Plasma levels of matrix metalloproteinase-9 in a normal population
- a psychoneuroendocrinological approach

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In memory of Olav Axelsson; 1937-2004

Read not to contradict and confute, nor to believe and take for granted... ...but to weigh and consider.
(Sir Francis Bacon; 1552-1626)
PREFACE

To understand any work that has been done, one will find many clues in the background of the conductor. My undergraduate background and interests put me academically somewhere in the interface between physiology and epidemiology. Thus, it came as a natural continuation in my academic training to further explore this interface.

My post-graduate training has been focused on how to plan, implement, and conduct a study in a normal population. Even though I would like to think that I once was able to conduct laboratory work with fair efficiency and accuracy, and hopefully will be able to update those skills one day, this dissertation is mainly a desktop product from my perspective. It has been performed without the need of a lab coat. It is my personal belief that the use of observational studies in normal populations is a crucial component in medical research as a whole, elucidating the findings from clinical and experimental designs.

The sections regarding biomarkers and pathophysiological pathways are kept to introductory overviews in this dissertation. I am well aware that simplifications have been made throughout those sections, which might appear somewhat incomplete for those dedicated to natural science and medicine. On the other hand, only a small fraction of researchers in the field of social medicine and public health science (the field comprising this dissertation) have a background in biology and chemistry. They are by far outnumbered by those with a background in sociology and behavioral sciences. For the latter group, this dissertation may be considered to be too detailed regarding its content on physiological mechanisms.

It is an inevitable clinch that has to be dealt with, a necessary price to pay whenever exploring the interface between adjacent disciplines.

I have tried to balance both standings in this dissertation, being stuck in the circular reasoning that there is no point in studying mechanisms without studying the determinants and equally pointless to study determinants without caring for the mechanisms. The attempt to balance these two throughout the dissertation is fuelled by the perhaps somewhat optimistic but sincere aim that both natural science reductionists as well as those that prefer qualitative data will find the dissertation to be of relevance.

It should be noted that this dissertation is just one piece in the puzzle. It is the first dissertation based on data from the Life conditions, Stress, and Health
(LSH)-study, but there are many more in the making from this rich data set. The analyses are for now limited to cross-sectional. Hopefully, even more intriguing results are yet to come, when prospective data on cardiovascular outcome are merged into the data set.

I was recruited in 2002 to the LSH-project, in the planning phase of the study, and have been working on it ever since. Thus, even though the dissertation as such is a big step in my academic development, since the beginning of my post-graduate training my concerns have included not only these few studies, but also the progress of the LSH-study as a whole.

During my research training, I have attended the multidisciplinary Graduate school of Health, Care, and Society, Linköping University and the seminar series held by Cardiovascular Inflammation Research Centre (CIRC), Linköping University, for students in post-graduate training. My research training is now reaching its end. My research as such however, has just begun. This dissertation is dedicated to the memory of Olav Axelson (1937-2004), late professor in epidemiology at Linköping University. With his sharp intellect, warmth, and caring support, he had a tremendous influence on me during my time as a Master’s student in public health. Professor Axelson was without a doubt the most influential person who inspired my choice to pursue post-graduate training.
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ABSTRACT

Several large-scale epidemiological studies have demonstrated the prognostic significance of psychosocial factors and stress for coronary artery disease (CAD). Observations of sudden changes in CAD incidence have led to the proposal of mechanisms regarding atherosclerotic plaque vulnerability. The collagen-degrading enzyme matrix metalloproteinase-9 (MMP-9) is increased in rupture-prone plaques with high inflammatory activity, and circulating levels of MMP-9 are raised in patients with acute coronary syndrome. However, the distribution of MMP-9 levels and its relations to psychosocial factors and the stress hormone cortisol have not been previously explored in a normal population.

The aim of this dissertation was to examine in a normal population the association of circulating levels of MMP-9 with traditional cardiovascular risk factors including levels of C-reactive protein (CRP), with psychosocial factors, and with saliva levels of cortisol. In addition, the reliability of a new method of ambulatory saliva sampling for assessment of cortisol levels was evaluated.

A sub-sample of the Life conditions, Stress, and Health (LSH)-study, a population based study exploring psychoneuroendocrinological pathways mediating the differences in CAD incidence over socioeconomic status, was used. Plasma levels of MMP-9 were examined in a sample randomly drawn from the LSH-study (n=400), aged 45 to 69 years at enrollment.

The main findings were: 1) there was a positive association between plasma MMP-9 levels and total risk load of cardiovascular risk factors. The findings were persistent after adjusting for CRP and could not be attributed to a single risk factor. 2) After adjusting for traditional cardiovascular risk factors and CRP, MMP-9 levels were positively associated with psychosocial risk factors and negatively associated with psychosocial resources. 3) Pooling saliva samples prior to laboratory analysis were as reliable as arithmetic means for assessment of diurnal cortisol variation in a field research setting. 4) There was a positive association between circulating levels of MMP-9 and saliva levels of cortisol, both diurnal peak level and evening level of cortisol.

The observed associations between MMP-9 and traditional cardiovascular risk factors, psychosocial factors, and saliva cortisol levels suggest a psychoneuroendocrinological pathway linking stress to plaque vulnerability and provide increased understanding of the association between psychosocial factors and CAD.
LIST OF PAPERS

Roman letters (I-IV) are used when referring to any of the four papers that constitute the base of this dissertation.

   running title: MMP-9 and cardiovascular risk factors

   running title: MMP-9 and psychosocial factors

    running title: Reliability of pooling cortisol

    running title: MMP-9 and cortisol
**WORD LIST AND ABBREVIATIONS**

The following words used in the dissertation may require explanation for those not academically skilled in natural science or medicine:

<table>
<thead>
<tr>
<th>Term</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>allostasis</td>
<td>Greek for “to stand in variability”, denoting stability through change. Term used for a system that responds to requirements by an adequate temporary physiological shift.</td>
</tr>
<tr>
<td>anabolic</td>
<td>Greek for “to build up”. A physiological process promoting growth and cellular synthesis.</td>
</tr>
<tr>
<td>angiogenesis</td>
<td>Greek for “to originate vessel”. Growth and formation of new blood vessels from pre-existing vessels.</td>
</tr>
<tr>
<td>atherosclerosis</td>
<td>Greek for “porridge hardening”, referring to accumulation of lipids and macrophages surrounded by fibrous caps in the artery walls.</td>
</tr>
<tr>
<td>basal lamina</td>
<td>A thin layer of connective tissue in the vessel wall, anchoring the endothelial cells that are in contact with the blood stream.</td>
</tr>
<tr>
<td>catabolic</td>
<td>Greek for “to break down”. A physiological process, promoting release of energy by breaking down large molecular structures in smaller units.</td>
</tr>
<tr>
<td>collagen</td>
<td>The main protein of connective tissue in animals.</td>
</tr>
<tr>
<td>endogenous</td>
<td>Greek for “arising from within”, referring to an internal process or synthesis in the body.</td>
</tr>
<tr>
<td>endothelial cells</td>
<td>A thin layer of cells in the interior surface of a blood vessel, serving as the interface between lumen and the vessel wall.</td>
</tr>
<tr>
<td>exogenous</td>
<td>Greek for “outside production”, referring to something that is not synthesized in the body.</td>
</tr>
<tr>
<td>extracellular matrix</td>
<td>The complex network of supporting and connective tissue that is not part of any cell.</td>
</tr>
<tr>
<td>homeostasis</td>
<td>Greek for “to stand equally”, denoting stability through constancy. Term used for a system that remains stable despite shifts in the environment.</td>
</tr>
<tr>
<td>in vivo</td>
<td>Latin for “within the living”, referring to an experiment or observed phenomenon in a living organism.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>in vitro</td>
<td>Latin for “within the glass”, referring to a study on tissue or specific cell types conducted outside a living organism.</td>
</tr>
<tr>
<td>ischemia</td>
<td>Greek for “restriction in blood”, referring to a shortage of oxygen in a tissue (causing permanent damage if prolonged).</td>
</tr>
<tr>
<td>leukocytes</td>
<td>Greek for “milk cell”. White blood cells, crucial for the immune system in inflammation.</td>
</tr>
<tr>
<td>lipoprotein</td>
<td>A macromolecular compound containing both lipids and protein. Lipoproteins transport cholesterol in the blood to and from peripheral tissue.</td>
</tr>
<tr>
<td>lumen</td>
<td>The inner space in blood vessels where blood is flowing.</td>
</tr>
<tr>
<td>macrophage</td>
<td>Greek for “big eater”. Specific type of white blood cell, which eliminate pathogens.</td>
</tr>
<tr>
<td>monocyte</td>
<td>Type of white cell blood type from which macrophages are derived.</td>
</tr>
<tr>
<td>myocardial</td>
<td>Referring to the muscular wall of the heart.</td>
</tr>
<tr>
<td>pathogen</td>
<td>Greek for “origin of suffering”. Any factor that is harmful for an organism and have the potential to trigger a disease.</td>
</tr>
<tr>
<td>platelets</td>
<td>Circulating cells in the blood stream, whose activation leads to blood clots.</td>
</tr>
<tr>
<td>plaque</td>
<td>An accumulation in artery walls containing lipids, macrophages, calcium, and connective tissue.</td>
</tr>
<tr>
<td>plasma</td>
<td>The liquid phase of blood, from where white cells, red cells and platelets are suspended through centrifugation (with no clotting allowed in preparation).</td>
</tr>
<tr>
<td>salutogen</td>
<td>Greek for “origin of well-being”. Any factor that is beneficial for an organism and have the potential to promote health.</td>
</tr>
<tr>
<td>serum</td>
<td>The liquid phase of blood from where white cells, red cells and platelets are suspended after the blood has been allowed to clot.</td>
</tr>
<tr>
<td>stenosis</td>
<td>Greek for “narrowing”. An abnormal narrowing in the blood vessel, commonly caused by a plaque.</td>
</tr>
<tr>
<td>stratification</td>
<td>From stratum, Latin for “layer”. The process of dividing a population in different groups according to a certain property.</td>
</tr>
</tbody>
</table>
The following abbreviations are used in the dissertation:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS</td>
<td>acute coronary syndrome</td>
</tr>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>Apo A1</td>
<td>apolipoprotein A1</td>
</tr>
<tr>
<td>Apo B</td>
<td>apolipoprotein B</td>
</tr>
<tr>
<td>ATC</td>
<td>anatomical therapeutic chemical classification system</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>CATS</td>
<td>cognitive activation theory of stress</td>
</tr>
<tr>
<td>CES-D</td>
<td>Centre for epidemiology studies depression scale</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CRH</td>
<td>corticotrophin-releasing hormone</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variance</td>
</tr>
<tr>
<td>DBP</td>
<td>diastolic blood pressure</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>diagnostic and statistical manual on mental disorders, fourth edition</td>
</tr>
<tr>
<td>EDTA-plasma</td>
<td>ethylene diamine tetraacetic acid treated blood plasma</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>GAS</td>
<td>general adaptation syndrome</td>
</tr>
<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>HPA</td>
<td>hypothalamus-pituitary-adrenal</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>ISSI</td>
<td>Interview schedule for social interaction</td>
</tr>
<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
</tr>
<tr>
<td>LSH-study</td>
<td>Life conditions, Stress, and Health-study</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
</tr>
<tr>
<td>PHC</td>
<td>primary health care</td>
</tr>
<tr>
<td>PHCC</td>
<td>Primary health care center</td>
</tr>
<tr>
<td>RA</td>
<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
</tr>
<tr>
<td>SOC</td>
<td>sense of coherence</td>
</tr>
<tr>
<td>TIMP</td>
<td>tissue inhibitor of metalloproteinases</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

1.1 Rationale for the dissertation

When the outline was drawn for this dissertation, it was based on the following four main arguments:

1. The inverse gradient in incidence of coronary artery disease over socioeconomic position cannot be explained by lifestyle alone.

A number of studies from more than eight decades has shown that there is an inverse gradient between socioeconomic position and health [1]. Life expectancy differs several years when stratified over socioeconomic position [2-6]. The differences in health are consistent regardless of how socioeconomic position is measured (in terms of income, education, occupation, employment, or immigrant status) [2,7,8]. The inverse gradient is apparent for a number of outcomes, with coronary artery disease (CAD) as one of the more prominent [1,2,7-9].

A clustering of cardiovascular risk factors has been reported in several studies, where people in lower socioeconomic position tend to smoke more, have a higher alcohol intake, a lower fruit and vegetable intake, and less physical activity [10]. However, the difference in CAD incidence can not be fully attributed to lifestyle [11-14]. Further, it has been shown that the widening of inequalities of CAD incidence over time had little or no relation to changes in social distribution of traditional cardiovascular risk factors [11]. It has therefore been suggested that psychosocial factors, at least in part, could explain the gradient in cardiovascular incidence over socioeconomic position [8-10,12].

2. There are a number of epidemiological studies which have shown an association between psychosocial factors and CAD.

There are a considerable number of epidemiologic studies pointing out the prognostic significance of psychosocial factors for CAD [15-24]. In particular, depression at clinical or sub-clinical levels has been shown to predict CAD in prospective studies [15-19]. The INTERHEART-study, the largest case-control
study conducted so far on myocardial infarction (n=15,152+14,820), points out that psychosocial factors is the third most influential risk factor next to smoking and dyslipidemia [25]. The independent association between psychosocial factors and CAD is however not widely recognized, as the suggested mechanisms as yet are tentative and not fully empirically verified [19,26,27]. Further studies on plausible mechanisms are needed to better understand and evaluate the epidemiological findings.

3. There are changes in CAD incidence that cannot be explained by changes in lifestyle.

The European East-West gap in CAD incidence as illustrated in Figure 1 with Sweden representing the Western European countries, can only in part be explained by a skew distribution of traditional CAD risk factors [13,14]. In fact, median levels of hypercholesterolemia prevalence were lower in the Eastern European countries than in the Western European countries [13]. It has thus, in concordance with the gradient of cardiovascular incidence over socioeconomic position, been hypothesized that psychosocial factors, at least in part, are attributable for the East-West gap in CAD incidence [13].

Figure 1. Standardized mortality rates in ischemic cardiovascular disease in ex-Soviet countries before and after the Eastern Bloc breakdown. Source: WHO/Europe, European mortality database [28].
Further, official statistics reveal that many ex-Soviet countries experienced an increased mortality in CAD shortly after the Eastern Bloc breakdown in 1990, accompanied by a rapid decline a few years later [28]. Lifestyle factors such as excessive alcohol intake, poor diet, and smoking are an insufficient explanation to the observations, since progression of atherosclerosis is considered to be a slow process, which would be reflected by a less dramatic fluctuation. It has been suggested that stress induced by the transition into a market economy, at least in part can be responsible for the rapid changes in incidence [29-31]. A similar pattern has been demonstrated in other settings, where dramatic societal or environmental changes of different origins, e.g. caused by political turbulence, caused by earth quakes, and caused by warfare, are accompanied by a sudden rise in CAD [29,32-35].

4. **The number of unexpected sudden cardiac deaths each year is high.**

Even though there are a number of risk factors for CAD that are well-established both clinically and epidemiologically, the risk factors identified so far still have poor predictive value on an individual basis [36-38]. Furthermore, even though the presence of at least one risk factor is common in individuals developing CAD [39] and that at least one stenosis are reported in many fatal events [40,41], a large proportion of acute coronary events are unexpected and strike many apparently healthy individuals, free from any of the major established risk factors [42] and with seemingly normal coronary arteries [43]. It is suggested that every year, up to 10,000 deaths in Sweden occur due to unexpected cardiac events [42]. This has led to suggestions of mechanisms affecting vulnerability in the circulatory system e.g. plaque vulnerability, where some individuals are more likely to develop future acute coronary syndrome (ACS) than others, despite having the seemingly same risk load [44].

Taken the four arguments together, it becomes strikingly clear that there are challenges which remain in risk stratification for ACS. Based on the arguments, it was hypothesized that there is a physiological pathway linking stress to plaque vulnerability. Such a pathway could at least in part explain the phenomena described in the arguments. Thus, the conceptual purpose of this dissertation was to investigate a novel marker of plausible relevance for plaque vulnerability using a psychoneuroendocrinological approach. The long-term goal with the underlying work is to increase the understanding on mechanisms and pathways that could explain the dramatic differences in
CAD incidence over time or between groups in a society and that could be prevented by intervening on determinants.

### 1.2 Coronary artery disease

Cardiovascular disease is one of the leading causes of premature death in Sweden [45] as well as worldwide [46,47]. Its global incidence and burden of disease is predicted to rise even further during the following decades [48,49]. CAD, characterized by an insufficient blood flow to the cardiac muscle thereby causing ischemia, is the most common manifestation of cardiovascular disease [45]. The occlusion preceding the insufficient blood flow is in most cases caused by a remodeling of the arterial wall, slowly narrowing the lumen, and a rupture, fissure, or erosion of the arterial wall stimulating thrombosis. See Figure 2.

*Figure 2. Different manifestations of CAD. Remodeling and rupture in coronary arteries.*

<table>
<thead>
<tr>
<th>Normal coronary artery</th>
<th>Stable angina</th>
<th>Destabilization, rupture causing thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>No occlusion in lumen</em></td>
<td><em>Reduced blood flow, potential ischemia</em></td>
<td><em>Severe occlusion due to blood clot</em></td>
</tr>
<tr>
<td>a) No apparent abnormalities</td>
<td>b) Visible plaque stenosis</td>
<td>a) No apparent abnormalities</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Visible plaque stenosis</td>
</tr>
</tbody>
</table>

Stable angina pectoris is characterized by a substantial occlusion in the coronary vessels reducing blood flow to the heart muscle. When demand on the heart increases, an ischemic state can occur. This state is however reversible and oxygen supply will return to sufficient levels within minutes if
the heart is allowed to rest. Typically, the plaques causing occlusions in stable angina pectoris are embedded in thick collagen-rich fibrous caps. This manifestation is not lethal in itself. Patients with stable angina pectoris can live with an occlusion for many years (although restricted to avoid vigorous physical activity).

A more serious event occurs if there is a destabilization of the vessel wall and an atherosclerotic plaque ruptures. When collagen from a ruptured fibrous cap comes in direct contact with the blood stream, platelets become highly activated, causing a blood clot. This reaction may cause a luminal occlusion, leading to a persistently impaired oxygen supply and a life-threatening ACS. Cardiac cells will die due to the oxygen deficit, causing an eventual myocardial necrosis, which defines a myocardial infarction. For survivors, the damage to the myocardium is permanent after a necrosis has occurred, leaving a collagen scar in the tissue and possibly permanently impaired function of the cardiac muscle.

Notably, as depicted in Figure 2, plaque instability is not necessarily connected to the size of the atherosclerotic plaque but may occur also in relatively small plaques.

### 1.2.1 Atherosclerosis – an inflammatory disease

For years the ruling paradigm has been that atherosclerosis is essentially promoted and maintained by an accumulation of lipids in the walls of large- and medium-sized arteries [50,51].

Over the last decade or so, advances in research have led to a shift in the paradigm, with the establishment of atherosclerosis as an inflammatory process [52-54]. Inflammation (Latin *inflammatio*, “to set on fire”) is a complex response to minimize damaging effects of potentially harmful stimuli. In the acute phase of inflammation, the stimulus and damaged tissue are removed and remaining tissue remodeled or regenerated. The inflammatory response is mainly regulated by leukocytes, in particular monocyte-derived macrophages, which migrate from the lumen of the blood vessels into the tissues. If the inflammation is prolonged, the acute state will be shifted towards a chronic state, characterized by simultaneous and persistent destruction and regeneration of the tissue. Whereas the acute inflammatory response is of utter importance to protect an organism from pathogens, chronic inflammation may be a double-edged sword. Due to double messages in local signaling
Introduction

(stimulating both tissue destruction and repair), there is a risk for breakdown of the vital tissue, further impairing its function. In a simplified model, inflammation in atherosclerosis can be described as an inflammatory response to any kind of harmful stimuli invading or adhering to an arterial wall. If the stimulus is eliminated during the acute phase, the inflammatory process will regress eventually. If the stimulus is persistent, a chronic state will develop with constant inflammatory activity at the local site. The accumulation of lipids is still considered to be a central component of the etiology of CAD, but attention is focused on the role of lipids in inflammation rather than the accumulation in itself. Modified lipoproteins, in particular oxidized low density lipoproteins (LDL), have been pointed out as a main trigger of inflammation in atherosclerosis [55-57]. A number of other inflammatory stimuli have been suggested as well, including bacterial infection [58,59], exogenous particles from air pollution [60,61], and adherent platelets [62,63].

