PAI-1 mass concentrations in plasma are usually measured (the equivalent terms t-PA antigen and PAI-1 antigen are also used). t-PA mass concentration is a mixture of active t-PA and inactive t-PA in complex with PAI-1 or other inhibitors such as C1-inhibitor\(^{168}\) (see **Figure 5**). The t-PA/PAI-1 complex constitutes the largest relative proportion of t-PA mass concentration in plasma while approximately 20% is in active, uncomplexed form\(^{169}\). t-PA mass concentration is inversely correlated with active t-PA\(^{170}\). Consequently, high mass concentrations of t-PA as well as PAI-1 are considered to reflect impaired fibrinolysis.
Figure 5. The relationship between t-PA activity and mass concentration, PAI-1 activity and mass concentration and t-PA/PAI-1 complex.

**Lipoprotein (a)**

Lipoprotein (a) consists of a low-density lipoprotein (LDL)-like particle and a unique, large glycoprotein, apolipoprotein (a) [apo(a)]\(^{171}\). Apo(a) is linked to the LDL-like particle by its surface apolipoprotein, apo B\(^{171}\). Plasminogen and apo(a) are both composed of structural loop-like domains called kringles\(^{172}\). However, while plasminogen consists of five different kringles (K1 to K5), the apo(a) particle is composed of a single copy of kringle 5 and a variable number of multiple repeats of kringle 4\(^{172}\). As a result, the different apo(a) isoforms vary in molecular weight from 300 to 800 kDa\(^{172}\). The variability in apo(a) size influences the levels of Lp(a) and the relation between apo(a) size and Lp(a) levels has generally been found to be inverse\(^{172}\). The Lp(a) particle is synthesized by the liver and possess a half-life in circulation of 3-4 days\(^{173}\). The site of catabolism for the Lp(a) particle is largely unknown, but renal clearance is plausible\(^{174}\). The concentration of Lp(a) vary by a thousandfold between individuals, but the variability has been shown to be relatively stable for a given individual\(^{175}\). Lp(a) levels are considered to be mainly genetically determined and are to a large extent unaffected by age, sex, and lifestyle factors\(^{175}\). The physiological function of Lp(a) is still unclear, and individuals with very low levels of Lp(a) have not been associated with any medical disorder\(^{172}\). The structural homology of Lp(a) and plasminogen has led to the suspicion of a pathophysiological role for Lp(a) since it has the ability to act as competitive antagonist of plasminogen\(^{176}\). In vitro studies have shown that Lp(a) binds to the surface of fibrin and inhibits the activation of plasminogen\(^{176}\). However, there is no clear evidence that Lp(a) interferes with plasminogen activation or inhibits fibrinolysis in vivo\(^{177}\). Other possible prothrombotic effects of Lp(a) have been suggested based on results from in vitro studies, including stimulation of PAI-1 expression and inhibition of t-PA synthesis by endothelial cells in culture\(^{178, 179}\). Lp(a) has also been implicated in the
inflammatory response as a potential inducer of IL-6\textsuperscript{180}. In addition, the LDL-like particle may promote atherogenesis by vascular accumulation of cholesterol\textsuperscript{181}.

**C-reactive protein**

C-reactive protein (CRP) is a circulating plasma protein and is considered the major acute-phase reactant in humans\textsuperscript{3}. This molecule was discovered in 1930 in the blood of patients with acute pneumococcal pneumonia and named for its binding to the C-polysaccharide of the pneumococcal cell wall\textsuperscript{182}. CRP consists of five identical non-covalent bound subunits arranged with cyclic pentameric symmetry in a disc-like configuration\textsuperscript{183}. The molecule has a calcium-dependent binding specificity for phosphocholine, a key ligand which is present in constituents of many bacteria, fungi, and parasites, and in the outer leaflet of most cell membranes\textsuperscript{183}. CRP promotes the elimination of pathogens and damaged cells by mediating phagocytosis by macrophages and neutrophils, and by activating the complement system\textsuperscript{3}. CRP is a member of the pentraxin family of proteins. Pentraxins belong to the lectin fold superfAMILY\textsuperscript{184}.

Circulating CRP is produced by the liver, although extrahepatic expression has been reported\textsuperscript{185}. Interleukin 6 (IL-6) is the main inducer of the CRP synthesis and a minor role is attributed to other pro-inflammatory cytokines such as IL-1 and tumour necrosis factor-\(\alpha\) (see Figure 6). CRP plasma levels may increase by 1000-fold in 24-48 hour in response to sepsis or other strong acute stimuli\textsuperscript{184}. The circulation half-life is about 19 hours in healthy individuals as well as in individuals with chronic diseases\textsuperscript{184}. The CRP molecule is eliminated from plasma and catabolised by hepatocytes exclusively\textsuperscript{186}. The term “high-sensitivity” CRP (or hsCRP) refers to the measurements of CRP over its normal range. Thus, the analyte designated as hsCRP is just CRP itself\textsuperscript{184}. However, in the interpretation of CRP values it is important to separate the modest increases in baseline values from the very low normal levels (usually measured by hsCRP methods) from the prolonged extensive increases in patients with systemic inflammatory diseases, chronic infections or a massive rise following sepsis or acute trauma\textsuperscript{184}.

**Interleukin 6**

Interleukin 6 (IL-6) is an inflammatory cytokine which consists of four long \(\alpha\)-helices\textsuperscript{187}. The molecule is expressed by different kinds of cells including activated macrophages, lymphocytes, fibroblasts, and vascular smooth muscle cells. IL-1 and TNF-\(\alpha\) are considered to be the main inducers\textsuperscript{3}. However, IL-6 has also been suggested as a myokine, defined as a cytokine that is produced and released by contracting skeletal muscle fibers\textsuperscript{188}. IL-6 is the first cytokine to increase in the circulation during strenuous exercise, since IL-1 and TNF-\(\alpha\) are generally not increased by exercise. IL-6 exerts its effects mainly by binding to hepatocytes and lymphocytes (see Figure 6) which express specific IL-6 receptors, but can also have effects on other cellular elements as there are soluble IL-6 receptors present in human serum\textsuperscript{187}. The binding of IL-6 to the IL-6 receptor promotes multiple inflammatory effects, including the synthesis of acute-phase proteins by hepatocytes and terminal differentiation of B-lymphocytes\textsuperscript{189}. In addition, IL-6 has been suggested to induce several metabolic effects such as increased lipolysis and to regulate glucose metabolism during exercise\textsuperscript{188}. After binding to the IL-6 receptor the IL-6 molecule is rapidly internalized and degraded within the target cell\textsuperscript{187}. The half-
life of IL-6 in circulation is shorter (<2 hours) than that of CRP, and the within-person variability is probably greater.

**Figure 6.** Overview of the effects of IL-6 on different cell types.

**Haemostatic and inflammatory markers and cardiovascular disease**

The physiological function of the haemostatic system is to arrest blood loss from injured blood vessels. Thrombosis is therefore considered as “haemostasis in the wrong place”. The presented haemostatic markers, which may reflect adhesion and aggregation of platelets (VWF) and activation of coagulation and fibrinolysis (D-dimer, t-PA, PAI-1, t-PA/PAI-1 complex, and possibly Lp(a) as an inhibitor of fibrinolysis), have been suggested to be associated with an increased thrombotic tendency and to have a potential causal role in CHD. Numerous studies have shown that haemostatic markers are associated with several established cardiovascular risk factors as well as CHD in general populations. A number of meta-analyses of the association between individual haemostatic markers and CHD have also been published. In these meta-analyses, the odds ratio for individuals with baseline plasma levels in the top third, compared with those in the bottom third, ranged from 1.4 to 1.8 except for PAI-1 which was 1.0 (see Table 1). t-PA/PAI-1 complex has shown to be associated with CHD and stroke, but is not widely studied and no meta-analysis has been published. Several of the haemostatic markers have also been suggested to be acute-phase reactants and thereby potential markers of inflammation. This has raised the question as to whether the associations of haemostatic markers and CHD are due to a direct role in atherothrombosis or simply reflect local or systemic inflammation. It is not clear to...
what extent adjustment for inflammatory markers may influence the associations between haemostatic markers and CHD.

Since inflammation is considered to play a key role in the atherosclerotic process and in destabilization of atherosclerotic plaque, circulating inflammatory markers such as CRP and IL-6 have been studied in relation to cardiovascular risk factors and outcomes. Cross-sectional studies have shown that higher concentrations of both CRP and IL-6 are correlated with several established cardiovascular risk factors, such as obesity and smoking. A large number of studies have found a clear association between CRP levels and future CHD events and in 2003, the American Centre of Disease Control and the American Heart Association published a joint statement supporting the use of hsCRP as a part of global coronary risk assessment. Subsequently, a meta-analysis of 22 population-based prospective studies involving 7068 CHD cases was published in 2004. In a comparison between individuals with CRP levels in the top third compared with those in the bottom third, the odds ratio after adjustment for established risk factors was 1.6 (95% CI, 1.5-1.7). The odds ratio in the meta-analysis was markedly lower compared to some of the early reports. Consequently, the recommendation of hsCRP for cardiovascular risk assessment in clinical practise by the American CDC/AHA has been questioned. The most recent updated meta-analyses of the association between CRP, IL-6, and CHD are presented in Table 1.

**Table 1.** The most recent meta-analyses on the association between CHD and haemostatic and inflammatory markers. Odds ratios for subjects with baseline values in the top third compared to those in the bottom third are presented.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Publication year</th>
<th>Prospective studies (N)</th>
<th>Total CHD cases (N)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer201</td>
<td>2001</td>
<td>7</td>
<td>1535</td>
<td>1.7 (1.3-2.2)</td>
</tr>
<tr>
<td>VWF202</td>
<td>2002</td>
<td>13</td>
<td>1622</td>
<td>1.5 (1.1-2.0)</td>
</tr>
<tr>
<td>t-PA204</td>
<td>2004</td>
<td>7</td>
<td>2119</td>
<td>1.5 (1.2-1.8)</td>
</tr>
<tr>
<td>PAI-1204</td>
<td>2004</td>
<td>5</td>
<td>833</td>
<td>1.0 (0.5-1.8)</td>
</tr>
<tr>
<td>Lp(a)203</td>
<td>2008</td>
<td>31</td>
<td>9870</td>
<td>1.4 (1.3-1.6)</td>
</tr>
<tr>
<td>CRP218</td>
<td>2004</td>
<td>22</td>
<td>7068</td>
<td>1.6 (1.5-1.7)</td>
</tr>
<tr>
<td>IL-6190</td>
<td>2008</td>
<td>17</td>
<td>5730</td>
<td>1.7 (1.4-1.9)*</td>
</tr>
</tbody>
</table>

*Odds ratio for extreme thirds received by personal e-mail correspondence, March 10, 2009 with Frances Wensley, Department of Public Health and Primary Care, University of Cambridge.

Even though associations have been found between haemostatic and inflammatory markers and CHD, this does not necessarily imply that these associations are causal. The simplest interpretation is that these markers may be considered simply as sensitive markers of the progression in atherosclerosis. They are also associated with established risk factors and may therefore be considered as confounders as well. In other words, haemostatic and inflammatory markers may be causes, consequences, or coincidences of CHD.
OBJECTIVES

The objective of the thesis was to evaluate the associations between certain lifestyle factors (physical activity and snuff use), biomarkers reflecting the haemostatic and inflammatory systems and risk of a first-ever myocardial infarction.

The specific aims of each paper were:

I. - to investigate the associations between commuting activity, occupational and leisure time physical activity and risk of myocardial infarction.

II. - to investigate the risk of myocardial infarction or sudden cardiac death among male snuff users without a previous history of smoking.

III. - to investigate the associations between haemostatic markers and risk of myocardial infarction with adjustment for established cardiovascular risk factors and inflammatory markers (primary aim).

- to investigate the predictive ability of haemostatic and inflammatory markers for future myocardial infarction above and beyond the established cardiovascular risk factors (secondary aim).

IV. - to explore the relative contribution of markers from different potential mediating pathways on the association between active commuting and risk of myocardial infarction.
MATERIAL AND METHODS

Study population
The studies in this thesis were based on the population of Västerbotten and Norrbotten, the two northernmost counties in Sweden. The area is sparsely populated with just over 500,000 inhabitants within an area of 154,300 km². The mean incidence rate in myocardial infarction (fatal and non-fatal, first and recurrent) was 400 per 100,000 in men aged 25-64 and 250 per 100,000 among women in the same age group during the study period.

The study base for this thesis, the Västerbotten Intervention Program (VIP) cohort and the Northern Sweden MONICA study cohort, are two subcohorts in the Northern Sweden Health and Disease Study (NSHDS) which also consists of a third subcohort, the local Mammography Screening Project (MSP). These three cohorts have been merged for research purposes. Since no data on cardiovascular risk factors other than tobacco consumption were collected in the MSP, this subcohort was not included.

