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FAMILY HISTORY IN RELATION TO MYOCARDIAL INFARCTION, AND ANALYSES OF GENE-ENVIRONMENT INTERACTIONS INVOLVING FACTORS OF HAEMOSTASIS

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Stockholm 2005

Published and printed by Repro Print, Stockholm, Sweden

ISBN 91-7140-396-5

Till Hasse

SUMMARY

Family history of coronary heart disease (CHD) has frequently been shown to increase the risk of MI. However, the mechanisms are not well understood. Probably, both genetic- and environmental effects contribute. It is possible that family history in combination with other cardiovascular risk factors is of particular importance in the aetiology of myocardial infarction (MI). Haemostatic factors seem to contribute in the causation of MI, although this is not established. Plasma fibrinogen and plasminogen activator inhibitor-1(PAI-1) are two potentially important risk factors, with their genetic variants possibly influencing effects. The potential involvements of these factors in interactions with other cardiovascular risk factors are poorly understood.

The aims of the present thesis were to assess the influence of family history of CHD on risk of first non-fatal MI in men and women, respectively, and to explore its potential role as a biologically interacting factor. The thesis also aimed to study the importance of fibrinogen and the G-455 A polymorphism, and PAI-1 and the 4G/5G polymorphism, in relation to risk of MI. Here a particular aim was also to explore potential synergistic effects for exposure combinations involving these factors regarding risk of MI. A final aim was to explore which cardiovascular risk factors may be most important for the long-term prognosis after a non-fatal MI.

Data are derived from the Stockholm Heart Epidemiology Program (SHEEP), a population-based case-control study of MI performed between 1992 and 1994 at the ten emergency hospitals within the county of Stockholm. The present analyses were restricted to 1643 men and women who had suffered a first-time non-fatal MI, and 2339 controls. Data on exposures were available from questionnaires, anthropometric measurements, blood samples, and medical records.

A family history of CHD (defined as ≥1 first-degree relative affected before the age of 65) was observed to be associated with risk of MI in both men and women. Synergistic effects were observed in women exposed to family history of CHD in combination with current smoking and with a high LDL/HDL quotient, respectively. In men, family history of CHD and diabetes mellitus seemed to act in synergy.

High level of plasma fibrinogen was associated with increased risk of MI in both men and women, although the OR decreased after adjusting for other cardiovascular risk factors. Presence of the A^{-455} allele was associated with increased fibrinogen level but not with increased risk of MI. No clear synergistic effects were observed. High plasma PAI-1 activity was associated with increased risk of MI, and in men it also interacted with smoking in increasing the risk synergistically. In women, presence of the 4G allele was associated although weakly with increased risk of MI.

Diabetes mellitus, job strain and abdominal adiposure had an impact on prognosis after MI in men. In women, prognostic importance was particularly noted for diabetes mellitus and for low level of Apolipoprotein A1. In both men and women the size of the initial infarction also had a prognostic value. In male survivors of MI, family history of CHD increased the risk of death from CHD during follow-up.

In conclusion, this thesis suggests the occurrence of several biological interactions between risk factors for MI. The involvement of family history in such interactions indicates that gene-environment interaction may be in operation. After MI, several primary and secondary exposures have an influence on the prognosis.

LIST OF PUBLICATIONS

This thesis is based on the following original articles/manuscript, which will be referred to in the text by their Roman numerals.

I. Leander K, Hallqvist J, Reuterwall C, Ahlbom A, de Faire U: Family history of coronary heart disease, a strong risk factor for myocardial infarction interacting with other cardiovascular risk factors: Results from the Stockholm Heart Epidemiology Program (SHEEP). Epidemiology 12; 215-221, 2001

II. Leander K, Wiman B, Hallqvist J, Falk G, de Faire U: The G-455A polymorphism of the fibrinogen Bβ-gene relates to plasma fibrinogen in male cases, but does not interact with environmental factors in causing myocardial infarction in either men or women. J.Int.Med. 252; 332-341, 2002

III. Leander K, Wiman, B, Hallqvist J, Sten-Linder M, de Faire U: PAI-1 level and the PAI-1 4G/5G polymorphism in relation to risk of non-fatal myocardial infarction. Results from the Stockholm Heart Epidemiology Program (SHEEP). Thromb.Haemost. 89; 1064-1071, 2003

IV. Leander K, Andersson T, Hallqvist J, Wiman B, Ahlbom A, de Faire U: Cardiovascular risk factors – importance for risk of recurrent myocardial infarction. Manuscript

CONTENTS

LIST OF ABBREVIATIONS

MI	Myocardial infarction
CHD	Coronary heart disease
CVD	Cardiovascular disease
OR	Odds ratio
CI	Confidence interval
S	Synergy index
RR	Relative risk
HR	Hazard ratio
PAI-1	Plasminogen activator inhibitor -1
LDL	Low density lipoprotein
HDL	High density lipoprotein
BMI	Body mass index
CRP	C-reactive protein
PTCA	Percutaneous transluminal coronary angioplasty
CABG	Coronary Artery By-pass Grafting

GENETIC TERMS[1-3]

Polymorphism	A locus where several allele combinations may occur
Genotype	In each individual, the specific pair of alleles at a locus
Allele	Each of the different states found at a polymorphic site
Chromosome	Linear DNA molecule that constitutes the basic physical block of heredity
Gene	DNA segment that is transcribed into messenger RNA and translated into a protein
Locus (plural: loci)	Position at a chromosome
Homozygous	Individual that carries two copies of the same alleles at the same site in the two homologous chromosomes of a given pair
Heterozygous	Individual that carries two different alleles at the same site in the two homologous chromosomes of a given pair
Haplotype	Set of allelic states found at neighbouring loci in a chromosome, as inherited from a parent
Heritability	Expresses the extent of which total phenotypic variation in a population is the result of genetic variation
Hardy Weinberg (H-W) Equilibrium	State in which the allele and genotype frequencies do not change from one generation to the next in a population. In H-W Equilibrium, allele and genotype frequencies are related through the H-W law: For a locus with two alleles P and Q at frequencies p and q, respectively, homozygotes for P are found at frequency p2, homozygotes for Q have a frequency q2, and heterozygotes are found at a frequency 2pq

1 INTRODUCTION

It is well known that coronary heart disease (CHD) aggregates in families. The underlying reason for this could be that the disease is genetically caused, or that the environmental exposures run in families. It is also possible that genetic and environmental influences interact, making some individuals more vulnerable to environmental risk factors than others. The biological mechanism by which a family history of CHD leads to increased risk of the disease is unclear. However, a certain part of its effect can be explained by an aggregation of cardiovascular risk factors within families[4]. Another part, but smaller, can be explained by the occurrence of familial single-gene disorders. The unexplained effect exerted by family history is likely to be composed of presently unidentified risk factors, genetic- or environmental, and/or of unknown interactive effects for certain combinations of risk factors. Studying family history in epidemiological analyses is a way of getting closer to the unidentified parts of the aetiology of CHD; the family history variable could be looked upon as a surrogate measure of unknown exposures and of unknown interactions between exposures.

Plaque rupture and thrombus formation have been identified as common mechanistic events in MI. In this connection, haemostatic factors are also believed to play an important role in the development of coronary heart disease[5-7]. However, their effects are still incompletely established[8, 9]. This thesis includes analyses of one coagulation factor, plasma fibrinogen, and one fibrinolysis factor, plasma plasminogen activator inhibitor-1 (PAI-1). For each of these haemostatic factors, one polymorphism of potential interest (as observed in earlier studies) was selected for analysis regarding its potential influence on risk of MI: the G-455A polymorphism of the fibrinogen Bβ-gene and the 4G/5G polymorphism of the PAI-1 gene.

The risk of recurrent CHD events after suffering a non-fatal MI is high. The adverse influence on prognosis exerted by diabetes mellitus has been frequently reported, as well as the influence from size of the initial MI. However, there seem to be many cardiovascular risk exposures that contribute to increasing the risk of recurrent CHD, both primary exposures and exposures secondary to the initial MI. There is a lack of knowledge regarding which exposures are most important for the prognosis. A number of potential prognostic factors, such as family history of CHD, have only been sparsely investigated.

1.1 MYOCARDIAL INFARCTION

1.1.1 Biology

The main underlying cause of cardiovascular disease (CVD) is atherosclerosis, which causes impaired blood circulation and ischemia. An understanding of athero-

sclerosis as an inflammatory process, rather than just a disease of lipid accumulation, has evolved during recent years[10, 11]. The term used for diseases where the coronary arteries are affected is coronary heart disease (CHD), where myocardial infarction (MI) is the most common disease. An MI occurs when part of the muscle tissue of the heart dies due to lack of oxygen. An MI generally ensues from a ruptured atherosclerotic plaque in a coronary artery, which brings about a thrombosis that occludes the vessel. The reason why a stable plaque suddenly becomes unstable and ruptures is still not known. In a stable plaque, the necrotic core of the plaque is covered by a thick fibrous cap, the smooth muscle cells are abundant, the matrix is collagen-rich, and the state of inflammation is mild to moderate. An unstable plaque, on the contrary, is characterized by a thin ruptured fibrous cap with thrombus, few smooth muscle cells, a collagen-poor matrix, and active inflammation[11-13]. It has also been pointed out, however, that unstable plaques, or "vulnerable plaques", are not the only culprit factors for the development of MI. Blood parameters and myocardial vulnerability may also be contributing factors[12].

1.1.2 Risk factors

Besides high age and male gender, the most well established primary risk factors for MI are tobacco use, hypertension, hyperlipidaemia, and diabetes[14-16]. Among other risk factors of importance according to epidemiological and clinical research are physical inactivity, overweight, dietary habits, diabetes mellitus, haemostatic factors, low socioeconomic status, and psychosocial strain[15, 17, 18]. Further, there is compelling evidence that elevated levels of inflammatory mediators (e.g. interleukin-6 and tumor necrosis factor alpha), cell adhesion molecules (e.g. intercellular adhesion molecule -1, P-selectine and E-selectine), and acute phase reactants (e.g. C-reactive protein, fibrinogen, and serum amyloid A) correlates with increased vascular risk[10]. A constellation of metabolic abnormalities resulting from the occurrence of obesity, sometimes called the metabolic syndrome[19], is also associated with increased cardiovascular risk[19-22]. Various definitions of the metabolic syndrome occur, but key components are: Much visceral fat, glucose intolerance, insulin resistance, dyslipideamia, and hypertension[19, 23]. Among factors clearly related to the metabolic syndrome are plasminogen activator inhibitor-1 (PAI-1)[24, 25], C-reactive protein and microalbuminuria[26, 27]. However, many variables occurring within the metabolic syndrome may be primarily a consequence to the presence of insulin resistance[19, 27]. About 200 risk factors of CVD have been discussed in scientific publications[28] but still its aetiology is far from well understood. Most likely, there is a large web of risk factors, independently important or important in combination with other factors, that cause CVD and MI.

1.1.3 Occurrence

Cardiovascular disease (CVD) is today the major cause of death in Sweden, accounting for 45% of all deaths in men and 44% in women (data from the year 2002)[29]. Also in a global perspective, CVD is a leading cause of death and dis-

ability[30]. However, the CVD mortality rate has been declining during the last 30 years[29, 31].

In the year 2002, the proportion of CVD deaths in the county of Stockholm caused by an MI was 30% in men and 23% in women[29]. The MI incidence (per 100,000) in the county of Stockholm was 413 in men and 280 in women in the year 2000. The same year, the MI mortality (per 100,000) was 173 in men and 132 in women[32].

Both incidence and mortality of MI are strongly related to sex and age. The risk of MI is about twice as high in men as compared with women. In individuals younger than 55 years, the risk of MI in men is 3-4 times higher than in women. With increasing age, from 50 years to 70 years, the risk of MI increases 6 times in men and 9 times in women[29].

The number of MI events yearly in the county of Stockholm amounts to about 6300 (3500 in men and 2700 in women) occurring in 5800 individuals (3300 men and 2500 women)[33].

1.2 RECURRENT CHD

After suffering an MI event the risk of recurrent CHD and death is high. However, a trend of decreasing recurrent event rate of MI between 1985 and 1998 was observed in the northern Sweden WHO MONICA (World Health Organisation Multinational Monitoring of Trends and Determinants of Cardiovascular Disese) area[34]. In the year 2000, in Stockholm County the proportion of first MI events leading to death within 1 year was 43% in men and 51% in women[32].

In 1990, the case fatality rate (death within 28 days after the MI) in the county of Stockholm was 42% in men and 46% in women, whereas in the year 2000 the corresponding case fatality rates were 35% in men and 39% in women[33].

A number of factors seem to contribute to increasing the risk of recurrent CHD. An interplay is likely to occur between primary risk factors, that continue to exert their effects after the initial MI, and secondary risk factors related to the MI event. Of importance for the prognosis are also interventions such as PTCA, CABG and thrombolysis, and medications such as ACE-inhibitors, beta blockers, and platelet activating agents [35-41]. In addition, changes in lifestyle factors in patients after MI, such as quitting smoking and improving dietary habits, also have prognostic effects[37, 42].