It has been somewhat debated if the inflammatory activity is centered locally in a culprit plaque causing the subsequent occlusion preceding ACS, or if the inflammatory process is more widespread in the arterial tree. Recent findings have suggested that an ischemic event is not a matter of “one bad atherosclerotic plaque”, but rather a consequence of ongoing inflammatory processes at multiple sites in the arteries [64-66]. This ongoing inflammation in atherosclerotic plaques may be reflected in circulating blood. Thus, measurements of inflammatory markers in serum or plasma may be used to predict the risk of future risk for ACS [67-69].

1.3 Traditional risk factors for coronary artery disease

Going back in history six or seven decades, mortality due to infectious disease came under control in the United States and Europe. There was a transition towards a new mortality pattern. Mortality rates for CAD had been steadily increasing in the Unites States since the beginning of the 20th century, and CAD became the leading cause of death around World War II. At that time, little was known about the etiology of CAD. Thus, there were few possibilities to reverse the continuously rising incidence. In 1948, the Framingham Heart Study was initiated by the American National Heart, Lung, and Blood Institute [70]. It was conducted on men and women aged 35 to 62 at enrollment and free from any apparent CAD. The study was conducted on the
normal population living in the small town of Framingham, Massachusetts (n=5,209). The participants were followed prospectively to outline risk factors for CAD. The study came to be a major contribution to CAD etiology as we know it today, and the project is still running, now following the third generation of participants from Framingham, Massachusetts. Studies on the first generation identified five major risk factors for CAD apart from age, namely hypertension, dyslipidemia, diabetes, obesity and smoking [70]. Today, there are numerous epidemiological, clinical, and experimental studies that strongly support the significance of these risk factors, but others have since then been added. Having the INTERHEART-study as a benchmark, nine main risk factors have been identified, estimated to attribute to more than 90% of the variance in incidence between groups categorized by exposure [25]. Each of the nine risk factors is described in more detail below. The first eight include hypertension, dyslipidemia, diabetes, obesity, smoking, alcohol intake, low physical exercise, and low fruit and vegetable consumption. These factors are only described briefly, as they are well established in cardiovascular epidemiology and clinical practice. As this thesis is based on a psychoneuroendocrinological approach, the ninth risk factor from the INTERHEART-study, psychosocial factors, is given a section of its own. A more in-depth explanation is needed to understand psychosocial factors as a risk factor for CAD as well as to provide adequate background for the results and discussion in this dissertation. In addition, one inflammatory marker, C-reactive protein (CRP) is presented briefly (apart from the risk factors studied in the INTERHEART-study), as it has been shown that CRP is a predictor of future coronary events [36,71].

1.3.1 Physiological characteristics

Hypertension

Hypertension refers to an abnormally high arterial pressure, where the systolic blood pressure (SBP) is defined as the peak pressure in the arteries, and the diastolic blood pressure (DBP) is the lowest pressure, just before the new pulse wave in the cardiac cycle. Estimated prevalence of hypertension varies with criteria defined, but regardless of definition, the prevalence is high. In Sweden, it is estimated that at least 10% of the population are hypertensive [72].
**Introduction**

Primary (or essential) hypertension constitutes about 95% of all diagnosed patients with hypertension [73]. The etiology for primary hypertension is not yet known (while secondary hypertension may be caused by for example kidney disease or excessive amounts of endogenous or exogenous steroids or stress hormones) [73]. Notably, the prevalence of primary hypertension increases markedly with age in Western populations [74] which is not the case in traditional societies [35,75].

In normal physiology, central arteries have an important buffering function by dampening pulse waves following cardiac output, reducing the potential damage that a harsh pulse wave would cause due to turbulent shear stress in more peripheral arteries. Subjects with hypertension are characterized by having stiff central arteries which limits this buffering function [76]. Thus, it has been suggested that more peripheral vessels adapt to the harsh pulse waves by becoming more stiff, further limiting the buffering function. The system will by this action adapt to a chronic state of hypertension [35]. The assumption of a chronic state is further strengthened by the fact that there are as of yet no medications that can reverse the hypertensive state. Pharmacological interventions are limited to reducing blood pressure by lifelong treatments.

**Dyslipidemia**

For almost a century, blood lipids have been suggested to play a crucial role in the development of CAD. In 1913, Antischkow fed rabbits with purified cholesterol and thereafter observed typical atherosclerotic lesions in the walls of the blood vessels. Hypotheses have changed throughout the years and mechanisms have been debated, but the central concept of the predictive value of dyslipidemia, i.e. a lipid imbalance, for future CAD events has remained [77-81]. Concordant with Antisckow’s early work, total cholesterol was long considered to be the driving force behind atherosclerosis. This was also recognized in the pioneering Framingham study [70]. Later, based on the density of lipoproteins carrying cholesterol, it was discovered that different lipoproteins have different physiological functions and different impacts on the etiology of CAD. A distinction between “bad cholesterol”, low density lipoprotein (LDL) and “good cholesterol”, high density lipoprotein (HDL), was made. A rule of thumb when distinguishing LDL from HDL is that LDL transports cholesterol and fatty acids from the liver to the peripheral tissues i.e. increase the fat content in peripheral tissues. HDL on the other hand transports cholesterol and fatty acids from the peripheral tissues back to the
Introduction

Liver for excretion or utilization elsewhere i.e. decreases the fat content in peripheral tissues. In concordance with this, high LDL has been shown to be positively associated with CAD incidence [82], whereas HDL is suggested to be protective and negatively associated with CAD incidence [83]. The ratio LDL/HDL has often been used to indicate a dyslipidemia where a high ratio is associated with cardiovascular disease.

The distinction of LDL and HDL has had a tremendous impact on clinical work, including pharmacological and other interventions targeting the bad cholesterol, trying to lower LDL levels.

In refined analysis, it has been shown that lipoproteins (including both LDL and HDL) are constituted by different types of apolipoproteins (apo) [84,85] as well as other proteins [86], with an inter-individual variation [86]. Two of these apolipoproteins, apo B and apo A1, have been shown to be better prognostic markers than LDL and HDL, respectively [87-89]. In the INTERHEART-study, the apo B/apo A1 ratio was superior to any of the cholesterol measurements for estimation of the risk of acute myocardial infarction [88].

In addition to cholesterol, levels of triglycerides are also taken into account when blood lipid profiles are estimated. Triglycerides are an important source of energy, transporting dietary fat as easy accessible fatty acids. Excessive levels of triglycerides are associated with CAD in univariate analysis. The strong association with obesity and invert relationship with HDL makes it however hard to disentangle the predictive value of triglycerides as a single risk factor [90].

**Diabetes mellitus**

Insulin is a hormone that has an anabolic effect, causing most of the body’s cells to take up available glucose from the blood stream, use it or store it intracellularly as an energy resource. A poor production of insulin in the pancreas (which is the case in diabetes mellitus type I), or insensitive receptors for insulin at a cellular level (which is the case in diabetes type II), causes high circulating levels of glucose and leads to the use of fat as an energy resource instead of glucose. In brief, apart from increase the susceptibility for dyslipidemia, obesity, and hypertension, the consequences of dysfunctional insulin signaling are suggested to be directly linked to inflammation and CAD, acting in multiple pathways. High glucose content in the blood may lead to a higher glucose uptake than normal in endothelial cells in the vessel wall, one of the few cell types where glucose uptake is not dependent by
Introduction

insulin. This in turn, leads to a higher production of glycoproteins in the extracellular matrix, making the vessel wall more prone to rupture [91]. The hyperglycemia in the blood stream also leads to a general glycation of many circulating proteins (e.g. LDL amongst others). The increased glycation leads to an increased vascular inflammatory burden [92,93]. Further, the uptake of fat by tissues normally using glucose as the primary energy source, is likely to cause accumulation of modified LDL, triggering peripheral inflammation as mentioned in section 1.2 [55-57].

In itself, diabetes is a strong risk factor for CAD, independent of the often co-varying risk factors of obesity, dyslipidemia, and hypertension. The risk for diabetic subjects to have an acute coronary event has been reported to be two-fold or higher [25]. However, the prevalence of diabetes is rather low in a normal population. According to WHO, the prevalence in the global population was 2.8% in 2000 [94]. Even though the prevalence is predicted to rise, and that the prevalence rises markedly with age [94], it is still low in comparison to other cardiovascular risk factors. The population attributable risk for CAD is therefore fairly low in comparison to other cardiovascular risk factors [25], see further section 1.3.3.

Obesity

A simple explanation why obesity is a risk factor for CAD is that a larger body requires more oxygen and forces the heart to constantly work harder. As an effect of increased blood volume and cardiac output, obesity is associated with abnormal cardiac function and hypertension [95,96].

Adipose tissue has in itself an important endocrine function, releasing pro-inflammatory cytokines, and triggers an inflammatory response. It has been suggested that the number of macrophages in adipose tissue is higher in obese persons [97]. The inflammatory activity in adipose tissue is not only a risk factor for CAD, but also associated with increased insulin resistance [98,99], and therefore links obesity with an increased risk for diabetes, providing an indirect pathway between obesity and CAD. In addition, its strong association with high triglycerides and low HDL bring further attribution to obesity as a risk factor for CAD.

According to existing guidelines, both body mass index (BMI) and waist circumference should be taken account when estimating obesity as risk factor for disease [100]. The prevalence of obesity is rapidly increasing worldwide. In Sweden, it is estimated that 45% of middle aged women (45-65 years) and 60% of middle aged men (45-65 years) are overweight. Further, approximately 12%
of the women and 15 % of the men in this age span were classified as obese [101].

**C-reactive protein**

CRP was discovered about eight decades ago in blood from patients with acute fever and illness [102]. When exposing the blood for *Streptococcus Pneumoniae*, a precipitation with a serum component was observed. The serum reaction could be seen in the acute phase of the disease and diminished as the patients recovered. From a biochemical perspective, the bacterial constituent causing the reaction could be identified as C-polysaccharides, hence the name C-reactive. Later on, it was discovered that CRP is not produced specifically in response to *S. Pneumoniae*, but is rather an acute phase protein that can be triggered by a large variety of stimuli in the early phases of an inflammatory reaction.

Like other acute phase proteins, CRP is essentially derived from the liver after induction by pro-inflammatory cytokines, predominantly interleukin-6 [103]. CRP is considered to be a central component of the immune system, by activating and regulating the complement pathway [104]. It binds to microbial surfaces, oxidized lipids, and apoptotic cells, facilitating clearance by phagocytosis [105]. In principle, the circulating level of CRP can be described as a marker indicating that some potentially harmful agent is present that the immune system wants to eliminate. It is clinically used to detect ongoing inflammatory processes. Even slight elevations of CRP in serum or plasma, reflecting a low-grade systemic inflammation are associated with several cardiovascular risk factors [106], but have also been shown to independently predict future coronary events [36,71]. Thus, CRP has been suggested to be a clinically useful biomarker in conjunction with the well-established cardiovascular risk factors, to increase accuracy in risk stratification.

**1.3.2 Behavioral risk factors**

In cardiovascular epidemiology, behavioral risk factors are often reduced to include smoking, alcohol intake, physical activity, and fruit and vegetable consumption. Even though there may be other factors of relevance denoting "behavior", it has been demonstrated in epidemiological studies that these four risk factors, in combination with SBP, dyslipidemia, diabetes and obesity, account for a large part of the risk load regarding CAD [25].
Smoking

Smoking is without controversy a risk factor for CAD. Numerous epidemiological studies show consistent findings of premature CAD mortality among smokers [25,107-109]. Smoking acts in multiple pathways to trigger a coronary event. It leads to an impaired ability to carry oxygen in the blood, thereby forcing the heart to work harder. It exerts an oxidative stress thereby damaging blood vessels, and it is strongly thrombotic causing platelet aggregation [110,111]. Further, smoking is tightly associated with inflammation, as the toxicity in the inhaled smoke triggers an acute inflammatory response [110,111]. Accordingly, long-term smoking has been associated with systemic inflammation in numerous studies [110,111].

Alcohol intake

The association between alcohol intake and CAD, on the other hand, has been more debated, for multiple reasons. First, alcohol intake was not identified as a risk factor in the high impact Framingham study [70]. Second, there is as of yet no consensus regarding mechanisms linking alcohol intake with CAD (although a variety have been suggested) [112]. In addition, several epidemiological studies have reported a phenomenon of J-shaped or U-shaped curves in mortality over dose-curves [113-115], further pointing out the complexity when studying alcohol as a risk factor for CAD.
A non-linear relationship has also been shown between alcohol intake and inflammatory markers, where a frequent (but relatively low) consumption is associated with a lower grade of inflammation in comparison to those reporting a lower alcohol intake, and excessive intake is associated with a higher grade of inflammation [116,117].
In the INTERHEART-study, moderate drinking was considered to be a protective factor for CAD [25]. Thus, the critical issue in the association between alcohol intake and CAD is to establish a cut-off point where the alcohol intake turns from beneficial to deleterious [112,115].

Physical activity

The impact of physical activity on CAD is more evident than the effects of alcohol intake, although some controversy remains. This is likely due to some uncertainties regarding how long time physiological effects remain of regular
physical exercise. The importance to separate the effects of being habitually physically active from having sporadic vigorous activity should be emphasized. Regular exercise has been shown to have a strong protective effect for future cardiovascular events in a number of epidemiological studies using different follow-up periods [118] whereas sporadic vigorous activity has been shown to trigger acute events, particular in otherwise sedentary people [119,120].

In randomized trials, physical activity interventions have been shown to increase levels of HDL [121], and reduce inflammation [122].

**Fruit and vegetable intake**

The predictive value of fruit and vegetables consumption on CAD is also somewhat debated. In theory, a high intake would be beneficial as the antioxidants would have a reducing impact on oxidative stress, limiting the damaging effect of free radical oxygen species in inflammatory processes. Several epidemiological studies have demonstrated an inverse association between intake of dietary antioxidants and cardiovascular risk [123-125]. However, there are some contradicting results from randomized trials studying the effect of vitamin supplements on future disease or mortality [126]. In fact, it has been suggested that excessive supplements of vitamins may increase mortality rather than reducing it [127]. It has been pointed out though, that doses in supplements often are substantially higher than normally found in a balanced diet, which could interfere with the functional use of free radicals as a part of the defense mechanism in the immune system [127]. Thus, an excessive intake of supplements should not be confused with potential effects of fruit and vegetable intake [127]. Evaluation of the findings in the observational studies [123-125] should rather concern the substantial co-variation with dietary pattern as a whole, as well as with other cardiovascular risk factors [123-125,128].

**1.3.3 Population attributable risk**

The issue of how much different risk factors contribute to the total CAD incidence is a matter of discussion. The reason for this is that cardiovascular risk factors can not be isolated and studied one by one in population studies. Several risk factors are not only clustered, as is the case in a sedentary lifestyle and dietary intake, but also, at least to some extent, share the same
pathophysiological pathways. This is discussed in literature on the metabolic syndrome in particular, a state characterized by hypertension, dyslipidemia, high levels of blood glucose, and a large waist circumference [129,130]. Three things determine how much of the total CAD incidence that can be attributed to a single cardiovascular risk factor:

- How common the risk factor is in the population.
- How strongly the risk factor is associated with future events.
- How large a proportion of the total incidence that is attributed to all known risk factors (and how large a proportion remains unexplained).

Given the methodological problems with the inability to disentangle different risk factors from each other, population attributable risks should be interpreted with caution. In absolute terms, the total population attributable risk often exceeds 100% in estimations. In relative comparisons however, population attributable risks may be a useful tool, comparing the impact of different cardiovascular risk factors on total CAD incidence. In Table 1, odds ratios and estimated population attributable risks are given, as reported in the INTERHEART-study [25].

_Table 1. Odds ratio and population attributable risk of myocardial infarction. Risk factors investigated in the INTERHEART-study. Table modified from Yusuf and colleagues, n=29,972 [25]._

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio (99% CI)</th>
<th>Population attributable risk, percent</th>
<th>Population attributable risk, rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyslipidemia</td>
<td>3.2 (2.8;3.8)</td>
<td>49.2%</td>
<td>1</td>
</tr>
<tr>
<td>Smoking</td>
<td>2.8 (2.5;3.2)</td>
<td>35.7%</td>
<td>2</td>
</tr>
<tr>
<td>Psychosocial factors</td>
<td>2.6 (2.2;3.3)</td>
<td>32.5%</td>
<td>3</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.6 (1.4;1.8)</td>
<td>20.1%</td>
<td>4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.9 (1.7;2.1)</td>
<td>17.9%</td>
<td>5</td>
</tr>
<tr>
<td>Fruit and vegetable intake</td>
<td>0.7 (0.6;0.8)</td>
<td>13.7%</td>
<td>6</td>
</tr>
<tr>
<td>Physical activity</td>
<td>0.9 (0.7;1.0)</td>
<td>12.2%</td>
<td>7</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.3 (2.0;2.7)</td>
<td>9.9%</td>
<td>8</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>0.9 (0.8;1.0)</td>
<td>6.7%</td>
<td>9</td>
</tr>
</tbody>
</table>
1.4 Psychosocial factors and coronary artery disease

The considerable number of epidemiologic studies pointing out the prognostic significance of psychosocial factors for acute coronary events [15-25], and the high population attributable risk as shown above [25] imply that psychosocial factors are an important risk factor for CAD. However, the collective use of the term psychosocial factors is diverging. This is probably due to its etymological origin. Combining the word *psyche* (Greek for soul or the self) and *social*, which refers to human society, open up for a variety of connotations over different research disciplines.

In this dissertation, the use of the psychosocial factors follows Marmot’s definition, stating that psychosocial factors are “psychological factors that are influenced by the social environment” [131]. More specifically, psychosocial factors are related to the internal process of stress response associated with potential triggers in a social context. Marmot and Wilkinson have depicted this in a simplified model [131] as seen in Figure 3. The model is based on the concept that there are a number of external determinants that are important for health, most of which are in interplay with psychology.

*Figure 3. Model suggesting a link between external factors and health. Modified from Marmot and Wilkinson [131].*

The ellipses in grey in Figure 3 constitute the concept of psychoneuroendocrinology. The “psycho” part is derived from psychology,
the “neuro” part derived from conscious and unconscious activity in the brain, and the “endocrine” part from the secretion of hormones in normal and pathophysiology. By unifying these parts in one concept, it is acknowledged that hormone secretion is highly influenced by psychological states and that an external load of stimuli is filtered by the brain before a physiological response is induced.

The psychological state may be influenced by heredity [132] and may be altered by different diseases [133]. However, in a normal population, it is thought that this psychological state can be modified, and is primarily attributed to psychosocial factors. The concept of psychosocial factors will be discussed more in detail in section 1.4.2. Before doing this, three basic frameworks in stress theory (the general adaptation syndrome, the concept of allostasis and the cognitive activation theory of stress) are introduced to further understand the role of psychosocial factors.

### 1.4.1 Psychosocial factors and the link to stress

Hans Selye is often claimed to be the father of the stress concept, referring to his pioneering work in the late 30s. Notably, Selye did not use the term “stress” himself in his early work. This is possibly because the medical establishment at the time regarded the term to be too unspecific [134]. Instead, Selye conceptualized stress as the general adaptation syndrome (GAS).