The Västerbotten Intervention Program
The Västerbotten Intervention Program (VIP) started in 1985 as a community intervention program for cardiovascular and diabetes prevention in the county of Västerbotten and is ongoing. The pilot program was gradually spread and covered the entire county in 1991. In the VIP, all men and women upon reaching 30, 40, 50 and 60 years of age were invited to participate in a health screening at their local primary health care centre (since 1996 at 40, 50 and 60 years of age). The participants undergo a health examination with focus on lifestyle and risk factors for cardiovascular diseases and diabetes and answer a comprehensive questionnaire. Each health screening concludes with health counselling. The VIP health examination, questionnaire, and blood sampling procedures were designed to be as similar as possible to the MONICA survey. The mean participation rate was 57% during 1990-96, but increased significantly from 1997 reaching a mean of 65% during the period up to 2008.

The Northern Sweden MONICA study
The multinational MONItoring of trends and determinants in CArdiovascular disease (MONICA) project was initiated in 1982 in 26 countries by the World Health Organization (WHO) with the aim of measuring trends in cardiovascular risk factors in parallel with cardiovascular end-points. In the framework of MONICA, population-based health surveys were performed in Norrbotten and Västerbotten counties in 1986, 1990, 1994 and 1999. In 1986 and 1990, a random sample of 250 women and 250 men for each of the age groups 25-34, 35-44, 45-54 and 55-64 years was invited to health examinations as related to cardiovascular risk factors. The surveys performed 1994 and 1999 also included the age group 65-74 years. The participation rate was between 75-80%.

The Northern Sweden Medical Research Bank
All participants in the VIP and the Northern Sweden MONICA study were requested to donate blood samples to be stored at the Northern Sweden Medical Research Bank at Umeå University Hospital for future research purposes. More than 90% donated
blood samples. At the first MONICA survey in 1986, only serum samples were obtained. Consequently, these samples were not suitable for analyses of the studied haemostatic and inflammatory markers.

**Study design**
All studies in this thesis were conducted with a prospective incident nested case-control study design\(^2\). The term *prospective* refers to the fact that in conformity with a cohort study, blood samples and information about exposure are collected before development of the outcome. In an *incident case-control* (or case-referent) study, new cases of a disease are compared to a control group of healthy subjects which is sampled from the entire study base that gave rise to the cases. This study design is often preferred in studies based on laboratory analyses, since a cohort study (in which the cases are compared to all subjects in the study base) is much more expensive and time-consuming. The term *nested* is often used to emphasize that the study subjects are participants in a larger study base, in this case the combined cohort of the participants in the VIP and the Northern Sweden MONICA study. The cases and controls in the studies in this thesis were identified through record linkage between the Northern Sweden MONICA Incidence Registry (presented below) and the study base (the participants in the VIP and the Northern Sweden MONICA study). The selection of cases can be described in four main steps:

*Step 1: Identification of all first-ever myocardial infarction cases occurring in Västerbotten and Norrbotten during the study period (January 1, 1985 to December 31, 1999).*

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**Figure 7.** Case finding by the Northern Sweden MONICA Incidence Registry.
Case definition
Cases of MI or suspected MI (ICD-8: 410-414, ICD-9: 410-414, and ICD-10: I20-25) occurring in-hospital or out-of-hospital in Västerbotten and Norrbotten were reported and recorded in the Northern Sweden MONICA Incidence Registry from January 1, 1985 to December 31, 1999. The recording of MI cases in the MONICA study included the age group 25-64 years. Additional recording was conducted for the NSHDS to include individuals over 64 years. All diagnosis were re-evaluated and confirmed through screening of hospital discharge records, general practitioners' reports, and death certificates by the Northern Sweden MONICA Incidence Registry in a standardized manner using MONICA methodology\(^{222, 223}\). A suspected coronary event was classified into one of five categories based on a combination of symptoms, ECG recordings, and enzyme measurements. The categories were: definite infarction, possible infarction, ischaemic cardiac arrest with successful resuscitation not fulfilling the criteria for definite or possible infarction, not infarction, and unclassifiable infarction. Subjects who died within 28 days from the onset of MI were recorded as fatal cases. The proportion of unclassifiable MIs was 1.5% for non-fatal MIs and 4.3% for fatal MIs during 1985 to 1997.

For non-fatal cases, only cases classified as definite infarction were included. Non-fatal cases were classified as definite infarction if they met one of the following criteria:

- Typical serial ECG progression (defined by the Minnesota codes).
- At least one measurement of elevated cardiac enzymes to more than twice the upper limit of normal in combination with typical symptoms and abnormal ECG.
- At least one measurement of elevated cardiac enzymes to more than twice the upper limit of normal in combination with an ECG progression labeled probable and atypical symptoms.

For fatal MIs we accepted diagnoses based on autopsies or confirmed by medical records as being caused by coronary heart disease (ICD-8 and ICD-9 codes 410-414, ICD-10 codes I20-I25). Fatal cases were classified as definite infarction if they fulfilled the criteria for definite non-fatal event or if they had visible evidence of recent MI or recent coronary occlusion found at autopsy. For fatal cases we also accepted “possible” MI defined as a history of ischemic heart disease, but not confirmed by autopsy, evidence of chronic occlusive ischemic heart disease or old infarction found at autopsy, or if there were typical or atypical symptoms before death and no evidence of other cause of death. Silent MI found on routine examination was not included because no accurate assignment of the date of occurrence could be made.

For SCD cases (paper II) we used two definitions: SCD with survival time <24 h and SCD with survival time<1 h. Survival time was defined as time from onset of the acute symptoms of a coronary event (or, in their absence, the fatal collapse of the person) to time of death. Data on survival time was obtained from patient records.
Step 2: Identification of all subjects who participated in health examination in the VIP or the Northern Sweden MONICA study during the study period.

Västerbotten (n=256 710 by Dec. 31, 1999)
95% of the study base

Norrbotten (n=258 094 by Dec. 31, 1999)
5% of the study base

Baseline health examinations
Jan. 1, 1985 to Dec. 31, 1999
- VIP health screening (n=66 200)
- MONICA health survey (n=7550)

Case finding
Jan. 1, 1985 to Dec. 31, 1999
MONICA Incidence Registry
First-ever MI cases (n=7337)
(abt 45% were cases from Västerbotten)

Figure 8. Baseline health examinations in the VIP and the Northern Sweden MONICA study.

Between January 1, 1985 and December 31, 1999, baseline health examinations were performed on a total of 66 200 subjects in the VIP and on a total of 7550 subjects in the Northern Sweden MONICA study.
Step 3: Record linkage between the cases in the Northern Sweden MONICA Incidence Registry and the participants in the VIP health screening or the MONICA health survey.

Figure 9. Record linkage for identification of first-ever MI cases with participation in health examination in the VIP or the MONICA study prior to the MI.

A total of 696 cases fulfilled the inclusion criteria for a first-ever MI with participation in health examination in the VIP or the MONICA study prior to the event (see Figure 9).
**Step 4: Exclusion of cases with cancer or previous stroke. Validation of the first-ever MI cases to exclude cases with MI before January 1, 1985 (when the documentation of the Northern Sweden MONICA Incidence Registry was initiated).**

![Diagram](image)

**Figure 10.** Validation of the first-ever MI cases and exclusion of cases with cancer or previous stroke.

Exclusion criteria for cases were previous MI or stroke, or a cancer diagnosis in the 5 years prior to or 1 year after diagnosis of MI, either according to the Northern Sweden MONICA Incidence Registry or the National Cancer Registry, or after validation if suggested by questionnaire data or patient records. After these exclusions 651 cases remained. Out of 651 cases (pilot study, n=77 and main study, n= 574), 546 had sufficient plasma samples.
The pilot study
Between January 1, 1985 and September 30, 1994, a total of 36,405 individuals participated in a health examination in the VIP or the Northern Sweden MONICA study. In a first record linkage between the study base and the Northern Sweden MONICA Incidence Registry covering the period January 1, 1985 to September 30, 1994, a total of 77 cases fulfilled the inclusion criteria of a first MI with participation in health examination in the VIP or the MONICA study prior to the event. These cases were included in a pilot study (see Figure 10). Several works from the pilot study have been previously published197, 225, 226.

The main study
The second record linkage between the study base and the Northern Sweden MONICA Incidence Registry covered the whole study period from January 1, 1985 to December 31, 1999. During this period, more than 73,000 individuals had participated in health examination in the VIP or the Northern Sweden MONICA study (see Figure 10). As a result of the study design a majority of the cases who fulfilled the inclusion criteria were diagnosed during the last part of the study period. In 1999, the last year of the study period, 172 cases of first-ever MI reported from Västerbotten were recorded in the Northern Sweden MONICA Incidence Registry. Out of these 172 cases, 116 were eligible cases in the main study.

Figure 11. Yearly frequency of first-ever myocardial infarctions in the Northern Sweden MONICA Incidence Registry with participation in health examination in the VIP or the MONICA study prior to the myocardial infarction during the study period, January 1, 1985 to December 31, 1999.
**Control definition**

Controls were randomly selected from the participants in health examinations in the VIP and the Northern Sweden MONICA study and matched for sex, age (±2 years), date of health examination (±4 months) and geographical region (see Figure 12). When possible, up to six controls per case were initially selected. This was done in order to enable that each case had at least one corresponding control left after exclusions. As for cases, exclusion criteria for the control group were previous MI or stroke, or cancer diagnosis in the 5 years prior to or 1 year after diagnosis with MI, either according to the Northern Sweden MONICA Incidence Registry or the National Cancer Registry, or after validation if suggested by questionnaire data or patient records. Controls were also excluded if they had died or moved out of the region (Västerbotten and Norrbottten) before the date of MI diagnosis for the corresponding case. In paper I and II, which were based on questionnaire data, as many controls as possible were included (up to six per case), whereas in paper III and IV, analyses of haemostatic and inflammatory biomarkers were performed on a maximum of two controls per case. The control-to-case ratio varied therefore between the studies from 1.6 (paper IV) up to 3.6 (paper I).

![Figure 12](image_url)

**Figure 12.** Simplified schematic illustration of control selection in a prospective nested incident case-control design. The 10 horizontal lines represent participants in the study base. Follow-up starts at baseline examination. For every case, two controls (or more) are selected from the study base that gave rise to that case.

**Cases and controls in paper I-IV**

**Paper I**

Cases (n=651) and controls (n=2238) from both the main study and the pilot study cohorts were included. Individuals were excluded if they indicated in the questionnaire the use of nitrates or other heart medicine during 14 days prior to examination, or greatly reduced exercise habits during the year prior to baseline. This was done in order to avoid potential bias by including individuals who had reduced their physical activity due to CHD or subclinical disease. A total of 583 cases and 2098 controls remained after exclusions.
Paper II
Cases and controls from both the main study and the pilot study cohort were included. All women were excluded since snuff use was rare among women (2.2%). A total of 525 cases and 1798 controls remained after exclusions.

Paper III
Only cases and controls from the main study cohort were included as plasma samples from the pilot study cohort had been used up during previous studies. Participants with insufficient plasma samples were also excluded. A total of 469 cases and 895 controls remained after exclusions.

Paper IV
Cases and controls from both the main study and the pilot study cohort were included (for the pilot study cohort, previously analyses of t-PA, t-PA/PAI-1 complex, VWF, and CRP levels were used\textsuperscript{197}). Only participants from the VIP were included, as data regarding commuting activity was not collected in the MONICA study questionnaire. Participants with insufficient plasma samples were also excluded. As in paper I, individuals were excluded if they indicated in the questionnaire the use of nitrates or other heart medicine during 14 days prior to examination, or greatly reduced exercise habits during the year prior to baseline. This was done in order to avoid reverse causation. In addition, individuals were also excluded if they were retired because of disability or old-age since they were not exposed to commuting activity during the study period. Individuals were excluded if information was missing on commuting activity, leisure time physical activity, occupational physical activity, smoking or educational level. A total of 204 cases and 327 controls remained after exclusions.

Study variables, collection and definitions
An overview of data sources for explanatory variables is presented in Table 2. An extract from questionnaires can be found in the Appendix which covers questions on physical activity and tobacco use. References to the appendix are shown in curly brackets.

Table 2. Overview of data sources for explanatory variables in paper I-IV.