Among the primary risk factors, a large body of evidence indicates that the presence of diabetes mellitus has an adverse effect on prognosis after MI[43-45]. Among other primary cardiovascular risk exposures suggested to influence the risk of recurrent CHD (and death) are hypertension and dyslipidemia[44, 46-48], low socioeconomic status[49] and different blood parameters[50, 51]. Among the secondary exposures de-

monstrated to worsen prognosis after MI are large size of the infarction, occurrence of heart failure and arrhythmias[52-56].

1.3 FAMILY HISTORY OF CORONARY HEART DISEASE

1.3.1 Definitions

The most common definition of a family history of CHD considers CHD events in first-degree relatives of the index case. Different age cut-off limits are used for the age of the relative when affected. A common cut-off limit is the age of 65. However, both higher and lower age limits occur in the literature[57]. According to current guidelines on CVD prevention, patients younger than 55 years (men) or 65 years (women) are likely to have close relatives that need to be examined for cardiovascular risk factors[58]. In the present thesis, family history of CHD is defined as having one or more first-degree relative(s) (biological parent, brother or sister) affected by CHD before the age of 65.

1.3.2 Prevalence

A relatively large proportion of the general population has a positive family history of CHD. In a cohort study of 7495 middle-aged men from the city of Göteborg in Sweden (the Multifactor Primary Prevention Study) the proportion was 26%[59]. This study did not use any cut-off age limit of first-degree relative when affected. Only first-degree relatives were considered, but no distinction between blood and non-blood relatives was made. A similar proportion of presence of family history of CHD was reported from a study of a population from West Scotland[60]. However, here the definition of family history was a parent having died from CHD. Of 3012 men included in the Northwick Park Heart Study (UK) the proportion was 33%. Here, all events of "heart attack" in relatives of the index case were considered regardless of kinship and age of relative when affected[61].

1.3.3 Association with cardiovascular risk

The first report of familial aggregation of CHD dates back to the 19th century. In the 1950s, in parallel with the development of new epidemiological methods, large scale studies on the importance of family history as a risk factor for cardiovascular diseases began. Two of the early studies were the ones carried out by Thomas and Cohen in 1955, and by Slack and Evans in 1966, both demonstrating evidence of increased frequency of CHD for individuals with a family history of the disease[62, 63]. From a study of young and middle-aged Finnish men, Rissanen reported in 1979 that a strong familial component in MI occurs at an early age and that this component decreases steeply with advancing age[64]. Friedlander reviewed the significance of familial clustering of CHD as a risk factor for the disease in 1994 and found support for a clear association between family history of CHD, mainly in young individuals, and risk of CHD. If several family members have suffered a coronary event the risk is especially high[65]. The effect could not be fully explained

by presence of the traditional cardiovascular risk factors in individuals with a family history of CHD. The relative risk reported in studies, with either a case-control or a cohort design, is most often approximate to 2.0[66-68]. Recent results from the cohort study performed in Göteborg mentioned above, demonstrate that a family history of CHD retains its importance as a cardiovascular risk factor also over a very extended follow-up up into old age[69]. In line with this are results from a large cohort study of Swedish twins suggesting that genetic factors affecting risk of CHD are in operation throughout the entire life span[70].

The following statement is taken from a textbook of cardiology: "Although nothing can be done to correct the family history, it is important to recognize that those with familial disorders are very susceptible to environmental influences. The other risk factors should be attended to particularly diligently"[71]. Indeed, prevailing clinical guidelines on CVD prevention recommend consideration of family history of premature CHD when assessing individual risk[58]. However, presence of family history of CHD is not included in the current version of the quantitative model for assessing risk of developing CVD[58, 72]. Nor do the Framingham Coronary Risk Functions include assessment of family history of CHD, although the utility of adding this variable is currently being examined[66]. Besides being an important risk indicator of future disease, a family history of CHD also defines the relatively small subset of families in the population that account for the most cases[73].

Some studies have reported different effects exerted by family history depending on whether the mother or the father has suffered from CHD. One hypothesis is that maternal influences may be stronger determinants of risk in offspring because smoking habits and other lifestyle factors in the mother may influence fetal development. Sesso et al. suggest that a maternal history of MI may be more strongly associated with increased risk of CVD than a paternal history[74]. In a recent study by Nilsson et al., increased risk of CVD morbidity and mortality was observed in men whose mothers had died from CVD before the age of 75[75]. However, others reported that paternal history of CHD is at least as important as maternal history[76]. Sesso et al. found that an early paternal history of CHD conferred a greater risk of CVD than if CHD occurred at an older age. However, for maternal history of CHD no such age-dependent effect was noted[74]. A recent study by Nasir et al. suggested that a history of premature CHD in siblings is more strongly associated with subclinical atherosclerosis than a parental history[77].

A small proportion of the family history of CHD could be explained by established genetic phenotypes such as the single-gene disorder familial hypercholesterolemia or hypertension from glucocorticoid-remediable aldosteronism and Liddle syndrome. Elevated cholesterol or hypertension caused by other factors, genetic and/or environmental, probably explains an additional proportion[64, 69]. Clearly, the family history variable captures effects of potential unmeasured exposures and interactions between exposures that family members have in common. Hunt et al. have expressed that "family history provides a surrogate measure of physiologic processes leading to CHD without requiring complete understanding of their underlying complexity"[73].

1.3.4 Assessment of family history

Most commonly, family history is assessed simply by asking the individual about the occurrence of disease in relatives. In some settings, however, it is also possible to interview all members of a family in order to obtain more accurate information. A third possibility is to use register data on disease or death in members of a family to obtain objective data. Registers that include data on CHD morbidity and mortality together with kinship data are of course useful. In Sweden, the Multiple Generation Register (MGR) was introduced in 1998 and this has enabled the use of register linkage analyses[75].

A "Family Risk Score" was introduced by Hunt and his co-workers in 1986 to quantify familial risk using information about number of family members as well as the age and sex for these[78]. Compared with more simple measures of family history, they found that Family History Score was more strongly related to risk of future CHD. Several other family history scores have been formulated, featuring different criteria for assigning each study participant an individual family risk score[79, 80,81]. Silberberg et al. reviewed 17 such family history scores in 1999, and recommended that the choice of a particular score should depend on the purpose of the study and characteristics of the data. However, no score offered a solution to the problems of few data. The authors conclude that when families are small and affected relatives are few, categorical definitions or simple counts are likely to be adequate[82].

1.4 GENETIC EPIDEMIOLOGY OF CHD

Persuasive evidence for a genetic component to the aggregation of coronary heart disease in families was presented in 1994 by Marenberg el al. in a large follow-up study of Swedish monozygotic and dizygotic twins. An increased concordance for death related to coronary artery disease was found in the monozygous group, and the effect was attenuated with age[83]. Using the same material, aiming to distinguish between environmental and genetic effects for death from CHD, Zdravkovic et al. report a heritability of CHD death of 45-69% in men and 26-50% in women[70]. Another finding that indicates possible genetic influences that are largely unknown, is the finding by Rosengren et al. that paternal, but not maternal, longevity appears to protect against coronary disease (a result based on studying middle-aged men from Göteborg in Sweden)[84].

Still more and more loci are being added to the list of possible genetic factors contributing to the genetic influences on CVD. However, for several years the prevailing opinion within this field of research has been that the genetic component of CVD in the general population is determined by multiple genes, each one of them explaining only a very limited proportion of the variability[85, 86]. In recent years, the analyses of combinations of genetic variants, i.e. haplotypes, have also become frequent. Further, for several years the occurrences of gene-environment interactions have been frequently proposed in research literature, and the current discussion still focuses on the potential causal effects due to combinations of genetic variants and environmental or biological factors[86-89]. However, the need for

increased attention to the choice of study design and the use of statistical methods has also been emphasized[86, 90, 91].

1.5 COAGULATION AND FIBRINOLYSIS

The haemostatic system contributes to a variety of body defense systems that are essential for a normal life. Amongst other factors, the system includes coagulation factors and their inhibitors, and fibrinolysis factors and their inhibitors. Perfect haemostasis means no bleeding and no thrombosis.

1.5.1 Fibrinogen

Fibrinogen is a large (340-kD) plasma protein which is formed of two α-, β- and γ-chains. The protein chains of fibrinogen are connected by several disulfide bonds. Fibrinogen is soluble in plasma but can be converted to insoluble fibrin in the process of coagulation (occurring at the end of the coagulation cascade under the influence of thrombin). The fibrin networks build up what we call "the clot"[92].

Elevated plasma fibrinogen is caused by increased synthesis and/or reduced removal of fibrinogen from the circulation. Fibrinogen is an acute phase protein, synthesized in the liver, and its levels rise in response to infections, inflammations and trauma[93]. Other factors that correlate with elevated levels include smoking[94, 95], family history of premature heart disease[96, 97], hypertension, age, obesity, cholesterol levels, hormonal changes and diabetes[98].

1.5.2 The G/A-455 polymorphism

The three polypeptide chains of the fibrinogen protein are encoded by three different genes clustered within a 50kb region located in the distal third of the long arm of chromosome 4q23-32[99, 100]. In vitro studies have suggested that β-chain synthesis limits the rate of the production of mature fibrinogen[101, 102]. Therefore, most studies within this field of research have focused on the β-chain. In the promoter region of the β-fibrinogen gene, a G/A sequence variation at position -455 was detected (using restriction enzyme HaeIII)[103, 104]. This polymorphism has been studied in many epidemiological studies. Generally, results show that the occurrence of the A allele at this polymorphic site is associated with increased levels of fibrinogen of about 0.3 g/L as compared with homozygotes for the G allele[105]. The A^{-455} allele is present in about 20% of the general population.

1.5.3 Plasminogen Activator Inhibitor -1

The fibrinolytic system is responsible for the lysis of fibrin clots (but also has other functions not discussed here). The central enzyme of the fibrinolytic system is plasminogen, and the two most important activators of this enzyme are tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA).

Fibrinolysis involves the binding of plasminogen and tPA to the fibrin surface where plasminogen is cleaved by tPA into its active form, plasmin. Plasmin then cleaves the fibrin filaments. The two activators t-PA and u-PA have a specific inhibitor, PAI-1.

PAI-1 is a 50 kDA glycoprotein of 379 aminoacids and belongs to the serine protease inhibitor (serpin) family. It is found in plasma and in thrombocytes. The PAI-1 that circulates in plasma is produced by a number of cell types including endothelial cells, hepatocytes, adipocytes and adipose tissue stromal cells. PAI-1 was first described in the early 1980´s[106, 107] and is considered to be the main regulator of fibrinolysis, due to its high specificity and fast action.

Being an acute phase reactant, PAI-1 increases in an acute state of inflammation but it can also rise during thrombosis[92]. Many factors regulate plasma PAI-1 levels, e.g. glucose and insulin[108], triglycerides and VLDL[109, 110], neurohumeral factors including the vasoconstrictor angiotensin II[111], aldosterone, and inflammatory cytokines (TNF alpha and IL-1), and thrombin[112]. The circadian variations of PAI-1 levels are considerable[113].

PAI-1 binds to t-PA and renders it inactive[114], forming the tPA/PAI-1 complex. Nordenhem et al. demonstrated a high correlation between level of PAI-1 activity and level of tPA/PAI-1 complex in plasma[115].

1.5.4 The 4G/5G polymorphism

The gene coding for PAI-1 has several polymorphic loci including a 4G/5G insertion/deletion –675 base pairs from the start site of the promoter[116]. Presence of the 4G allele at this polymorphic site has been reported to correlate with increased level of PAI-1, but study results are not consistent[117]. Further, the 4G/5G promoter site has also been suggested to exhibit genotype-specific responses to triglycerides, with the highest level of PAI-1 in 4G homozygous individuals with elevated triglycerides[118, 119]. It has been reported that metabolic features of the insulin resistant state account for a larger proportion of the PAI-1 variance in men as compared to genetic influences[120].

1.5.5 Fibrinogen and PAI-1 - associations with CHD

In the 1980s, the importance of thrombi in cardiovascular disease became apparent[121-123]. Meade et al. were among the first to report an association between plasma levels of fibrinogen and cardiovascular disease in the Northwick Park Heart Study[122]. This prompted others to test the hypothesis that increased levels of coagulation factors (or fibrinolysis inhibitors) would be associated with increased risk of cardiovascular events. Several prospective studies have reported an association between high level of plasma fibrinogen and risk of CHD[9, 94, 123, 124]. Evidently, fibrinogen is involved in the process that leads to thrombosis, and it seems to have several functions such as a role as a substrate for fibrin formation, as a mediator of platelet aggregation, and as a determinant of plasma viscosity[9, 125]. A

meta-analysis performed in 1998 by Danesh et al. using data from 18 different reports, showed that fibrinogen levels in the top third were associated with an OR of 1.8 (95% CI 1.6-2.0)[126]. Results from the PRIME (Prospective Epidemiological Study of Myocardial Infarction) Study performed in four centres of the MONICA program also provide epidemiological evidence for a role of fibrinogen in the pathogenesis of CHD[6]. High fibrinogen is certainly a consistent risk factor in several studies. However, fibrinogen level clusters with other risk factors, and although an elevated level could have effects on atherogenesis and thrombogenesis, the opinion of many investigators is that there is still no proof that fibrinogen is a cause of CHD[5]. The haemostatic factors are intimately correlated; thus, focusing on one factor to the exclusion of others may be inappropriate[8].