**The general adaptation syndrome**

Based on experimental animal studies, Selye postulated that exposure to stressors, or “non-specific noxious agents eliciting a syndrome with characteristic morphological and chemical alterations” as he put it, is followed by three stages as shown in Figure 4. Similar patterns arise regardless of the nature of the stressor [135]. Phase one, the alarm phase or acute phase, is triggered by exposure to any kind of potentially harmful stimulus. This phase has been described by Cannon as the well-known catchphrase “fight or flight”, when the body quickly mobilizes energy to handle a potential threat [136]. An acute response is characterized by a hormonal shift towards a catabolic state. The stress hormones cortisol, epinephrine, and norepinephrine rise, whereas anabolic hormones promoting repair and growth such as insulin and sex steroids decrease [35]. After this rapid mobilization, the physiological response will decline [137]. The second stage is referred to as stage of
resistance, or stage of adaptation, when the physiological response is high in order to meet the demands of the prolonged stressor at hand. If the duration of exposure is further prolonged, the body will eventually reach the third stage, the stage of exhaustion. The exposure to stressors has now triggered a dysfunctional state and hormonal imbalance, where the physiological response is weak, despite being exposed to a stressor normally triggering a strong response.

*Figure 4. The three phases of General Adaptation Syndrome. Modified from Selye [135,137]*

The GAS model illustrates how prolonged exposure to a certain stressor will eventually lead to a dysfunctional stage of exhaustion. It should however be noted that the model is based on animal studies in strict experimental settings. When transferred to laboratory stress tests or ambulatory sampling in normal populations in everyday life, individuals that are in the exhaustion stage according to GAS should in theory be less responsive when exposed to acute stressors. This is supported by empirical data, comparing groups with clinical or subclinical signs of fatigue and exhaustion with groups with less or no signs of exhaustion, the latter group showing a higher responsiveness [138-140]. Thus, the response to an acute stressor is determined more by individual characteristics and the history of previous and current stressors, than by the
actual tested acute stressor itself. It is well established that different individuals will have different responses to the same stressor, dependent on earlier experiences from similar situations [134]. These observations have led to the incorporation of cognitive function in stress theory, an important contribution, not originally included in Selye’s early work.

**The concept of allostasis**

In 1988, Sterling and Eyer introduced the concept of allostasis in stress theory. The coined term allostasis literately means “to stand in variability”, denoting stability through change. It was introduced as an antonym to the well-spread term homeostasis meaning “to stand equally”, denoting stability through constancy. Allostasis is based on the observation that most physiological variables have a diurnal variation, determined by specific behavioral states and environmental events [35]. Sterling and Eyer argue that the term homeostasis may be misleading as it wrongly implies that different systems are kept constant at a “normal level”. They claim that more important for maintaining health is the ability to respond thereby causing an appropriate arousal when facing an environmental challenge. Therefore, a more adequate terminology, according to Sterling and Eyer, should address the variation rather than the chronic state that homeostasis implies. In their allostatic model, health is defined as a state of responsiveness. [35] It is thus suggested that detrimental effects of stress occur only when the ability to respond is lost. A key concept in the allostasis model is the ability to restitute. An insufficient restitution leads to a sustained arousal which in turn inevitably leads to the inability to respond appropriately. Thus, according to Sterling and Eyer, in order to remain or regain allostasis people should be encouraged to “rest and play in proportion to their work and striving to increase predictability, control, and feedback in their lives” [35].

As pointed out by McEwen (widely known for having introduced the concept of allostasis in medicine), homeostasis applies to a limited number of systems essential for life such as maintenance of body temperature, pH, and oxygen tension [141]. According to McEwen, allostasis is a necessary process to support homeostasis in the mentioned systems [141].
**The cognitive activation theory of stress**

There are a number of existing stress theories, somewhat overlapping, which incorporate cognitive function in frameworks to understand stress and how a stimulus is translated into a physiological response. One of the more widespread is the cognitive activation theory of stress (CATS), formalized by Ursin and Eriksen [134]. In their theory, they divide the term stress into four different entities, as seen in Table 2.

*Table 2. Four formal definitions of stress according to Ursin and Eriksen [134].*

<table>
<thead>
<tr>
<th>Entities of stress</th>
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<tbody>
<tr>
<td>• The exposure (stressor, stimulus)</td>
</tr>
<tr>
<td>• The experience and feelings of the situation (based on self-reports)</td>
</tr>
<tr>
<td>• The psychoneuroendocrinological activation</td>
</tr>
<tr>
<td>• The experience and feelings of the somatic response</td>
</tr>
</tbody>
</table>

These four meanings of stress could and should, according to Ursin and Eriksen, be measured separately to further understand the concept of stress and the role of psychoneuroendocrinology in health and disease [134].

The main component in CATS is the feedback to the brain from the outcome of the response, which will alter both the exposure to the stimulus and the perception of the stimulus in similar situations henceforth. See Figure 5. In other words, whether a stimulus is considered pleasant or threatening depends on previous experiences and expectations of the outcome [134]. The process is dynamic, where the stressor and outcome are evaluated and re-evaluated in similar situations to come.
Consonant with this, the feeling of being stressed can both be linked with positive outcome expectancy and negative outcome expectancy. Thus, according to CATS, there is no point in trying to measure stress by objectively focusing on the external load of exposure. Attempts to measure stress should rather be focused on the subjective experience and feelings elicited by the stressors and the stress response [134].

CATS has implications on GAS as it leads to the conclusion that it is not possible to make a general dose-response association between load of exposure and stage of exhaustion. Lazarus and Folkman have summed up the importance of cognitive function in filtering stress load with the statement that “it is not stress per se [referring to exposure to a given stimulus], but how people cope with it, that affects health and well-being” [142]. This is where psychosocial factors come into place. Whereas Selye did focus on the link between two of the stress denotations in Table 2, exposure and psychoneuroendocrinological activation, psychosocial factors are linked to the other two meanings of stress according to Ursin and Eriksen, namely the experience of the situation and the experience of the somatic response. Metaphorically, people have a “black box” formed by earlier experiences that highly alters the association between stress load and psychoneuroendocrinological response. Thus, if prolonged exposure to a stressor leads to an eventual stage of exhaustion or not is more influenced by the black box than by the stress load itself. When opening the black box, a
complex web of psychosocial factors folds out. These are described more in detail in section 1.4.2.

1.4.2 Psychosocial factors – positive and negative aspects

The principal concept of psychosocial factors is depicted in Figure 6.

Figure 6. Psychosocial factors, altering the association between load of stimuli and psychoneuroendocrinological response.

The impact of an acute stressor is dependent on the subjective experience on the total load of stressors i.e. a combination of other stressors at hand (regarding both duration and intensity), and previous experiences regarding the acute stressor.

The psychoneuroendocrinological response of the acute stressor exposure is modified by the psychosocial factors, determining whether the response will be either adequate or dysfunctional in everyday life. See section 1.7 for further introduction on physiological consequences.
Introduction

The person’s individual characteristics regarding psychosocial factors are formed in interplay with the social environment in dynamic processes. In addition, as pointed out in CATS, the feed-back from the outcome leading to positive or negative outcome expectancy has a further impact on psychosocial factors.

An important distinction is whether the psychosocial factors swaying the stress response are influenced mainly by a state, i.e. a temporary condition (that may be triggered by a recent positive or negative event in everyday life), or a trait, i.e. a more long-term personality feature (reflected by long-term learning processes and life conditions). In general, all psychosocial factors can be influenced by both state and trait components.

When assessing psychosocial factors, most instruments (typically structured questionnaires or interviews) are designed to minimize the impact of a temporary state in favor of capturing more stable trait properties. Questionnaires compiled to assess psychosocial factors can be divided into those focusing on external factors such as exposure in work environment, those focusing on external social resources, and those focusing on individual psychosocial characteristics. As argued for in CATS [134], focusing on exposure does not add much in psychoneuroendocrinology. Thus, the following introduction includes social support, individual psychosocial resources, and individual psychosocial risk factors.

Social support

The role of social ties in health and disease has long been discussed. Explanatory models span from the macro level such as theories of social capital, to the micro level such as group psychology to facilitate changed or maintained habits. Several observational studies have linked various indicators of social isolation with increased mortality [143,144]. The associations with all-cause mortality are accompanied by an increased CAD incidence in groups with low social support [145,146]. These findings remain after adjustment for other risk factors, suggesting that differences in health behavior in groups with different social activity can not explain the increased risk of CAD in socially isolated groups [145,146]. It is generally agreed that external resources in terms of functional social ties are beneficial when dealing with acute or chronic stressors. Hawkley and Cappicio have suggested that it is not the stress response per se that differs, but rather the perception of the load, where lonely individuals appear to be more likely to perceive events in daily life as stressful events [147].
Commonly a separation is made between the quantity of social ties and the quality of social ties. In other words, the size of your social network should not be confused with the emotional support provided by someone who truly cares for you. This separation is based on the observation that a large social network with many social contacts does not necessarily provide the availability of someone close to confide in. Both the quantity, i.e. the social network, and the quality, i.e. the emotional support, have been shown to be associated with reduced risk of CAD [145].

**Psychosocial resources**

Psychosocial resources are defined as factors that have a positive impact on how a person copes with prolonged exposure to stressors. In other words, psychosocial resources can be said to be resilience factors, providing a buffering function when exposed to stressors, and modifying the person’s perception of the situation. Key concepts such as coping, self-esteem, and perceived control have been established in a number of theories when describing psychosocial resources [148]. These concepts, with overlapping constructs, are highly a function from cognition, characterized by a sense of meaningfulness, predictability, and free will. Psychosocial resources are generally considered to be personality traits, formed by long-term learning. The driving force for both building up and maintaining psychosocial resources is the ability to handle a stressor. By mastering a challenge, the perceived control of similar situations to come is increased, knowing that everything went well the last time the stressor occurred. The success of resolving a potentially threatening situation builds up the self-esteem and affects the free will to face other upcoming equal or even more demanding challenges. Taken together, coping with stressors result in positive outcome expectancy. In this way, success breeds success. Psychosocial resources are preserved, as mastering a challenge once increases the ability to master it again (with less effort required).

On the other hand, if the positive expectation is lost, reduced psychosocial resources can also be negatively preserved in vicious circles. Failure to master a situation leads to an eventual reduction of self-esteem. The persistence of different situations that not are resolved leads to an up-load of stressors, bringing more failures to come. This will lead to an eventual state of hopelessness, characterized by negative outcome expectancy, knowing that it is no point of trying as it probably just will lead to another failure. Thus, both meaningfulness, and free will are affected by loss of psychosocial resources.
Introduction

Even though there are a number of epidemiological studies linking different aspects of psychosocial resources with health in general, prospective studies that study the association between psychosocial resources and reduced risk for ACS are few (not counting those studying social support as a psychosocial resource). However, two independent major epidemiological studies have demonstrated such an association. In the EPIC-Norfolk study (n=20,425) coping as assessed by Pearlin’s mastery scale (described more in detail in section 3.3.2) were independently associated with a reduced cardiovascular mortality [24]. In the INTERHEART-study, there was a statistically significant inverted association between risk for ACS and perceived control, an instrument assessing one’s perception of control over circumstances in everyday life [22,149].

Psychosocial risk factors

In contrast to the more closely inter-related psychosocial resources, psychosocial risk factors are in principle derived from two more distinct domains. The domain of vital exhaustion and depression is tightly linked with the negative emotions accompanying long-term exposure to stressors that can not be resolved. In other words, this domain represent a state where total stress load has for some time been exceeding the individual’s ability to master them, and is characterized by a sense of helplessness, and loss of meaningfulness.

The other domain of anger, hostility and cynical distrust are linked to negative emotions that may evolve in a social interplay. In cardiology, this was first acknowledged by Friedman and Rosenman in the late 50s, coining the widely spread expression “Type A behavior pattern”. They observed that there was unproportionally high number of CAD-patients having a personality trait characterized as competitive, overt aggressive, and with difficulties to relax. [150]. Friedman and Rosenman spent the following decades to find out the pathophysiological mechanisms explaining this high over-representation in CAD-patients. The impatience when things take longer time than it should, and hostility that may be triggered also by minor events, have implications in stress research. An individual showing a Type A behavioral pattern is more likely to regard events in everyday life as stressors, and more likely to respond with a sustained arousal.

Both the domain of depression [15-19] as well as the domain of hostility and cynical distrust [16,20,21,23] have been shown in extensive literature to have predictive value for future CAD events.
In the INTERHEART-study, a questionnaire was used aiming to measure clinical depression according to the fourth edition of Diagnostic and statistical manual on mental disorders (DSM-IV) [22]. An increased score on the questionnaire was positively associated with a higher risk for myocardial infarction. The domain of hostility was not assessed.

1.5 Cortisol

As mentioned in section 1.4, cortisol is one of the main stress hormones, essentially preparing peripheral organs for action. The release of cortisol is mediated by the so called hypothalamus-pituitary-adrenal (HPA)-axis. In short, the principal stimulus of the HPA-axis is the corticotrophin-releasing hormone (CRH), first isolated and identified by Vale and colleagues in 1981 [151]. CRH is locally produced in hypothalamus. When facing an acute stressor, CRH increases markedly, stimulating a secretion of adrenocorticotrophic hormone (ACTH) in the pituitary gland. As the name suggest, circulating ACTH stimulates the cortex of the adrenal glands, stimulating the cortisol production and release into circulation.

In normal physiology, most individuals exhibit a typical pattern with a distinct diurnal variation in cortisol levels, peaking approximately 30 to 45 minutes after awakening and declining throughout the day, with lowest levels in the night, around 4.00 AM [152,153]. However, the intra-individual diurnal variation is considerable. The correlation between cortisol levels over consecutive days has been reported to be around r=0.5 in several studies [139,154,155]. This intra-individual variation are thought to, at least in part, be explained by fluctuations throughout the day when exposed for physical or emotional stressors, i.e. are influenced by temporary states which may vary from day to day [139,155,156].

Given the rise of CRH by acute stressors, a dysregulation of the HPA-axis with cortisol as the end-point, have been suggested to be closely linked with psychosocial factors [157-159]. More specifically, individuals with a poor score on psychosocial instruments tend to have a higher baseline of cortisol levels and/or poorer response to laboratory stress test [138], a higher diurnal total output of cortisol, and/or a more flattened diurnal variation of cortisol throughout the day, with lower peaks and higher levels in the evenings [139,159]. Cortisol acts on intracellular nuclear receptors found in most cell types throughout the body and regulating the transcription of target genes [160].
Thus, in contrast to the other stress hormones epinephrine and norepinephrine, which exert immediate actions, the effects of cortisol are generally slower but more persistent [161]. The discovery in the late 40s that a synthesized cortisol derivate could reverse inflammation in rheumatoid arthritis (RA) [162] have rendered a Nobel Prize. The anti-inflammatory properties have led to the wide-spread clinical use of exogenous cortisol (or hydrocortisone as the synthetic form is named). However, while pharmacological doses (much higher than physiological doses) indeed are anti-inflammatory, the role of endogenous cortisol is far more complex. It has been suggested that cortisol modulate the expression in approximately 10% of our genes [161]. This enormous palette has puzzled scientists for many years. From an evolutionary perspective, it has been suggested that the effects that cortisol exert have evolved to minimize effects of water losing intestinal diseases. In other words, all effects of cortisol converge to prevent a certain death due to cholera or other infections causing severe diarrhea [163]. The multi-faceted role of cortisol has been demonstrated by numerous studies, associating a dysfunction in the HPA-axis with a number of pathophysiological conditions. These include studies on e.g. autoimmune diseases [164], cancer [165], and psychiatric disorders [159]. Focusing on CAD, imbalance in energy regulation as a consequence as a of a dysregulated HPA-axis has been suggested to lead to obesity, diabetes, and hypertension [35,141,161,166]. Further a dysregulation of the HPA-axis, and a high total cortisol output have been associated with arterial stiffness [167-169]. Moreover, it has been suggested that the ability of cortisol to dampen effects of inflammatory processes and keep them from spreading [170-172] could be lost if the HPA-axis is dysregulated. Insufficiency to suppress ongoing inflammation by expressing “inappropriately low cortisol levels” has accordingly been suggested as an additional mechanism linking a dysregulation of cortisol with CAD [173,174]. Nijm and colleagues have given some empirical support to the suggestions, demonstrating that CAD patients had a significantly higher total diurnal output of cortisol as well as a lower possibility to respond with a rise in cortisol levels to a stressor in a laboratory stress test [174]. There is however a lack of prospective population studies, why the long-term effects of a dysregulated HPA-axis on CAD incidence as of yet are unclear. Taken together, although the entire chain has not been demonstrated in the same study, altered cortisol levels due to stress has been suggested as a strong
candidate mechanism when explaining the observed link between psychosocial factors and CAD.

1.6 Matrix metalloproteinase-9

The MMP enzyme family was originally described in 1962 by Gross and Lapiere [175]. In their work, they studied the metamorphosis in frogs with the underlying assumption that such a rapid and yet precise remodeling requires a tightly regulated enzyme system. In their experiments, they found a collagenolytic factor, simply referred to as “the tadpole collagenase” [176]. Further research revealed that this was just one of several enzymes with a common protein domain structure and with similar specificity. In humans, there are 23 different enzymes in the family [177]. In the current nomenclature [178], the name of the enzyme family is derived from the following:

- **Matrix**, referring to the network of supporting tissues that is not part of any cell. It is mainly constituted by fibrils of different types of collagen.
- **Metallo**, referring to the metal ion (typically the zinc ion Zn²⁺) on which the enzyme is dependent to form a functional active site of catalyzation.
- **Proteinase**, referring to the enzymatic function which catalyses the breakdown of proteins.

All of the MMPs have the ability to breakdown different collagens and thereby have the potential to degrade most extracellular and connective tissues [177,179,180]. Since MMPs are the only known enzyme family capable of degrading collagens in mammals, they are of importance in virtually any process that involves remodeling, ranging from angiogenesis and cell proliferation to wound healing and degradation of dysfunctional tissue [177,179,180].

To prevent misdirected degradation of vital tissue, the activity of MMPs requires tight regulation at different levels. As a first regulating step, all MMPs are synthesized as inactive forms and released as pro-enzymes. To function as degrading enzymes, all MMPs require activation from other MMPs or other regulatory agents [180]. As a next step in the control of MMP activity, humans have four different endogenous MMP inhibitors, the so called tissue inhibitor of metalloproteinases (TIMPs) 1, 2, 3, and 4. It has been postulated that the ratio of MMP/TIMP may provide information on whether the overall activity leans towards degradation or vegetative growth, respectively. In theory a higher ratio would speak for a high extent of degradation [181-183]). However, TIMPs not only inhibit MMP activity, but also act as transporters and carriers...
of the enzyme. Therefore, levels of MMPs are generally highly correlated with levels of TIMPs in vivo [184,185].

Being active in many processes, MMPs can be produced, stored, and released by a number of cell types. Leukocytes are often mentioned as the cell type most important for MMP regulation [186-189], but smooth muscle cells, endothelium, and platelets also produce, and/or store various MMP’s [189-191].

One important member in the enzyme family is MMP-9. Before the introduction of the current nomenclature [178], MMP-9 was known as 92 kDa gelatinase or gelatinase B, since the enzyme is able to break down gelatin, a hydrolyzed form of collagen. It has later been shown that MMP-9 also degrades a number of related proteins in connective tissue, briefly overviewed in Table 3. For an extended list of MMP-9 substrates, see Björklund and Koivunen [192].