<table>
<thead>
<tr>
<th>Data</th>
<th>Paper I</th>
<th>Paper II</th>
<th>Paper III</th>
<th>Paper IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health examination and questionnaire</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Plasma analyses of frozen samples</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pilot study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health examination and questionnaire</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma analyses of frozen samples</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

(previously analysed)\textsuperscript{197}
**Occupational and leisure time physical activity**

Statements on physical activity were obtained from the questionnaire. Since the questionnaire design differed regarding physical activity between the MONICA survey and the VIP screening, responses were compiled into three categories (paper I and IV). Individuals stating “almost never physically demanding work” or “sedentary work” were categorised as “low occupational physical activity” {F1:4, 97:1}. Individuals stating “often physically demanding work” or “heavy physically demanding work” were categorised as “high occupational physical activity” {F1:1, 97:4}. Remaining individuals represented an intermediate group categorised as “moderate occupational physical activity”. Individuals stating “no exercise for the last three months” or who were “never physically active during leisure time for the last year” were categorised as “low leisure time physical activity” {G6:1, 96:1}. Individuals stating “exercise at least two to three times a week” or “at least three hours a week” were categorised as “high leisure time physical activity” {G6:4 and 5, 96:5 and 6}. Remaining individuals represented an intermediate group categorised as “moderate leisure time physical activity”. Occupational and leisure time physical activity were used as dichotomous variables (low versus moderate/high) in analysis of combined effects of different types of physical activity. Leisure time physical activity was also used as a dichotomous variable (low versus moderate/high) in multivariate analyses in paper II.

**Commuting activity**

Data on individual commuting habits were obtained from the questionnaire for subjects participating in the VIP screening. Separate answers were given for the four seasons {G1}. Questions concerning commuting habits were not present in the MONICA questionnaire and these subjects were not included in analysis. The commuting activity variable was compiled into three categories; bus, walking or bicycling every season (“active commuting”), car commuting 1-3 seasons (“irregular commuting habits”), and car commuting every season (“car commuting”). Commuting by bus was considered to be associated with higher energy expenditure than car commuting, as most bus commuters have to walk in order to arrive at their bus stop. In analysis of combined effects of different types of physical activity, commuting activity was dichotomized as “car to work at least one season” or “active commuting all seasons”.

**Tobacco use**

Information on tobacco use was self-reported in the questionnaire at the baseline health examination. Smoking was defined as current daily smoking of cigarettes, cigars or tobacco pipe {H1:2-4, 8:1, 18:1, and 20:1}. Former smokers were defined as non-smokers (paper I, III, and IV). Occasional smokers were categorised as non-smokers. Snuff consumption (paper II) was defined as current daily use of snuff {H4:3-6, 23:2-5}. In paper II smoking and snuff use were categorised into eight groups: (1) never used tobacco, (2) never smoked, current snuff user, (3) former smoker, current snuff user, (4) current smoker, no current snuff use, (5) current smoker, current snuff user, (6) never smoked, former snuff user, (7) former smoker, never used snuff, (8) former smoker, former snuff user.
Consumption of fruits and vegetables
Consumption of fruits and vegetables was obtained and computed from a large number of questionnaire data. Consequently, the categorisation was coarse. The frequencies were categorised in two groups: (1) ≤ once a day and (2) > once a day.

Education level
Data on education level were self-reported in the questionnaire and categorised in three groups: (1) elementary school, (2) upper secondary school, and (3) university degree.

Blood pressure and hypertension
In the VIP, blood pressure was measured after five minutes of rest in a supine position. An adjustment was made for sitting posture based on a comparison between sitting and supine position in 1850 subjects from the VIP health screening. In the MONICA survey, blood pressure was measured twice in every subject after a five-minute rest in a sitting position. The mean value of the two measurements was registered. Hypertension was defined as systolic blood pressure ≥ 160 or diastolic ≥ 95 mmHg, or antihypertensive medication during a period of 14 days before the health examination in paper I and II. In paper IV, hypertension was defined as systolic blood pressure ≥ 140 or diastolic ≥ 90 mmHg, or use of antihypertensive medication during a period of 14 days before the health examination, in order to conform with current clinical definition.

Body mass index
Body mass index (BMI) was calculated after measurement of body weight and height, as weight (kilograms) divided by height (meters squared). Subjects were weighed in indoor clothing without shoes. Height was measured without shoes.

Diabetes, fasting glucose and glucose tolerance
Statements on diabetes were obtained from the questionnaire. In paper I, II, and III, a participant was categorised as diabetic if a history of diabetes was reported. Fasting glucose concentrations were measured in plasma with a Reflotron bench-top analyser (Boeringer Mannheim GmbH, Mannheim, Germany) at the time of the health examination. A two-hour glucose tolerance test using 75 g glucose dissolved in 300 mL water was performed for a majority of the participants. In paper IV, a participant was categorised as diabetic if a history of diabetes was stated in the questionnaire and/or if diabetes was diagnosed at the health examination by the following definition: fasting plasma glucose ≥ 7.0 mmol/L and/or postload plasma glucose ≥ 11.0 mmol/L (≥ 12.2 mmol/L in the VIP, as capillary plasma was drawn).

Cholesterol
Venous blood serum samples for lipid measurements were obtained after fasting 4 hours or more. Total cholesterol was measured by enzymatic methods with Reflotron benchtop analyzers at each health centre in the VIP and by an enzymatic method at the Umeå University Hospital laboratory in the MONICA survey (both methods from Boehringer Mannheim GmbH, Mannheim, Germany). The two methods were evaluated by measurements on 180 subjects. The mean value for each method differed by 0.04 mmol/L and the correlation coefficient between the methods was 0.90. Total cholesterol was used for lipid adjustments in paper I and II.
Plasma analyses of haemostatic and inflammatory markers and apolipoproteins

Venous blood samples were obtained after fasting 4 hours or more. The samples were drawn in a sitting position, with a minimum of stasis, into evacuated glass tubes (Venoject) containing 1/100 volume of 0.5 mmol/L EDTA. Plasma was obtained by centrifugation at 1500g for 15 minutes, aliquoted, and then frozen within one hour. The plasma samples were initially stored frozen at -20° C for up to one week and then transferred to the Northern Sweden Medical Research Bank and stored at -80° C until analysis after the study period. Plasma analyses were conducted at the Department of Clinical Chemistry, Skellefteå County Hospital in 2004 (VWF, t-PA, PAI-1, t-PA/PAI-1 complex, CRP), the Division of Cardiovascular Medicine and Medical Sciences, University of Glasgow in 2005 (D-dimer, Lp(a), IL-6), the Department of Clinical Chemistry, Umeå University Hospital in 2007 (apo A-1, apo B) and in 1996 for analyses in the pilot study (t-PA, t-PA/PAI-1 complex, VWF and CRP).

Enzyme-linked immunosorbent assays (ELISAs) were used for measurements of D-dimer (Hyphen BioMed, Paris, France), VWF (DAKO, Copenhagen, Denmark), t-PA (Imulyse tPA Biopool, Umeå, Sweden and TintElize tPA, Biopool, Umeå, Sweden in 153 of the samples, which had antibodies causing disturbances in the Imulyse analysis), PAI-1 (TintElize PAI-1, Biopool, Umeå, Sweden), t-PA/PAI-1 complex (TintElize tPA/PAI-1, Biopool, Umeå, Sweden), high-sensitivity (hs)CRP (IMMULITE, Diagnostic Products Corporation, USA), IL-6 (R & D Systems, Oxford, UK). Lp(a) levels were measured using an enzyme immunoassay (ELITEST-Lp[a]) and an assay standard (Hyphen BioMed, Paris, France). This ELISA based system uses a monoclonal anti-Lp(a) antibody for capture and a polyclonal anti-Apo(B) antibody for detection. The method is not sensitive to variations in the number of repeat domains in apo(a).

Subsequent to the publication of paper I and II, but prior to statistical analyses in paper III and IV, apolipoprotein A-1 and B were analysed using frozen plasma samples. We considered apolipoprotein A-1 and B to reflect dyslipidaemia more adequately than total cholesterol (HDL was not analysed at the health examination on all participants and the proportion missing values was substantial). We also wanted to enable comparisons with the global INTERHEART study, in which apo B/apo A-1 ratio was used. Apolipoprotein A-1 and B were determined by immunoturbidimetry (Dako, Glostrup, Denmark).

Plasma specimens were analyzed in triplets including one case and two controls. The position of the cases varied at random within each triplet to avoid systematic bias and inter-assay variability. Measurements of biochemical markers were made by laboratory staff unaware of participant’s case and control status. Intra- and inter-assay coefficients of variation (CVs) are presented in Table 3.
Table 3. Intra- and inter-assay CVs in our hands for plasma analyses of frozen samples.

<table>
<thead>
<tr>
<th></th>
<th>Intra-assay CV (%)</th>
<th>Inter-assay CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer</td>
<td>4.7</td>
<td>5.2</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>4.9</td>
<td>7.3</td>
</tr>
<tr>
<td>t-PA (TintElize t-PA)</td>
<td>4.3 (6.5)</td>
<td>10.7 (4.2)</td>
</tr>
<tr>
<td>PAI-1</td>
<td>3.5</td>
<td>4.7</td>
</tr>
<tr>
<td>t-PA/PAI-1 complex</td>
<td>6.0</td>
<td>6.3</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>4.2</td>
<td>4.7</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>5.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>7.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Apo A-1</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Apo B</td>
<td>1.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Statistical analyses

To compare baseline characteristics between groups, the Mann-Whitney two independent samples test or the Kruskal–Wallis Several Independent test (paper I) were used for continuous variables and the Chi-square test for categorical variables. In order to evaluate the associations between the study variables and MI, conditional logistic regression analysis was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) in univariate and multivariate models (paper I-IV). The Chi-square linear-by-linear association test for trend was used for trend analyses (paper I). In paper II, the change-in-estimate method was used with a 10% limit (on a priori basis) to determine whether a possible confounder should be included in the multivariate analyses231. In paper III, natural log–transformed values were used to achieve approximately normal distributions of all markers except for Lp(a) (since log-transformation of Lp(a) did not lead to a less skewed distribution). Associations between parameters were then examined using Pearson’s correlation. One-way AnOVA was used to obtain mean levels of measured continuous variables by thirds of the distribution of levels of each of the exposure variables in controls, and tests for trend across the 3 groups of each variable were obtained for dichotomous variables. To estimate the discriminative value of the haemostatic and inflammatory markers in predictive models the area under the receiver operating characteristic (AUROC) curve was calculated for each exposure variable and compared to a model including smoking, systolic blood pressure, body mass index, apolipoproteins A-1 and B, and history of diabetes. The AUROC curve ranges from 0.50, indicating no discrimination beyond random chance, to 1.0, indicating perfect discrimination between cases and non-cases. Generally, AUROC curve between 0.70 and 0.80 are considered acceptable, and those between 0.80 and 0.90 are considered excellent232.

In paper IV, we used a method described by Ditlevsen et al233 which was developed to estimate how much of the effect of an exposure on an outcome is explained by a third, intermediate variable (see Figure 13). We used Spearman’s rank correlation coefficient to investigate the correlations between exposure variables. Variables considered to be confounders rather than mediators were included in a multivariate model called “baseline model”. Variables were identified by the extent of their correlation with commuting activity (P≤0.10), and included in the multivariate model as potential mediators. The potential mediators were added one at a time to the
baseline model and then together in an overall model to assess their relative contribution to the effect of active commuting on risk of MI. A larger change in OR from the baseline model by this procedure was interpreted as a larger mediating effect. To enable direct comparisons between multivariate models, complete case data were used. The following formula, developed for a similar analyses of mediators of physical activity and CHD\textsuperscript{234}, was used to calculate the proportions of the association between commuting activity and MI that could be explained by each potential mediator or group of variables from one or multiple proposed mediating pathways: 

$$\frac{(\text{OR}_{\text{baseline model}} - \text{OR}_{\text{adjusted model}})}{(\text{OR}_{\text{baseline model}} - 1) \times 100\%}.$$ 

\textbf{Figure 13.} Schematic diagram of the conceptual differences between confounding and mediating variables\textsuperscript{233, 235}.

Detectable odds ratios for different samples in this thesis are presented in \textbf{Table 4}. Statistical analyses were performed using SPSS version 11.5, 15.0, and 17.0 (USA) and Stata, version 9.2 (USA).
Table 4. Detectable odds ratios for different samples. Calculations based on a statistical power of 80% at the significance level of 5% and a control-to-case ratio of 2.0.

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Prevalence of exposure variable 10%</th>
<th>Prevalence of exposure variable 30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>651 cases with health examination and questionnaire data (paper I)</td>
<td>&gt;1.51</td>
<td>&gt;1.33</td>
</tr>
<tr>
<td>403 cases with health examination, questionnaire data, and complete plasma samples (paper III)</td>
<td>&gt;1.67</td>
<td>&gt;1.43</td>
</tr>
<tr>
<td>145 cases with health examination, questionnaire data and complete plasma samples (paper IV)</td>
<td>&gt;2.24</td>
<td>&gt;1.81</td>
</tr>
<tr>
<td>69 female cases with health examination, questionnaire data and complete plasma samples (paper III)</td>
<td>&gt;3.04</td>
<td>&gt;2.33</td>
</tr>
<tr>
<td>49 cases of SCD with survival time &lt;1 h (paper II)</td>
<td>&gt;3.61</td>
<td>&gt;2.72</td>
</tr>
</tbody>
</table>

Ethical considerations

The study protocol for all studies in this thesis was approved by the Research Ethics Committee of Umeå University and the corresponding data handling procedures were approved by the National Computer Data Inspection Board. All subjects were informed at the time of the health examination and gave written consent to future use of data from health examination and donated blood samples for research purposes. In addition, all cases with non-fatal MI gave informed consent subsequent to the MI for registration in the MONICA incidence registry. A small minority of subjects were also contacted by telephone for validation of questionnaire data and patient records if any uncertainty remained concerning previous MI before January 1, 1985. After the cases and controls had been selected, all data handling procedures and analyses were conducted using an anonymous dataset and no further contact was made with the subjects. As a result of these procedures the possibility of violation of the subject’s integrity must be considered to be low.