An impaired fibrinolytic function, as measured by increased PAI-1 activity, has been found to increase the risk of MI[127] and CHD[6, 7]. However, evidence has accumulated, indicating that PAI-1 predominates in the insulin resistance syndrome[23-25]. Therefore, the association between PAI-1 and cardiovascular disease is probably to some extent mediated by vascular risk markers present in the insulin resistance syndrome[7, 128].

Although presence of the A allele at the G-455 A polymorphism of the β-fibrinogen gene seems to correlate with elevated levels of plasma fibrinogen (as stated above), most investigators have not found evidence for an association between the presence of this allele and increased risk of CHD. Out of 13 studies evaluating this association reviewed by Vischetti et al., only 5 reported the presence of an association[105]. In a pooled analysis of case-control studies, Boekholdt et al. even found presence of the A^{-455} allele to be protective against MI[129].

Study results give conflicting evidence on the strength of the relation between PAI-1 gene polymorphisms and risk of MI[130]. A meta-analysis performed by Iacoviello et al. in 1998 studied the effect of PAI-1 genotype on risk of MI and demonstrated a weak effect regarding presence of the 4G allele[131]. Similar results were obtained by Boekholdt et al. performing a pooled analysis of 7 case-control studies; the 4G allele was associated with a slightly increased risk of MI, OR 1.2 (95% CI 1.0-1.4)[129].

1.6 OTHER EXPOSURES CONSIDERED

Apart from family history of CHD, plasma fibrinogen, plasma PAI-1, and two specific genetic exposures (all described in earlier paragraphs), the analyses in this thesis also include a number of other established cardiovascular exposures. These have been analysed regarding their potential interactive effects in combination with other exposures for the risk of MI, and also regarding their possible role as confounding factors to the observed associations.

Variables mainly based on questionnaire data include current smoking, overweight, diabetes mellitus, physical inactivity, job strain and socioeconomic position.

Variables mainly based on data from the physical examination include hypertension, hypercholesterolemia, high LDL, low HDL, high LDL/HDL quotient, hypertriglyceridemia, high apolipoprotein B, low apolipoprotien A1, high Lp(a), high C-reactive protein, high fasting insulin, high tPA/PAI-1 complex, and high von Willebrand factor.

Exposures secondary to the initial MI (collected for hospitalised cases) include high peak levels of acute cardiac enzymes (Creatine kinase (CK), CK-MB, CK-B and Lactate Dehydrogenase (LD)), heart failure, ventricular arrhythmia, supraventricular arrhythmia, atrioventricular block, thrombolytic therapy, Percutaneous Transluminal Coronary Angioplasty (PTCA), coronary surgery, and administration of beta blockers, diuretics, ACE inhibitors, nitrates, platelet inhibitors, calcium antagonists and statins.

For definitions of the variables enumerated above, see section under Methods. Here, comments on selected definitions of exposures will be given.

Firstly, the definition of diabetes mellitus used in all four papers did not include blood glucose criteria; for this reason a number of undetected cases of diabetes mellitus may have been classified as unexposed. However, at the time when the SHEEP data were collected, our definition was widely applied. The classification of exposure to hypercholesterolemia was also considerably more conservative as compared with the definition of today. (According to current clinical guidelines, cholesterol levels above 5.0mmol/L are considered elevated[132]). Further, the classification of exposure to hypertriglyceridemia is also somewhat conservative, using a fixed cut-off limit 0.6 units higher than the cut-off limits suggested by current clinical guidelines[132]. The exposure to high peak level of acute cardiac enzymes should indicate the size of the initial MI. At the time when the SHEEP cases were hospitalised, the measurement of CK-MB was not routinely performed at all participating hospitals. Further, the use of statins had not yet become widespread.

2 AIMS OF THE THESIS

In men and women respectively, the aims of the thesis were:

- to assess family history of CHD as a risk factor for non-fatal first-time acute MI.

- to study the haemostatic factors plasma fibrinogen and plasma PAI-1 regarding their potential associations with increased risk of non-fatal first-time acute MI. Further, to study the possible influence on MI risk of the G-455A polymorphism at the fibrinogen Bβ-gene and of the 4G/5G polymorphism at the PAI-1 gene, as well as to assess their associations with plasma levels of fibrinogen and PAI-1 respectively.

- to explore potential indications of biological interactions in causing MI, involving the genetic- and environmental factors under study.

- in patients who survived an initial MI, to assess the importance of a large number of cardiovascular risk exposures in relation to risk of recurrent non-fatal MI or death from CHD in the years after their first MI.

3 MATERIAL AND METHODS

3.1 THE SHEEP STUDY

The thesis is completely based on material from the Stockholm Heart Epidemiology Program (SHEEP), a large population-based case-control study. The study population comprised all Swedish citizens living in the county of Stockholm who were 45 to 70 years of age and had no previous clinical diagnosis of MI.

3.1.1 Cases

All first-time acute MI events in the study population were eligible for identification as cases. The criteria for MI diagnosis were specified by the Swedish Association of Cardiologists in 1991[133] and included (1) certain symptoms, according to case history information; (2) specified changes in blood levels of the enzymes serum creatine kinase and serum lactate dehydrogenase; and (3) specified electrocardiogram changes. Male cases were identified during a 2-year period, 1992-1994, and female cases during a 3-year period, 1992-1994. During the period January 1 to October 31, 1992, the upper age limit for subjects was 65; from November 1, 1992, and onwards it was 70 years.

The cases identified in SHEEP were classified as non-fatal if they survived at least 28 days after the day of their diagnosis (which is an internationally accepted definition), and as fatal if they did not. The non-fatal cases were mainly identified through a special organization set up at the 10 emergency hospitals within the county of Stockholm (89% of male cases and 80% of female). The remaining proportions of male and female non-fatal cases included in SHEEP were identified by checking in discharge registers. The fatal cases were mainly identified through preliminary death certificates (85%). The sources of identification of the remaining fatal cases were (1) the special hospital organization mentioned above (9%), and (2) the discharge registries (6%). These proportions were identical for men and women.

3.1.2 Controls

One control per case was randomly sampled from the study base within 2 days of the case occurrence, using the computerized registers of the population of Stockholm. In order to increase the efficiency of the study, the random sampling of controls was restricted to occur within strata of individuals with the same sex, age (within a 5-year interval) and residential area as the case in question. Each control candidate was checked for previous MI events since 1975 using the computerized hospital discharge register for the county of Stockholm (ICD9-codes 410, 412, or corresponding codes in previous ICD revisions). Five control candidates were sampled at the same time, so that a potentially non-responding control could be

replaced by another control who belonged to the study base at the time of the case occurrence. Occasionally, both the initial- and a substitute control were included, owing to a late response from the initial control. Therefore, more controls than cases were finally included.

3.1.3 Collection of exposure data

Postal questionnaires covering a wide range of exposure areas were distributed to non-fatal cases and to their controls. Non-respondents were reminded at least four times. Occasionally, missing answers appeared on separate questions and these were asked for in a supplementary telephone interview. The questionnaires of fatal cases were distributed to a close relative 6 to 12 months later. Here, no reminders were given. However, a supplementary telephone interview was occasionally carried out.

Non-fatal cases were invited to a health examination, which included blood sampling, blood pressure measurements and anthropometrical tests, about 3 months after disease onset (and inclusion in SHEEP). This time interval was chosen in order to allow for cases to regain a metabolic stable state[134, 135]. The examination date for the controls was set as close as possible to that of the corresponding case in order to avoid bias due to seasonal variation in the blood parameters. All subjects were asked to fast overnight before attending the physical examination that always took place in the morning hours. The nurses responsible for the physical examinations were unaware of the individuals´ status as case or control. The blood pressure values were recorded as the mean of two readings taken in supine position after 5 minutes of rest. After 10 minutes of rest in the supine position, the patients had blood drawn from an antecubital vein into evacuated tubes (containing sodium citrate [final concentration 0.129 mol/L], EDTA, or nothing for serum samples) with use of minimal stasis. The citrated blood samples were centrifuged within 30 minutes, and plasma was immediately frozen in aliquots and stored in -70° C until they were transported in appropriate freezer-bags to the central SHEEP biobank. Samples were then analysed (within one month) by trained laboratory technicians at the Department of Clinical Chemistry, Karolinska Hospital, in a random, blinded manner to reduce any bias. Cholesterol and triglycerides were analysed in serum from fresh samples. Plasma fibrinogen was determined in samples that had been kept frozen at -70° C. At the same department, DNA was extracted from blood for the purpose of genotyping.

3.1.3.1 Methods used for the analyses of different parameters

Total cholesterol	Enzymatic colometric method (Kodak Echtachem)
Triglycerides	Same as above
HDL cholesterol	Same as above
LDL cholesterol	Calculated according to the Fridewald formula[136-138]
Fibrinogen	Fibrin polymerisation test according to method described by Vermylen et al.[139]

G/A-455 polymorphism	Genotyping using a method described elsewhere[140], with some modifications, see paper II
PAI-1 activity	Spectrolyze PAI-1 kit (Biopool AB)
4G/5G polymorphism	Genotyping using a method described elsewhere[141]
tPA/PAI-1 complex	Biopool TintElize tPA and TintElize tPA/PAI-1
C-reactive protein	Immunonephelometric system (Dade-Behring, Marburg, Germany)
ApoA1, Apo B, Lp(a)	Immunichemical techniques
vWF	Enzyme-linked immunosorbent assays (ELISA)
Insulin	RIA kits[142]
Interleukin-6	ELISA method

3.1.4 Collection of data on secondary exposures

When a case fulfilling the SHEEP criteria was admitted to a participating hospital, the attending physician or nurse received a specific SHEEP form to fill out regarding details about the MI diagnosis, the occurrence of complications, interventions, and the administration of medical substances. The form was to be signed by the attending physician.

3.1.5 Numbers of participants

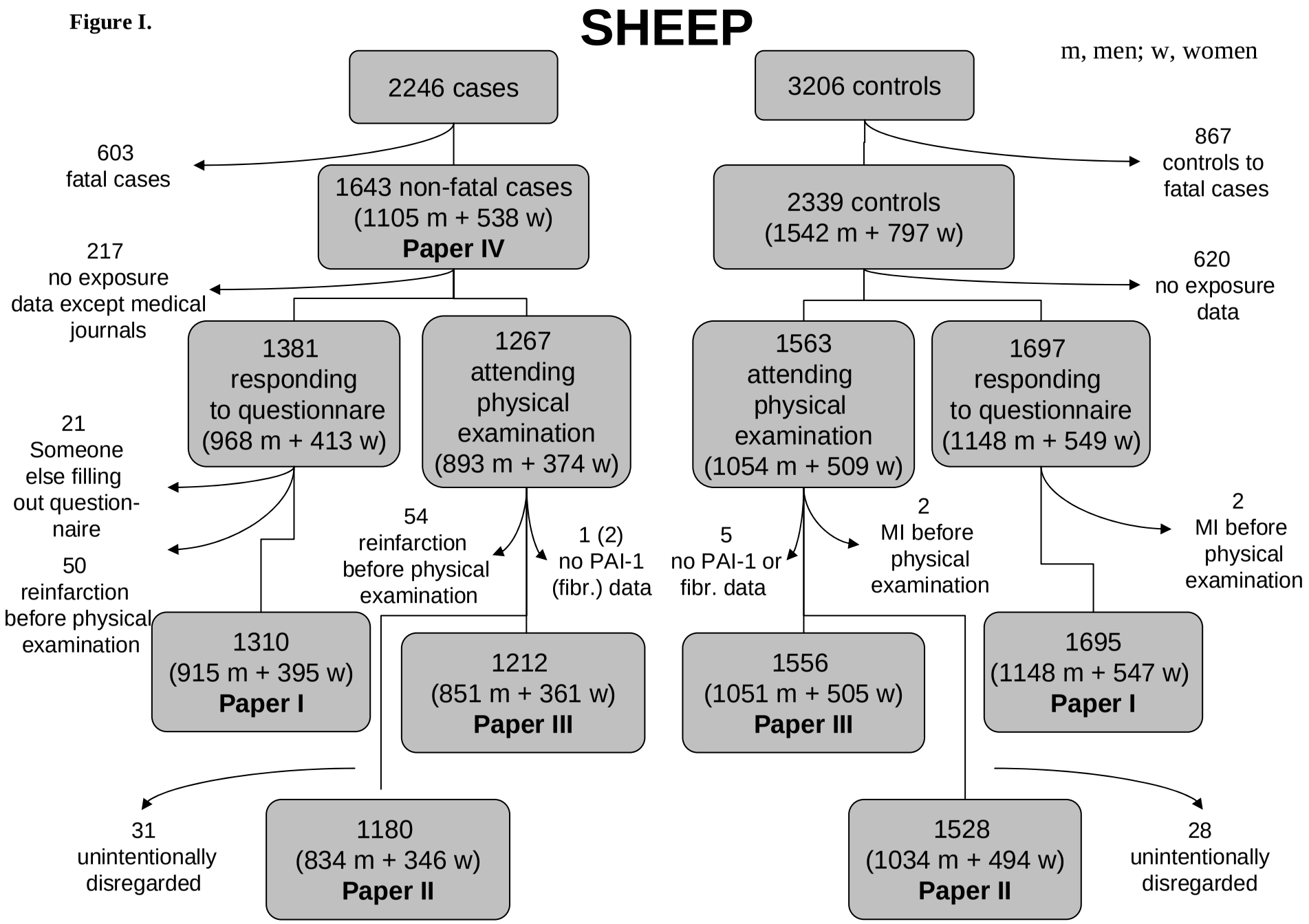
In total, 2246 cases were identified in the SHEEP and 3206 controls were included. For the four present sub-studies of SHEEP, only patients who survived at least 28 days after their MI were included because analyses within the papers are mainly based on blood samples (naturally not available from the fatal cases). The total number of identified non-fatal cases was 1643 (1105 men and 538 women) and the corresponding controls amounted to 2339 (1542 men and 797 women). The participation rates amongst these (either in questionnaire or health examination) were 87% (90% in men and 80% in women) and 73% (75% in men and 70% in women). More detailed information on numbers of participants and selection criteria for paper I-IV is shown in figure I.