Table 3. Substrates for MMP-9.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen type IV</td>
<td>Binds with other collagen type IV molecules to form sheets. Collagen type IV mainly constitutes the basal lamina in blood vessel walls, anchoring the endothelial cells surrounding the lumen.</td>
</tr>
<tr>
<td>Collagen type V</td>
<td>A collagen type that forms fibrils with collagen type I, the most abundant collagen type, to build fibrous structural proteins in connective tissue and extracellular matrix.</td>
</tr>
<tr>
<td>Gelatins</td>
<td>Irreversibly hydrolyzed collagens (partly denatured) abundant in connective tissue.</td>
</tr>
<tr>
<td>Laminin</td>
<td>A non-collagenous protein which forms sheets in the basal lamina and extracellular matrix. Associated with collagen type IV via the protein enactin (also a substrate for MMP-9).</td>
</tr>
</tbody>
</table>

The synthesis and secretion of MMP-9 is induced during the inflammatory response, primarily by the pro-inflammatory cytokine interleukin-1 (IL-1) [193], or other pro-inflammatory pathways [186,194]. Like many other MMPs, MMP-9 is produced and stored in a broad variety of cells but is primarily associated with leukocytes [186,187,194,195], especially macrophages [188,189,194,196]. MMP-9 activity is suppressed by exogenous cortisol and other glucocorticoid derivates [197,198]. The suppression may be indirect, mediated by the inhibition of IL-1 expression [198], or direct via the intracellular transcription factor activator protein-1 [197]. It has moreover been
suggested that glucocorticoids up-regulate the MMP-9 inhibitor TIMP-1, thereby further reducing the effect of MMP-9 activity [199].

1.6.1 Plausibility of MMP-9 in coronary artery disease

Patients with acute cardiac events have been shown to have elevated plasma levels of MMP-9 in a number of studies [177,189,200-202]. Most studies so far have compared levels of MMP-9 in ACS patients with those in stable angina patients. It has also been shown that circulating MMP-9 is an independent predictor of new cardiac events in patients with stable CAD [200], but little is known about MMP-9 before onset of disease. There are a few cross-sectional studies in selected populations linking increased levels of MMP-9 with hypertension [181,203], diabetes [204], smoking [205], and excessive alcohol intake [206] but due to a lack of prospective data before onset of disease, the predictive value of MMP-9 is merely speculative at this point.

Of the ones mentioned, the link to hypertension is the most outlined. It has been suggested that MMP-9 is up-regulated in stiffened central arteries [203,207], in order to remodel the stiff, collagen-rich vessel walls into more flexible structures with an increased buffering capacity. Further, when a vessel wall is remodeled, there is a need for neovascularization within the vessel wall as it grows to supply oxygen to all the cells within the wall. Hence, MMPs are activated to facilitate new microvascular growth [208].

Most literature regarding MMP-9 and CAD is focused on the destabilization of atherosclerotic plaques. The basic concept is that MMP-9 facilitates the plaque rupture or erosion by attacking the collagen-rich fibrous cap and basal lamina which surrounds it [55,189,209,210]. It has been shown that unstable plaques rich in macrophage-derived foam cells, have a higher MMP-9 activity than stable plaques with a lower grade of inflammation [211-213]. To sum up, there are an extensive number of both observational and experimental studies that associates increased levels of MMP-9 with CAD. Further, the impact of stress hormones on MMP-9 regulation make MMP-9 a prime candidate marker when studying the relation between stress and ACS.
1.6.2 MMP-9 in other conditions and diseases

Even though the number of studies on MMP-9 in cardiovascular disease is growing rapidly, these studies are by far outnumbered by those linking MMP-9 to cancer. MMPs are known to play several important roles in cancer development. In tumor-induced angiogenesis, there is an up-regulation of MMP-9 and other MMPs in the extracellular matrix to facilitate neovascularization and thereby provide oxygen to the growing tumor [214-216]. MMPs also promote tumor growth and invasion, partly by stimulating the release of growth factors such as fibroblast growth factor and vascular endothelial growth factor, partly by degrading the stroma surrounding the tumor [192,217]. Thus, various cancer treatment strategies have focused on how to inhibit the activity of MMPs and thereby reduce the growth of malignancies.

Another common group of diseases where MMP-9 has been shown to be of relevance is autoimmune diseases such as RA [218], where it is thought that MMP-9 activity increases the degradation of cartilage and joint tissues [219].

Further, the ability to degrade different collagens brings implications to different surgical and orthopedic settings. Activation and inhibition of MMPs have been linked to a number of different applications, e.g. tendon ruptures, tissue ulcers, and wound healing [220,221].

It has also been suggested that MMP-9 could be a marker in diabetes, as MMP-9 has been shown to be up-regulated in hyperglycemia in vitro [222] and up-regulated by insulin in an animal experiment in vivo [223]. There is however a lack of consistency in human observational studies where some studies show significant differences between diabetic subjects and controls [204,224], while other report no difference between the groups [225,226].

1.7 A psychoneuroendocrinological model

A central assumption when studying the etiology of CAD is that the cardiovascular risk factors do not operate in isolation, but acts in conjunction with one another. Indeed the physiological response that follows takes the same pathway regardless if the stressor is of physical or emotional nature. In this sense, there is no existing binary opposition of a “physio” and a “psycho” pathway, they should be viewed upon in an integrative system.
The psychoneuroendocrinological model in Figure 7 constitutes the framework for this dissertation. The model is combining the concept of allostasis as described by Sterling and Eyer [35] the concept of CATS [134]. Using the model, it is hypothesized that stress and psychosocial factors is linked with ACS via loss of allostasis accompanied by a dysregulation of remodeling in vessel walls.

Figure 7. A psychoneuroendocrinological model linking psychosocial factors with ACS.

The acute stress is filtered by the psychosocial factors modifying the perception of the situation. The stress response, regardless of trigger, is driven by a hormonal shift, in particular by an increase the stress hormones cortisol, epinephrine and norepinephrine. The ability to respond is modified by intrinsic factors such as the genome, proteome and body posture as well as lifestyle factors such as physical activity, diet, smoking, and alcohol intake. An increase of the stress hormones leads to a suppressed inflammatory response, increased oxygen supply, and rise in blood glucose and fatty acids to provide energy in the acute phase [35]. A dynamic response refers to an achievement of allostasis, characterized by a functional rise in stress hormones which in
Introduction

turn leads to a mastered challenge and a period of restitution. This is beneficial for the individual as a psychophysiological learning process will lead to positive expectancy, control and predictability of the stress load, i.e. will strengthen the psychosocial resources. In addition, a possibility to rest after a mastered challenge will lead to restitution and beneficial training for the physiological systems. As part of an adaptation to the environment, a well-regulated remodeling occurs, preparing the system for similar situations to come.

If the initial response of the stress hormones is not sufficient to meet the demand of the situation, a higher stress response will be induced in order to master the situation. The sustained arousal following a situation like that may be a double-edged sword. On one hand, a sustained elevation of stress hormones provides the ability to respond functionally to a prolonged stressor. On the other hand, a sustained arousal can only be functional for a given time. If not followed by restitution, a state of sustained arousal will eventually lead to a state of non-responsiveness, and to a stage of fatigue and exhaustion. One explanation for this transition is the receptors at a cellular level, which will eventually be down-regulated in sustained arousal to slow down the effects of the stress hormones [227,228]. This leads to an increased hormone secretion in an attempt to reach an adequate physiologic response, which in turn, leads to a further down-regulation of receptors. By these vicious circles, the ability to achieve allostatics is lost in a maladaptive system due to sustained arousal. If this state is prolonged, it has been suggested that excessive cortisol secretion leads to manifestations of the cardiovascular risk factors hypertension, diabetes and obesity [35].

When allostatics is lost, the link between cortisol and inflammation and remodeling may be of particular relevance, as the suggested links to hypertension, diabetes and obesity can not explain the rapid fluctuations in CAD incidence and a high incidence of unexpected cardiac deaths as described in the rationale. Importantly, the association between a dysregulated HPA-axis and inflammation are not limited to markers in circulating blood. It has also been demonstrated that cortisol has direct effects on macrophages, which becomes maladaptive when the HPA-axis is dysregulated [228-231]. This interplay between sustained arousal and inflammation provides a plausible mechanism where stress may interact with other pathogens and thereby modify inflammation in atherosclerosis. In other words, regardless of what triggers inflammation in the first place, it is plausible that sustained arousal may cause a situation where invaded macrophages do not respond properly to the endogenous stress hormones, thus making it harder to dampen
the inflammation. In concordance, the ability of cortisol to suppress MMP-9 activity [197-199], might diminish in situations where the ability to achieve allostasis is lost. This might lead to a dysregulation of remodeling, losing the vital impact of cortisol mediating the MMP-9 activity. Thus, according to the model, MMP-9 might be of plausible relevance studying mechanisms linking stress and psychosocial factors with ACS.

1.8 Outline of the dissertation

This dissertation focuses on studying circulating levels of MMP-9 in a normal population. A principal model of the associations investigated is depicted in Figure 8. While different associations with MMP-9 are examined in Paper I, II and IV, a potential method enhancement in cortisol assessment is presented in Paper III.

*Figure 8. Outline for the dissertation. Number in grey ellipse refers to respective section in the introduction.*
2. AIMS

2.1 General aim

The general aim of this dissertation was to study circulating levels of MMP-9 in relation to cardiovascular risk factors and CRP as an inflammatory marker, to psychosocial factors, and to salivary cortisol in a normal population.

2.2 Specific aims

The specific aims were to examine the following:

**Paper I, MMP-9 and cardiovascular risk factors:**
- How circulating levels of MMP-9 are distributed in a normal population without known CAD.
- If circulating levels of MMP-9 are associated with traditional cardiovascular risk factors.
- If circulating levels of MMP-9 are associated with total cardiovascular risk load or if a possible association to total risk load is driven by a single risk factor alone.

**Paper II, MMP-9 and psychosocial factors:**
- If circulating levels of MMP-9 are associated with psychosocial risk factors and resources, independent of cardiovascular risk factors, ongoing medication, or known diseases.
- If associations between MMP-9 and psychosocial risk factors, for example depression and hostility, are mirrored as overlapping constructs or independent from each other.

**Paper III, Reliability of pooling cortisol:**
- If pooling cortisol samples from distinct time points over consecutive days is as reliable as using arithmetic means.

**Paper IV, MMP-9 and cortisol:**
- If there is an association between circulating levels of MMP-9 and ambulatory saliva samples of cortisol in a normal population.
The papers and results are related as shown in Figure 9, illustrating the internal aim for each paper in the dissertation.

Figure 9. Relation of the papers in the dissertation.
3. MATERIAL AND METHODS

3.1 The LSH-study

The LSH-study is an abbreviation for the Life conditions, Stress, and Health-study. It was designed to study a normal population, both in cross-sectional analyses and later on in prospective analyses, with the possibility to incorporate register data on mortality and morbidity at an individual level. The work in this dissertation is based on a subset from the LSH-study (see section 3.5). Thus, the aims and findings in this dissertation constitute some but not all of the pieces in the puzzle.

3.1.1 Aims and model of the LSH-study

The main aim of the LSH-study is to test if psychoneuroendocrinological pathways mediate the association between socioeconomic status and incident cardiovascular disease. It is hypothesized that loss of allostasis lead to an increased general vulnerability in susceptible subjects. The study design is based on the assumption that life conditions as well as lifestyle and psychosocial factors have an impact on psychoneuroendocrinological mechanisms and inflammation. There are multiple analyses currently underway in the LSH-study. The principal aims can be divided into six levels, illustrated in Figure 10.
Material and methods

Figure 10. Principal model and aims of the LSH-study. Boxes and ellipses in grey mark the scope in this dissertation.

Aims

1. To study the associations between socioeconomic position, life conditions, behavioral risk factors, and psychosocial factors respectively.
2. To study the association between traditional CAD risk factors and plausible biomarkers related to stress, inflammation, and vulnerability.
3. To study the association between psychosocial factors and plausible biomarkers.
4. To study the interaction between behavioral risk factors and psychosocial factors regarding mentioned plausible biomarkers.
5. To study life conditions, behavioral risk factors, and psychosocial factors as predictors for outcome in different diagnoses.
6. To study biomarkers as predictors for outcome in different diagnoses.

This dissertation is mainly comprised of the work from levels 2 and 3. See Figure 8 in section 1.8 for comparison.
3.1.2 Study design

The study was initiated in 2002. The first data collection took place between October 2003 and June 2004 and was conducted in the county of Östergötland in the southeast of Sweden. The study base was defined as the population living in the catchment area for any of the ten collaborating Primary health care centers (PHCCs). The participating PHCCs covered a wide range of socioeconomic positions and represented both rural and more urban areas of Östergötland, as shown in Figure 11.

*Figure 11. The catchment areas in the LSH-study.*

**Participating Primary Health Care Centers:**

- Kolmården PHCC
- Hageby PHCC (Norrköping)
- Kungsgatan PHCC (Norrköping)
- Skärblicka PHCC
- Åtvidaberg PHCC
- Berga PHCC (Linköping)
- Lambohov PHCC (Linköping)
- Lyckornas PHCC (Motala)
- Vadstena PHCC
- Ödeshög PHCC

The study design was set to include participants (n=1,000) evenly distributed in sex and age, ranging from 45 to 69 years at enrollment. An exclusion criterion was self-reported severe disease that hindered the possibility to participate, e.g. terminal cancer, severe dementia, or severe psychiatric disorders. Severe difficulties understanding Swedish was set as the other exclusion criterion.

3.1.3 Study population

There were 1,007 participants enrolled in the LSH-study (502 men and 505 women).
The response rate for the initial baseline study was 62.5%. The sample was representative for the population in terms of educational attainment, employment rates, and immigrant status.

### 3.1.4 Random sample procedure

The LSH-study was designed to examine a stratified random sample of the study population. The procedure was based on the methodology used in the HAPIEE-study [232] and was performed in the following steps:

1) The catchment areas for each of the ten PHCC were defined, using geographical maps matched with corresponding postal codes.

2) The population that was living in any of the ten catchment areas, that is had a registered address within any of the listed postal codes on December 31, 2002 was identified using the official register of the total population.

3) All inhabitants born between January 1, 1933 and December 31, 1957 were selected.

4) All inhabitants were stratified by PHCC, sex, and 5-year categories (born 1933 – 1937, 1938 - 1942, 1943 - 1947, 1948 - 1952, and 1953 - 1957 respectively), using the postal codes and the Swedish personal identity number.

5) Every person in each stratum was assigned an arbitrary decimal, $a_{stratum}$, in the interval between 0.000 000 and 1.000 000 by a random number generator. The participants in each stratum were sorted in ascending order according to $a_{stratum}$.

6) The design was set to include 10 persons from each stratum. By dividing by 20, the random sample procedure provided for a participation rate of 50%. The total number of persons in each stratum, $n_{stratum}$, was thus divided by 20. The ratio was rounded to the nearest integer, $x_{stratum}$, interpreted as “one out of $x_{stratum}$ persons in every stratum will be invited to the LSH-study”.

7) An integer, $i_{stratum}$, within the interval of 1 to $x_{stratum}$, was computed for each stratum using a random number generator.

8) Using the lists sorted by $a_{stratum}$, persons who were invited to the LSH-study were selected by $i_{stratum} + x_{stratum}$, where the additions of $x_{stratum}$ were iterated 19 times. Thus, the first person in each stratum invited to the
LSH-study had the post corresponding to $i_{stratum}$ and the twentieth person the post corresponding to $i_{stratum} + 19(x_{stratum})$.

9) Participants were invited consecutively, starting from the top of the list until there were ten participants that had accepted the invitation in each stratum.

10) There were a few strata in which the participation rate was lower than 50%. In those cases, the steps 7 to 9 above were repeated, using an altered $i_{stratum}$, to enable an inclusion of 10 participants in each stratum.

### 3.1.5 Data collection

The data collected were acquired from measurements of physiological characteristics (see section 3.2), an extensive set of questionnaires (see section 3.3) and biological samples in saliva, blood, and urine (see section 3.4). After the random sample procedure, invitations were sent out, together with the first set of questionnaires. If the respondent was willing to be a participant in the study, salivettes and a vial for a urine sample were sent out together with an appointed time for a PHCC visit. If there was no answer, a reminder was sent out after about two weeks.

### 3.1.6 Ethical considerations

Every participant in the study has given written consent stating that the data from the questionnaire, the measurements on physiological characteristics, and the biological samples can be used for research. The participants were informed that they, at anytime upon request and without any further explanation, can drop out of the study and ask to have their collected biological samples destroyed immediately.

All data were treated with confidentiality. The code-numbers used for the participants were not identifiable to a higher extent than age, sex, and catchment area.

The study design was approved by the local ethics committee in Linköping (No. 02-324).
3.2 Primary health care center visit

The participants came to the PHCCs in the morning. The participants’ first set of questionnaires (see section 3.3) and the saliva samples (see section 3.4.1) were submitted to the assistant nurses. Participants with clinical symptoms of infection were instructed to come back to the PHCC after recovery. All blood samples were taken between 6.30 and 9.00 AM in a fasting state. After the measurements and blood sampling, the participants were offered a complimentary breakfast. Based on the measurements, and the data from the questionnaires regarding smoking and physical activity, all participants were given direct feedback on their results. The nurses assisting in the study had specific guidelines regarding the discovery of abnormal measures on blood pressure, blood lipids and/or blood glucose. If abnormally high, the participants were scheduled to see a nurse or a physician for further check-up. A modified version of the health profile developed in Habo PHC, Sweden [233] was used as an educational tool in a health dialogue between the nurse and the participant. When applicable, a brief intervention was conducted, in terms of a motivating interview focusing on diet, physical exercise, and smoking cessation. If the participant had multiple risk factors, well defined criteria according to existing guidelines (based on the SCORE-system) [234] and the risk estimation algorithm developed in the software Precard (also based on the SCORE-system) [235] were used as a golden standard for intervention recommendations. Feed-back on blood lipids were sent to the participants when the results had returned from the laboratory.

All nurses collecting data were trained together and the laboratory equipment used was calibrated to ensure standardization.

3.2.1 Physiological characteristics

Blood pressure (SBP and DBP) were measured in a sitting position in two minutes interval after five minutes of rest. The mean of second and the third measurement (Omron M5-1 digital) were used as estimates for SBP and DBP. Weight, height, and waist-hip measurements in standing position were collected. Plasma glucose (HemoCue), triglycerides, and lipids (ADVIA 1650) were analyzed directly after sample collection, and LDL-cholesterol was calculated using Friedewald’s formula [236]. This formula is based on the
assessment of total cholesterol, HDL, and triglycerides, using the approximation shown in Figure 12.

Figure 12. Friedewald’s formula used to estimate LDL concentrations (mmol/l) in blood.

\[
[\text{LDL}] = [\text{Total cholesterol}] - [\text{HDL}] - (0.456 \times [\text{Triglycerides}])
\]

3.3 Questionnaires

All participants were asked to fill in an extensive set of questionnaires. The first set of questionnaires was sent by mail to the participant. The other set was distributed at the PHCC. The filled-in questionnaires were collected either at the PHCCs or sent back by mail. The following sections (3.3.1 to 3.3.3) present the items that have been used in this dissertation. Data in the LSH-study questionnaires not analyzed in this dissertation are omitted.

3.3.1 Behavioral risk factors for cardiovascular disease

**Smoking**

Smoking was addressed using the single item “Do you currently smoke?” with the four alternatives [No, never have] [No, I quit in the year….] [Yes, sometimes, less than a cigarette per day] and [Yes, on a regular basis], respectively. A dichotomy was generated, congregating the first two and the two latter alternatives.

**Alcohol**

Alcohol intake was based on a food frequency questionnaire adopted from the Swedish Mammography Cohort study [237] combining frequency and typical amounts of different beverages. By combining the frequency with the
beverage, a sum score of alcohol in grams per week was calculated. Based on the sum score, three ordinal categories were computed (less than 110 g/week, between 110-170 g/week, and more than 170 g/week respectively). In addition, there were two categories for never-users and participants that had quit drinking alcohol.

**Physical activity**

The measurements of physical activity were adopted from the population surveys previously conducted by the Public Health Science Centre in the county of Östergötland [238]. The two questions given in Table 4 were used to assess daily activity.

*Table 4. Items on physical activity.*

**Considering the last 12 months:**

1. How much physical exercise do you normally have in your “every day life” (like walking or biking to work, snow shuffling, work in the garden or similar)? [None] [A few occasions per week] [Several occasions per week] or [Daily or close to daily]

2. How much physical exercise do you have voluntarily, apart from the physical exercise above? [Hardly anything] [Hardly anything, but walk a few times a week] [Light activity at least once a week] [Moderate activity at least once a week] [Vigorous activity on a regular basis]

The items were combined into an index, as suggested from the authors, with the following four categories: hardly any physical activity, some physical activity, could be more physically active and enough physical activity respectively [238].