A possible ethical dilemma may occur with the present study design if subjects are found to have clearly pathological result in blood samples analysed subsequent to the health examination. If that were to occur, a secure data key kept by the Northern Sweden Medical Biobank could be used to identify individuals. However, an identification of such a subject would only be a possible alternative if the pathological result is strongly associated with a considerable health hazard, an efficient treatment for the pathological state is at hand, and the subject is still alive and could benefit from such a treatment. Such measures are not conceivable in the present studies since extreme levels of the studied variables have not shown to be that strongly and coherently associated with health hazards. Nevertheless, the main results of these studies should be trustworthily communicated to the population in northern Sweden. This can be done through local media and through educational meetings with health care personnel, particularly those involved in the health screening in the ongoing VIP.
RESULTS

Characteristics of the study population
The total study population consisted of 651 cases and 2238 controls. Baseline characteristics for cases and controls are presented in Table 5. The study populations differed between the studies as a result of the different exclusion criteria. The separate study populations are presented in each paper I-IV.

Follow-up times ranged from less than one year to a maximum of 14 years with a median of 4.0 years. VIP represented 86.8 % of the total study population and the Northern Sweden MONICA study represented 13.2 % of the total study population.

### Table 5. Baseline characteristics for cases and controls in the total study population.
The separate study populations and characteristics of the studied variables are presented in each paper I-IV.

<table>
<thead>
<tr>
<th></th>
<th>Both sexes</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases n=651</td>
<td>Controls n=2238</td>
<td>Cases n=525</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.2</td>
<td>53.9</td>
<td>53.9</td>
</tr>
<tr>
<td>University degree, %</td>
<td>8.6</td>
<td>14.5</td>
<td>8.1</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>36.7</td>
<td>20.2</td>
<td>37.9</td>
</tr>
<tr>
<td>Low consumption of fruits and vegetables*, %</td>
<td>23.2</td>
<td>18.7</td>
<td>25.5</td>
</tr>
<tr>
<td>Diabetes**, %</td>
<td>10.3</td>
<td>4.5</td>
<td>10.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.0</td>
<td>25.9</td>
<td>27.0</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.68</td>
<td>6.19</td>
<td>6.61</td>
</tr>
<tr>
<td>Apo B/apo A-1 ratio, %</td>
<td>0.99</td>
<td>0.85</td>
<td>1.00</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>140.6</td>
<td>134.4</td>
<td>139.6</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>87.9</td>
<td>84.8</td>
<td>87.8</td>
</tr>
<tr>
<td>Antihypertensive medication, %</td>
<td>16.9</td>
<td>10.7</td>
<td>14.5</td>
</tr>
<tr>
<td>Nitrates or other heart medication, %</td>
<td>4.1</td>
<td>1.8</td>
<td>3.6</td>
</tr>
</tbody>
</table>

*≤ once a day. **history of diabetes or diagnosed at baseline health examination.
RESULTS

**Commuting activity, occupational and leisure time physical activity and risk of myocardial infarction (paper I)**

In univariate analysis, significant associations with MI were seen for commuting activity and leisure time physical activity for men and both sexes combined. Occupational physical activity was not associated with risk of MI in univariate analysis. In multivariate analysis, similar trends in risk were seen in men and women for commuting activity, but the association between car commuting and MI was not significant for women. Men who were car commuters 1-3 seasons/year or every season had an increased risk of MI after multivariate adjustment (OR 2.04; 95% CI, 1.18-3.53 and OR 1.68; 95% CI, 1.10-2.56, respectively) compared to men who were active commuters (went to work by bus, walked or cycled) every season. The trends in risk for occupational physical activity differed between men and women, showing a significantly lower risk of MI for moderate compared to low occupational physical activity for men in multivariate model 2 (OR 0.70; 95% CI, 0.50-0.98), which included educational level. In multivariate model 1, with adjustment for other forms of physical activity, high compared to low leisure time physical activity was associated with lower risk of MI for men (OR 0.68; 95% CI, 0.47-0.97), but this association was lost after further adjustment in multivariate model 2. A non-significant trend for lower risk for moderate and high compared to low leisure time physical activity was seen in women in both multivariate models (see Table 6).

Several different food frequency questionnaires were used during the study period. Data compilation was complex and time-consuming and was not finalized until the end of this project. Hence, consumption of fruits and vegetables was not included in any of the papers. In an analysis subsequent to the publication of paper I, we attempted to assess the potential residual confounding effect on the main findings by this variable. This was achieved by including it in one multivariate model together with commuting activity, and another model together with leisure time physical activity. Only small alterations in OR were observed compared to the crude OR for commuting activity and leisure time physical activity (OR for car commuting every season in both sexes altered from 1.71; 95% CI, 1.18-2.47 to 1.70; 95% CI, 1.16-2.50, and OR for car commuting 1-3 seasons in both sexes altered from 1.74; 95% CI, 1.09-2.76 to 1.73; 95% CI, 1.06-2.81. OR for high leisure time physical activity in both sexes altered from 0.69; 95% CI, 0.50-0.95 to 0.68; 95% CI, 0.48-0.96, and OR for moderate leisure time physical activity altered from 0.85; 95% CI, 0.67-1.09 to 0.89; 95% CI, 0.69-1.15).
Table 6. Risk of MI using conditional logistic regression progressively adjusting for potential confounders.

<table>
<thead>
<tr>
<th></th>
<th>Matched for sex and age</th>
<th>Multivariate model 1*</th>
<th>Multivariate model 2**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95 % CI)</td>
<td>OR (95 % CI)</td>
<td>OR (95 % CI)</td>
</tr>
<tr>
<td><strong>Commuting activity:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both sexes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bus, walking or bicycle every season</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Car 1-3 seasons</td>
<td>1.74 (1.09-2.76)</td>
<td>1.84 (1.18-2.87)</td>
<td>1.90 (1.18-3.05)</td>
</tr>
<tr>
<td>Car every season</td>
<td>1.71 (1.18-2.47)</td>
<td>1.90 (1.34-2.70)</td>
<td>1.74 (1.20-2.52)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bus, walking or bicycle every season</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Car 1-3 seasons</td>
<td>1.99 (1.17-3.38)</td>
<td>1.95 (1.17-3.24)</td>
<td>2.04 (1.18-3.53)</td>
</tr>
<tr>
<td>Car every season</td>
<td>1.76 (1.17-2.66)</td>
<td>1.87 (1.26-2.78)</td>
<td>1.68 (1.10-2.56)</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bus, walking or bicycle every season</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Car 1-3 seasons</td>
<td>1.08 (0.40-2.94)</td>
<td>1.32 (0.50-3.47)</td>
<td>1.30 (0.45-3.78)</td>
</tr>
<tr>
<td>Car every season</td>
<td>1.73 (0.73-4.12)</td>
<td>1.54 (0.67-3.54)</td>
<td>2.24 (0.89-5.65)</td>
</tr>
<tr>
<td><strong>Occupational physical activity:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both sexes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Moderate</td>
<td>1.07 (0.82-1.40)</td>
<td>0.97 (0.74-1.26)</td>
<td>0.81 (0.60-1.09)</td>
</tr>
<tr>
<td>High</td>
<td>1.09 (0.74-1.63)</td>
<td>0.89 (0.60-1.31)</td>
<td>0.71 (0.46-1.09)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.98 (0.73-1.31)</td>
<td>0.87 (0.65-1.17)</td>
<td>0.70 (0.50-0.98)</td>
</tr>
<tr>
<td>High</td>
<td>1.08 (0.70-1.66)</td>
<td>0.88 (0.58-1.35)</td>
<td>0.67 (0.42-1.08)</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Moderate</td>
<td>1.55 (0.84-2.85)</td>
<td>1.36 (0.74-2.51)</td>
<td>1.19 (0.59-2.39)</td>
</tr>
<tr>
<td>High</td>
<td>1.10 (0.41-2.99)</td>
<td>0.78 (0.29-2.10)</td>
<td>0.84 (0.29-2.50)</td>
</tr>
<tr>
<td><strong>Leisure time physical activity:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both sexes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.85 (0.67-1.09)</td>
<td>0.86 (0.67-1.10)</td>
<td>1.03 (0.79-1.34)</td>
</tr>
<tr>
<td>High</td>
<td>0.69 (0.50-0.95)</td>
<td>0.69 (0.50-0.95)</td>
<td>0.94 (0.66-1.34)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.88 (0.67-1.15)</td>
<td>0.89 (0.68-1.16)</td>
<td>1.08 (0.80-1.45)</td>
</tr>
<tr>
<td>High</td>
<td>0.66 (0.46-0.96)</td>
<td>0.68 (0.47-0.97)</td>
<td>0.98 (0.66-1.46)</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.74 (0.41-1.32)</td>
<td>0.68 (0.37-1.24)</td>
<td>0.86 (0.43-1.72)</td>
</tr>
<tr>
<td>High</td>
<td>0.81 (0.38-1.73)</td>
<td>0.68 (0.31-1.47)</td>
<td>0.74 (0.31-1.74)</td>
</tr>
</tbody>
</table>

*Multivariate model 1: Commuting activity and occupational and leisure time physical activity in the same model. **Multivariate model 2: Adjusted as in model 1 and for smoking, BMI, cholesterol, diabetes, hypertension and educational level.

Combined effects of commuting activity and occupational and leisure time physical activity on risk of MI
In order to explore the combined effects of commuting activity and occupational and leisure time physical activity on the risk of myocardial infarction we dichotomized all three types of activity. Occupational and leisure time physical activity were dichotomized in low versus moderate and high. Commuting activity was dichotomized in car to work at least one season versus active commuting all seasons.
Subjects were classified into eight categories based on different combinations of physical activity. The effect on risk of myocardial infarction was compared between the categories using the combination of low occupational physical activity, low leisure time physical activity, and car to work at least one season as a reference category in a conditional multivariate logistic regression analysis with adjustment for smoking, history of diabetes, hypertension, cholesterol, BMI and educational level. We found a significant risk reduction in two categories; combined low occupational physical activity, moderate or high leisure time physical activity, and bus, walking or bicycle every season (OR 0.24; 95% CI, 0.08-0.69), and combined moderate or high occupational physical activity, moderate or high leisure time physical activity, and bus, walking or bicycle every season (OR 0.38; 95% CI, 0.18-0.82) (see Figure 14). This analysis was not included in paper I.

**Figure 14.** Combined effects of commuting activity and occupational and leisure time physical activity on the risk of MI. ORs are adjusted for smoking, hypertension, BMI, cholesterol, history of diabetes and educational level.
Snuff use and risk of myocardial infarction and sudden cardiac death (paper II)

Snuff use and myocardial infarction
The association between snuff use and MI was investigated in univariate and multivariate models. For snuff users without a previous history of smoking the univariate (only age-matched) OR was 0.90 (95% CI, 0.52-1.55) compared to non-tobacco users. In a multivariate model including body mass index, leisure time physical activity, educational level, and cholesterol level, the odds ratio was 0.82 (95% CI, 0.46-1.43). The risk among smokers with no current snuff use was clearly increased (OR 2.60; 95% CI, 1.91-3.54) in the same multivariate model (see Table 7).

Table 7. Conditional logistic regression showing the risk of MI in relation to three categories of tobacco habits with progressive adjustment for potential confounders.

<table>
<thead>
<tr>
<th></th>
<th>Matched for age</th>
<th>Multivariate model 1*</th>
<th>Multivariate model 2**</th>
<th>Multivariate model 3***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never used tobacco</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Never smoked, current snuff user</td>
<td>0.90 (0.52-1.55)</td>
<td>0.87 (0.50-1.51)</td>
<td>0.90 (0.52-1.56)</td>
<td>0.82 (0.46-1.43)</td>
</tr>
<tr>
<td>Current smoker, no current snuff use</td>
<td>2.66 (1.98-3.57)</td>
<td>2.82 (2.09-3.82)</td>
<td>2.78 (2.05-3.78)</td>
<td>2.60 (1.91-3.54)</td>
</tr>
</tbody>
</table>

*Multivariate model 1: Adjusted for BMI. **Multivariate model 2: Adjusted for BMI, leisure time physical activity, and educational level. ***Multivariate model 3: Adjusted for BMI, leisure time physical activity, educational level, and cholesterol level.