As indicated in figure I, 60 SHEEP individuals were “unintentionally disregarded” in Paper II. Plasma fibrinogen values for these individuals were actually available, but they were unintentionally disregarded when merging SAS data-files over SHEEP data. However, this did not affect results.

Although participants attending the physical examination were requested to have fasted overnight before their arrival, a small proportion (173 participants) stated that they had not fasted overnight or gave uncertain information regarding this matter. These individuals were not considered in analyses including triglycerides or insulin.

More details about the SHEEP study are found elsewhere[143, 144].

Figure I.


SHEEP
m, men; w, women
2246 cases
3206 controls
603 fatal cases
867 controls to fatal cases
1643 non-fatal cases (1105 m + 538 w) Paper IV
2339 controls (1542 m + 797 w)
217 no exposure data except medical journals
620 no exposure data
1381 responding to questionnare (968 m + 413 w)
1267 attending physical examination (893 m + 374 w)
1563 attending physical examination (1054 m + 509 w)
1697 responding to questionnaire (1148 m + 549 w)
21 Someone else filling out question-naire
50 reinfarction before physical examination
54 reinfarction before physical examination
1 (2) no PAI-1 (fibr.) data
5 no PAI-1 or fibr. data
2 MI before physical examination
2 MI before physical examination
1310 (915 m + 395 w) Paper I
1212 (851 m + 361 w) Paper III
1556 (1051 m + 505 w) Paper III
1695 (1148 m + 547 w) Paper I
31 unintentionally disregarded
1180 (834 m + 346 w) Paper II
1528 (1034 m + 494 w) Paper II
28 unintentionally disregarded

3.2 REGISTER DATA

For the purpose of paper IV, register data was used to obtain information on non-fatal recurrent CHD events, CHD mortality and total mortaliy. The register used was the National Acute Myocardial Infarction Register in Sweden that was initiated in 1996 by record linkage between the Hospital Discharge Register and the Cause of Death Register. (The register comprises all persons with MI, reported to either the Hospital Discharge Register or the Cause of Death Register.) The Hospital Discharge Register includes all patients discharged from public hospitals in Sweden. The Cause of Death Register comprises all deaths where the deceased was registered as a Swedish resident, whether or not the death occurred in Sweden.

3.3 DEFINITIONS OF EXPOSURES

3.3.1 Family history of CHD

Data on family history of CHD was collected through the use of questionnaires. In the beginning of the questionnaire section, subjects were informed that all questions were only concerned with biological first-degree relatives. It was specifically stated that half-siblings should not be considered. The questions forming the basis for the assessment of family history of CHD are shown in figure II (translated from Swedish). Predefined answers in bold were considered when assessing exposure to family history of CHD.

Family history of CHD was defined as having one or more first-degree relative(s) (biological parent, brother or sister) affected by CHD before the age of 65. Individuals who lacked knowledge of CHD occurrence in either a parent or a sibling were deleted from the main analyses in paper I but included (with “don´t know” answers set to “no”) in paper IV.

Figure II.

Question 161. Does your father live?

☐ Yes ⇒ If yes, how old is he? _____years

☐ No ⇒ If no, at what age did he die? _____years

⇒If no, what caused his death?

☐ **Infarction of the heart**

☐ Stroke (in brain)

☐ Other. What? _____

☐ Don´t know

Question 162. Was your father affected by any health problem before the age of 65?

☐ No, generally he was healthy

☐ **Infarction of the heart**

☐ **Vascular spasm in heart**

☐ Diabetes

☐ High blood pressure

☐ High blood lipids

☐ Stroke (in brain)

☐ Other. What? _____

☐ Don´t know

Question 165. Do you have any siblings? (do not count half siblings)

☐ Yes ⇒ Please answer the next question

☐ No

Question 166. How many are your siblings? *Number of siblings:* _____

Question 167. Please indicate if any of your siblings were affected by, or died from any of the following cardiovascular diseases before the age of 65:

Sibling number:	*1*	*2*	*4*	*5*	*6*
Infarction of the heart	☐	☐	☐	☐	☐
Vascular spasm in heart	☐	☐	☐	☐	☐
Stroke (in brain)	☐	☐	☐	☐	☐
High blood pressure	☐	☐	☐	☐	☐
Diabetes	☐	☐	☐	☐	☐
High blood lipids	☐	☐	☐	☐	☐
Don´t know	☐	☐	☐	☐	☐

3.3.2 Fibrinogen and PAI-1

Individuals with a plasma fibrinogen level above the 90th percentile value (in some analyses above the 75th percentile value) among the controls (male and female respectively) were classified as exposed.

Individuals with a plasma PAI-1 activity value above the 90th percentile value (in some analyses above the 75th percentile value) among the controls (male and female respectively) were classified as exposed.

3.3.3 Genetic variables

Individuals with presence of the A allele at the fibrinogen Bβ gene G-455A polymorphic site were classified as exposed.

Individuals with the 4G allele present at the PAI-1 gene 4G/5G polymorphic site were classified as exposed.

3.3.4 Other exposures

Variables based on data from questionnaire or physical examination

Diabetes mellitus:
Subjects who reported that they were controlling diabetes with diet, insulin, or other drug treatment were classified as exposed.

Overweight:
In papers I-III overweight was determined by using data on height and weight from the physical examination (or if not available, questionnaire data) to calculate Body Mass Index (BMI), cut-off value 28 kg/m2, corresponding to the 75th percentile value of the control group. In paper IV, overweight was determined by using the individual waist/hip ratio, cut-off 1.0 in men and 0.9 in women (corresponding to the 75th percentile value of the male and female control group).

Cigarette smoking:
Current smoking was defined as persons who smoked or had stopped smoking within the last two years. Ex-smoking was defined as having smoked daily but having stopped more than two years prior to examination.

Job strain:
Job strain was determined as having low decision latitude but high psychosocial demands as measured by questions derived from the Karasek-Theorell questionnaire[145].

Physical inactivity:
Individuals who reported inactive leisure time were defined as exposed.

Low socioeconomic position:
Using questionnaire data on the individuals´ occupation 10 years before inclusion in SHEEP, the exposed group included unskilled and skilled manual workers, low-grade non-manual workers, self-employed individuals, students and housewives.

Hypertension:
In paper I-III, individuals who received anti-hypertensive drug therapy or those with a systolic blood pressure (BP) ≥160 mm Hg or a diastolic BP ≥90 mm Hg were classified as exposed. The limit for systolic BP was changed to 140 mm Hg for paper IV to accommodate to changed clinical guidelines for classification of hypertension.

Hypercholesterolemia:
Individuals with total cholesterol levels ≥6.5mmol/L or receiving lipid-lowering medication were classified as exposed. This fixed cut-off was chosen according to the current guidelines at the time when the SHEEP data was collected.

Hypertriglyceridemia:
Individuals with fasting serum triglyceride levels ≥2.3 mmol/l were classified as exposed.

Other blood parameters:
All variables were dichotomised. The 75th value of the male and female control group, respectively, was used as cut-off when determining exposure to high Low-Density Cholesterol (LDL), high Apolipoprotein B, high Lipoprotein (a), high C-reactive protein, high von Willebrand factor, high tissue plasminogen activator (t-PA)/PAI-complex, high insulin, and high interleukin-6. The 25th percentile value of the male and female control group, respectively, was used as cut-off regarding exposure to low High-Density Cholesterol (HDL) and low Apolipoprotein A1. Cut-off value for exposure to high LDL/HDL quotient was 4.0.

Medications:
In papers I-III, data from questionnaires on the current use (or the use one week before the MI occurred) of lipid-lowering- and antihypertensive medications was used.

<u>Variables based on data from the hospitalisation (only concerns cases)</u>

High peak levels of cardiac enzymes:
The variables Creatine kinase (CK), CK-B, CK-MB, and Lactate Dehydrogenase (LD) were dichotomised. The 75th value of the male and female control group, respectively, was used as cut-off limit when determining exposure. Those classified as "exposed" were individuals where at least one of the four peak enzyme values was above the cut-off limit.

Complications:
"Exposure" to heart failure (degree II, III or IV), supraventricular arrhythmia, ventricular arrhythmia, and atrio-ventricular (AV)-block II-III was simply stated as yes or no.

Interventions:
"Exposure" to thrombolysis, PTCA, and coronary surgery was simply stated by yes or no.

Medications:
"Exposure" to beta blockers, diuretics, ACE inhibitors, nitrates, platelet inhibitors, calcium antagonists, and statins was simply stated by yes or no.

3.4 STATISTICAL ANALYSES

All statistical analyses were performed using SAS (versions 6.11; 6.12; 8e)[146].

Mean values of continuous variables were compared using the t-test (Proc ttest). Median values of variables with skewed distribution (PAI-1) were compared using the Kruskal Wallis test (Proc npar1way wilcoxon). The geometric mean plasma fibrinogen values in paper II, standardized for age, were compared using ProcGLM (model ANOVA). The analyses of Hardy Weinberg equilibrium were performed using the Chi-Square Test (chisq exact under Proc Freq).

In papers I-III, odds ratios (OR) were calculated as estimates of relative risks. The statistical model used to adjust for the influence of potential confounders was the logistic regression model (Proc Logistic), yielding OR with 95 % CI. All logistic regression models were unconditional (did not keep case-control pairs). Age and residential area were adjusted for by using indicator variables (5-year strata for age). Both these variables were included in all the logistic models because of their property as design variables in the study. Sex was also a design variable, but all results reported are gender specific.

In paper IV, hazard ratios (HR) with 95% CI using the Cox regression model (Proc Phreg) were calculated. The Cox regression model, also called the Proportional Hazards model, is designed for analyses of survival data, featuring the calculation of risks over a time period with changing incidence rates. Probabilities of surviving through each successive time interval are calculated. Age (in days) was chosen as the underlying time scale involving an adjustment for age in all the regression models. The influences of other potential confounding factors were considered by including these variables in the models.

The strategy used when considering potential confounding effects on the associations under study was the same in all four papers. Single adjustments for covariates were performed, as well as adjustments for combinations of covariates, in order to evaluate their impact on the results obtained. The covariates included in

multivariate models in previous studies of the particular association were also taken into account when deciding what covariates to include in the final model.

3.4.1 Biological interaction

We have defined biological interaction between two risk factors in accordance with Rothman and Greenland, i.e. the two risk factors (component causes) must share the causal responsibility[147]. In the absence of either one of the two risk factors certain cases of the disease would not occur. Thus, the empirical criterion of interaction is that the effect of the combined exposure on the two risk factors is of a different magnitude from what could be expected from the effects exerted by each one of them.

To assess biological interaction, we calculated synergy index scores (S) and the 95% CI, based on the ratio of the combined effects to the sum of the separate effects of the two risk factors[148, 149], see figure III. An S score ≠ 1.0 indicates departure from an additive effect between the two variables. An S score exceeding 1.0 indicates a synergistic effect, whereas an S score below 1.0 indicates an antagonistic effect. Potential influences of confounders on the different ORs in the model are adjusted for through the use of logistic regression (papers I-III)[150] or Cox regression (concerns paper IV where the SAS program used corresponds to the program used in papers I-III but was modified to Phreg/Cox regression).

Figure III. Model for analysis of biological interaction between two risk exposures (according to Rothman 1986[148]).

	Absence of exp. A	Presence of exp. A
Absence of exp. B	RR=1 (reference category)	RR_A
Presence of exp. B	RR_B	RR_{AB}

Synergy index score = $(RR_{AB}-1) / (RR_A + RR_B-2)$

Exp=Exposure; RR=Relative risk

4 RESULTS

4.1 PAPER I

Family history of CHD was present in about 50% of cases and 30% of controls as determined from answers to the questionnaire.

As expected, the presence of a family history of CHD (i.e. having any first-degree relative who were affected by CHD before the age of 65 years) was clearly associated with risk of MI in both men and women, adjusted OR (95% CI) 2.0 (1.6-2.6) and 2.1 (1.5-3.0) respectively.