**Fruit and vegetable intake**

The measurement of fruit and vegetable intake utilized the previously mentioned food frequency questionnaire developed by the Swedish Mammography Cohort study [237]. The questionnaire is constituted by detailed items, estimating a sum score of daily intake of fruit and vegetables. The following fruits and vegetables are included: orange/ citrus fruits/ orange juice/ grapefruit juice/ apple/ pear/ banana/ berries (fresh or frozen)/ jam/ marmalade/ purée/ fruit compote/ fruit soup/ lettuce/ cabbage/ cauliflower/
broccoli/ sprouts/ tomato/ tomato juice/ bell pepper/ spinach/ green peas/ onion/ leek/ garlic/ mixed vegetables/ pea soup/ beans/ lentils/ soya products/ cooked potatoes/ fried potatoes/ French fries/ carrot/ beetroot.

A sum score in grams per day was computed, using the algorithm designed for the instrument [237]. Data were used as ordinal categories, as attempts to quantify total intake more exactly would be based on assumptions that could not be validated in the LSH study.

The participants were divided into three subgroups: Low intake (less than mean minus one SD), moderate (mean plus/minus one SD), and high intake (more than mean plus one SD), with the underlying assumption that such a stratification would discriminate individuals having a high daily consumption of fruit and vegetables from individuals having a low daily consumption.

### 3.3.2 Psychosocial instruments

The psychosocial instruments were chosen with the criteria that they should be validated and previously tested in a substantial number of studies. Furthermore, outcome on the instruments should have been associated with cardiovascular disease [15,16,23,24,146,239,240], and/or have been associated with inflammatory markers in previous studies [241,242]. Nine different instruments were used. None of them focus on the external load of stressors, but rather the experience of the stressful situation and the stress response. All but two (availability of attachment and availability of social integration) are constructed as Likert scales, that is based on a number of statements or questions on which the respondent is asked to agree or disagree in a fixed number of answer alternatives. Typically, there are both positive and negative statements and/or questions in the same instrument. The responses to the items are summed into a non-weighted index where each of the items has the same proportional contribution to the summed score.

In our analyses, there was an allowance for a partial loss with missing values on one item for instruments with less than 15 items, and missing values on two items for instruments with more than 15 items. Higher partial losses were regarded as missing data. An imputation process was performed for participants with an acceptable partial loss in the instruments tested, where an arithmetic mean of the items with data was computed on an individual basis and used as an approximate response in the items with missing values.
Material and methods

Availability of attachment

Orth-Gomér’s instrument known as availability of attachment (also called emotional attachment or emotional support) has its origin in the comprehensive Interview schedule for social interaction (ISSI) [243]. Aiming at feasibility in self-administered questionnaires to use in cohort studies (with CAD as the main outcome), ISSI was reduced to two subsets of items, one of which is availability of attachment [244]. The instrument is thought to measure what a person’s social network provide in terms of emotional support, e.g. if the respondent has someone close to confide in and to lean on in real hardships. In other words, it is thought to address the quality of social ties.

Availability of attachment differs in construction from the other instruments used, as the answer alternatives are simply restricted to “yes” or “no” rather than a grading Likert scale. Six items are used. The number of positive answers is summed to an index, giving a range from 0 to 6. By construct, the distribution of scores will be highly skewed to the right in a normal population, as most respondents do at least have someone close to lean on in real hardships [145]. Availability of attachment is thought to be stable over time. Closely related instruments have demonstrated high test-retest stability in various settings (r=0.7 to 0.9) [245]. The full instrument may be seen in Appendix II.

Availability of social integration

Availability of social integration is the other abbreviated subset derived from ISSI [244]. The items concern number of people that the respondent interacts with in daily life during a week e.g. that share interests or that can visit at any time. In other words, it is thought to address the quantity of social ties.

Like availability of attachment, availability of social integration is not a Likert scale, but a summed index ranging from no social ties to a large amount of social ties. It has been pointed out by the constructors that availability of social integration should not be regarded as a continuous scale, as the change of one unit in the summed score is not the same at all points at the summed index [145]. Like availability of attachment, availability of social integration is thought to be stable over time. Closely related instruments have demonstrated high test-retest stability in various settings (r=0.7 to 0.9) [245].
The instrument is constituted by six items with six answer categories, giving a range in the sum score from 6 to 36. The full instrument may be seen in Appendix III.

**Mastery**

The questionnaire of mastery was constructed by Pearlin in 1978 [148]. It aims to capture ability to cope with potential stressors in everyday life. Pearlin emphasizes that mastery is a resource (as opposed to a strategy) which buffers “social experiences that adversely penetrate people’s emotional life”. A central concept is that mastery does not refer to what people do in a given situation but rather what people are regardless of context. In other words, mastery is based on the idea of general psychosocial resources that protects the individual from potential stressors that could cause a negative affect. Pearlin describes mastery as being one of three vital components of coping with stressors. The items in the questionnaire concern the extent to which one regards one’s life chances as being under one’s control in contrast to being fatalistically ruled [148]. Although the other two components (self-esteem, see section below, and self-denigration) are omitted, the mastery scale is frequently used in questionnaires as a proxy for the concept of coping. The test-retest stability have been shown to be high, in concordance with the underlying theory of mastery as a stable trait (r=0.85 comparing a situation with acute stress with a supposedly non-stressful situation 3 months afterwards) [246]. The instrument are constituted by seven items with four answer categories, giving a range in the sum score from 7 to 28. The full instrument may be seen in Appendix IV.

**Self-esteem**

The questionnaire of self-esteem was also constructed by Pearlin in 1978 [148]. In Pearlin’s definition, self-esteem is another aspect of coping besides mastery. The instrument aims to capture the positiveness of one’s attitudes towards one self. As with mastery, the questionnaire is designed to reflect what people are rather than what people do. The items were not invented by Pearlin himself, but are derived from a factor analysis from Rosenberg’s self-esteem scale [148]. By definition, self-esteem is linked to outcome expectancy following a stressor. As with mastery, the test-retest stability has been shown to be high (r=0.85 comparing a situation with acute stress with a supposedly non-stressful
situation 3 months afterwards) [246]. The instrument is constituted by ten items with four answer categories, giving a range in the sum score from 10 to 40. The full instrument may be seen in Appendix V.

**Sense of coherence**

The concept of sense of coherence (SOC) was presented by Antonovsky 1987 [247]. It is based on the idea that there are salutogene factors that contribute to good health despite harsh conditions in daily life. The idea is based on observations of Israeli women and men who survived concentration camps in World War II and, despite the experiences there, managed to maintain apparent mental and physical health. Antonovsky proposed that the salutogenic SOC is based on three interrelated concepts of meaningfulness, comprehensibility and manageability. SOC is defined as a stable trait in theory. Its stable properties can be confirmed in test-retest stability performed in various settings (being around \( r=0.5 \) in a ten year follow-up) [248], even though its pointed out that SOC is less stable than initially proposed [248]. The SOC-scale differs somewhat in principle from the other Likert scales used. Whereas the others are constructed with the same fixed answer alternatives for every item, typically stating a number of days per week, or ranging from minor to major extent, the answer alternatives in SOC vary. The possible answers are constructed based on opposable pairs (such as very often versus never) which give the highest versus and the lowest score on the specific item. The categories in between are not defined but are treated as a continuous scale between lowest to highest score on each item. Several versions of the questionnaire have been used in studies with different purposes [248]. In this study, we have used the widespread 13-item version with seven answer categories, giving a range in the sum score from 13 to 91. The full instrument may be seen in Appendix VI.

**Hostile affect**

The Cook-Medley hostility scale was developed in 1954 [240]. It was originally designed to identify teachers who had difficulties getting along with their students. Over the years, the instrument has been refined for use as a more general instrument to capture attitudes towards others. In 1988, Barefoot and colleagues suggested a categorization of six subsets of items, that could be used as predictors in survival analyses, where poor outcome was associated
with higher mortality [240]. Hostile affect is one of those six subsets, referring to the experience of negative emotions associated with social contacts. The items aim to capture an admittance of anger, impatience, and reluctance when facing other people. It is a questionnaire focusing on the affect and frustration in silence rather than the aggression, as it does not imply that the respondent actually acts on the basis of these emotions (there is another subscale in the original measurement, hostile aggression that captures overt aggression) [240]. Hostile affect is supposed to be a trait. A high test-retest stability (r=0.84 in a follow-up period of four years) has been demonstrated [249]. The instrument is constituted by five items with five answer categories, giving a range in the sum score from 5 to 25. The full instrument may be seen in Appendix VII.

**Cynicism**

Cynicism, like hostile affect, is one of the subsets that Barefoot and colleagues derived from the Cook-Medley hostility scale [240]. The items are focused on perception of other’s behavior in everyday life and aim to capture a negative view of mankind, beholding people in general as unworthy, deceitful, and selfish. Like hostile affect, cynicism is considered to be a stable trait formed under a considerable amount of time (r=0.84 for the entire Cook-Medley hostility scale for a four-year period, r=0.76 for a ten-year period) [250,251]. The correlation with Hostile affect has been shown to be around r=0.4 [240] The instrument is constituted by twelve items with five answer categories, giving a range in the sum score from 12 to 60. The full instrument may be seen in Appendix VIII.

**Depression**

Centre for Epidemiological Studies Depression Scale (CES-D) was developed by Radloff in 1977 [252]. It was designed to measure depressive symptoms in a normal population by condensing items from a pool of previously validated depression scales. The aim of the instrument was to provide a useful tool for epidemiological studies, capturing the major components in depressive symptomatology. It was not designed to capture a clinical depression per se, by using a discriminating cut-off value, but rather to compare the current level of depressed mood between different groups, with emphasis on the affective component i.e. on diffuse and unspecific state of sadness, worthlessness and hopelessness. Despite being designed to measure symptoms at a given short
time point, thereby asking for a temporary state, CES-D has been shown to have a acceptable test-retest stability \( r=0.54 \) in a normal population without any severe life events between assessments) [252].

The instrument is constituted by twenty items with four answer categories, giving a range in the sum score from 0 to 60. The full instrument may be seen in Appendix IX.

**Vital exhaustion**

The Maastricht questionnaire of vital exhaustion was developed by Appels in 1987 [239]. It was designed to measure mental and physical fatigue as a risk factor for myocardial infarction, based on clinical observations that many patients report “a lack of energy” prior to a cardiac event. It is thought to measure a state following a long-standing problem that the respondent has not been able to resolve. In other words, it is tightly linked to a state of chronic stress, and has been described as a minor depression or a state of demoralization [239]. As with CES-D, even though the questionnaire items are focused to capture a temporary state, the test-retest stability is generally high \( r=0.85 \) over a follow-up time of 4 years) [253].

A slight modification of the original scale was used, based on 19 items instead of the original 21, with a somewhat modified phrasing in a few items. Three answer categories were used, giving a range in the sum score from 19 to 57. The full instrument may be seen in Appendix X.

**3.3.3 Ongoing medication and previous diagnoses**

To capture any previous events of myocardial infarction, the question “Have you ever had any event of myocardial infarction diagnosed by a medical doctor? [Yes][No][Don’t know]” was used. Questions regarding angina pectoris, stroke, diabetes, and cancer were constructed similarly. The diagnosis of RA and other chronic inflammatory diseases was captured using the question “Have you ever had any other chronic or long-term disease diagnosed by a medical doctor? [Yes][No][Don’t know] If yes, please specify”.

The participants were further asked to specify any ongoing medication, taken on a regular basis. All medications were listed and grouped by the Anatomical Therapeutic Chemical (ATC) classification system.
3.4 Measurements of biomarkers

Aliquots (0.5 ml) of plasma for analysis of MMP-9 and serum for analyses of CRP were stored in -70°C approximately 16 to 22 months before laboratory analysis. Aliquots of saliva for analysis of cortisol were stored in −70°C approximately six months before analysis.

3.4.1 Matrix metalloproteinase-9

The concentrations of MMP-9 were measured in EDTA-plasma. All analyses were conducted by the same laboratory assistant using an ELISA method with human Biotrak ELISA systems (Amersham Biosciences, Uppsala, Sweden). The assay for MMP-9 measures MMP-9, Pro-MMP-9 and the ProMMP-9/TIMP-1 complex. The lower detection limit was 0.6 ng/ml. The interassay coefficient of variance (CV) was 7.2 to 7.9%. The analyses of MMP-9 were performed in two runs, where preliminary statistical analyses were performed on half of the participants (n=201), before deciding if analysis should be performed on the entire sub-sample. The same batch of the kit was however used in both runs. The mean difference of storage time prior to analyses between the two runs was estimated to be around six months.

Tissue inhibitor of metalloproteinases-1

TIMP-1 was measured in half of the participants (n=201) using the same EDTA-plasma samples as for analysis of MMP-9. An ELISA method with human Biotrak ELISA systems (Amersham Biosciences, Uppsala, Sweden) was used. The assay recognizes free TIMP-1, and TIMP-1 complexed with MMPs and has a lower detection limit of 1.25 ng/ml and a CV of 6.8%.

3.4.2 Cortisol

The assessment of cortisol was designed to capture an average diurnal variation in everyday life. As cortisol in saliva has been shown to reflect concentrations in serum with good precision [254,255], the assessment of cortisol was performed using a non-invasive procedure. Cortisol was sampled at home, using specific salivettes designed for the purpose.
Material and methods

Study design was set to include sample collection during three consecutive days, with sampling three times a day (9 samples in total). The participants were instructed to start sampling preferably on a Tuesday and end on a Thursday (with the exception of night-shift workers who were instructed to start any day of convenience, collecting samples three days in a row with the same working hours). Samples were collected immediately after awakening (t1), thirty minutes after awakening before breakfast (t2), and just before going to bed in the evening (t3), aiming to sample in proximity to the highest (t1 + t2) and lowest (t3) cortisol levels in the diurnal variation. The participants were instructed to avoid physical exercise, food intake, and smoking one hour prior to sampling. Each participant was asked to fill in a protocol for the samples collected, giving information on the estimated time point for sampling. There was also a space for the participant to write comments (e.g. “been smoking” or “recently been eating”) regarding circumstances that could affect the interpretation of the results. To minimize the risk of participants taking the salivettes in the wrong order, the salivettes were marked with a unique number for t1, t2, and t3, respectively, and different colors were used to indicate day 1 to 3. If salivettes nonetheless were taken in the wrong order, there was a given space on the sampling protocol where this could be indicated. It was thus possible to take non-compliance to the protocol into account. Further, the participants reported normal awakening time, making it possible to adjust for deviation from normal awakening time in the analyses.

The participants were instructed to keep the salivettes in a refrigerator as soon as a saliva sample had been collected. The samples were thereafter brought to the closest PHC centre (typically on the first Friday following the days of sampling), where they were centrifuged. 150 μl from each salivette (or as much as the salivette with least content if less than 150 μl), were pooled into cryogenic vials as follows: one vial for awakening samples (pool- t1), one for samples taken thirty minutes after awakening (pool- t2), and one for evening samples (pool- t3). If one salivette was empty, equal volumes of saliva from the other two were pooled together. Remaining saliva in each salivette was transferred to separate vials, giving in total 12 vials per participant. These were kept in a freezer (−70°C) until analysis. The duration in the freezer prior to analysis have been suggested not to affect cortisol levels to any major extent [256]. All samples were thawed and analyzed in duplicate using a radioimmunoassay method (Diagnostic Products Corporation, Los Angeles, CA). The intra-assay coefficient of variation was 9.4% at 5 nmol/l and 9.8% at 12 nmol/l. The analysis of cortisol was performed in one run.
3.4.3 C-reactive protein

CRP was measured in serum according to standard accredited laboratory routines. All samples were analyzed in the same laboratory, using a highly sensitive latex-enhanced turbidimetric immunoassay (Roche Diagnostics GmbH, Vienna, Austria) with a lower detection limit of 0.03 mg/l and CV of 1.7%.

3.5 Specific study designs

In an initial phase, it was decided that MMP-9 should be explored in a subset of the LSH-study. Applying basic assumptions regarding the prevalence of CAD risk factors and MMP-9 distribution in a normal population, power calculation yielded an estimation of 400 participants to be sufficient in order to show significant associations between CAD risk factors and MMP-9. Plasma samples from 402 participants were randomly drawn from the first 1,007 participants in the LSH-study, without any given criteria for stratification such as sex or age. All ten participating PHCCs were represented in the subset in an even distribution. Paper I, II, and IV are based on this drawn sub-sample (n=402).

Depending on the specific study aims, the subset was treated somewhat differently in the different papers. In Paper I, as we wanted to explore the distribution of MMP-9 in relation to cardiovascular risk factors before onset of disease in a normal population, participants with a self-reported history of CAD and participants unsure of diagnosis were excluded from the analyses. Participants using cholesterol lowering medication were excluded as well, further pushing the healthy selection towards a population free from known CAD.

In Paper II and IV, all participants were included in the crude analyses, with the exception of two outliers on MMP-9 (>200 ng/ml) discussed in section 5.5.3. Exclusions were made in specific sub-analyses to minimize possible confounding from disease and ongoing medication.

In Paper III, a pilot study aiming at enhancing methodology of ambulatory saliva cortisol assessments, a smaller sub-sample was drawn from the LSH-study (n=30). Again, there were no criteria given for stratification. The participants were drawn from eight of the ten collaborating PHCCs. The conceptual purpose differs from that of the other three papers, as the others
have MMP-9 as the main outcome, investigating associations with other factors. In Paper III, for each respective time point (t₁, t₂, and t₃), cortisol levels in pooled saliva samples from three days were compared with arithmetic means of cortisol levels derived from saliva samples from each day. See Figure 13.

Figure 13. Principal design of the pilot study in Paper III. The same volume was taken from each of three vials collected in consecutive days and transferred to a fourth vial.

3.6 Statistical methods

The statistical analyses are all based on well established methods, mainly using the software Stata 6.0 (College Station, Stata Corporation). Some additional analyses have been conducted in SPSS version 14.0 and 15.0 (SPSS Inc), and VassarStats (Richard Lowry, Vassar college). The power calculations were conducted using EpiInfo 2000 (the Centers for Disease Control and Prevention).

Continuous data on plasma MMP-9 were set as the outcome in Paper I, II, and IV. Cortisol levels in pooled saliva samples were set as the outcome in Paper III.

An overview of the statistical methods is found in Table 5.
Table 5. Overview of the statistical methods used.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td><strong>Significance testing on differences between groups</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>t-test</td>
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<td>✗</td>
<td>✗</td>
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<td><strong>Correlation between variables</strong></td>
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<tr>
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</tr>
</tbody>
</table>

A 2-sided probability value of $p \leq 0.05$ was considered as statistically significant. In concordance, 95% confidence intervals have been used in combination with calculated estimates.

The analyses were performed using the variables listed in Table 6.

Table 6. Variables used in the regression and partial correlation analyses.