Snuff use, fatal myocardial infarction and sudden cardiac death
In paper II we also studied the risk of fatal MI and sudden cardiac death among snuff users. Two definitions of SCD was used; SCD with survival time <24 hour and SCD with survival time <1 hour from the onset of symptoms. BMI, leisure time physical activity, educational level, and cholesterol level were considered as confounders and adjusted for. We found no increased risk of fatal MI or SCD for current snuff users without a previous history of smoking when compared to non-tobacco users. The only category with significantly increased risk was current smokers with no current snuff use (OR 4.54; 95% CI, 1.55-13.25 for SCD with survival time <1 hours, see Table 8).
**RESULTS**

**Table 8.** Conditional logistic regression showing the risk of fatal MI and SCD in relation to three categories of tobacco habits.

<table>
<thead>
<tr>
<th></th>
<th>Fatal MI within 28 days</th>
<th>SCD with survival time &lt;24 hours</th>
<th>SCD with survival time &lt;1 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Crude OR (95% CI)</td>
<td>Adjusted OR* (95% CI)</td>
</tr>
<tr>
<td>Never used tobacco</td>
<td>30</td>
<td>1.00 (1.00)</td>
<td>1.00 (1.00)</td>
</tr>
<tr>
<td>Never smoked, current snuff user</td>
<td>7</td>
<td>1.02 (0.37-2.83)</td>
<td>1.12 (0.38-3.29)</td>
</tr>
<tr>
<td>Current smoker, no current snuff use</td>
<td>37</td>
<td>3.33 (1.80-6.14)</td>
<td>3.53 (1.83-6.84)</td>
</tr>
</tbody>
</table>

Controls are matched for sex and age.

* Adjusted for BMI, leisure time physical activity, educational level, and cholesterol level.

**Haemostatic and inflammatory markers and risk of myocardial infarction (paper III)**

Paper III evaluated the associations between haemostatic markers and the risk of MI with adjustment for established cardiovascular risk factors and inflammatory markers. Correlations between haemostatic and inflammatory markers in controls are presented in **Figure 15** (for details see paper III, Supplementary table 1). As expected, the haemostatic markers t-PA, PAI-1, and t-PA/PAI-1 complex showed correlations with each other and t-PA and t-PA/PAI-1 complex were negatively correlated with Lp(a). D-dimer and VWF were correlated, and VWF was also correlated with t-PA/PAI-1 complex. The inflammatory markers CRP and IL-6 were correlated with each other and with the haemostatic markers t-PA, PAI-1, t-PA/PAI-1 complex, D-dimer, and VWF, but not with Lp(a).
The association with MI for each of the haemostatic and inflammatory biomarkers are presented in Table 9. All studied variables showed significant associations in the sex- and age-matched analysis. With the exception of VWF, the associations between haemostatic markers and MI were attenuated when adjusted for established risk factors, as well as in the model with adjustment for inflammatory markers. However, the haemostatic markers D-dimer, VWF, t-PA and t-PA/PAI-1 complex were associated with myocardial infarction even after adjustment for established risk factors and inflammatory markers (CRP and IL-6). The associations between inflammatory markers and MI were also attenuated when adjusted for established risk factors, and similarly when adjusted for haemostatic markers. IL-6, D-dimer, and VWF remained significantly associated with MI in a final model after adjustment for established risk factors and all other studied variables. In the sex-specific analyses, women tended to have somewhat higher odds ratios than men for several of the studied markers, but test for heterogeneity were insignificant (P>0.10) for all markers (for details see paper III, Supplementary table 2 and Supplementary figure 1).
**RESULTS**

**Table 9.** Odds ratios of extreme thirds of haemostatic and inflammatory markers on risk of myocardial infarction with progressive adjustment for potential confounders.

<table>
<thead>
<tr>
<th>Haemostatic markers</th>
<th>n cases/ n controls</th>
<th>Crude OR (95%CI)</th>
<th>Model 1* (95%CI)</th>
<th>Adjusted for model 1 and inflammatory markers*** (95%CI)</th>
<th>Adjusted for model 1 and for all other variables in table (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D-dimer</strong></td>
<td>408/727</td>
<td>2.61 (1.18-3.77)</td>
<td>2.31 (1.53-3.50)</td>
<td>1.97 (1.27-3.04)</td>
<td>2.06 (1.30-3.26)</td>
</tr>
<tr>
<td><strong>VWF</strong></td>
<td>415/745</td>
<td>2.52 (1.82-3.48)</td>
<td>2.71 (1.89-3.90)</td>
<td>2.52 (1.72-3.67)</td>
<td>2.42 (1.63-3.60)</td>
</tr>
<tr>
<td><strong>t-PA</strong></td>
<td>413/739</td>
<td>3.01 (2.11-4.28)</td>
<td>1.90 (1.27-2.83)</td>
<td>1.63 (1.07-2.48)</td>
<td>1.39 (0.79-2.46)</td>
</tr>
<tr>
<td><strong>PAI-1</strong></td>
<td>415/745</td>
<td>1.76 (1.28-2.43)</td>
<td>1.09 (0.75-1.56)</td>
<td>0.95 (0.65-1.38)</td>
<td>0.66 (0.43-1.02)</td>
</tr>
<tr>
<td><strong>t-PA/PAI-1 complex</strong></td>
<td>415/745</td>
<td>3.33 (2.35-4.72)</td>
<td>1.96 (1.31-2.92)</td>
<td>1.64 (1.08-2.49)</td>
<td>1.51 (0.83-2.72)</td>
</tr>
<tr>
<td><strong>Lp(a)</strong></td>
<td>404/714</td>
<td>1.36 (1.01-1.85)</td>
<td>1.33 (0.95-1.86)</td>
<td>1.39 (0.98-1.97)</td>
<td>1.41 (0.98-2.03)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inflammatory markers</th>
<th>n cases/ n controls</th>
<th>Crude OR (95%CI)</th>
<th>Model 1* (95%CI)</th>
<th>Adjusted for model 1 and haemostatic markers** (95%CI)</th>
<th>Adjusted for model 1 and for all other variables in table (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CRP</strong></td>
<td>414/740</td>
<td>3.04 (2.17-4.26)</td>
<td>1.92 (1.31-2.82)</td>
<td>1.58 (1.03-2.41)</td>
<td>1.43 (0.91-2.24)</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>405/720</td>
<td>3.46 (2.40-5.01)</td>
<td>2.23 (1.49-3.33)</td>
<td>1.67 (1.08-2.60)</td>
<td>1.63 (1.02-2.60)</td>
</tr>
</tbody>
</table>

All models are matched for age and sex. Thirds are calculated based on distribution of marker in controls.

*Model 1: adjusted for smoking, systolic blood pressure, apolipoprotein A-1, apolipoprotein B, body mass index and history of diabetes; **haemostatic markers: t-PA, PAI-1, t-PA/PAI-1 complex, D-dimer, VWF and Lp(a); ***inflammatory markers: CRP and IL-6.

**Predictive ability of haemostatic and inflammatory markers**

The AUROC curve for each of the eight biomarkers, above and beyond the established risk factors smoking, systolic blood pressure, apolipoproteins A-1 and B, history of diabetes, and body mass index, are presented in Figure 16. Combined, the established risk factors had an AUROC curve of 0.76 (SD 0.015). None of the individual markers showed significant predictive ability over the established risk model with the exception of VWF (AUROC curve increased to 0.79; SD 0.014). When markers were used in combination they showed a stronger predictive ability over the established risk factor model. A selected panel of haemostatic markers including D-dimer, VWF, t-PA, PAI-1, t-PA/PAI-1 complex, and Lp(a) showed a stronger predictive ability (AUROC curve 0.81; SD 0.014) than a similar model of the inflammatory markers CRP and IL-6 (AUROC curve 0.78; SD 0.014), although both appeared to be significantly different from the baseline model. When all eight markers were included the predictive ability of the model, as expected, increased further (AUROC curve 0.82; SD 0.013).
Baseline model

- plus D-dimer
- plus VWF **
- plus t-PA *
- plus PAI-1
- plus t-PA/PAI-1 complex *
- plus Lp(a)
- plus CRP *
- plus IL-6 *

- plus panel of haemostatic markers **
- plus panel of inflammatory markers **
- plus all novel biomarkers **

Figure 16. Relative predictive ability of MI for different markers above and beyond established risk factors using the AUROC curve. Dotted lines represent AUROC curve and 95% confidence intervals for a baseline model including age, sex, smoking, systolic blood pressure, apolipoproteins A-1 and B, history of diabetes and body mass index. *P<0.05 for \( \chi^2 \) heterogeneity test comparing models with and without variables of interest. **P<0.001 for \( \chi^2 \) heterogeneity test comparing models with and without variables of interest. Calculations are based on information from 397 cases and 693 controls with data on all variables included to enable direct comparison between variables.

Potential mediators of risk reduction associated with commuting activity (paper IV)

Correlations between exposure variables and potential mediators of risk reduction associated with commuting activity were analysed in cases and controls. Car commuting was correlated with several established and novel risk factors including smoking, low educational level, high occupational and low leisure time physical activity, apo B/apo A-1 ratio, BMI, IL-6, t-PA, and t-PA/PAI-1 complex (P-value ≤0.10), but not hypertension, diabetes, CRP, VWF or D-dimer. A set of variables were, on an a priori basis, considered as potential confounders rather than mediators of the effect of commuting activity on MI risk (smoking, educational level, leisure time physical activity, and occupational physical activity). These variables were included in a baseline model. Since hypertension and diabetes were not correlated with car commuting, they were not explored as potential mediators. However, as these variables are considered established risk factors, hypertension and diabetes were included in the baseline model.
The OR for car commuters compared to active commuters was 1.77 (95% CI, 1.05-2.99) after adjustment for variables in the baseline model. A significantly increased crude OR was also seen in subjects with irregular commuting habits, but the OR decreased and the significance was lost after multivariate adjustment. To enable direct comparisons between multivariate models, the risk for MI in car commuters compared with active commuters were analysed using complete case data (see Table 10). The potential mediators, selected by their correlation with car commuting, were analysed individually and in combination. The table also shows the proportion of the effect that can be explained by each of the potential mediators. We found that apo B/apo A-1 ratio appeared to be the largest contributor among the established risk factors, explaining 26% of MI risk related to commuting activity. IL-6 and t-PA/PAI-1 complex were major contributors and explained 27.6% and 33.6%, respectively. An overall combined model which included apo B/apo A-1 ratio, BMI, IL-6, t-PA, and t-PA/PAI-1 complex appeared to explain 40.1% of the MI risk related to car commuting.

Table 10. Odds ratio for MI adjusted for baseline model and potential mediators of the effect of commuting activity. Proportions of MI risk related to commuting activity that is explained by potential mediators, individually and in combination, are presented. Calculations based on 145 cases and 212 controls with complete data sets.

<table>
<thead>
<tr>
<th></th>
<th>Car commuting versus active commuting</th>
<th>Proportions of MI risk related to commuting activity that is explained**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline model*</td>
<td>1.66 (0.94-2.95)</td>
<td></td>
</tr>
<tr>
<td>Baseline model + apo B/apo A-1 ratio</td>
<td>1.49 (0.82-2.71)</td>
<td>26.0%</td>
</tr>
<tr>
<td>Baseline model + BMI</td>
<td>1.54 (0.88-2.70)</td>
<td>18.7%</td>
</tr>
<tr>
<td>Baseline model + apo B/apo A-1 ratio + BMI</td>
<td>1.49 (0.82-2.69)</td>
<td>26.4%</td>
</tr>
<tr>
<td>Baseline model + IL-6</td>
<td>1.48 (0.84-2.61)</td>
<td>27.6%</td>
</tr>
<tr>
<td>Baseline model + t-PA</td>
<td>1.52 (0.86-2.66)</td>
<td>23.1%</td>
</tr>
<tr>
<td>Baseline model + t-PA/PAI-1 complex</td>
<td>1.44 (0.82-2.54)</td>
<td>33.6%</td>
</tr>
<tr>
<td>Baseline model + IL-6 + t-PA + t-PA/PAI-1 complex</td>
<td>1.40 (0.79-2.48)</td>
<td>39.7%</td>
</tr>
<tr>
<td>Baseline model + all risk factors and inflammatory and haemostatic markers above</td>
<td>1.40 (0.76-2.57)</td>
<td>40.1%</td>
</tr>
</tbody>
</table>

*Baseline model: Smoking, educational level, leisure time physical activity, occupational physical activity, hypertension and diabetes. **Calculated using the following formula: (OR_{baseline model} - OR_{adjusted model})/(OR_{baseline model} - 1) \times 100\%.
Methodological considerations

Study design
The prospective nested case-control study design used in this thesis has several general advantages\textsuperscript{224}. First, this design avoids the potential biases of other case-control designs that are unable to include fatal cases. Second, the prospective design minimizes the risk of recall bias, since under- and over-reporting of health behaviours can be expected to be evenly distributed among cases and controls. Third, the prospective design is less susceptible to reverse causality since plasma samples were collected prior to the outcome and not affected by the subsequent myocardial infarction. Last, a case-control study is less expensive and less time consuming than a cohort study because only a limited number of controls are analysed, instead of the entire study base\textsuperscript{235}. A cohort study design would have been a reasonable alternative in papers I and II, since no analyses were performed on frozen plasma samples in these two studies. However, questions concerning previous MI were not included in all baseline questionnaires and a cohort study design with more than 70,000 participants would have necessitated a large amount of work to exclude all individuals with MI prior to January 1, 1985, the inception date for the incidence registry.