A family history of CHD, defined as having at least 2 affected first-degree relatives (who were affected by CHD before the age of 65 years) yielded even stronger associations, with ORs of 3.4 (95% CI 2.1-5.9) in men and 4.4 (95% CI 2.4-8.1) in women.

The presence of a family history of CHD (≥1 first-degree relative affected before the age of 65) was observed to interact synergistically with certain other cardiovascular risk factors in increasing the risk of MI. In women these factors were 1) High LDL/HDL quotient, S 3.8 (95% CI1.5-9.7) 2) Current smoking, S 2.9 (95% CI 1.2-7.2) and 3) Job strain, S 2.1 (95% CI 0.7-6.2). In men, family history of CHD and the presence of diabetes mellitus seemed to act in concert in increasing the risk of MI, S 2.8 (95% CI 1.0-7.9). The ORs for single exposures and combined exposures to the interacting risk factors are shown in table I.

Table I. Odds ratios (OR) with 95% Confidence Intervals for single exposures as well as combined exposures in five different combinations of cardiovascular risk exposures, all combinations including family history of CHD (Exposure A).

	Exposure constellation	Exposure B			
Women					
		LDL/HDL ≥4	Current smoking	Job strain	Diabetes mellitus
	A but not B	1.8 (1.2-2.7)	1.7 (1.1-2.6)	1.8 (1.2-2.7)	2.3 (1.6-3.2)
	B but not A	2.1 (1.3-3.5)	1.7 (1.1-2.7)	1.3 (0.8-1.9)	6.7 (2.6-17)
	A and B	8.3 (4.5-15)	5.2 (3.2-8.6)	3.3 (2.0-5.4)	4.7 (1.7-13)
	Reference category (not A, not B)	1	1	1	1
	S	3.8 (1.5-9.7)	2.9 (1.2-7.2)	2.1 (0.7-6.2)	0.5 (0.1-2.7)
Men					
	A but not B	2.3 (1.7-3.1)	2.3 (1.7-3.1)	2.0 (1.6-2.6)	2.0 (1.5-2.5)
	B but not A	2.7 (2.0-3.6)	2.3 (1.8-3.1)	1.3 (0.9-1.8)	2.4 (1.5-4.1)
	A and B	4.5 (3.2-6.3)	3.8 (2.7-5.4)	2.5 (1.7-3.7)	7.7 (3.7-16)
	Reference category (not A, not B)	1	1	1	1
	S	1.2 (0.7-1.9)	1.1 (0.6-1.7)	1.1 (0.5-2.4)	2.8 (1.0-7.9)

S, Synergy index score
All data adjusted for age, residential area, current smoking, ex-smoking, job strain, physical inactivity, overweight, diabetes mellitus, hypertension, hypercholesterolemia, and low socioeconomic position (except for the factor under analysis).

4.2 PAPER II

High plasma fibrinogen was associated with increased risk of MI in both genders even though the associations were weakened after adjusting for possible confounding factors. Using the 90th percentile value in the control group as cut-off limit, the OR in men was 1.6 (95% CI 1.2-2.3) after adjustment for the potential confoun-

ding effects from age, residential area, smoking, hypercholesterolemia, physical inactivity, overweight, diabetes mellitus, and hypertriglyceridemia. In women, the corresponding OR was 1.5 (95% CI 0.9-2.6).

The presence of the A allele at the G-455A polymorphic site of the fibrinogen Bbeta-gene was associated with a higher level of plasma fibrinogen than the presence of the G allele. However, this difference was only significant for male cases. No evidence for an association between the presence of the -455 A allele and increased risk of MI was found.

There were no clear signs of biological interactions for the co-exposure to the -455 A allele and various environmental cardiovascular risk factors. However, diabetes mellitus in men and current smoking in women both yielded S score point estimates above 1.0 when combined with presence of the -455 A allele, S 3.1 (95% CI 0.7-14.1) and S 1.4 (95% CI 0.6-3.2), respectively.

No observation of a potential biological interaction involving high level of plasma fibrinogen was detected.

4.3 PAPER III

In crude analyses (but adjusted for age and residential area being study design variables) exposure to high plasma PAI-1 was associated with increased risk of MI in both genders, OR (95% CI) 2.1 (1.6-2.8) in men and 2.0 (1.3-3.2) in women, using the 90th percentile value in controls as cut-off limit. Further adjustments for smoking, hypercholesterolemia, physical inactivity, and high C-reactive protein led to lower OR: 1.9 (95% CI 1.4-2.8) in men and 1.5 (95% CI 0.9-2.5) in women. Even lower ORs were obtained after additional adjustments for the variables hypertension, overweight, diabetes mellitus and hypertriglyceridemia, all of which have been known to be part of the metabolic syndrome.

An interesting finding was a strong indication of a synergistic interaction between high plasma level of PAI-1 and current smoking in men. This result was robust, even with respect to risk factors included in the metabolic syndrome.

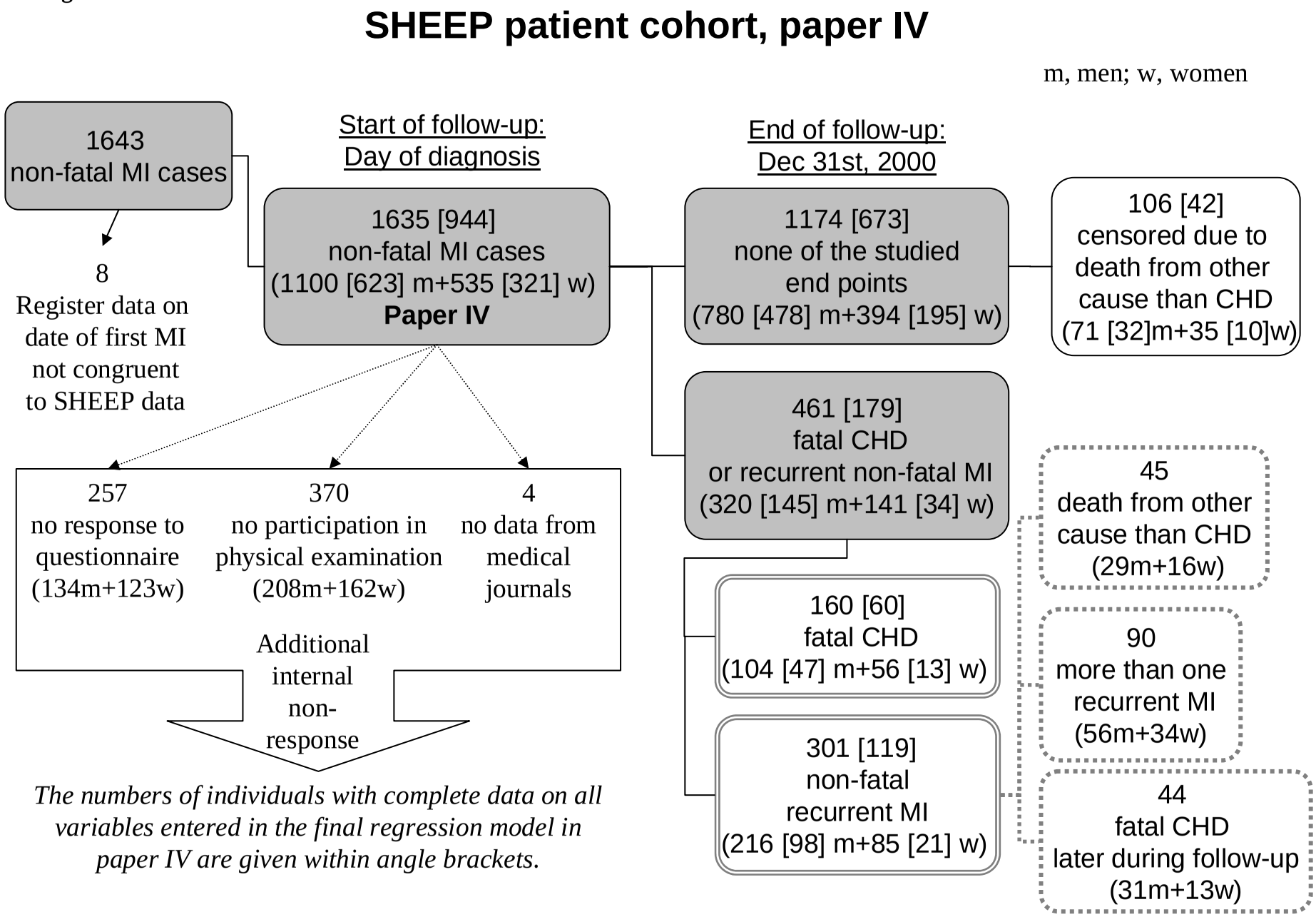
In male cases and female controls, presence of the 4G allele of the PAI-1 4G/5G polymorphism was related to higher plasma PAI-1 levels as compared to presence of the G allele. Presence of the 4G allele was slightly associated with increased risk of MI in women, OR 1.4 (95% CI 1.0-2.0), but not in men.

From the analyses of potential gene-environment interactions involving the PAI-1 4G/5G polymorphism, no clear indications of synergistic effects were obtained. However, the presence of the 4G allele in combination with e.g. presence of physical inactivity and overweight yielded S score point estimates substantially above 1.0, but with wide confidence intervals.

4.4 PAPER IV

An outline of study participants in paper IV is presented in figure IV.

Figure IV.



The numbers of individuals with complete data on all variables entered in the final regression model in paper IV are given within angle brackets.

In patients who survived at least 28 days after their MI, a number of the primary risk exposures were observed to continue exerting their effects. A summary of results for the variables most strongly associated with increased risk of the composite end point, recurrent non-fatal MI or death from CHD (in either men or women), is presented in table II.

Table II. Hazard ratios (HR) for recurrent non-fatal MI or death from CHD in men and in women. A selection of variables with prognostic importance according to the results of paper IV.

	Men		Women	
	HR (95% CI)	n	HR (95% CI)	n
Diabetes mellitus	1.6 (1.0-2.4)	623	2.5 (0.9-6.9)	229
Family history of CHD	1.4 (1.0-2.0)	623	1.0 (0.5-2.0)	229
Job strain	1.5 (1.0-2.1)	621	1.1 (0.5-2.4)	226
Apolipoprotein A1	0.9 (0.6-1.3)	622	2.3 (1.1-5.0)	228
High waist/hip ratio	1.4 (1.0-2.0)	623	0.8 (0.4-1.8)	229
High peak cardiac enzymes	1.3 (0.9-1.9)	623	4.4 (2.0-9.7)	229
Heart failure degree III-IV	2.2 (1.2-4.0)	623	1.0 (0.3-3.7)	229

Results adjusted for age (by using age as the underlying time scale), family history of CHD (defined as ≥ 1 first-degree relative affected by CHD before the age of 65), current smoking, ex-smoking, job strain, physical inactivity, high waist/hip ratio, diabetes mellitus, hypertension, hypercholesterolemia, low socioeconomic position, high peak cardiac enzyme, heart failure (degree II-IV), beta-blocker therapy, and thrombolysis (except for the factor under analysis).

The risk of suffering a non-fatal recurrent MI or of dying from CHD during follow-up was most strongly influenced by the presence of diabetes mellitus with multi-variate adjusted HR of 1.6 (95% CI 1.0-2.4) in men and 2.5 (95% CI 0.9-6.9) in women. Job strain in men also increased the risk of this end point, HR 1.5 (95%

1.0-2.1), whereas in women the risk was most strongly influenced by a low level of apolipoprotein A1, HR 2.3 (95% CI 1.1-5.0).

In men, the risk of dying from CHD during follow-up was particularly influenced by the presence of a family history of CHD, HR 2.0 (95% CI 1.1-3.6). In women, hypertriglyceridemia indicated increased risk of dying from CHD, even though the confidence interval was wide (HR 3.5; 95% CI 0.8-14.8).

In men, the risk of non-fatal recurrent MI was most strongly influenced by a high waist/hip ratio, HR 1.6 (95% CI 1.0-2.4). In women, high HR point estimates were noted for a number of variables, including low socioeconomic position and hypertension, but the confidence intervals were wide.

Exposures related to the incident MI (secondary exposures) also influenced the prognosis after MI. Size of the infarction as measured by heart failure in men and peak enzyme level in women showed the strongest influences.

Apart from weak indications of biological interactions in men between smoking and high PAI-1 activity, and between smoking and the presence of heart failure, no synergistic or antagonistic effects were observed. However, the power to detect such biological interaction was rather low, especially in women. Thus, important undetected biological interactions may still be in operation.

5 DISCUSSION OF RESULTS

5.1 PAPER I

5.1.1 Effects exerted by family history of CHD

Family history was observed to be associated with risk of MI in both genders even after adjustments for the effects of potential confounding factors. This agrees with several other studies[65, 66, 68]. Most of the earlier studies indicate that they aim to assess whether the effect is *independent* of other cardiovascular risk exposures. However, this choice of words must be questioned since it is in the nature of the family history variable that its occurrence depends on some other factors, genetic or environmental. Thus the effects exerted by a family history can never be independent. The interpretation of findings of "independent effects" would be that there are other factors in operation that are not accounted for in the analyses. From a primary prevention perspective the assessment of an "independent effect" may also seem paradoxical[73] because a large percentage of CHD is due to known risk factors that tend to cluster in families[73, 151]. Nevertheless, from a perspective of disease aetiology and mechanisms, it must be useful to try to refine and describe the effects exerted by a family history of CHD as far as possible.