<table>
<thead>
<tr>
<th>Variables tested</th>
<th>Paper I</th>
<th>Paper II</th>
<th>Paper III</th>
<th>Paper IV</th>
<th>Described in section</th>
</tr>
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<tbody>
<tr>
<td><strong>Outcome</strong></td>
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<td>MMP-9</td>
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<td>✗</td>
<td>✗</td>
<td>✗</td>
<td>1.6 &amp; 3.4.1</td>
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<td></td>
<td>✗</td>
<td>1.5 &amp; 3.4.2</td>
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<tr>
<td><strong>Variables tested</strong></td>
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<td>Age</td>
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<td>✗</td>
<td>✗</td>
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<td>Sex</td>
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<td>✗</td>
<td>✗</td>
<td>3.1.1</td>
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<td>Physiological characteristics</td>
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<td>✗</td>
<td>✗</td>
<td>1.3.1 &amp; 3.2.1</td>
</tr>
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<td>Behavioral risk factors</td>
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<td>✗</td>
<td>✗</td>
<td>1.3.2 &amp; 3.3.1</td>
</tr>
<tr>
<td>Psychosocial factors</td>
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<td>✗</td>
<td>1.4 &amp; 3.3.2</td>
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<td>Self-reported diagnoses</td>
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<td>Ongoing medication</td>
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<td></td>
<td>✗</td>
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<tr>
<td>CRP</td>
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<td>✗</td>
<td>✗</td>
<td>✗</td>
<td>1.3.1 &amp; 3.4.3</td>
</tr>
<tr>
<td>Cortisol peak</td>
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<td></td>
<td></td>
<td>✗</td>
<td>3.4.2</td>
</tr>
<tr>
<td>Cortisol evening</td>
<td>✗</td>
<td></td>
<td></td>
<td>✗</td>
<td>3.4.2</td>
</tr>
<tr>
<td>Deviation from self-reported awakening time</td>
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<td></td>
<td></td>
<td>✗</td>
<td>3.4.2</td>
</tr>
<tr>
<td>Non-compliance to protocol</td>
<td>✗</td>
<td></td>
<td></td>
<td>✗</td>
<td>3.4.2</td>
</tr>
</tbody>
</table>
Most variables were used as collected (as ordinal categories or continuous data). There are however a few variables that were modified as follows, to minimize possible biases in regression analyses:

- To deal with reversed causation, participants that had quit smoking within five years prior to the examination due to health problems (n=13) or had reduced alcohol intake due to health problems within five years prior to examination (n=12) were included in the top ordinal category.
- Due to skewness in the distribution of CRP, continuous data was divided into ordinal categories (quartiles). Even though it was stated in the instructions that no samples should be collected during an ongoing acute inflammation, there were a few participants (n=10) with CRP>10 mg/l. These were excluded in specific sub-analyses but otherwise included in the highest ordinal category.
- Continuous data on body mass index (BMI) were arranged as interval dummies. This was based on the assumption that relationships to body mass index are non-linear, where both abnormally low and abnormally high BMI are related to poor health.
- To deal with potential bias of non-compliance in timing of morning samples of cortisol, a peak value (i.e. the highest values of awakening, t_1, and thirty minutes after awakening, t_2) was used as a proxy for the highest diurnal level instead of the more commonly used +30 minutes after awakening [152,153].
- Cortisol levels from the ambulatory saliva sampling were aggregated into quintiles rather than continuous data on cortisol in Paper IV. A peak level of saliva cortisol >60 nmol/l (n=6) and an evening value of cortisol >15 nmol/l (n=9) were discarded as outliers.

Further, total risk load of cardiovascular risk factors was computed in Paper I, summing up dichotomies of the eight factors mentioned in 1.3, using the criteria in Table 7.
Table 7. Total cardiovascular risk load model used in Paper I. For each criterion fulfilled one point was assigned yielding a summed index ranging from 0 to 8.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Criteria</th>
<th>( n ) matching criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>SBP&gt;160 and/or DBP &gt;100 mm Hg and/or using antihypertensives</td>
<td>81</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>LDL/HDL ratio &gt;3</td>
<td>54</td>
</tr>
<tr>
<td>Obesity</td>
<td>BMI&gt;3</td>
<td>67</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Self-reported known diagnosis</td>
<td>22</td>
</tr>
<tr>
<td>Smoking</td>
<td>Current smoker, or quit within the last five years due to health problems</td>
<td>57</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>More than 170 g/week or reduced intake within the last five years due to health problems</td>
<td>37</td>
</tr>
<tr>
<td>Physical activity</td>
<td>All other than top ordinal category</td>
<td>272</td>
</tr>
<tr>
<td>Fruit and vegetable</td>
<td>All other than top ordinal category</td>
<td>307</td>
</tr>
</tbody>
</table>

CRP was not used in the index but included as an adjustment in the regression model testing the association between MMP-9 and total risk load.
4. RESULTS IN BRIEF

4.1 General findings on MMP-9

Results from Paper I, Paper II, and Paper IV yielded findings on circulating levels of MMP-9 as follows:

- MMP-9 could be detected in plasma in all participants. The mean and standard deviation (n=400) were 39.2 ng/ml (SD 22.8 ng/ml), ranging from 2.9 to 143.9 ng/ml.

- Circulating levels of MMP-9 were significantly higher in participants with a prior myocardial infarction and/or angina pectoris. In a univariate analysis (n=381), the difference between mean levels for the group with self-reported myocardial infarction and/or angina pectoris (n=16) and the other participants was 14.0 ng/ml (mean: 52.7 (SD 34.5) vs. 38.7 (SD 22.1) ng/ml, p=0.015).

- Circulating levels of MMP-9 were significantly lower in women than in men. In a univariate analysis (n=400), the difference between mean levels for men and women was 8.3 ng/ml (mean: 43.4 (SD 24.5) vs. 35.1 (SD 20.3) ng/ml p<0.001).

- There was a significant but rather weak correlation between MMP-9 and CRP. The partial correlation between MMP-9 and CRP (adjusting for age and sex) was r=0.12 (n=389, p<0.001). After exclusion of individuals with CRP>10 mg/l the coefficient was still fairly low, r=0.19 (n=379, p<0.001).
4.2 Specific findings

The results from each paper are presented in brief, in relation to the aims.

4.2.1 Paper I, MMP-9 and cardiovascular risk factors

The main finding in this study was the significant correlation (p<0.001) between plasma levels of MMP-9 and total load of traditional cardiovascular risk factors in a population-based sample without any known CAD and free from cholesterol lowering medication. Total risk load summed up by dichotomies of the eight factors hypertension, dyslipidemia, diabetes, obesity, smoking, high alcohol intake, low physical activity, and low fruit and vegetable intake, were positively associated with MMP-9 (+ 5.7 ng/ml (95% CI 3.6;7.7) per additional risk factor, p<0.001), see Figure 14. The findings were persistent when divided by sex (not shown in Figure: men n=157, p=0.005, women n=167, p<0.001).

Figure 14. Association between MMP-9 and total cardiovascular risk in a population without known CAD (n=328). The total risk load was summed up by the eight risk factors hypertension, dyslipidemia, diabetes, obesity, smoking, high alcohol intake, low physical activity and low fruit and vegetable intake.
Importantly, the association was not driven by one single risk factor alone. When removing one risk factor at a time from the total risk load, the associations between risk load and MMP-9 were highly significant (p<0.001) in all eight models tested, regardless of adjusting for CRP or not. In univariate analysis, levels of MMP-9 were associated with a number of cardiovascular risk factors, see Table 8.

Table 8. MMP-9 and its association with traditional cardiovascular risk factors (n=335 to 345). Regression coefficients expressed as increase of MMP-9 in ng/ml per standard deviation increment or category. Models adjusted for age and sex.

<table>
<thead>
<tr>
<th>Variable (SD increment)</th>
<th>Mean or prevalence</th>
<th>Beta (increase in MMP-9 level)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (4.3 kg/m²)</td>
<td>26.5</td>
<td>2.3 ng/ml</td>
<td>0.053</td>
</tr>
<tr>
<td>Waist circumference (13.0 cm)</td>
<td>90.8</td>
<td>3.4 ng/ml</td>
<td>0.013</td>
</tr>
<tr>
<td>SBP (19 mm Hg)</td>
<td>131.5</td>
<td>2.9 ng/ml</td>
<td>0.022</td>
</tr>
<tr>
<td>DBP (12 mm Hg)</td>
<td>83.5</td>
<td>2.1 ng/ml</td>
<td>0.075</td>
</tr>
<tr>
<td>Heart rate (10.4 bpm)</td>
<td>66.3</td>
<td>2.7 ng/ml</td>
<td>0.024</td>
</tr>
<tr>
<td>HDL (0.35 nmol/l)</td>
<td>1.6</td>
<td>-2.5 ng/ml</td>
<td>0.046</td>
</tr>
<tr>
<td>Triglycerides (0.79 nmol/l)</td>
<td>1.3</td>
<td>3.7 ng/ml</td>
<td>0.002</td>
</tr>
<tr>
<td>Smoking (yes/no)</td>
<td>17; 83%</td>
<td>17.0 ng/ml</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol intake (3 ordinal categories)</td>
<td>80; 9; 11%</td>
<td>7.2 ng/ml</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical exercise (3 ordinal categories)</td>
<td>5; 75; 20%</td>
<td>-6.4 ng/ml</td>
<td>0.010</td>
</tr>
<tr>
<td>Fruit and vegetable intake (3 ordinal categories)</td>
<td>21; 68; 11%</td>
<td>-4.1 ng/ml</td>
<td>0.050</td>
</tr>
<tr>
<td>CRP (quartiles, mg/l)</td>
<td>0.8 (0.3-2.1)*</td>
<td>4.3 ng/ml</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* CRP expressed as median and interquartile range

Diabetes, total cholesterol, LDL, and LDL/HDL-ratio were tested as well, without any significant or close to significant associations with MMP-9. In multivariate analyses based on the risk factors in Table 8, significant positive associations of MMP-9 levels remained with SBP, smoking, alcohol intake, and CRP, run in the same model adjusting for age and sex.
4.2.2 Paper II, MMP-9 and psychosocial factors

There were high correlations between several of the psychosocial instruments tested, as shown in Table 9. Cynicism showed lowest correlations to other instruments tested, although significant in all but CES-D.

Table 9. Partial correlations between different psychosocial instruments adjusted for age and sex (n = 368 to 386).

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CES-D</td>
<td>1.00</td>
<td>0.73</td>
<td>0.17</td>
<td>0.08</td>
<td>-0.51</td>
<td>-0.50</td>
<td>-0.56</td>
<td>-0.30</td>
<td>-0.38</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.132</td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Hostile affect</td>
<td>1.00</td>
<td>0.20</td>
<td>0.11</td>
<td>-0.55</td>
<td>-0.55</td>
<td>-0.64</td>
<td>-0.21</td>
<td>-0.34</td>
<td>-0.17</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td>p=0.026</td>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Cynicism</td>
<td>1.00</td>
<td>0.42</td>
<td>-0.25</td>
<td>-0.25</td>
<td>-0.38</td>
<td>-0.16</td>
<td>-0.01</td>
<td>-0.17</td>
<td>-0.13</td>
</tr>
<tr>
<td>p=0.007</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

In regression models adjusting solely for age and sex, all psychosocial factors apart from vital exhaustion (p=0.127) were significantly associated with levels of MMP-9.

The main finding in this study was that, after adjustment for self-reported known diagnoses (CAD, cancer, and RA), cardiovascular risk factors including CRP, and ongoing medication, there were significant positive associations between MMP-9 and CES-D (p=0.022), hostile affect (p=0.016), and cynicism (p=0.006) and a significant negative association with SOC (p=0.046), see Table 10. Further, close to significant negative associations were found between MMP-9 and mastery (p=0.051) and emotional support (0.084). When run in the same model, MMP-9 was significantly associated with both CES-D and
cynicism, independent of each other (p=0.018 and p=0.006, respectively) and independent of other potential confounders.

Table 10. Association of MMP-9 with psychosocial risk factors. Beta coefficient expressed as increase of MMP-9 in ng/ml per standard deviation increment of psychosocial score. a) n=377-391, b and c) n=325-338.

<table>
<thead>
<tr>
<th>Psychosocial factors (SD)</th>
<th>a) Age, sex and diagnoses</th>
<th>b) Age, sex, diagnoses, and cardiovascular risk factors</th>
<th>c) Age, sex, diagnoses, cardiovascular risk factors and medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>CES-D (7.4)</td>
<td>2.9 (0.6;5.3)</td>
<td>3.2 (0.7;5.7)</td>
<td>2.9 (0.4;5.4)</td>
</tr>
<tr>
<td>Vital exhaustion, (7.2)</td>
<td>1.9 (-0.4;4.2)</td>
<td>1.8 (-0.7;4.5)</td>
<td>1.6 (-1.0;4.3)</td>
</tr>
<tr>
<td>Hostile affect (2.8)</td>
<td>2.6 (0.4;5.0)</td>
<td>2.7 (0.2;5.2)</td>
<td>3.0 (0.5;5.6)</td>
</tr>
<tr>
<td>Cynicism (8.1)</td>
<td>3.4 (1.1;5.8)</td>
<td>3.1 (0.6;5.7)</td>
<td>3.5 (1.0;6.2)</td>
</tr>
<tr>
<td>Mastery (3.4)</td>
<td>-2.6 (-4.9;0.3)</td>
<td>-2.6 (-5.1;0.2)</td>
<td>-2.4 (-4.9;0.0)</td>
</tr>
<tr>
<td>Self esteem (4.5)</td>
<td>-2.5 (-4.9;0.3)</td>
<td>-2.4 (-4.8;0.2)</td>
<td>-1.9 (-4.4;0.5)</td>
</tr>
<tr>
<td>SOC (10.2)</td>
<td>-2.6 (-4.9;0.3)</td>
<td>-2.3 (-4.9;0.2)</td>
<td>-2.5 (-5.0;0.0)</td>
</tr>
<tr>
<td>Emotional support (1.1)</td>
<td>-1.8 (-4.2;0.5)</td>
<td>-2.0 (-4.5;0.3)</td>
<td>-2.0 (-4.5;0.3)</td>
</tr>
<tr>
<td>Social integration (5.8)</td>
<td>-2.3 (-4.6;0.0)</td>
<td>-1.8 (-4.2;0.6)</td>
<td>-1.3 (-3.8;1.0)</td>
</tr>
</tbody>
</table>

Rerunning the analyses in Table 10, adjusting for age and sex but excluding participants with a history of CAD, RA, or cancer with ongoing treatment, did not alter the significant associations in Table 10.

Further, analyses were performed post-hoc to rule out if the significant regressions in Table 10 were possibly driven by one or a few outliers. As depicted in Figure 15 (not shown in Paper II), a poor score on the psychosocial instruments, i.e. high scores regarding psychosocial risk factors and low scores regarding psychosocial resources, was consistently associated with an increase in MMP-9 throughout the whole range of MMP-9 levels.
4.2.3 Paper III, Reliability of pooling cortisol

For each time point (t1, t2, and t3) cortisol measured in pooled samples corresponded well with the calculated arithmetic mean. The correlation coefficient between samples pooled prior to analysis and arithmetic means was $r=0.97$ (0.93;0.99 $p<0.001$) for the awakening samples (t1), $r=0.98$ (0.95;0.99, $p<0.001$) for the samples thirty minutes after awakening (t2), and $r=0.66$ (0.39;0.83, $p<0.001$) for the evening samples (t3). An overall correlation between pooled values and arithmetic means is shown in Figure 16. The pooling of saliva from a distinct time point over three consecutive days was as reliable as using arithmetic means derived from the three samples.
Paper III also indicates that pooling samples from three days provides a better proxy for general diurnal variation of cortisol than samples from one day. The partial correlation coefficients between cortisol levels from one of the consecutive days to another adjusting for age, sex, and non-compliance to the protocol ranged from \( r = 0.46 \) (0.08;0.73, \( p = 0.033 \)) to \( r = 0.77 \) (0.55;0.89, \( p < 0.001 \)). The partial correlation coefficients using the same adjustment between pooled values to any of the consecutive days ranged from \( r = 0.74 \) (0.51;0.89, \( p < 0.001 \)) to \( r = 0.87 \) (0.73;0.94, \( p < 0.001 \)).

### 4.2.4 Paper IV, MMP-9 and cortisol

In Paper IV, we found significant trends regarding levels of MMP-9 and peak cortisol levels and evening cortisol levels. These trends were persistent adjusting for age and sex solely (peak \( n = 315 \), beta +1.9 ng/ml per quintile, \( p = 0.029 \); evening \( n = 315 \), beta +2.1 ng/ml per quintile, \( p = 0.017 \)) or adjusting for myocardial infarction, angina pectoris, RA, diabetes, cancer with ongoing treatment, chronic obstructive lung disease, osteoporosis and hypothyroidism, traditional cardiovascular risk factors, and CRP (peak \( n = 314 \), beta +1.8 ng/mL per quintile, \( p = 0.035 \); evening \( n = 313 \), beta +1.6 ng/ml per quintile, \( p = 0.047 \)). Mean of MMP-9 levels over quintiles is shown in Figure 17.
Figure 17. Means of MMP-9 (± standard error of mean) over categories of cortisol.
5. GENERAL DISCUSSIONS

5.1 The choice of MMP-9 as a biomarker

There are a number of biomarkers that could be of relevance when studying the role of psychoneuroendocrinology in ACS. A cornerstone in the reasoning when choosing a plausible marker in this dissertation was that the marker should have been associated with plaque vulnerability. Five additional requirements as shown in Table 11 were set prior to the analyses to identify a plausible marker.

Table 11. Requirements stated to find a plausible marker.

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Supported in reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Plausible to have a direct impact on plaque vulnerability</td>
<td>[55,189,209,210,257,258]</td>
</tr>
<tr>
<td>• Associated with patients diagnosed with angina or myocardial infarction</td>
<td>[177,189,200-202,258]</td>
</tr>
<tr>
<td>• Associated with cardiovascular risk factors before onset of disease</td>
<td>[181,203,205,206]</td>
</tr>
<tr>
<td>• Associated with inflammation</td>
<td>[186,188,189,193-196,259]</td>
</tr>
<tr>
<td>• Associated with stress hormones</td>
<td>[197,198,260]</td>
</tr>
<tr>
<td>• Possible to measure after thawing frozen samples</td>
<td>[261]</td>
</tr>
</tbody>
</table>

When matching requirements with a literature search over theories and empirical data, the enzyme family of matrix metalloproteinases (MMPs) emerged as a group of relevance. See section 1.6 for further details. The choice of MMP-9 as the main outcome was based on the fulfilling of each of the requirements in Table 11. Moreover, the substrate specificity to collagen type IV and other proteins in the basal lamina hinted at a potential proximal role in plaque rupture.
5.2 On general findings of MMP-9

It is noticeable that earlier studies investigating levels of MMP-9 in a normal population are sparse. The design and analyses conducted in the LSH-study, contributes with information from a middle-aged normal population, which might be a fruitful addition to existing knowledge, otherwise to a large extent based on experimental and clinical designs.

In some earlier studies there has been a large proportion of participants with undetectable levels of MMP-9 [184,262]. It is an undesirable situation to have a biomarker that is not detectable in some participants, as it is not possible to disentangle if the lack of this marker is a proper reflection of a functional (or dysfunctional) physiology, if samples have been treated differently, or if it is due to insensitivity in the laboratory analyses. A detection rate of 100% of the study population, as was the case in these studies, implies that a sensitive laboratory method was used, and that MMP-9 has a vital and important function.

The full detection rate in this study is a noteworthy difference from earlier population based studies. This could possibly be due to a relatively short period of time passing between sample date and analysis, where results in other studies may be biased by the degradation of MMP-9 in a prolonged storage, even at low temperatures [261]. Although the ELISA technique were slightly different, a comparison of our results with the Framingham study [16] is appropriate as the population characteristics according to traditional risk factors, age, and sex were in the same range in both studies. As MMP-9 could be detected in only 20% of the participants in the Framingham study [16] (using a 2-site sandwich ELISA assay with a detection level of 4 ng/ml), the authors concluded that MMP-9 was unlikely to be an informative biomarker in a low-risk population. Our detection rate of 100% rejects this argument suggested in the Framingham material as invalid.

The significant difference of MMP-9 levels between the CAD-patients and participants free of known disease is previously described and well established [177,189,200-202]. The finding as such does not add much in terms of novel knowledge, but serves as an important validation tool of the data in this study.