Internal validity

Internal validity is the extent to which the results of an investigation accurately reflect the true situation of the study base\textsuperscript{235}.

How representative were the cases included in the studies of all cases in the study base? How representative were the controls of the entire study base?
A small proportion of cases and controls were excluded because of previous cancer or stroke (see Figure 10) verified by inclusion in other research studies based on the Northern Sweden Medical Biobank. In addition, 0.4% of all surviving cases recorded in the MONICA Incidence Registry did not give consent to personal recording\textsuperscript{223}. Additionally, in the studies using frozen plasma a small proportion of cases and controls were excluded because of insufficient sample volume. Since these exclusions are limited they are not likely to have a major impact on the internal validity. It should be noted that only cases with a first-ever myocardial infarction were included. This was done to reduce potential reverse causality from post-MI alterations in lifestyle, medical therapy, and biomarkers related to the MI itself.

Since the controls are analyzed, and not the entire study base, their selection may introduce bias\textsuperscript{235}. This potential drawback is usually not an issue in case-control studies nested within well-defined cohorts, like the VIP and the Northern Sweden MONICA study\textsuperscript{221, 223}, as this insures that the controls adequately represent all subjects in the study base at risk for becoming a case\textsuperscript{224}. In the present studies, controls were free of MI, stroke, and cancer during the whole study period (they would otherwise have been registered as cases in studies monitored by the Northern Sweden Medical Biobank). This may result in an “extra-healthy” control group. However, no clear indication was found for such an effect when the baseline data of
controls in the present studies were compared with data from the Northern Sweden MONICA population survey\textsuperscript{23, 236}.

\textit{Was the assessment of the exposure variables adequate?}
As in other large-scale studies, the assessment of physical activity was based on self-reports and thereby susceptible to misclassification. Participants in health examinations may be influenced by the social desirability of reporting particular behaviours, like regular exercise and neglect other behaviours, such as watching television\textsuperscript{28}. It is not clear to what extent social desirability may affect responses on commuting activity specifically, but environmental awareness and health consciousness may have lead to over-reporting of active commuting in paper I and IV. Seasonal variation in physical activity may also have caused some misclassification, particularly in the VIP cohort (the health examinations in the MONICA study were conducted during the same season at all four surveys). Generally, misclassification of physical activity reduces the apparent magnitude of any benefits from physical activity\textsuperscript{28}, which should be considered in the interpretation of the results.

The assessment of physical activity was also limited by the fact that the physical activity questionnaires did not allow separate reporting of intensity and duration. Furthermore, the questionnaires did not enable any clear estimation of total physical activity. Total physical activity is difficult to measure though, particularly in epidemiological studies. An accurate but simple classification of physical activity patterns may be more appropriate than an attempt to estimate overall energy expenditure\textsuperscript{237}. Nevertheless, the assessment of physical activity has to be considered as a weakness in this thesis. In 2008, a validation study of the VIP physical activity questionnaire was conducted in 184 subjects using the Actiheart\textsuperscript{27}, a combined heart rate and movement sensor designed for assessment of energy expenditure. The data from the validation study have not been analysed yet (March 2009).

A review of the questionnaires was made after paper I had been published. In 21 subjects from the pilot study cohort (with health examination in the MONICA study) the occupational physical activity had been slightly misclassified. This was due to the fact that different categorisation had been used in the pilot study dataset and the main study dataset, which was not noted initially. However, the questionnaire in the MONICA study did not include questions on commuting activity. Thereby, the univariate analyses for occupational physical activity were marginally biased, but the misclassification had no effect on results from the multivariate analyses (data not shown).

In the present studies, a single health examination and blood sampling was performed on each participant which makes within-person variability a potential limitation. Fluctuations in biomarkers, as well as changes in lifestyle and medication, may dilute the association between baseline values and MI. The VIP provides health counselling after the examination for every participant which may contribute to such changes. Longitudinal studies using the MONICA population surveys indicate rather clear alterations in the population as a whole during the study period in several variables, including lifestyle factors\textsuperscript{120, 236}. Increased statistical precision could have been achieved by multiple measurements and calculations of regression dilution bias\textsuperscript{190, 238}.
Diurnal and seasonal variation has been observed in both haemostatic and inflammatory markers\textsuperscript{239}. Whereas seasonal variation may have led to slightly weakened associations with MI for some of the biomarkers (controls were matched for date of health examination, ±4 months), the impact of diurnal variation was probably low since blood samples were obtained in the morning for a majority of the study population. The plasma samples were stored between 5 and 20 years (median 12 years) at -80°C. The effect of storage-time was modest, explaining only 0.8-2.0\% in a study of the changes in values of t-PA, PAI-1 and t-PA/PAI-1 complex over time\textsuperscript{240}.

Individuals with type 0 blood group have about 30\% lower mean VWF levels\textsuperscript{241}. This effect was not controlled for as data on AB0 blood groups was not available. The variation in VWF between blood groups may therefore have diluted the association between VWF and MI somewhat. VWF concentrations may also be influenced in individuals with von Willebrand's disease, but the impact on our results from this is probably minor since the prevalence of von Willebrand's disease is estimated to about 1-3\% of the general population\textsuperscript{242}.

The main pathophysiological role of Lp(a) is suggested to be the ability to bind to the surface of fibrin and act as a competitive antagonist of plasminogen\textsuperscript{176}. A previous study have shown that only small size apo(a) isoforms display high affinity binding to fibrin\textsuperscript{243}. Thus, it has been proposed that the impact of Lp(a) on cardiovascular risk depends more on the relative concentration of Lp(a) particles containing small apo(a) isoforms than on the plasma concentration of Lp(a)\textsuperscript{176}. In our study the relevance of different apo(a) isoforms was not investigated.

\textbf{How were confounding variables controlled for?}
Confounding variables were controlled for by matching and multivariate adjustments. The controls were matched for sex, age, date of health examination, and geographical region. The restricted number of variables that were matched for reduces the risk for “over-matching”, i.e. correlation of the exposure with the variable matched for and thereby losing the power to detect a difference in odds of exposure\textsuperscript{224}. Furthermore, the age-matching was performed with a narrow age span to minimize the risk of residual case-control differences. A disadvantage with matching as an approach to controlling for confounding variables is the possibility of evaluating risk associated with the matched variables is lost\textsuperscript{244}.

Several potential confounders were statistically adjusted for in each study, including established cardiovascular risk factors and educational level. We did not find any indication of residual confounding when the main findings were adjusted for consumption of fruit and vegetables. Residual confounding may be present, however, resulting from different distribution between cases and controls regarding alcohol consumption, psychosocial factors, waist-to-hip ratio, and other potential confounding variables that we could not control for.

\textbf{Methodological commentary regarding paper III}
The most commonly used method for evaluation of predictive ability is comparisons of the AUROC curve with and without the novel marker or markers. The AUROC curve (equivalent to C-statistics) represents the ability of a test to discriminate cases from non-cases and is a function of the sensitivity and specificity of that test across the entire spectrum of possible cut-off values\textsuperscript{232}. However, the reliance on AUROC
curves alone for evaluation of predictive ability in novel markers has been criticized for being insensitive. The method requires that a single novel marker is very strongly associated with disease and poorly correlated with the established risk factors in order to significantly improve the AUROC curve\(^{232}\). Additional methods such as net reclassification improvement, integrated discrimination improvement, calibration, and global fit have therefore been proposed\(^{245}\). These calculations were not tested in this study.

**Methodological commentary regarding paper IV**

The method we used to calculate the portion of an exposure effect on outcome that can be explained by a third, intermediate variable have been described by Ditlevsen et al\(^{233}\). They refer to this measure as “mediation proportion”. This method has primarily been used in social epidemiology (in path analysis and structural-equations modelling). There are two major limitations of this method. First, it assumes a causal relationship between an exposure and an outcome\(^{233}\). Consequently, in our case we have assumed a causal relationship between physical activity and MI. For ethical and practical reasons no primary preventive randomized trials on physical activity with CHD as clinical outcome exists. However, the large amount of observational studies in different populations, using a variety of physical assessment methods, indicate that CHD is inversely related to physical activity in a dose-response manner\(^{45}\). Second, the method is interpretable under the assumption that no effect modification between the exposure and the intermediate variable exists (i.e. that there is homogeneity of the risk difference in strata of the intermediate variable)\(^{246}\). We controlled for this in our study by calculating the OR for car commuting versus active commuting in different strata of the potential mediators (stratified in tertiles with limits defined by the controls). We found some variance in OR in the different strata, but no clear indication of interaction on an additive scale (data not shown). Mediating effects present a general epidemiological problem, particularly in the studies of lifestyle factors which are considered as underlying causes of established and novel risk factors (see Figure 2). The method used in paper IV, the calculation of mediation proportion, should only be viewed as a partial solution to the general problem with intermediate variables and mediating effects\(^{233}\).

**External validity**

_External validity_ is the extent to which the results of a study are applicable to other populations\(^{235}\).

**How representative is the population of northern Sweden in terms of coronary heart disease and lifestyle in a national and international perspective?**

Comparisons of incidence rates of myocardial infarction have been made between the different populations (mainly European) in the international MONICA collaboration. Between 1986-99, northern Sweden was above the median in incidence rates for both men and women in an international comparison and clearly higher than the Gothenburg MONICA population in the southwest of Sweden\(^{247}\). During the study period the morbidity and mortality related to MI have decreased in Sweden. The benefit has been greater for men compared to women, and the decrease has occurred at a quicker pace in northern Sweden where initial incidence rates exceeded the general population of Sweden as a whole\(^{220, 248}\). The population in northern Sweden has also experienced a clear decline in total cholesterol values during the period 1986-99, beginning from a level which was one of the highest in the international MONICA collaboration\(^{236}\). In contrast, blood pressure levels and BMI remained on an
intermediate level. The proportion of the population on treatment with lipid-lowering drugs in the Northern Sweden MONICA study increased from 0.4% in 1990 to 3.1% in 1999, while the proportion on antihypertensive drugs remained unchanged during the same time period. The lifestyle in northern Sweden is characterized by a low prevalence of smoking and a high prevalence of snuff use, especially in men (see Figure 3). Exercise habits have been compared on a national level with no clear indication of geographical differences, and the seasonal variation in leisure time physical activity was consistent in all Swedish regions. The dietary patterns in northern Sweden have followed the national trends, but some regional differences have been shown such as a lower intake of fruit and vegetables when compared to the Stockholm region, and a correspondingly higher intake of fat and saturated fat in men.

Are the participants in VIP and the Northern Sweden MONICA study a valid study base for studies on the population of northern Sweden?
Selection bias in social and health factors in the VIP was assessed for the participants in 1992 and 1993. During these years the mean participation rate was 57%. The 1990 Population and Housing Census was used to get information on social factors for non-participants. The MONICA surveys in 1990 and 1994 were used as a reference population for health-related factors. Unemployed individuals and individuals with low income or younger age were somewhat less prone to participate, but the overall differences were small between participants and non-participants. There were also generally small differences in health-related factors, but significant differences were seen in the mean level of total cholesterol, which were lower in VIP participants when compared to MONICA participants. Blood pressure, on the other hand, was higher in VIP participants compared to MONICA participants.

The participants in the Northern Sweden MONICA study were randomly selected from a population register and the participation rate was between 75-80%. A comparison was made between participants and non-participants in the 1986, 1990, and 1994 surveys. Data for non-participants were based on telephone interviews. No difference in educational level was found between participants and non-participants, but the proportion of regular smokers was somewhat higher and body mass index slightly lower among non-participants. Participation rates and comparisons between participants and non-participants altogether indicate that the combination of the VIP and the Northern MONICA study is a sufficient population-based study cohort and representative of the population of northern Sweden. However, it should be noted that the health examinations in the VIP and the MONICA study were performed on participants 30-60 years and 25-74 years, respectively, which limits the generalisability to these age groups. Other studies have indicated that the predictive value of established risk factors such as dyslipidaemia and hypertension weakens with advancing age. Hence, it is uncertain if the findings in our studies are applicable to populations outside these age boundaries.