Synergistic effects were observed for presence of family history in combination with current smoking in women. Thus, some components of the family history, genetic or environmental, interact biologically with the effects exerted by current smoking in increasing the risk of MI in women. A further investigation into which component of the family history may interact with current smoking in women is warranted. Some evidence for a biological interaction between smoking and other major cardiovascular risk factors such as dyslipidemia has been reported[152]. Evidently, a further investigation must also include an exploration of synergistic interactions between genetic markers and current smoking in women. Interestingly, both the genetic markers studied in this thesis (papers II and III) were indicated to be involved in a biological interaction with current smoking in women (point estimates however being unstable).

A corresponding discussion to the one above is relevant for the other exposures that were observed to interact synergistically with the presence of a family history of CHD, i.e. a high LDL/HDL quotient and job strain, respectively, in women and diabetes mellitus in men.

An association between high levels of fibrinogen and family history of CHD has been reported[96, 97, 153, 154]. However, fibrinogen showed no association with family history in a large study of middle-aged men of Irish or French descent by Yarnell et al. from 2003[155]. It has been suggested that fibrinogen may be of particular importance in subjects who, other than in connection with their family history, appear to

be at low risk in terms of conventional coronary artery disease risk factors[153]. Adjustments for high plasma fibrinogen in the analyses of family history of CHD in paper I did not alter results.

Considering the large proportion with a family history of CHD in the population and also the magnitude of the relative risk for this exposure, it may be of interest to estimate the proportion of MI cases in the population that are attributed to this exposure. Using formulas described by Rothman[156], firstly the "attributable fraction" (the proportion of disease burden among the exposed that is caused by the exposure) is calculated as (OR-1) / OR. Secondly, the "attributable fraction" is multiplied by the proportion of all cases in the total population that are exposed. Applying this formula to our data would involve the following calculation. Firstly, inserting the adjusted OR of 2.0 (in women the adjusted point estimate was 2.0 but this is rounded off) yields an "attributable fraction" of 50%. The proportion of exposed cases is 50%, which yields a proportion of 25% of all MI cases in the population being attributable to the exposure. In this discussion it is also highly relevant to consider that a proportion of the disease is likely to be attributed to synergistic effects between risk factors.

5.1.2 Bias adherent to the family history variable

The assessment of exposure to family history of CHD neither considers the size of the family nor the risk factor profile of family members. The probability that an individual is "exposed" to a family history of CHD depends on how many family members could possibly be affected. The background risk for the disease in the base population could also influence the prevalence of family history. From the SHEEP data information about family size is available. No clear difference regarding number of siblings was noted comparing cases and controls. A correlation between a large family and occupation as a farmer was noted. A spontaneous idea may be to adjust for family size as a potential confounding factor in the regression model. However, family size does not fulfill the epidemiological criteria for a confounding factor, i.e. being associated both with the exposure under study and with the study outcome. Belonging to a large family does not necessarily increase the risk of CHD although it increases the probability of having a positive family history of CHD. Including family size in the statistical model would therefore involve an adjustment for other potential effects related to both large family size and risk of MI, such as perhaps low socioeconomic position. A way to consider the aspect of family size may be to give different weights after the proportion of siblings that is affected when assessing a family history.

Restricting the analyses to include data on occurrence of CHD only in parents (not considering CHD in siblings) is a way of setting aside the influence of family size. As reported in paper I, such analyses yielded results similar results to those when data on siblings were included. Sibling data may however give important information about the degree of presence of family history. Furthermore, a recent study showed that the presence of disease in siblings could be particularly important for the risk of coronary artery calcification[157].

The potential influence of recall bias regarding CHD in close relatives must be considered. This problem has been studied by many investigators who have found that the influence of recall bias is generally small[78, 158, 159, 160]. Bensen et al. reported from a large validation study, as part of the NHLBI´s Family Heart Study, a sensitivity of 85% and a specificity of 93% for the proband´s report regarding CHD status in a parent (the parent´s self-report set as the gold standard)[159]. Another study by Watt et al. performed in West Scotland, reports a specificity of offspring reports of parental CHD deaths of 86%[60].

An investigation of 61 men and women from the Scottish MIDSPAN Study explored what factors may affect whether people regard themselves as having a family history of CHD or not. Results from qualitative interviews revealed that men, particularly working-class men, required a greater number of close relatives to be affected for them to perceive that they had a family history[161]. It has also been suggested that men may be less likely than women to report a family history of heart trouble[60, 162]. Such influences on the assessments of family history would most likely be non-differential.

It has recently been suggested that individuals who report a family history of CHD might be more likely to practise CHD-risk-reducing behaviour than those who do not report a family history[163]. Further, individuals with a family history of heart disease may be more likely to have their level of cholesterol measured as compared with individuals without a family history of heart disease[164]. Contradictory to these findings, a study by Watt et al. in 2000 showed that adult sons and daughters with experience of a parent having died from CHD generally did not perceive this as a family weakness attributable to heart disease[60]. Nevertheless, the potential occurrence of risk-reducing behaviours in individuals with a family history of CHD would probably be equally frequent in cases and controls, with a possible dilution of family influences.

5.1.3 Aspects of prevention

The importance of considering a family history of CHD in primary prevention seems unquestionable. It has been suggested that substantial advancements may be achieved by focusing on the family as a specific target for disease prevention[163, 81, 79]. Interventions with focus even on mild risk factors in individuals with a family history may have more preventive benefit than expected due to a removal of a potential synergistic effect.

Although there may be important opportunities to improve the efficiency of preventive actions, one must also consider the potential negative effects of an increased awareness of influence of family history on risk. People deal differently with thoughts, beliefs and information about their own inherited predisposition. When learning about the risks associated with a family history of CHD, people may tend to become fatalistic about health and disease. However, this kind of data are still scarce[162].

5.2 PAPERS II AND III

5.2.1 Effects exerted by fibrinogen and PAI-1

Several studies on the association between plasma fibrinogen and risk of MI have reported an effect of fibrinogen that could not be explained by presence of other factors[165]. The results of paper II confirm these findings. However, considering the number of biological processes where plasma fibrinogen seems to be involved, it is still possible that fibrinogen is merely a risk marker for MI and not a risk factor itself.

High PAI-1 activity has also been associated with risk of MI[7]. However, its´ close relation to the factors included in the metabolic syndrome seems established. In paper IV, the decreased risk estimates after including metabolic factors in the regression models indicate the presence of PAI-1 in the metabolic syndrome.

An increased susceptibility to environmental cardiovascular risk factors in individuals carrying the A-455 allele at the fibrinogen Bβeta gene G/A-455 polymorphic site has been suggested[105]. Reviewing gene-environment interactive mechanisms with special focus on plasma fibrinogen, Vischetti et al. conclude that in multifactorial diseases such as CHD, genetic variability influences the risk of disease by determining a different individual susceptibility to environmental risk factors[105]. Even though the present thesis shows no clear signs of biological interactions involving presence of the A-455 allele, nor involving presence of the 4G allele at the PAI-1 gene 4G/5G polymorphic site, the possibility of gene-environmental interactions involving these polymorphisms should not be ruled out. Clearly, even more large study materials than the present are needed to obtain more stable estimates regarding biological gene-environment interactions.

We observed a weak association in women between presence of the 4G allele at the PAI-1 4G/5G polymorpohic site and increased risk of MI. No association between presence of the fibrinogen Bβeta gene -455 A allele and risk of MI was observed. These results agree with the findings reported by Boekholdt et al. reviewing case-control studies including these genetic variants[129]: The pooled result of two case-control studies, including a total of 983 patients and 1121 controls, associated homozygosity for the –455A allele (compared with GG homozygosity) with decreased risk of MI (OR 0.66 95% CI 0.44-0.99). Further, the pooled result of seven case-control studies, including 2813 patients and 3358 controls, revealed that homozygosity for the PAI-1 4G allele was slightly associated with increased risk of MI, OR 1.2 (95% CI 1.04-1.39). No gender-specific data was presented[129].

5.2.2 Measurements of exposure

The method used for measuring PAI-1 activity in plasma is highly dependent on cautiousness while handling samples. However, all links in the handling of SHEEP blood samples were carefully performed and supervised. Specific samples intended for analyses of fibrinogen and PAI-1 were frozen to -20° immediately after

sampling, and frozen to (and kept at) -70° within 24 hours. Except for imprecise PAI-1 measurements of values near zero, measurements of both plasma fibrinogen and PAI-1 were reliable. Nevertheless, some misclassification may have occurred due to measuring errors, but they would be of a non-differential character. Misclassification of genetic exposures seems unlikely.

Some patients may have stopped smoking after the MI, which in turn perhaps would lower their level of plasma fibrinogen. Further, the taking of fibrates and statins in cases would have a possible lowering effect on their fibrinogen levels. The possible misclassification of exposure introduced would yield an underestimation of results. However, fibrates and statins were only prescribed to a small proportion of the SHEEP patients because these drugs were relatively new.

5.3 PAPER IV

Studying factors that influence the prognosis after MI is an important research area. Considering that MIs are fairly common among middle-aged men and women and that the affected individuals are easily reached and often highly motivated to reduce their risk of recurrent events, epidemiological data on prognostic variables may have large preventive impact. Such data may also guide us into possible biological mechanisms being in operation, for example by putting focus on those primary risk factors that seem to continue to exert their effect after a MI event. The research on prognostic factors after MI has apparent gaps that should be filled.

According to follow-up data on the patients with non-fatal MI, several primary cardiovascular risk exposures, as well as exposures secondary to the incident MI were associated with an adverse prognosis after MI. A difficult (and time-consuming) but important task was to decide which potential confounding factors to enter in the final multivariate regression model. No dramatic confounding effect by any specific covariate was observed. However, a number of covariates had moderate influence on results, but often only in combination with other covariates. Evidently, different influences from covariates on results were expected depending on the main variable under study. However, a final general model was chosen including a large set of covariates that all seemed important with respect to the entire spectrum of variables under study.

The large number of covariates included in the chosen model for adjustment of confounding effects unfortunately involved the exclusion of a substantial proportion of individuals from the analyses. Apart from the proportion of individuals that did not respond to the questionnaire or did not attend the physical examination, the 28% of men and 36% of women were excluded due to a missing value in any of the 13 variables included in the final model. Among these 13 variables, missing values occurred most frequently regarding heart failure (15% in both men and women) followed by hypercholesterolemia in men (5%) and thrombolysis in women (11%).

In order to address the question of a potential selection bias introduced by the proportion of individuals excluded due to internal missing values, crude results were reanalysed with the same restriction criteria of individual complete data in all 13 variables. These results were very similar to the crude results without the restriction. Despite the additional missing proportion introduced by including the large number of covariates in the model, it thus seems fair to point out the benefit of simultaneously considering all these potential confounding factors. The confidence intervals around the HR point estimates are not extremely wide (except for a few results in women).

The diabetes mellitus definition used is conservative as it does not consider blood glucose values. Thus, individuals with undetected diabetes are not defined as exposed and neither are a possible number of borderline diabetics. It has been shown that undetected diabetes may occur frequently in patients with MI [166]. Further, stress hyperglycaemia increases the risk of in-hospital mortality in patients with and without diabetes[167]. It has been suggested that a newly diagnosed glucometabolic state would also increase the long-term risk of cardiovascular events after MI, but data are still limited[168].

Although it was available, follow-up information after December 31st 2000 was not used because of an introduction of the new diagnostic criteria in the hospital care.

It could be speculated upon that a non-fatal recurrent MI would more likely be related to a progressing atherosclerotic process as compared to a recurrent MI that was fatal. The latter would perhaps be more related to the extent of the heart muscle damage. Unfortunately, however, we lacked a marker of the degree of atherosclerosis. Yet, by separately analysing the risk of recurrent non-fatal MI and the risk of fatal CHD, respectively, indications of different causal mechanisms may be discovered through comparing the observed importance of prognostic factors for each of these outcomes. Comparing a fatal outcome and a non-fatal outcome, our results do indicate a different pattern of prognostic importance for the variables considered. However, the power of these analyses is not sufficiently high to draw any strong conclusions.

In men, a prognostic effect of family history of CHD was observed. As no prognostic effects were observed for dyslipidemia or hypertension (factors likely to be rather frequent in individuals with a family history) this may indicate that non-lipid components of the family history influences the prognosis after MI in men.

The post-MI patient population is changing, a fact which limits the possibility to generalise results. For several years, the case fatality rate has declined, largely explained by an increased use of effective therapies such as thrombolytic and beta-blocker therapy[36-38]. The long-term prognosis after MI has also improved ever since the mid-60s, mainly explained by improvements of treatments[37, 169]. A further issue to consider when discussing whether study results are general is the possible difference between hospitals, regarding their tendency to use different therapies and regarding their secondary prevention strategies. However, in Sweden, such differences seem likely to be rather small[35].