The significant difference of MMP-9 levels between women and men goes together with the CAD incidence, which is substantially lower for women than men in this age-span. Thus, our data reveals an ecological association between MMP-9 and CAD incidence.
As CRP has been shown to induce production of MMP-9 in several experimental studies [263-265] we were expecting a stronger correlation between MMP-9 and CRP than we found in the material. However, it has been argued for that measurement of CRP is not sufficient as an adequate proxy for inflammatory processes [266], being to reductionistic in its simplification of a complex inflammatory response. Kaysen and colleagues argue that the release of CRP and other hepatic acute phase proteins is one pathway in inflammation, but not the only one. Other activation may contribute separately to outcomes [266]. The surprisingly weak correlation in our findings support that CRP and MMP-9, at least to some extent, could be markers of different physiological pathways, or different stages in an inflammatory process. The low correlation between MMP-9 and CRP led to some post-hoc analyses in the material, not presented in any of the papers. The association between CRP and psychosocial factors were examined. Using the same regression model as in Table 10c (Paper II, section 4.2.2,) setting continuous data on CRP levels <10 mg/l as the outcome instead of MMP-9 levels, there were no significant associations between CRP and any of the nine psychosocial factors tested (p=0.153 to p=0.956).

It may be noted that this in coherence with some large-scale observational studies which recently have concluded that no association between psychosocial factors and inflammatory markers can be found [267,268]. In a recent study from the Whitehall II study on civil servants free from clinically validated coronary heart disease (n=6,396), CRP was investigated together with other markers of inflammation. Nabo and colleagues state that “Our findings suggest that psychological factors do not affect inflammation although they predict incident coronary heart disease” [267]. In that light, the weak correlation between CRP and MMP-9 in our data might be of particular interest to further understand the role of inflammation and/or remodeling in the link between psychosocial factors and CAD. See section 5.4.2 for further discussions.

The association between CRP and cortisol levels was examined as well on our data. Using the same fully adjusted regression model as in Paper IV (section 4.2.4) with CRP <10 mg/l as the outcome instead of MMP-9, the associations between CRP and peak levels of cortisol (n=302, beta-0.1 mg/l per quintile, p=0.053), and between CRP and evening levels of cortisol (n=303, beta-0.1 mg/l per quintile, p=0.069) were marginally significant, pointing at the opposite direction than associations with MMP did. The absolute difference of CRP
levels between the top quintile versus the bottom were however low, about 0.5 mg/l for both peak and evening levels of saliva cortisol. The post-hoc analyses on cortisol brought further support that CRP and MMP-9, at least to some extent, could be markers of different physiological processes.

5.3 On specific findings

5.3.1 Paper I, MMP-9 and cardiovascular risk factors

As most previous studies have examined ACS patients [177,189,200-202], it has not been possible to outline whether raised levels of MMP-9 are a primary phenomenon, reflecting ongoing remodelling involved in the pathogenesis of ACS, or if the raised levels are a second phenomenon, reflecting remodeling triggered by the damage caused by an ischemic state. Our data demonstrating a significant association with total risk in a normal population free from known CAD implies that elevation of MMP-9 levels may occur prior to a cardiac event.

The multiple linear regressions positively associating MMP-9 with SBP, smoking, alcohol intake, and CRP alcohol intake, confirm earlier findings, as all independent variables have been suggested to be associated with MMP-9 levels before [181,186,193,194,203-206]. However, most of the previous studies showing these associations were performed either in patient groups or in cross-sectional analyses on selected high-risk study populations. By our study, these findings have been confirmed in a normal population.

One possible controversy is the positive association between MMP-9 and alcohol intake. The reported mean alcohol intake in the top ordinal category was 270 g/week. This equals approximately three drinks or glasses of wine per day, a level usually considered to be high but not extreme in cardiovascular epidemiology [112,115]. On one hand, our data points towards a dose-depending gradient between alcohol intake and MMP-9 levels rather than a U-shaped association that is implied in other studies on inflammatory markers [116,117]. On the other hand, a note of caution should be at place as smoking and alcohol intake, the behavioral factors that remained significant in a multivariate analysis, were highly clustered to other risk factors. Only a handful of participants (n=5) had smoking or alcohol intake as the only present
behavioral risk factor. In most of the participants, these factors appeared in combination with at least one of the other tested risk factors. Hence, it cannot be excluded that an effect from e.g. low physical activity and/or low fruit and vegetable intake as found in the regression models solely adjusting for age and sex, would be hidden in a multivariate analysis due to co-variation to alcohol and smoking.

Further, in contrast to most variables tested, total cholesterol, LDL and LDL/HDL ratio did not reveal any statistically significant or close to significant association with MMP-9 levels. On one hand, it has been suggested before that MMP-9 does not relate to the lipid profile [195]. On the other hand the variation of the lipid profile variables was likely to be truncated by the exclusion criteria and the selection towards a healthy population.

### 5.3.2 Paper II, MMP-9 and psychosocial factors

To our knowledge, the association between psychosocial factors and MMP-9 levels has not been described previously. The novel findings could be of relevance from a psychoneuroendocrinological view as the assumed pathway studied is in line with previous observations of rapid fluctuations of CAD incidence (described in the rationale, section 1.1).

Due to the considerable correlations in Table 9, analyses were not performed to outline which psychosocial factor matters the most for an elevation of MMP-9. Instead, the more important observation was that there seem to be a consistency when MMP-9 was tested against psychosocial factors. All psychosocial resources (including social integration and emotional support) were pointing towards a negative association with levels of MMP-9, and all psychosocial risk factors were pointing towards a positive association with levels of MMP-9.

As cynicism was not significantly correlated to CES-D, they could be run in the same model. The persistent significant associations with both cynicism and CES-D in the same model adjusted for other cardiovascular risk factors suggest that the positive association of MMP-9 levels with cynicism, and depressive mood, are independent and not merely an effect of co-variation or promotion of established cardiovascular risk factors.
5.3.3 Paper III, Reliability of pooling cortisol

The correlation coefficients between pooled saliva samples and arithmetic means were extremely high. The evening cortisol levels (t₃) had a lower correlation than had the other two time points for assessment (t₁ and t₂). This is likely explained by the low variation in the evening values (where even a small deviation between a pooled sample and its corresponding arithmetic mean will have a relatively high impact on the correlation coefficient). In absolute terms the agreement was high also for evening values. Intuitively, it is not perhaps a stunning surprise that pooling saliva from three samples days provide the same information as using arithmetic means derived from each of the samples. Therefore, the results need to be seen in its context. Internally, this study was performed to explore if it was feasible to pool samples instead of calculating arithmetic means, thereby reducing expenditure and allowing for analyses on more participants. As the LSH-study was conducted in 10 PHCCs, and involved more than one laboratory assistant per PHC in several cases, a pilot study had to be performed to study if the implementation regarding pooling procedure was successful. The satisfactory findings in Paper III led to the use of the method throughout the LSH-study, exemplified by Paper IV.

In more general terms, the findings in this technical note imply that the method may be an attractive alternative for field research projects, where a high number of samples are required. There is at current an ongoing discussion in the field of psychoneuroendocrinology on how to gather information on diurnal cortisol variation in large scale clinical or population based studies, without spending a fortune on the laboratory analysis. The well-known intra-individual day-to-day variation [139,154,155] brings shortcomings to the use of single day measurements. The commonly used sampling from consecutive days calculating arithmetic means on the other hand multiplies the costs. Thus, in the debate on how to assess cortisol in large scale studies saving as much as possible of both information and expenditure, the idea of pooling saliva samples prior to laboratory analysis could be worth implementing.

5.3.4 Paper IV, MMP-9 and cortisol

The main finding in this study was that MMP-9 was associated with a dysregulated secretion pattern of cortisol. With knowledge on the MMP-9-
suppressive properties of exogenous cortisol, as demonstrated by suppressed MMP-9 levels after one cortisol injection in a human experimental study [197], it might be suspected that high levels of cortisol in a population sample would be associated with lower levels of MMP-9. However, and importantly, the paradox of high cortisol levels, supposed to be anti-inflammatory, in combination with a ongoing chronic inflammatory process has been recognized in earlier studies, proposing a model where long-term elevation of cortisol output eventually leads to a resistance to cortisol-mediated signaling in monocytes [230,231]. The empirical findings in this study go well with the proposed psychoneuroendocrinological model (section 1.7), where a state of sustained levels of cortisol might lead to increased MMP-9 levels in a dysregulated remodeling.

5.4 Implications of the findings

5.4.1 The psychoneuroendocrinological model revisited

Going back to the framework of this dissertation, the question is: Does the empirical data fit into the suggested model? To illustrate this, a modified version of the psychoneuroendocrinological model originally presented in Figure 7 (section 1.7), is depicted in Figure 18.
MMP-9 was chosen as it has a central role in tissue remodeling and an assumed role in the development of vulnerable plaques. Starting from the top of the model, we have found the following associations with MMP-9:

- A positive association with psychosocial risk factors
- A positive association with cortisol levels
- A positive association with behavioral risk factors (lifestyle)
- A positive association with hypertension
- A positive association with obesity
- A positive association with systemic inflammation (as assessed by CRP)
- A positive association with previous ACS

Taken together, empirical observations in our studies support the model as it was proposed. None of the associations studied contradict this model. In addition, there are previous studies that bring further support to other links in
the model, not studied in this dissertation. The link between psychosocial factors and dysregulation in the HPA-axis, as suggested in an extensive number of studies, is of crucial importance in the model [139,157-159]. It is a possible drawback that the associations between psychosocial factors and cortisol in our data are as of yet not studied. For now, the model relies on the assumption that the empirical data others have reported on cortisol and psychosocial factors are valid also in this study material. It may be noted that the model was drawn in the context of stress as a risk factor for ACS. Lifestyle (i.e. behavioral risk factors for CAD) was set as a mediator of cortisol response. This should not be interpreted as the only pathway in which behavioral factors operate. It is acknowledged that behavioral risk factors act in multiple pathways converging in an increased risk for ACS. The psychoneuroendocrinological model, supported by our empirical data, suggests that a dysregulation of remodeling is not only mediated through acute phase inflammatory mediators, but also directly via a diminished impact of cortisol. The suggested mechanism that loss of allostasis leads to a dysregulated remodeling may be of relevance not only for ACS, but in psychoneuroendocrinology in general, as discussed below.

5.4.2 Specificity of cardiovascular disease?

MMP-9 was measured as a plausible marker to further understand the underlying mechanisms in the association between psychosocial factors and CAD. However, even though our associations found in combination with previous empirical data support that MMP-9 may be a marker of relevance in this context, it should be pointed out that it is unlikely that MMP-9, as well as any biomarker involved in remodeling, would be a specific marker for cardiovascular disease. Allowing speculations beyond the findings in this dissertation, this has two main implications. 1) There are several diseases and medications that need to be taken into account as adjustments in cardiovascular epidemiology when further exploring levels of MMP-9. 2) The non-exclusive role of MMP-9 in cardiovascular disease implies that stress leading to an eventual dysregulation in remodeling may have an impact in a number of diseases. There is an ongoing debate on the potential role of stress in the progression of tumors [269]. Interestingly, Sephton and colleagues have demonstrated that a
dysregulation in the HPA-axis (assessed as a flat diurnal variation of cortisol) is associated with a reduced survival in breast cancer patients [165]. As there is an up-regulation of MMP-9 levels in tumor-induced angiogenesis [214-216], the link between a dysregulated HPA-axis and a diminished control of remodeling would also possibly be applicable for cancer. In parallel, dysfunction of the HPA-axis in RA should be mentioned. Many patients with RA exhibit a low cortisol secretion, described as a relative adrenal insufficiency, not being able to dampen the ongoing inflammation [270]. MMP-9 activity is thought to worsen the progress of RA, causing a degradation of cartilage and joint tissues [218,219]. Moreover, it has been suggested that psychosocial factors predict the long-term course of disease activity in early RA [271]. In that light, although highly speculative, the associations found between psychosocial factors and levels of MMP-9 fits well with its proposed role in the dysregulation in remodeling also in diseases and conditions other than ACS. This should be further studied as this notion, should it be confirmed, opens up for a number of hypotheses, shedding new light on the old ideas of general susceptibility to disease [272]. Going back to the rationale, the inverse gradient of morbidity and mortality over socioeconomic position applies in a number of outcomes [1,2,7-9]. It is an intriguing thought that at least some of the observed differences in incidence are attributable to loss of allostatic stability and general susceptibility, as hinted at in the proposed psychoneuroendocrinological model.

5.5 Methodological considerations

It should be emphasized that all results are based on cross-sectional analyses. It comes with the design that it is not possible to draw any conclusions on temporality or causality. Just by studying associations alone with the biological marker MMP-9, it is not possible to elucidate the role of MMP-9 in pathogenesis. However, as studies on normal populations are sparse, the approach in this dissertation adds to the existing knowledge derived from clinical and experimental studies.
5.5.1 Participation rate and possibility to generalize

The participation rate of 62.5% is in the same range as other studies conducted at the time in Sweden, using extensive questionnaires [273,274]. The sample was representative for the population in Östergötland in terms of educational attainment, employment rate and immigrant status. However, it is likely that there was a healthy selection in the study, i.e. that invitations are rejected to a higher extent by individuals having a present disease or a high risk load [275]. Thus, it could be suspected that the inter-individual variation is reduced by this bias. As a consequence, there is a risk that regression models fail to show any significant associations between the variables tested, even though such an association is true when studying the population as a whole. In other words, the supposed healthy selection is more likely to possibly reduce the true associations rather than introducing artifacts. Thus, it is not presumably critical in this dissertation, as it leads to a conservative interpretation of the analyses conducted. The associations found in this population are likely to be applicable in other similar populations as well.

5.5.2 Exclusions in the analyses

The original exclusion criteria for participation in the LSH-study were drawn on hindrance of participation rather than medical reasons. For example, a cancer diagnosis was not an exclusion criterion per se, whereas a late stage terminal cancer was.
Depending on the research question, specific exclusions were made (either in all analyses as in paper I or in specific sub-analyses as in paper II and IV) on the basis on self-reported diagnoses and medication. It has been argued that self-reported data regarding CAD diagnosis has both high specificity and sensitivity [276], implicating that self-reported diagnoses were feasible in this setting. However, no diagnoses were clinically verified in the LSH-study, and therefore it can not be excluded that an unknown number of participants could have been misclassified in the stratifications regarding diagnoses (CAD or any other disease) or medication.
On the other hand, as both self-reported diagnosis and medications were reported, a participant had to be non-compliant on both diagnosis and medication to be wrongly classified. Thus, it was concluded that a possible misclassification of diagnoses was not likely to be a major bias in this data set.
5.5.3 Assessment of MMP-9

Plasma versus serum

In previous studies, both sera and plasma are common when analyzing levels of MMP-9. In our ELISA analyses, we decided to use plasma (treated with EDTA). It is suggested that sera might provide artificially high levels, as MMP-9 is released by leukocytes and platelets during preparation [190,277,278]. Thus, it was assumed that levels in plasma would be a better proxy for circulating levels of MMP-9 (even though that choice makes it difficult to compare absolute levels with previous studies using sera).

Effect of storage in the freezer

An important possible bias, especially when comparing absolute levels between different studies, is how long the aliquots have been in the freezer prior to analysis. Ruoy and colleagues have described a dramatic degradation of MMP-9 in relatively short duration periods in the freezer [261]. This could have implications in our studies, especially since the samples were analyzed in two runs, with an approximately six month gap between the two runs. The mean difference in duration time in the freezer prior to analysis was however shorter, as the first run was mainly constituted by the samples collected in an early phase of data collection (October 2003 to February 2004), and the second run mainly constituted by samples from a later phase (January 2004 to April 2004). There was no significant difference of mean level of MMP-9 between the first (n=201) and the second (n=201) run of analyses (p=0.491). Thus, no effect of duration in freezer prior to analysis could be found in the material.

Outliers

The levels of MMP-9 were distributed as shown in Figure 19. Based on the distribution it was decided that two values should be discarded as outliers (224.4 and 246.1 ng/ml, respectively). Both of the discarded outliers were more than three standard deviations higher than the highest included value in the analyses (143.4 ng/ml).
It could not be revealed if the levels were artificially high due to inadequate laboratory analysis or if there are participants in this material with circulating levels of MMP-9 this high. Regardless of cause, the two outliers would have a big impact on all analyses, irrespective of using continuous or logarithmically transformed data. Thus, exclusion of these two outliers facilitated interpretation of associations found in the continuous range covering the vast majority of the participants.

**Assessment of TIMP-1**

Some authors have suggested that including/adjusting for the endogenous inhibitor TIMP-1 would enhance the physiological information of MMP-9 [181-183]. Thus, we tested this in a randomly selected sub-sample (n=201). However, the addition of TIMP did not change the associations between MMP-9 and total risk load used in Paper I. Moreover, the correlation between MMP-9 alone and the ratio MMP-9/TIMP-1 is very high, $r=0.95$. As TIMP-1 did not add any information to alter the conclusions, analyses on TIMP-1 were not performed in the remaining plasma samples.
5.5.4 Assessment of cortisol

The cortisol assessment was designed to capture a general ability to respond and restitute in everyday life. The marked diurnal variation [152,153] in combination with the intra-individual day to day-variation [139,154,155] leads to the requirement of a large set of samples. Measurements were conducted during three consecutive days as our group as well as other researchers have previously used three days with satisfactory compliance [139,165]. Moreover, earlier research in our group supports that mean cortisol levels over three days seem to be more valid, as they corresponded more strongly to psychosocial measures than any single day measurements [139].

As we were interested in estimating the peak value and the lowest level, it was decided that three time points should be sufficient. The first two samples at awakening and thirty minutes after awakening (t1 + t2) were in proximity to the assumed highest cortisol level, and the evening sample just before going to bed (t3) was in proximity to the assumed lowest cortisol level in the diurnal variation.

Pooling samples – misclassification of information?

An important question raised is whether information of value is lost when pooling saliva samples prior to laboratory analysis. The usefulness of pooling saliva samples depends on the research question at hand. A pooling procedure is inappropriate if the research question concerns the difference in cortisol secretion between different days [155].

As long as the hypothesis concerns responsiveness in cortisol levels in everyday life, and the alternative would be to calculate arithmetic means, pooling is highly recommended.

However, a major threat to the concept of pooling is the presence of possible outliers. Abnormally high outliers distorting the analyses may exist at all distinct time-points but will have the highest impact when studying evening values (as the absolute level and intra-individual variation is normally low at this time-point [152,153]).

In Paper III, the partial correlations suggested that deviation from normal estimated awakening time and non-adherence to the protocol did not affect the correlation coefficient to any large extent, whereas outliers in the night values did. It seems therefore to be important to carefully monitor possible exposures that could raise the cortisol secretion in the evening and to provide
careful instructions to the participants to minimize such exposures. In refined designs, aliquots from every sample taken before pooling should be kept in a freezer until the pooled vials are analyzed. This provides the opportunity to analyze the samples constituting a pooled sample if an abnormal level is found, thus being able to rule out if the value can be explained by a single sample, or if the abnormality is consistent throughout all the samples.

**Outliers**

The levels of cortisol were distributed as shown in Figure 20. Based on the distribution it was decided that a cortisol peak level exceeding 60 nmol/l (n=6, in ascending order 62, 66, 70, 102, 111 and 403 nmol/l, respectively) and an evening cortisol level exceeding 15 nmol/l (n=9, in ascending order 15, 24, 42, 42, 43, 58, 91, 224 and 550 nmol/l, respectively) should be discarded in the analyses.

*Figure 20. Distribution of cortisol in Paper IV.*

It is suspected that the most abnormally high cortisol values are artifacts due to contamination of blood in the saliva. There is however no consensus on
where to draw the line for physiologically irrelevant saliva cortisol levels, making the cut-offs discriminating outliers somewhat arbitrary. Moreover, there is a risk that ambulatory saliva sampling of cortisol in itself introduces some artifacts to the data, such as taking the samples in the wrong order, or being non-compliant to the time point when you were supposed to collect saliva. If the participant had reported any type of non-compliance to the protocol, they were excluded from the analyses in Paper IV. However, it was not possible to determine if more participants than those reporting it were non-compliant.