How do the cases registered by the Northern Sweden MONICA incidence registry correspond to all cases in northern Sweden?
The Northern Sweden MONICA Incidence Registry aims to register all suspected coronary events in Västerbotten and Norrbotten. To insure a complete registration, computer-based lists of discharge diagnoses from acute-care hospitals and nursing homes are screened annually for additional cases. All reported events are re-evaluated using MONICA methodology. Quality assessments have shown that
the Northern Sweden Incidence Registry was one of the MONICA units with a maximum data quality score during the whole study period\textsuperscript{255}.

When studies using MONICA methodology are compared with studies that were directly based on routine hospital diagnoses and the Causes of Death Register during this time period there is a discrepancy which may have to be considered. The MI cases recorded in the MONICA Incidence Registry 1987-2000 were compared with the MI events for the age group 25-64 years registered in the Swedish heart statistics (directly based on the routine hospital diagnoses and the Causes of Death Register) from the National Board of Health and Welfare. With the MONICA Incidence Registry as a standard, 9.3\% of the MI cases in the routine hospital diagnoses and 16.3\% of the MI cases who died out-of-hospital and were registered in the Causes of Death Register were found to be false positive (personal correspondence by e-mail, February 19, 2009, Max Köster, the National Board of Health and Welfare).

**Sample size and power**
The studies in this thesis are based on a fairly large sample of cases. Despite this, sample size was an issue, particularly in analyses of combined effects of different types of physical activity, snuff use and SCD, several analyses in female cases, and for analyses of potential mediators of risk reduction associated with commuting activity (detectable limits for different samples are presented in Table 4). In paper IV, the final analyses were conducted on 145 cases and 202 controls with complete data sets enabling direct comparisons between models. Using complete case data the association between commuting activity and MI was no longer statistically significant, which can be explained in part by wider confidence intervals. A power calculation based on the current exposure prevalence and a control-case ratio of 2 shows that it would require at least 500 cases with complete data sets to show statistical significance at OR 1.40. However, our aim was to explore the relative contribution of markers from different potential mediating pathways for the association between active commuting and MI. For this objective, which focuses on evaluation of the possible etiological link between commuting activity and MI, the significance level of the association is not as essential as in a study which investigates the magnitude of such an association. Nevertheless, the statistical uncertainty was large for the calculated mediation proportions and the findings in paper IV, together with analyses of combined effects of different types of physical activity, snuff use and SCD, and several of the analyses in female cases should be considered as hypothesis-generating. The associations need to be further evaluated in larger studies.
Main findings -interpreted in the light of current knowledge

Commuting activity
In paper I we found that active commuting was associated with a decreased risk of MI. The risk estimates were similar for both sexes but significant in men only, probably due to the fact that the study was underpowered for women. The association with CHD for this form of physical activity has only been investigated in two previous prospective studies. Wagner et al.41, found no significant association between active commuting and CHD in a cohort study with 167 CHD cases from France and Northern Ireland. In 2007, Hu et al.67 presented the results of a large cohort study from Finland including 4660 CHD cases with a mean follow-up of 18.9 years. They found that active commuting was associated with a decreased risk of CHD in women, but not in men. In contrast to the Finnish study, the association between active commuting and CHD was not confounded by occupational and leisure time physical activity in our study.

The association between active commuting and cardiovascular risk was recently investigated in a meta-analysis based on eight studies, including paper I in this thesis. The meta-analysis included prospective studies with different cardiovascular outcomes (CVD mortality, CHD, MI, stroke, hypertension, and diabetes). Active commuting was associated with an overall 11% reduced risk of cardiovascular outcomes in the adjusted model. However, there was a clear heterogeneity among the individual study results, especially for men. These divergent results may be related to differences in the protective effect for different outcomes, or may be related to residual confounding in some of the studies. A general issue in studies of health effects of active commuting is that commuting habits are complex behaviours that seem to be affected by a variety of individual characteristics, such as age, sex, socioeconomic status, health and environmental consciousness, interest in physical activity, and attitudes toward cars and bicycles. Psychosocial factors like social support and self-efficacy are probably also relevant. Several environmental characteristics such as presence of pavements and footpaths, green spaces, and lack of heavy traffic may have an impact on commuting habits. It has been suggested that the physical environment may affect transportation activity more than recreational activity. Some of the factors that influence commuting habits are likely to be related to health as well, and may thereby act as potential confounders. Consequently, a deeper understanding in the determinants of active commuting may be needed to enable adequately control for confounding in observational studies.

Leisure time physical activity
The association between leisure time physical activity and CHD has been evaluated in a large number of studies and several meta-analyses have been published. In our study the association did not remain significant after multivariate adjustment. However, since several of the co-variates adjusted for may be considered as mediators to the effect of physical activity (a reasoning which was further developed in paper IV), one has to evaluate the association in uni- and multivariate analyses as a whole. Nevertheless, the association was somewhat weaker in our study compared to a recent meta-analysis by Sofi et al which included 26 cohort studies (relative risk 0.88 for moderate LTPA and 0.73 for high LTPA compared with low LTPA). A possible explanation for the weaker association may be that most other studies have
included commuting activity in the assessment of leisure time physical activity. The fact that the questionnaires used in the VIP and the MONICA study focused on exercise during leisure time may also have diluted the association with MI since a substantial part of the energy expenditure (and indirect physical activity) on leisure time has shown to be related to non-exercise activities.\textsuperscript{265}

Our analysis of the combined effects of the different forms of physical activity indicated a potential to markedly reduce the risk of MI if active commuting and moderate to high leisure time physical activity are combined. The association remained significant for this combination after adjustment for established risk factors and education level. This finding is generally in line with similar analyses on Finnish population, particularly in Finnish women.\textsuperscript{34, 67}

**Occupational physical activity**

A weak association was observed between occupational physical activity and MI, but only after multivariate adjustment including education level. The lack of association in women may reflect sex differences in professions and work tasks, but we cannot rule out residual confounding from socioeconomic factors in women. There is a clear socioeconomic gradient in health, particularly in relation to CHD.\textsuperscript{266} Previous studies by Anand et al.\textsuperscript{267} and Thurston et al.\textsuperscript{268} indicate that adverse socioeconomic characteristics may be stronger associated with CVD in women than in men. Control for additional socioeconomic factors such as income may therefore be required to fully control for social disadvantage in both sexes.

Other studies on occupational physical activity have been inconclusive with beneficial effects\textsuperscript{34, 58-60, 62, 64}, null effects\textsuperscript{38, 41, 61}, and potentially adverse effects\textsuperscript{59, 63} on CHD risk. A more detailed questionnaire on occupational physical activity was used in a study by Fransson et al.\textsuperscript{59}. They found a protective effect on MI risk for jobs that included walking and standing, whereas having a job that included a lot of lifting or carrying was associated with increased risk of MI. Their findings indicate that a questionnaire which better discriminates between more aerobic occupational physical activity (such as walking) and more anaerobic occupational physical activity (such as heavy lifting) may be required to further evaluate the association with MI.

**Snuff use**

We found no increased risk of MI among snuff users without a previous history of smoking. Our findings are in line with a meta-analysis based on Swedish studies published up to 2005.\textsuperscript{269} The meta-analysis found the risk estimate 1.06 (95\% CI, 0.83-1.37) for CHD (5 studies) and 1.17 (95\% CI, 0.80-1.70) for stroke (2 studies) among snuff users.\textsuperscript{269} In addition, our study gave no support for the hypothesis that the risk of SCD is increased among snuff users. The SCD cases were few, however, and we did not have statistical power to detect small differences in risk of SCD (see Figure 4).

Regarding fatal MI, the results of previous studies are more incongruent. The risk of fatal MI was shown to be increased among snuff users in a large study of construction workers,\textsuperscript{122} but not in studies that were based on general populations.\textsuperscript{125, 128, 129} The reason for the disparity is not clear, but may in part be explained by the fact that information on snuff exposure in the construction worker study was obtained from the 1970s to the early 1990s, which implicates exposure to snuff with a different
chemical composition than today. In particular, the level of TSNA\textsuperscript{s} in snuff have decreased since the 1980s\textsuperscript{117}.

We did not find any association between snuff use and MI, but we cannot exclude that snuff use may have other effects on cardiovascular health. High snuff consumption may be associated with metabolic health hazards. In a study on the association between several lifestyle factors and the metabolic syndrome, Norberg et al\textsuperscript{137} found that ten-year development of metabolic syndrome was associated with high-dose consumption of snuff at baseline, even when controlling for smoking. Persson et al\textsuperscript{142} found that heavy use of snuff was associated with an increased risk of type 2 diabetes in a cross-sectional study. Their findings indicate that dose-stratification in snuff consumption may be essential, particularly when snuff use is evaluated in relation to metabolic outcomes. It is, however, a methodological challenge to adequately control for other unhealthy behaviour which may be present in individuals with high snuff consumption.

Previous studies on the long-term effect of snuff use on blood pressure are inconsistent. In a large prospective study on construction workers, Hergens et al\textsuperscript{123} found that snuff users had a higher risk for increased blood pressure and for being diagnosed with hypertension during follow-up. Several smaller cross-sectional studies on general populations have not detected any adverse effects on blood pressure levels during non-exposure periods, nor an increased prevalence of hypertension\textsuperscript{127, 134-138}. This divergence may be due to less statistical power to detect such associations in the population-based studies. In addition, prolonged snuff use during the day (leading to high total snuff consumption) may be a more socially acceptable behaviour among manual workers than in other professions. This may further explain as to why an association between snuff use and hypertension has been found among construction workers, but not in general populations. Furthermore, it cannot be ruled out that prolonged snuff use during the day may have adverse effect on mean blood pressure which is not detected by use of standardized resting blood pressure measurements during periods of non-exposure. Such an adverse effect from snuff use was indicated in the first and only published study using ambulatory 24-hour blood pressure monitoring by Bolinder et al\textsuperscript{133}

**Haemostatic and inflammatory markers**

In paper III we found that several haemostatic markers (D-dimer, VWF, t-PA, t-PA/PAI-1 complex) were associated with MI even after adjustment for established risk factors and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers. In a model which included established risk factors and all studied haemostatic and inflammatory markers (CRP and IL-6). Similarly, CRP and IL-6 were associated with MI independent of established risk factors and haemostatic markers.
pathways in CHD. Epidemiologic studies alone cannot resolve this question. One of the benefits of experimental studies is that local vascular models can be studied without the influence of systemic inflammation, which has many biological actions and may confound local inflammatory effects on the epidemiological research level. For example, a possible link between inflammation and fibrinolysis has been suggested by Robinson and co-workers based on findings in experimental research. They found that in men with stable CHD, intra-arterial infusion of the pro-inflammatory cytokine TNF-α caused a rise in t-PA activity and t-PA mass concentration in the infused arm, but not in the non-infused arm.

The associations with MI found in our study were similar or somewhat higher in magnitude than those found in previous meta-analyses of the studied markers. The consistency of several of the associations between biomarkers and CVD has fueled expectations of the feasibility of biomarkers to improve cardiovascular risk assessment. In particular CRP, has been suggested for this purpose. During the last few years, however, a number of studies have shown that despite the association between the CRP and CHD, the predictive ability of CRP over and above established risk models may be questioned. In our study we found that the inclusion of several biomarkers in combination may improve predictive ability above and beyond that of a model with established risk factors only. Similar results have been found in a few other studies of CVD and cardiovascular mortality that have evaluated predictive ability of a combination of biomarkers from several potential mechanistic pathways.

Mediators to the effect of active commuting

Regular physical activity has beneficial effects on several of the established cardiovascular risk factors, including BMI, hypertension, dyslipidaemia, and diabetes. However, with few exceptions the beneficial effect of physical activity on CHD risk persists even after the established risk factors are controlled for. This indicates that the impact of physical activity is not fully accounted for by reduction of established risk factors, and that additional mediating mechanisms may contribute to the effect of physical activity on CHD risk.

In paper IV we found that established risk factors (apo B/apo A-1 ratio and BMI) together with inflammatory and haemostatic markers (IL-6, t-PA, and t-PA/PAI-1 complex) appeared to explain a substantial proportion (40%) of the reduction in MI risk related to active commuting. Moreover, the inflammatory and haemostatic markers seemed to be potential major mediators of the protective effect. Few previous attempts have been made to estimate the relative contribution of established and novel risk factors, representing different mechanistic pathways, to the effect of physical activity on CVD. In a previous work from the Women’s Health Study, Mora et al. used similar methods. They found that a combination of inflammatory and haemostatic markers (CRP, fibrinogen, soluble intracellular adhesion molecule-1) appeared to be the largest contributor to the effect of leisure time physical activity on risk of CVD, explaining 32.6% of the protective effect.