The CHD mortality in Sweden is still considerably higher in comparison with many other countries, i.e. those in the Mediterranean region[29]. Thus, there is probably a potential for decreasing the rate of CHD mortality.

6 GENERAL DISCUSSION

6.1 CARDIOVASCULAR RISK EXPOSURES

Biological interaction between risk factors in causing MI has been very little explored as yet. Studies aiming to differentiate between synergistic (or antagonistic) effects on one hand, and additive effects on the other, are warranted. It was pointed out by Hallqvist et al. in 1996 that many studies aiming to analyse interactive effects do not perform these analyses in a strict epidemiological manner[170]. A survey of recent practice in the analysis and reporting of epidemiological data showed that interactions tests are rare[171]. However, the awareness of the problem is increasing[73, 91, 172]. With increasing knowledge of synergy effects of exposure to certain combinations of risk factors, genetic or environmental, we would be better able to pinpoint which individuals would benefit most from changing their lifestyle habits, such as giving up smoking or improving dietary habits. Furthermore, knowledge of potential interaction effects would certainly contribute to direct experimentally orientated research towards important biological mechanisms. Interestingly, this thesis identifies a number of possible synergistic interactions between various cardiovascular exposures.

No clear indications of effects on MI risk exerted by the genetic variables under study were observed. However, it is still possible that such effects may exist but that they could only be detected in an even larger body of data than the present material.

The potential different aetiology for MI comparing men and women has been much discussed. As an example, the gender difference in the age of onset of CHD is as yet unexplained[173]. Further, it has been suggested that the genetic influences on CHD death are only marginally mediated through the presence of traditional cardio-vascular risk factors among men, but more so among women[151]. In all four papers forming this thesis, sex was clearly an effect modifier, indicating gender-specific causal chains for MI.

As stated earlier, a number of individuals stated that they had not fasted or gave imprecise information regarding this matter when they arrived for the physical examination. Despite the fact that levels of PAI-1 may be somewhat affected by recent food intake, the non-fasting individuals are still included in the analyses of PAI-1 (papers III and IV). There was an equal proportion of non-fasting individuals among cases and controls. However, because of a possible misclassification of PAI-1 in the non-fasting individuals, data was reanalysed and restricted to fasting individuals. The results of reanalyses of papers III and IV were almost identical to those presented. Unfortunately, it was stated in paper III that only values of fasting individuals were considered, but this sentence is incorrect and should be deleted(see also errata to this thesis).

As explained under the heading Material and Methods, a number of 31 cases and 28 controls were unintentionally disregarded for inclusion in paper II. It is unlikely that this introduced any systematic bias. However, including also these individuals in the analyses, the geometric mean values for plasma fibrinogen remained the same and ORs for exposure to high level of plasma fibrinogen were almost identical with the ones that were published.

6.2 METHODOLOGICAL CONSIDERATIONS

6.2.1 Strengths

The major strengths of the SHEEP material are the large number of subjects included with extended information from questionnaires and biological parameters. Further, the study was carefully designed, well performed and clearly described in all details (much appreciated by later users such as myself).

Early descriptions of the case-control design indicated that these studies were less valid than cohort studies. However, this outlook has changed in parallel with the realization that a well-designed case-control study corresponds to a hypothetical cohort study from the same study base, the only difference being that the case-control study, instead of measuring exposures in all individuals included in the study population, uses a sample of the study population (that generated the cases) to estimate the exposure frequencies in this population. As cited from a recently published textbook in epidemiology: "Case-control studies represent a high achievement of modern epidemiology, and if conducted well, they can reach the highest standards of validity"[156].

Another important methodological strength is the complete follow-up of surviving MI patients that was possible with the use of the national MI register (linked with cause of death).

6.2.2 Information bias

A common drawback in case-control studies is the retrospective collection of data as it may involve information bias.

In paper I, data on family history of CHD is likely to be somewhat influenced by recall bias: Cases may reflect more, as compared with controls, over their "genetic" background in order to find explanations for the MI occurrence. This might then more frequently lead to a "better" recall of CHD events in close relatives, which would involve overestimated study results. Over-reporting of CHD in relatives is also possible because cases may try to find an explanation for their disease and thus may falsely remember events of CHD. Validation studies on the matter indicate that recall bias in case-control studies of family history of CHD are unlikely to exert any substantial bias on the results[158, 160]. Studies have demonstrated a sensitivity of 68%

to 86%, and specificities ranging from 86% to 98%, for reported family history of CHD[158, 159, 174].

In papers II and III, the retrospective collection of data on fibrinogen and PAI-1 respectively must be considered. However, the genotype variables are not affected by this potential source of error. Patients and controls were invited to the physical examination about three months after the SHEEP inclusion of the case. Fibrinogen and PAI-1 may initially be increased because of the inflammatory response due to the MI event, but repeated measurements of orosomucoid have shown that the acute inflammatory reaction is gone by three months[134, 135, 175, 176].

Values of blood parameters measured in cases should in general reflect the exposure before the MI occurred; It has been shown that after three months MI patients have regained metabolic stability[134, 135, 175]. However, the systolic and diastolic blood pressure may still be lower three months after the MI. The reduction in blood pressure has been correlated to indices of "infarction size"[175]. Blood pressure values of SHEEP cases were in general lower as compared with controls. Apart from the possible reductive influence on blood pressure of the initial infarction, the use of medications with an antihypertensive effect was more common among cases than among controls.

It is possible that lifestyle changes as well as interventions in cases during the first three months could have influenced the observed frequencies of exposures. Such influences would probably involve underestimation of results. Furthermore, most exposures considered were markedly higher among cases as compared with controls.

6.2.3 Non-participation

The response rates for cases as well as controls were similar in different age groups. Further, the response rates across different residential areas were similar. This should in general reduce the risk of bias due to age or residential area.

Even though the proportion of non-responders in SHEEP is relatively low, it is possible that non-responders differ from responders with regard to their pattern of exposure. Probably, however, the reasons for non-participation are similar for the group of cases and for the controls. Still, the fact that the response rate differed between cases and controls must be considered. The lower response rate in controls as compared with cases may have several explanations. One hypothesis would be that individuals who have experienced CHD in their close family might be particularly motivated to participate in a study about MI. If so, such a motivating effect is likely to occur in both cases and controls, but is perhaps even more likely to occur in cases since they have just experienced an MI themselves. The hypothetical differential non-participation introduced would involve study results that are underestimated.

It is notable that the participation rate among cases is correlated to the outcome after MI, as indicated from the follow-up data in paper IV. The non-response was higher in patients who suffered a recurrent MI or died from CHD during follow-up as compared with the other patients. Thus, the severity of the disease seems to have had an effect on the decision to respond, and thus the exposure pattern may differ between the groups. It is difficult to evaluate how this would influence results, but it seems reasonable that family history, high fibrinogen, high PAI-1 and other exposures, if anything, would occur more frequently in individuals with more severe CHD. If so, study results are underestimated.

Men were more likely to agree to participate than women; this naturally means that there is less uncertainty regarding the influence of non-response in men compared with women.

6.2.4 Patients with fatal MI

The exclusion of cases in SHEEP who died within 28 days after the day of their diagnosis of MI may be a limitation. It is possible that this group of MI patients was differently exposed as compared with surviving cases, i.e. the causal chain of disease mechanisms may be different for a fatal MI event in comparison with a non-fatal one. In an effort to evaluate this hypothetical difference, questionnaire data from close relatives to fatal cases were used. The response rate for relatives was 62%. Some characteristics of the patients with fatal MI are shown in table III.

6.2.4.1 Characteristics

The female patients with fatal MI were on average 1.7 years older than the males. Further, the female patients in general seemed to be more exposed to traditional cardiovascular risk factors compared with men. Similar to the difference in exposure frequencies between non-fatal cases and their controls, the fatal cases were substantially more exposed than their controls. Comparing the fatal cases with the non-fatal cases, the proportions of those who were exposed differed for some of the variables. Family history of CHD was somewhat less frequent in fatal cases, whereas physical inactivity, diabetes mellitus, job strain, and current smoking (the latter only in women) were more frequent.

Table III. Characteristics of SHEEP patients with fatal MI (as described by close-relatives to patients).

	Men				Women			
	Fatal cases	n	Controls	n	Fatal cases	n	Controls	n
Age	60.9± 6.8	380	60.9± 6.9	546	62.6± 6.2	223	63.1± 6.2	321
Family history of CHD, ≥1*	39%	137	16%	325	41%	87	31%	184
Family history of CHD, ≥2†	10%	93	4%	249	20%	64	10%	140
Current smoking	51%	236	30%	390	61%	137	36%	228
Ex-smoking	31%	236	33%	390	12%	137	21%	228
Physical inactivity	63%	227	37%	386	83%	134	43%	228
Diabetes mellitus	18%	233	7%	390	24%	136	3%	230
Job strain	31%	210	20%	389	49%	118	35%	223
Overweight	18%	233	7%	390	24%	136	3%	230

* ≥1 biological parent or sibling affected by CHD before the age of 65 according to questionnaire data.
† ≥2 biological parents or siblings affected by CHD before the age of 65 according to questionnaire data.

6.2.4.2 Risk of MI

In order to estimate if the impact of exposure to a family history of CHD was different comparing risk of a fatal MI with risk of non-fatal MI, ORs were calculated based on the fatal cases and their controls. Two sets of analyses were performed: 1) Analyses based on individuals who were able to give complete information about CHD in their mother, father and siblings, and 2) Analyses based on all individuals who had given some information about CHD in either a parent or a sibling. The results are presented in table IV. Overall, a family history of CHD was less strongly associated with risk of a fatal MI.

Table IV. Odds ratios (OR) for selected exposures in the SHEEP patients with fatal MI and their controls.

		Men			Women		
		OR	95%CI	n	OR	95% CI	n
Family history of CHD, ≥1 close relative affected	Crude*	1.8	1.2-2.8	462	1.5	0.9-2.6	271
	Crude* ("Don´t know" set to "no")	1.5	1.0-2.1	609	1.8	1.1-2.8	360
	Adjusted†	1.8	1.1-3.0	439	1.4	0.7-2.7	251
	Adjusted† ("Don´t know" set to "no")	1.4	0.9-2.1	565	1.5	0.9-2.7	324
Family history of CHD, ≥2 close relatives affected	Crude*	2.2	0.9-5.8	342	2.9	1.2-7.3	204
	Crude* ("Don´t know" set to "no")	2.1	0.9-4.8	462	3.1	1.4-6.8	272
	Adjusted†	2.0	0.7-6.0	327	3.3	1.0-10.3	188
	Adjusted† ("Don´t know" set to "no")	1.7	0.7-4.5	432	3.0	1.2-7.9	244

*Adjusted for age and residential area.
†Adjusted for age, residential area, current smoking, ex-smoking, physical inactivity, job strain, overweight, and diabetes mellitus.

6.2.5 The chosen model to analyse interaction

Although it is reasonable to assume that biological interactions between risk factors contribute to the causation of CHD[89, 172], there is still a lack of consensus on which epidemiological and statistical models are most appropriate for studying such mechanisms. In fact, the term interaction has no generally accepted definition. In many publications it is used to indicate effect-measure modification, a term which

has its own definition[156]. The term interaction is also frequently used to refer to statistical interaction, which is another concept[91].

This thesis uses a model for analysing presence of biological interaction in epidemiological material, first introduced by Rothman in 1974[177] and further described by Rothman in 1976[178] and 1986[148]. This method requires large sample sizes, which of course is a disadvantage. The crucial issue is often to get a sufficient number of individuals that are simultaneously exposed to both of the exposures under analyses. However, the method has important advantages, as it evaluates both synergistic and antagonistic effects from a combined exposure.

7 CONCLUSIONS AND FUTURE PERSPECTIVES

The results of this thesis not only confirm what earlier works have shown regarding an association between presence of a family history of CHD and increased risk of MI, but it also extends our knowledge on the influences of family history of CHD as strong risk factor for MI. The observed relative risk of a non-fatal first-time acute MI was approximately doubled in both men and women with at least one first-degree relative affected by CHD before the age of 65. Having at least two such relatives yielded a relative risk of more than 3 in men and more than 4 in women.

The results also suggest the occurrence of several biological interactions between risk factors for MI, including family history of CHD. The interactive effect of a family history of CHD is interesting because it indicates that gene-environment interactions are in operation that could perhaps be further explored biologically. In addition, knowledge about biological interactions involving a family history of CHD may open out for preventive considerations.

Elevation of the fibrinolytic factor plasma PAI-1 was clearly associated with risk of MI, with an observed relative risk of about 2 in men and women respectively. Most striking was the strong synergistic effect observed for the combination of exposure to current smoking and high plasma PAI-1 activity in men. This result may have clinical implications in the way that male smokers with elevated level of PAI-1 could constitute a particular group for effective primary preventive actions. However, it is important to realize that these results must be repeated in other epidemiological materials before firm conclusions can be made and consequently transferred into clinical usefulness.