5.5.5 Assessment of psychosocial factors

The instruments used for assessment of psychosocial factors were designed to capture one of the main categories of social support, psychosocial resources, or psychosocial risk factors. Work-related psychosocial instruments, which have been frequently used when studying psychosocial factors, were deliberately omitted from the analyses. Many of the most frequently used work related instruments aim to capture load of stressors. As we wanted to capture the feelings related to the stressors rather than the stress load itself (see section 1.4.2) it was decided that no work-related psychosocial instruments should be used in these analyses. In Paper II, social support was referred to as a psychosocial resource, although this factor is by definition not internal. However, as it is supposed that social support acts indirectly to improve psychosocial resources, being a “coping assistant”, it has been suggested that social support should also be included when studying psychosocial resources [279,280]. Moreover, social support may be a proxy for a personality trait regarding how one reacts and responds in a social context [280], further motivating the inclusion of social support when measuring psychosocial resources.

Unique entities or overlapping constructs?

There has for long been a discussion on the usefulness of dividing different psychosocial factors into separate concepts. Whereas they in theory can be described as different entities, e.g. how stressors are perceived and what the reaction to a prolonged stressor is, the entities may be hard to disentangle in statistical analyses, due to a considerable co-variation.
The complexity is derived from several levels. First, the temporality issue is critical, as poor coping with a stress load accompanied by an insufficient restitution will eventually lead to fatigue or exhaustion, as pointed out in CATS [134]. Second, there is the issue of construct validity, as there are a number of examples when comparing psychosocial instruments, where it appears that psychosocial risk factors are simply reflected by inverse psychosocial resources and vice versa. As an example, two items from two different psychosocial instruments are compared. The first item “At times I think I am no good at all” is taken from Pearlin’s self-esteem scale (see Appendix V). The inverted score of the item is summed together with other items in an instrument aiming to assess self-esteem, a psychosocial resource. The other item “I felt I was just as good as other people” is taken from Centre of Epidemiology Studies Depression Scale (see Appendix IX). The inverted score of the item is summed together with other items in an instrument aiming to assess depression, a psychosocial risk factor. The comparison is made to pin-point difficulties in separating different domains of psychosocial factors. As psychosocial factors are defined by several tightly linked domains, it might be necessary to use instruments that cover more than one domain in the same instrument. The complexity calls however for an awareness when interpreting results where two or more psychosocial instruments are run in the same analysis, as there is a risk that the instruments are partly unique in construct and partly have an inter-questionnaire overlap. This is further exemplified by a comparison between depression and vital exhaustion. From a clinical perspective, it could be argued that depression and vital exhaustion are different entities [281-283]. However, in normal populations where most participants report sub-clinical levels, correlations are generally high (around $r=0.7$) between questionnaires assessing depression and vital exhaustion [283-285]. The conversion in depressive symptomatology makes it challenging to distinguish the entities when using questionnaires.

Third, the raised question of unique entities or overlapping constructs is not only a matter of temporality or item compilation, but also further complicated by the fact that plausible psychoneuroendocrinological pathways are limited. Thus, if different psychosocial domains lead to the same pathways, it is a relevant discussion if different aspects of psychosocial strain have additive or shared contribution to cardiovascular risk. So far, the literature is inconclusive [286-288], and further investigations are warranted to evaluate chronic stress and psychosocial factors as risk factors for CAD.
The high correlations between different psychosocial instruments as shown in Table 9, is an inevitable challenge and calls on a cautiousness when trying to disentangle different effects that specific psychosocial entities may exert.

5.5.6 Assessment of traditional risk factors

The assessment of all traditional risk factors followed general guidelines as previously established either in epidemiological studies and/or in clinical practice. The protocol for the physiological risk factors followed the protocol in the HAPIEE-study [232].

Total risk load

The total risk load algorithm was not based on an established index. It should be mentioned that it was not designed to be used in predictive models of ACS. It was nevertheless useful in this early phase of explorative analyses of MMP-9 levels in normal populations. The major advantage using a sum score where each risk factor was equally weighted was that one risk factor at a time could easily be subtracted, providing the possibility to study if the association between MMP-9 levels and total risk load was driven by one single risk factor or if multiple risk factors contributed to the MMP-9 level.

Reversed causation for smoking and alcohol intake

There is always a risk in cross-sectional analyses that results might be biased by reversed causation. In this dissertation, the possibility of reversed causation was taken into account regarding the variables smoking and alcohol intake. Participants that had recently quit smoking and/or recently reduced alcohol intake due to health problems were assigned to the top category (regardless of category assessed in the questionnaires).

An arbitrary cut-off for a behavioral change regarding smoking and/or alcohol intake was set as five years prior to examination. The choice of a relatively long period was motivated by the intention to capture a reversed causation for a number of outcomes in the LSH-study and not only levels of MMP-9. The mean level of MMP-9 for participants that matched this criterion in regard to smoking was 55.8 ng/ml (n=13). The mean level of MMP-9 for this group was in the same range as for current smokers (n=50, 55.4 ng/ml, p=0.96), and was
significantly higher than the mean MMP-9 level in non-smokers (n=323, 36.4 ng/ml, p<0.001).
The mean level of MMP-9 for participants that matched this criterion in regard to alcohol intake was 59.4 ng/ml (n=12). The mean level of MMP-9 for this group was in the same range as for other participants in the top category (n=38, 51.0 ng/ml, p=0.39), and was significantly higher than the mean MMP-9 level in the lowest category (n=316, 36.8 ng/ml, p<0.001).
The participants matching the criteria for reversed causation did not drive the associations between MMP-9 and smoking and alcohol intake respectively. Excluding these participants in regressions on MMP-9 adjusting for age and sex, there were still significant associations both with smoking (p<0.001) and with alcohol (p=0.004).
Thus, adjusting for reversed causation seemed to be of relevance in the analyses.
6. FUTURE RESEARCH

The study of MMP-9 in normal populations is in an early phase of exploration. Paper I, II, and IV present some promising results, but there are many studies that need to evolve in order to bring conclusions to a higher level. The findings in this dissertation carry a number of implications for future research, which taken together will increase the knowledge and outline the possible usefulness of MMP-9 in cardiovascular epidemiology and in psychoneuroendocrinology in general. In particular, three issues have emerged which warrant further studies. These concern the intra-individual stability over time of circulating levels of MMP-9, the predictive value of MMP-9 for ACS in a normal population, and perhaps the most intriguing, if circulating levels of MMP-9 could be reduced by intervention programs on stress management and behavioral risk factors.
7. CONCLUDING REMARKS

Taken together, the studies performed in a normal population indicate that plasma MMP-9 could be an informative biomarker when studying the association between stress and cardiovascular risk.

The following conclusions were made:

- In a middle-aged population without reported symptomatic CAD, MMP-9 levels were associated with total cardiovascular risk load as well as with single risk factors. This was found also after adjustment for CRP levels.
- MMP-9 levels were independently associated with psychosocial factors after adjustment for cardiovascular risk factors and circulating levels of CRP. All psychosocial resources (including social integration and emotional support) pointed towards a negative association with levels of MMP-9, and all psychosocial risk factors pointed towards a positive association with levels of MMP-9. In particular, depression and cynicism were associated with high levels of MMP-9.
- Pooling saliva samples prior to laboratory analysis was as reliable as arithmetic means over consecutive days when assessing diurnal cortisol variation in a field research project.
- A dysregulated diurnal pattern of cortisol, in particular reflected by elevated evening values, was associated with elevated levels of MMP-9.

The observed associations between MMP-9 and traditional cardiovascular risk factors, psychosocial factors, and cortisol levels suggest a psychoneuroendocrinological pathway that may link stress to plaque vulnerability and increase the understanding of observed associations between psychosocial factors and CAD.
8. SUMMARY IN SWEDISH


Syftet med denna avhandling var att studera associationen mellan cirkulerande nivåer av MMP-9 och etablerade riskfaktorer för hjärt- och kärlsjukdom, inklusive nivåer av C-reaktivt protein (CRP), mellan MMP-9 och psykosociala faktorer, samt mellan MMP-9 och kortisol i saliv i en normalpopulation. Utöver detta testades tillförlitligheten i en nyutvecklad metod för att mäta kortisol i saliv.

Ett slumpmässigt urval från LSH-studien (Livsvillkor, stress och hälsa) användes (n=400). LSH-studien är en populationsbaserad studie designad för att studera om psykoneuroendokrinologiska mekanismer kan mediera de skillnader som finns över en socioekonomisk gradient vad gäller insjuknande i hjärt- och kärlsjuklighet. Plasmanivåer av MMP-9 studerades i urvalet som bestod av män och kvinnor som var 45-69 år gamla när studien påbörjades.


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10. REFERENCES


References


References


References


[103] Li SP, Goldman ND. Regulation of human C-reactive protein gene expression by two synergistic IL-6 responsive elements. Biochemistry 1996;35:9060-8.


References


[119] Mittleman MA, Maclure M, Tofler GH, Sherwood JB, Goldberg RJ, Muller JE. Triggering of acute myocardial infarction by heavy physical


References


[136] Cannon WB (1929) Bodily changes in pain, hunger, fear and rage; an account of recent researches into the function of emotional excitement, 1929, pp. xvi , 1 l., 404, D. Appleton and company, New York and London.,


References


References


References


[204] Derosa G, D’Angelo A, Scalise F, Avanzini MA, Tinelli C, Peros E, Fogari E, Cicero AF. Comparison between metalloproteinases-2 and -9


References


References


[266] Kaysen GA, Levin NW, Mitch WE, Chapman AL, Kubala L, Eiserich JP. Evidence that C-reactive protein or IL-6 are not surrogates for all inflammatory cardiovascular risk factors in hemodialysis patients. Blood Purif 2006;24:508-16.


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Ett deltagande i studien omfattar följande:
• Att Du fyller i bifogade frågeformulär.
• En insamling i hemmet av salivprov och urinprov.
Vi kommer också att följa din hälsoutveckling över tiden, och kommer att återkomma, första gången efter två år med nya frågor.
Du kommer att få svar på resultaten av blodtryck, blodsocker och blodfetter, vilket innebär att besöket kan ses som en hälsokontroll.
Vi är tacksamma för ditt svar i bifogade svarsbrev, så snart du kan, senast inom en vecka.
Med vänliga hälsningar!

Margareta Kristenson
Överläkare/Univ. Lektor
Institutionen för Hälsa och Samhälle
Linköpings Universitet
Har du frågor; ring telefon 013-xxxxxx

APPENDIX
Invitational letter to the LSH-study (in Swedish)

Välkommen att medverka i en studie om Östgötens Hälsa!

Ett deltagande i studien omfattar följande:
• Att Du fyller i bifogade frågeformulär.
• En insamling i hemmet av salivprov och urinprov.

Vi kommer också att följa din hälsoutveckling över tiden, och kommer att återkomma, första gången efter två år med nya frågor.
Du kommer att få svar på resultaten av blodtryck, blodsocker och blodfetter, vilket innebär att besöket kan ses som en hälsokontroll.
Vi är tacksamma för ditt svar i bifogade svarsbrev, så snart du kan, senast inom en vecka.
Med vänliga hälsningar!

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Xxyy
Vårdcentralchef
Vårdcentral Z

xxyy
Sjuksköterska
Vårdcentral Z
Orth-Gomér’s Availability of Emotional support

Principal construct of instrument:  

<table>
<thead>
<tr>
<th>Item 1 to 6</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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</table>

Scoring of positive statements with two alternatives  
(all items)  

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Items in questionnaire:  

Do you have

1. Someone special whom you can lean on?  
2. Someone who feels very close to you?  
3. Someone to share your feelings with?  
4. Someone to confide in?  
5. Someone to hold and comfort you?  
6. Someone at home who really appreciates what you do for him/her?
Orth-Gomér’s Availability of Social integration

Principal construct of instrument:

<table>
<thead>
<tr>
<th>None</th>
<th>1-2</th>
<th>3-5</th>
<th>6-10</th>
<th>11-15</th>
<th>More than 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item 1 to 6</td>
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<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

Scoring of positive statements (all items)

1. Number of people with whom you share interests.
2. Number of people met during an ordinary week.
3. Number of friend who at anytime can come and visit your home who would not embarrassed if it were untidy.
4. Number of friends or family members with whom you can talk frankly.
5. Someone available whom you can ask small favors.
6. Someone available – apart from family- to whom you can turn in times of difficulties.
Pearlin’s Mastery scale

*Principal construct of instrument:*

<table>
<thead>
<tr>
<th>Item 1 to 7</th>
<th>Not at all</th>
<th>To a minor extent</th>
<th>To some extent</th>
<th>To a major extent</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Scoring of positive statements (2 items)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Scoring of negative statements (5 items)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
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</tbody>
</table>

*Items in questionnaire:*

To what extent are the following statements accurate for you?

1. There is really no way I can solve some of the problems I have.
2. Sometimes I feel that I’m being pushed around in life.
3. I have little control over the things that happen to me.
4. I can do just about anything I really set my mind to do.
5. I often feel helpless in dealing with the problems of life.
6. What happens to me in the future mostly depends on me.
7. There is little I can do to change many of the important things in my life.
Pearlin’s Self-esteem scale

Principal construct of instrument:

<table>
<thead>
<tr>
<th>Item 1 to 7</th>
<th>Not at all</th>
<th>To a minor extent</th>
<th>To some extent</th>
<th>To a major extent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scoring of positive statements (5 items)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Scoring of negative statements (5 items)</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Items in questionnaire:

To what extent do you agree to the following statements?

1. I feel that I’m a person of worth, at least on an equal plane with others.
2. I feel that I have a number of good qualities.
3. All in all, I am inclined to feel that I’m a failure.
4. I am able to do things as well as most other people.
5. I feel I do not have much to be proud of.
6. I take a positive attitude toward myself.
7. On the whole, I am satisfied with myself.
8. I certainly feel useless at times.
9. I wish I could have more respect for myself.
10. At times I think I am no good at all.
Antonovsky’s Sense of coherence

Principal construct of instrument:

<table>
<thead>
<tr>
<th>Item 1 to 13</th>
<th>Lowest alternative</th>
<th>Highest alternative</th>
</tr>
</thead>
</table>

Scoring of positive statements (8 items)

1 | 2 | 3 | 4 | 5 | 6 | 7 |

Scoring of negative statements (5 items)

7 | 6 | 5 | 4 | 3 | 2 | 1 |

Items in questionnaire:
Considering the last couple of months:

1. Do you have the feeling that you don’t really care about what goes around on you? [Very seldom or Never] vs. [All the time]
2. Has it happened in the past that you were surprised by the behaviour of people whom you thought you knew well? [It has never happened] vs. [Many times]
3. Has it happened that people whom you counted on disappointed you? [It has never happened] vs. [Many times]
4. Until now your life has had… [no clear goals or purpose at all] vs. [very clear goals and purposes]
5. Do you have the feeling that you are treated unfairly? [All the time] vs. [Very seldom or Never]
6. Do you have the feeling that you are in an unfamiliar situation and don’t know what to do? [All the time] vs. [Very seldom or Never]
7. Doing the things you do every day is… [A source of deep pleasure and satisfaction] vs. [A source of pain and boredom]
8. Do you have very mixed-up feelings and ideas? [All the time] vs. [Very seldom or Never]
9. Does it happen that you have feelings inside you would rather not feel? [All the time] vs. [Very seldom or Never]
10. Many people – even those with a strong character – sometimes feel like sad sacks (losers) in certain situations. How often have you felt this way in the past? [Never] vs. [All the time]
11. When something happened, have you generally found that…? [You overestimated or underestimated its importance] vs. [You saw the things in its right proportions] continued on next page
12. How often do you have the feeling that there’s little meaning in the things you do in your daily life? [All the time] vs. [Very seldom or Never]

13. How often do you have feelings that you are not sure you can keep under control? [All the time] vs. [Very seldom or Never]

Cook Medley’s Hostile affect scale

**Principal construct of instrument:**

<table>
<thead>
<tr>
<th>Item 1 to 5</th>
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<th>To a minor extent</th>
<th>Neither nor</th>
<th>To some extent</th>
<th>To a major extent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scoring of negative statements (4 items)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Scoring of positive statements (1 item)</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

**Items in questionnaire:**

*To what extent do you agree to the following statements?*

1. It makes me impatient to have people ask my advice or otherwise interrupt me when I’m working on something important.
2. Some of my family have habits that bother and annoy me very much.
3. People often disappoint me.
4. I am not easily angered.
5. There are certain people whom I dislike so much that I am inwardly pleased when they are catching it for something they have done.
Cook-Medley’s Cynicism scale

Principal construct of instrument:

<table>
<thead>
<tr>
<th>Item 1 to 12</th>
<th>Not at all</th>
<th>To a minor extent</th>
<th>Neither nor</th>
<th>To some extent</th>
<th>To a major extent</th>
</tr>
</thead>
<tbody>
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</table>

Scoring of negative statements (all items)

Items in questionnaire:

To what extent do you agree to the following statements?

1. I have often had to take orders from someone who did not know as much as I did.
2. I think a great many people exaggerate their misfortunes in order to gain the sympathy and help of others.
3. It takes a lot of argument to convince most people of the truth.
4. I think most people would lie to get ahead.
5. Most people are honest chiefly through fear of being caught.
6. Most people will use somewhat unfair means to get profit or an advantage rather than to lose it.
7. No one cares much what happens to you.
8. It is safer to trust nobody.
9. Most people make friends because friends are likely to be useful to them.
10. Most people inwardly dislike putting themselves out to help other people.
11. I have often met people who were supposed to be experts who were no better than I.
12. People generally demand more respect for their own rights than they are willing to allow for others.
### Centre for Epidemiological Studies Depression scale

*Principal construct of instrument:*

<table>
<thead>
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<th>1-2 days</th>
<th>3-4 days</th>
<th>5-7 days</th>
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<tbody>
<tr>
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</tr>
</tbody>
</table>

*Scoring of negative statements (16 items):*

0  1  2  3

*Scoring of positive statements (4 items):*

3  2  1  0

*Items in questionnaire:*

*During the past week:*

1. I was bothered by things that do not usually bother me.
2. I did not feel like eating; my appetite was poor.
3. I felt that I could not shake off the blues even with help from my family or friends.
4. I felt I was just as good as other people.
5. I had trouble keeping my mind on what I was doing.
6. I felt depressed.
7. I felt that everything I did was an effort.
8. I felt hopeful about the future.
9. I thought my life had been a failure.
10. I felt fearful.
11. My sleep was restless.
12. I was happy.
13. I talked less than usual.
15. People were unfriendly.
16. I enjoyed life.
17. I had crying spells.
18. I felt sad.
19. I felt that people dislike me.
20. I could not get “going”.
Maastricht Questionnaire of Vital exhaustion

Principal construct of instrument:

<table>
<thead>
<tr>
<th>Item 1 to 19</th>
<th>Never</th>
<th>Sometimes</th>
<th>Often</th>
</tr>
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<tbody>
<tr>
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<td>☐</td>
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</table>

Scoring of negative statements (all items) 1 2 3

Items in questionnaire:

Considering the last couple of months:

1. Do you often feel tired?
2. Have you felt less confident lately?
3. Do you have a feeling that you haven’t accomplished much lately?
4. Do you believe that you have come to a “dead end”?
5. Do you feel more listless recently than before?
6. Do you have the feeling that you can’t cope with everyday problems as well as you used to?
7. Do you sometimes feel like your body is like a battery losing its power?
8. Do you feel cast down?
9. Do you feel like you are losing your self-restraint?
10. Have you ever had e feeling lately, like “I do not achieve enough, I could achieve more if only I were healthier, not so weak, not so limp?"
11. Have you noticed lately that it takes a longer time than before to “get going”?
12. Do you lately think more often about acquaintances or relatives that are deceased?
13. Do you have e feeling that nobody can help you with those problems deep inside?
14. Are you becoming less satisfied with yourself?
15. Do you feel less capable of doing something useful these days?
16. Do minor hassles irritate you easily in these days?
17. Would you want to be dead at times?
18. Can you bring yourself less and less to leave the house and go for a visit?
19. Do you have the feeling these days that you don’t have what it takes anymore?