It has previously been suggested that the protective effect from regular physical activity may in part result from the favourable effects on haemostasis. In an earlier cross-sectional study from the Northern Sweden MONICA Study among adults aged 25–64 years, lower t-PA mass concentrations and PAI-1 activity was found in those who exercised regularly. In a 20-year follow-up of men in the British
Regional Heart Study, regular leisure time physical activity showed significant and inverse dose-response relationships with t-PA mass concentration, VWF, D-dimer, fibrinogen, plasma and blood viscosity, platelet count, coagulation factors VIII and IX, CRP, and white cell count even after adjustment for potential confounders. An examination of changes in physical activity between baseline and 20 years later showed that inactive men who took up at least light physical activity had levels of blood variables approaching those who remained at least lightly active, whereas those who became inactive showed levels more similar to those who remained inactive. Stratton et al studied the effects of a 6-month endurance training on a number of haemostatic markers in 10 young (24-30 years) and 13 older (60-82 years) male subjects. The subjects in both the young and older group increased their maximum oxygen consumption after the training period by 18% and 22%, respectively. No effect was seen in haemostatic markers among the younger subjects, whereas in the older subjects considerable and statistically significant changes were found; t-PA mass concentrations decreased by 39%, t-PA activity increased by 39%, and PAI-1 activity decreased by 58%.

Strenuous bouts of physical activity result in a short-term increase in levels of IL-6 and several other pro-inflammatory cytokines which is followed by a transient increase in acute-phase reactants such as CRP. Previous studies have shown that the increase in IL-6 is the earliest and most prominent of the cytokine responses during strenuous physical activity. This effect seems to be independent of muscle damage and directly related to the intensity, duration, and mass of muscle recruited for the activity. Moreover, the effect appears to be more pronounced in untrained individuals at the same intensity level. Parallel with this acute inflammatory response, several protective anti-inflammatory effects have been observed, including increased levels of the cytokine inhibitors, IL-1 receptor antagonist, and soluble TNF-α receptors and decreased levels of leukocyte adhesion molecules. These effects may counter-regulate the inflammatory state. It has been suggested that the long-term anti-inflammatory effect associated with regular physical activity may be ascribed to this same mechanism elicited by intermittent acute bouts of physical activity.

In our study, IL-6, but not CRP, was negatively correlated with active commuting. The reason for this is not clear, but one possible explanation might be that IL-6 levels may be more sensitive than CRP levels to regular physical activity of low to moderate intensity, as in active commuting. No firm conclusion can be drawn based on current knowledge, but the results from a number of previous studies lend some support to this hypothesis. A previous cross-sectional study indicated that regular walking was associated with lower levels of pro-inflammatory cytokines such as IL-6, independent of vigorous physical activity. In a sample of 4072 participants from the population-based American National Health and Nutrition Examination Survey (NHANES) III, the relationship between elevated inflammatory markers and various forms of exercise was examined. After controlling for possible confounding factors, only regular participation in activities with generally higher intensity such as jogging and aerobic dancing was associated with lower levels of CRP, whereas activities with generally relatively lower intensity such as cycling, swimming, and gardening was not associated with CRP levels. A few physical activity intervention studies with focus on walking as the primary mode of exercise have shown significant reductions in IL-6 levels, but not in CRP levels. It is possible that IL-6 may be more closely linked to regular physical activity than CRP simply because IL-6 is produced and released by the actual contracting skeletal muscle fibers during physical activity, whereas CRP is
released by down-stream effects of IL-6 and other pro-inflammatory cytokines following physical activity.$^{188}$

A large proportion of the effect of active commuting on MI risk could not be explained in our study. Several additional effects have been suggested to explain the 30-50% reduction in CHD risk associated with regular physical activity.$^{274}$ Increased bioavailability of nitric oxide$^{280,281}$ and enhanced parasympathetic tone$^{282}$ may constitute such beneficial effects. These were not investigated as potential mediating mechanisms.

**Clinical utility**

The findings in our studies give further support for promotion of regular physical activity in the prevention of CHD. Active commuting may be a feasible way to achieve the recommended guidelines of 30 minutes daily physical activity. This mode of regular physical activity has several advantages; it is environmentally friendly, inexpensive, and can be integrated in daily life. Furthermore, active commuting can be jointly promoted by health care, employers, traffic planners, and environmental agencies.

The positive effects of regular physical activity on coronary risk seem to be mediated through several mechanistic pathways and only partly through established risk factors. A patient who takes up regular physical activity should therefore be encouraged even if the established risk factors remain unchanged. There seems to be no single marker that can characterize these health benefits or serve as a valid surrogate measure for the effects of physical activity on coronary risk. Thus, for clinical assessment and monitoring of a physical activity intervention, other tools which aim to measure the actual physically active behaviour, such as pedometers and self-registration of physical activity, may be more adequate than biochemical testing.

Whereas smoking is clearly associated with increased risk of MI, no increased risk for MI has been found among snuff users without a previous history of smoking. However, other health hazards have been associated with snuff use$^{283-285}$. From a public health perspective the avoidance of tobacco products is the only sound long-term goal.

We found that the inclusion of several biomarkers in combination may improve predictive ability beyond that of a model utilizing only established risk factors. However, it must be stressed, that before a multimarker panel is introduced in clinical practice, this panel must satisfy the requirement of showing consistently improved predictive ability in addition to established risk factors, as well as achieve clear benefits in health economic analyses. Until this is proven, prediction of cardiovascular risk in clinical practice should be based on the established risk models.

**Future perspectives**

The established cardiovascular risk models serve several purposes; they help us to predict cardiovascular risk, educate the public on how to avoid health hazards, guide intervention in high risk individuals, and serve as a basis for research of new pharmaceutical agents. The global INTERHEART study identified nine modifiable risk factors, including established risk factors, that account for over 90% of the risk of MI.$^{18}$ This landmark study consolidated the established risk models as the foundation
of cardiovascular risk, but also emphasized the benefits of a healthy lifestyle. The studies in this thesis support the importance of a healthy lifestyle for cardiovascular health. An important objective in future research will be to translate this knowledge into clinical practise and to define optimal means for lifestyle interventions.

The established risk factors are used to predict the risk of cardiovascular disease and have been integrated in risk scores\textsuperscript{15, 16}. Our study suggests that consideration of biomarkers from different mechanistic pathways may improve the prediction of coronary risk beyond that of established risk factors alone. We also found that regular physical activity was inversely associated with future MI independent of established risk factors. This raises the following question: can prediction of coronary risk be further improved by an assessment of physical activity or physical fitness? This may be an interesting research question for future studies.

During the last few decades snuff use has increased among both men and women in Sweden, whereas smoking has declined\textsuperscript{120}. As a result, even small increases in health hazards associated with snuff use may have great impact on public health. This trend underscores the need to pool data from several population-based studies for analyses of the associations between snuff use and different health-related outcomes. This would yield a higher statistical precision and increase the likelihood of detecting potential minor health hazards among snuff users. In addition, there is a need to further evaluate the impact on mean blood pressure in snuff users, preferably using ambulatory 24-hour blood pressure monitoring.

Several important causal discoveries have been made using observational studies, including cholesterol and CHD. However, observational studies have also suggested cardioprotective effects of vitamins\textsuperscript{286} and hormone replacement therapy\textsuperscript{287} that could not be confirmed in RCTs\textsuperscript{288, 289}. Confounding, reverse causation and other biases have limited the application of observational epidemiology and the progress in research in several areas. Despite the volume of observational studies showing increased risk of CHD in individuals with increased levels of haemostatic and inflammatory markers, it is still controversial whether these associations represent a cause, a consequence or confounding. Understanding the causal relationships is a key to identifying potential targets of intervention. Different research strategies have been suggested to increase evidence for a causal effect of a modifiable factor or provide evidence against causal effects for associations found in observational studies. One such strategy is to design specific inhibitor drugs, such as the CRP specific inhibitor drug 1,6 (bis)-phosphocholine hexane currently being developed for clinical testing\textsuperscript{290}. If an inhibitor drug is able to specifically block CRP function for sustained periods, the effects of the CRP inhibition could be studied in order to elucidate the role of CRP in CHD. Another strategy for strengthening (or weakening) causal inferences from observational studies is the use of Mendelian randomization\textsuperscript{291}. This approach uses polymorphisms or haplotypes in the regulatory region of a biomarker’s gene, for example the CRP gene, which has been reliably associated with circulating levels of CRP. Since the inheritance of genetic variants should be subject to the random assortment of maternal and paternal alleles, and not associated with other genetic variants (Mendel’s second law), the effects of these genetic variants can be studied with a study design similar to that of a randomised trial. By this strategy the usual biases resulting from confounding and reverse causation may be avoided. As for CRP, a few studies have been conducted using genetic variants in the CRP gene as un-biased proxies for circulating CRP levels\textsuperscript{292–294}. 
The genetic variants have shown significant associations with CRP levels, but no association with CHD outcome. This indicates that the association between CRP and CHD is not causal, but may reflect effects on CHD which are more proximal in the inflammatory cascade. Mendelian randomization has also been used in the studies of lifestyle factors and provided evidence that alcohol intake increases the risk of esophageal cancer. Indeed, new discoveries of genetic variants in the human genome may give us helpful surrogates for further evaluation of the lifestyle factors and novel biomarkers beyond the established risk factors.
CONCLUSIONS

- A clearly increased risk of myocardial infarction was found for car commuting compared to active commuting. High versus low leisure time physical activity was associated with decreased risk of myocardial infarction. Low occupational physical activity was associated with risk of myocardial infarction in men.

- The risk of myocardial infarction or sudden cardiac death was not increased among male snuff users without a previous history of smoking compared to non-tobacco users. However, for sudden cardiac death the study did not have statistical power to detect small differences in risk.

- The haemostatic markers D-dimer, VWF, t-PA, and t-PA/PAI-1 complex were associated with myocardial infarction after adjustment for established risk factors and inflammatory markers (CRP and IL-6).

- The addition of eight haemostatic and inflammatory markers could improve the predictive ability for future myocardial infarction beyond that of a model utilizing only established risk factors.

- A combination of established risk factors, haemostatic and inflammatory markers appeared to explain a substantial proportion (40%) of the difference in risk for myocardial infarction between active commuters and car commuters. IL-6, t-PA, t-PA/PAI-1 complex, apo B/apo A-1 ratio, and BMI seemed to be the largest potential mediators when tested individually.
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I thought of that while riding my bicycle.

Albert Einstein
1879-1955

-on the theory of relativity.
APPENDIX

Extract from questionnaires

Questions on physical activity

VIP:

F1. Is your work physically demanding?
   1. Yes, often
   2. Yes, sometimes
   3. No, rarely
   4. No, almost never

G1. Mark in the table below how you usually travel to work for each season.
   1. Car (spring, summer, autumn, winter)
   2. Bus (spring, summer, autumn, winter)
   3. Walking (spring, summer, autumn, winter)
   4. Bicycle (spring, summer, autumn, winter)

G6. How often have you trained or exercised in training clothes during the last three months to improve your fitness and/or to feel healthy?
   1. Never
   2. Now and then—not regularly
   3. 1 time per week
   4. 2-3 times/week
   5. More than 3 times/week

MONICA:

96. How much have you been physically active or exerted yourself physically during leisure time for the last year?
   1. Hardly anything
   2. Mostly sedentary, sometimes a walk or similar activity
   3. Lighter physical exertion at least 2 hours per week
   4. More exertive exercise 1-2 hours per week
   5. More exertive exercise at least 3 hours per week
   6. Hard training or competition regularly or several times per week with heavy physical exertion

97. How much have you been physically active or exerted yourself physically during work for the last year?
   1. Sedentary work
   2. Light, but partly mobile work
   3. Moderately physically demanding work
   4. Heavy physically demanding work
Questions on tobacco use

VIP :

H1. Do you smoke currently?
   1. No, I have never smoked
   2. Yes, I smoke cigarettes
   3. Yes, I smoke cigars
   4. Yes, I smoke pipe
   5. Yes, I smoke occasionally (not daily)
   6. Not now, but I used to smoke regularly
   7. Not now, but I used to smoke occasionally

H4. Have you ever used snuff?
   1. No
   2. Yes, previously but not currently
   3. Yes, less than 2 packages per week
   4. Yes, 2-4 packages per week
   5. Yes, more than 4 but less than 7 packages per week
   6. Yes, 7 packages or more per week

MONICA:

8. Do you smoke cigarettes currently?
   1. Yes, regularly (1 cigarette or more per day)
   2. No
   3. Sometimes (less than 1 cigarette/day)

12. Have you ever smoked cigarettes regularly previously?
   1. Yes, regularly
   2. No

18. Have you ever smoked a pipe?
   1. Yes, at present regularly
   2. No
   3. I smoke a pipe sometimes (less than 1 time/day)
   4. Previously, but not now

20. Have you ever smoked cigars or cigarilles?
   1. Yes, at present regularly
   2. No
   3. I smoke cigars/cigarilles sometimes (less than 1 time/day)
   4. Previously, but not now

23. Have you ever used snuff?
   1. Yes, previously but not currently
   2. Yes, less than 2 packages per week
   3. Yes, 2-4 packages per week
   4. Yes, more than 4 but less than 7 packages per week
   5. Yes, 7 packages or more per week
   6. No