No clear indication of association with risk of MI was observed for either of the two haemostatic genetic variants under study, although some weak effects were noted. Potential associations between genotype and MI, possibly through interaction with certain environmental factors such as smoking, could be detected in an even more large study material than the SHEEP. Even though the epidemiological case-control design is well suited to analyse associations between genetic factors and disease, the analyses of biological interactions require very large study materials to elicit distinct evidence of synergistic or antagonistic effects. It must be realised, however, that in a complex multifactorial disease such as MI, numerous genes are involved, either increasing or decreasing individual susceptibility to environmental factors.

8 SUMMARY IN SWEDISH

Hjärtkärlsjukdomar är ett av våra största folkhälsoproblem – det orsakar mycket lidande och stora vårdresurser krävs. Bland kärtkärlsjukdomarna domineras dödsorsaksstatistiken av kranskärlssjukdom, där hjärtinfarkt är den vanligaste diagnosen. Sedan länge har vikten av förebyggandet av dessa sjukdomar insetts, både primärt och sekundärt (hos redan drabbade individer). Dock är hjärtkärlsjukdomarnas etiologi långt ifrån klarlagd.

Den underliggande sjukdomen hos merparten av hjärtinfarktpatienterna är ateroskleros i hjärtats kranskärl. Detta innebär att det på blodkärlens insida finns fettrika inlagringar (s.k. plack) och sannolikt att inflammatoriska processer pågår. Blodcirkulationen blir sämre och ischemi kan uppstå. Av okänd anledning kan en spricka uppstå vid ett plack i kärlväggen med en intimablödning som följd. Detta aktiverar blodets koagulationsmekanismer och en trombos kan bildas. Trombosen förhindrar tillförseln av nytt syrerikt blod till ett visst område av hjärtat, och hjärtinfarkten är ett faktum.

Förekomst av kranskärlssjukdom hos nära anhöriga, s.k. familjaritet för kranskärlssjukdom, förekommer oftare hos individer som drabbats av hjärtinfarkt jämfört med sådana som ej drabbats. Familjaritet anses också vara en s.k. oberoende risk faktor för kranskärlssjukdom och hjärtinfarkt, då dess effekt inte helt har kunnat förklaras av anhopning av traditionella kardiovaskulära riskfaktorer i de drabbade familjerna. I flera studier har en relativ risk omkring 2.0 redovisats för förekomst av familjaritet - detta efter justering för ett antal faktorer som skulle kunna ”störa” analysen av familjaritetens effekt såsom högt blodtryck, höga blodlipider, rökning etc. Familjaritetens betydelse för uppkomsten av hjärtinfarkt kan vara genetiskt betingad men kan också bero på att ännu icke identifierade omgivningsmässiga faktorer ansamlas i vissa familjer. Förekomsten av interaktioner mellan genetiska och omgivningsmässiga faktorer är också en trolig förklaring till dess betydelse. Individer med vissa genetiska anlag skulle kunna vara särskilt känsliga för viss typ av omgivningsmässig exponering, dvs ha en genetisk predisposition för hjärtinfarkt. Kunskaperna om eventuella interaktiva effekter är idag mycket ofullständiga men intresset för interaktionsanalyser är växande då de kan ge värdefull information för hypotesbildning om sjukdomsmekanismer. Dessutom kan kunskapen om vilka riskfaktorer som förstärker varandras effekter ge förbättrad teoretisk grund för preventionsarbete.

Ett viktigt led i hjärtinfarktsjukdomens orsakskedja utgörs troligen av benägenheten för trombosbildning. Balansen mellan koagulationsfaktorer och faktorer som motverkar koagulationsprocessen, s.k. fibrinolysfaktorer, har i tidigare studier visat sig vara betydelsefull. I denna avhandling ingår epidemiologiska analyser av plasmanivåer av fibrinogen och plasminogen activator inhibitor-1 (PAI-1) samt för var och en av dessa faktorer analyser av en genetisk polymorfi som i tidigare studier visat

sig kunna ha betydelse för hjärtinfarktrisk (fibrinogen-Bbeta-genens G/A-455 polymorfi respektive PAI-1-genens 4G/5G-polymorfi).

Med tillgång till ett stort epidemiologiskt material, SHEEP (Stockholm Heart Epidemiology Program), som inkluderar både män och kvinnor har vi haft möjligheter att studera ovan nämnda faktorer och förekomsten av potentialla interaktioseffekter för olika kombinationer av riskfaktorer, genetiska såväl som omgivningsmässiga. Studiebasen för SHEEP inkluderar alla individer som tidigare inte haft kliniskt diagnosticerad hjärtinfarkt, var svenska medborgare och bosatta inom Stockholms läns landstings upptagningsområde. Studiebasen innefattade både män och kvinnor under en period av ca 2 år (1992-1993) och endast kvinnor under ytterligare ett års tid (år 1994). Under de första 10 månaderna (jan-okt 1992) innefattades individer i åldrarna 45-65 år emedan den återstående tiden innefattade åldersintervallet 45-70 år.

Avhandlingsarbetet baseras på de 1643 fall av hjärtinfarkt (förstagångsinsjuknanden) som identifierades i SHEEP där patienten överlevde minst 28 dagar efter datumet för diagnos, samt de kontrollpersoner som slumpmässigt valdes ur studiebasen i samband med fallets insjuknande, totalt 2339 individer. Exponeringsinformation insamlades i huvudsak via frågeformulär och genom en hälsoundersökning som ägde rum ca 3 månader efter fallets insjuknande. Hälsoundersökningen inkluderade blodprovstagning. För fallen insamlades även information från medicinklinikerna beträffande interventioner, eventuella komplikationer etc. Etiska tillstånd för projektet har erhållits.

Denna avhandling bekräftar att familjaritet för CHD är en stark riskfaktor för hjärtinfarkt för både kvinnor och män. Den ålders- och sjukvårdsområdesjusterade oddskvoten för hjärtinfarkt hos män med minst en nära anhörig som drabbats av CHD före 65 års ålder är 2,0 (95% konfidensintervall [KI] 1,6-2,5) jämfört med män utan familjaritet för CHD. Motsvarande oddskvot för kvinnor är 2,1 (95% KI 1,6-2,9). Då man istället klassar individer med minst två nära anhöriga som drabbats av CHD före 65 års ålder som exponerade blir oddskvoten för hjärtinfarkt 3,4 (95% KI 2,1-5,9) hos män och 4,4 (95% KI 2,4-8,1) hos kvinnor.

Kriteriet för interaktion, enligt den valda modellen för analys av potentiella synergistiska eller antagonistiska effekter, uppfylldes då exponering för familjaritet för CHD bland kvinnor kombinerades med förekomst av rökning respektive förekomst av en hög kvot mellan LDL och HDL kolesterol. Den synergistiska effekten som observerades var stark för båda dessa kombinationer av exponering. För män tycktes familjaritet för CHD interagera synergistiskt med förekomst av diabetes mellitus.

Hög nivå av fibrinogen i plasma var relaterat till ökad risk för hjärtinfarkt hos både män och kvinnor. Efter justering för en mängd tänkbara faktorer som skulle kunna påverka det observerade sambandet mellan högt fibrinogen och hjärtinfarktrisk, observerades oddskvoten 1,6 (95% KI 1,2-2,3) för män och 1,5 (95% KI 0,9-2,6) för kvinnor (definitionen av högt fibrinogenvärde baserad på det 90:e percentilvärdet hos den manliga respektive kvinnliga kontrollgruppen).

Hög PAI-1-aktivitet i plasma var relaterat till ökad risk för hjärtinfarkt hos både män och kvinnor. Oddskvoten för hjärtinfarkt hos män med en PAI-1-nivå högre än det 90:e percentilvärdet hos de manliga kontrollerna var 1,9 (95% KI 1,4-2,8) jämfört med män med lägre PAI-1-nivåer. Den motsvarande oddskvoten för kvinnor var 1,5 (95% KI 0,9-2,5). Dessa resultat erhölls efter justering för rökning, hyperkolesterolemi, fysisk inaktivitet och högt C-reaktivt protein. Det kunde vidare påvisas att hög PAI-1-aktivitet hos män i kombination med rökning ger en synergistiskt ökad risk för hjärtinfarkt. Detta resultat påverkades inte i någon större utsträckning av justering för potentiella s.k. confounding-faktorer, inte heller för justering för faktorer ingående i det metabola syndromet.

Utnyttjande data från det nationella hjärtinfarktregistret gjordes en uppföljning av de patienter i SHEEP vars hjärtinfarkt var icke-fatal. Syftet var att studera vilka faktorer som påverkar risken för återinsjuknande i hjärtinfarkt och död i CHD. Av de 1635 patienter som följdes upp (1100 män och 535 kvinnor) återinsjuknade 461 (320 män och 141 kvinnor) i hjärtinfarkt (fatal eller icke-fatal) eller dog i annan CHD. Bland de primära riskfaktorer som studerades visade sig diabetes mellitus vara en viktig prognostisk faktor för både män och kvinnor, hazard ratio 1,6 (95% KI 1,0-2,4) respektive 2,5 (95% KI 0,9-6,9). Hos kvinnor noterades även en negativ prognostisk effekt av låg nivå av apolipoprotein AI respektive lågt HDL-kolesterol. Hos män visade sig ”job strain”, dvs en kombination av höga krav och lågt beslutsutrymme i arbetslivet, ha en negativ inverkan på prognosen. Separat analys av risk för fatal CHD under uppföljningstiden visade att bland de primära exponeringarna hade familjaritet för CHD störst betydelse, men endast hos män. Hos kvinnor tycktes istället höga triglycerider öka risken för fatal CHD men konfidensintervallet var brett. Separat analys av risk för icke-fatal hjärtinfarkt under uppföljningstiden indikerade en ökad risk beträffande förekomst av övervikt (hög kvot mellan midje- och stussmått) hos män. Bland exponeringarna sekundära till den första hjärtinfarkten noterades en negativ prognostisk effekt för förekomst av hjärtsvikt hos män och höga maximum-nivåer av hjärtenzymer hos kvinnor. Båda dessa variabler kan ses som indikatorer på den initiala hjärtskadans storlek. Även förekomst av supraventrikulär arrytmi hos män tycktes öka risken för återinsjuknande.

Sammanfattningsvis, visade sig familjaritet för CHD vara en viktig riskfaktor för hjärtinfarkt hos både män och kvinnor. Det kunde också visas att familjaritet i kombination med ett antal andra etablerade kardiovaskulära riskfaktorer ger en synergistiskt ökad risk att drabbas av hjärtinfarkt. Detta tyder på förekomsten av biologiska interaktionsmekanismer där troligen genetiska faktorer bidrar. Förhöjda nivåer av fibrinogen respektive PAI-1 i plasma indikerade ökad risk för hjärtinfarkt. För de två genetiska polymorfier som studerades, potentiellt förknippade med fibrinogennivåer respektive PAI-1-nivåer, observerades vissa samband mellan genotyp och plasma-nivåer, emedan inga tydliga interaktionseffekter avseende hjärtinfarktrisk kunde påvisas. Efter en icke-fatal hjärtinfarkt tycks ett flertal primära exponeringar ha prognostisk betydelse liksom exponeringar sekundära till den initiala hjärtinfarkten.

9 ACKNOWLEDGEMENTS

This work has been performed at the Institute of Environmental Medicine, Karolinska Institutet. I would like to express my sincere gratitude to the following people who have contributed to this thesis.

First of all, my deep gratitude to my principal supervisor Ulf de Faire for introducing me to epidemiological cardiovascular research and for being an excellent supervisor. Thank you for believing in me from the start, for your encouragement and friendship, for making it possible for me to accomplish this thesis, for always having your door open, and for providing a pleasant research environment.

I am also deeply grateful to my co-supervisor Johan Hallqvist. Thank you for sharing with me your epidemiological knowledge and interesting ideas, for being an excellent source of information regarding the SHEEP material, and for taking great pains to assist me when needed.

All SHEEP patients and controls, thank you for making this research possible.

Gunnel Gråbergs, my colleague and SAS programming expert, thank you for providing skilful help, for always checking variables carefully, and for the last-minute assistance before print of the thesis. Thank you also for being such a good and reliable friend.

Annika Gustavsson at the SHEEP centre, thank you for all your valuable help with the SHEEP data.

Professor Björn Wiman at the Department of Clinical Chemistry, KS, my co-author, thank you for your valuable insights, your encouraging words and for your captivating enthusiasm regarding research.

Tomas Andersson, my co-author and an expert on statistical issues, thank you for all your interesting and useful insights, comments and advice. Christina Reuterwall, my co-author, thank you for your interest and valuable comments. Also, thank you both for all those badminton and indoor bandy games that were great fun!

My other co-authors Professor Anders Ahlbom, Gunnar Falk and Margareta Sten-Linder, thank you for your work with the SHEEP data that took place before I started my thesis project, and for your valuable contributions to the manuscripts.

Professor Sam Schulman, Professor Hans Lithell and Docent Maria Feychting, thank you for your valuable comments at my half-time seminar.

Many thanks to all my colleagues at the Institute of Environmental Medicine for contributing to such a pleasant working environment. Apart from those earlier mentioned, I also appreciate the assistance while working with this thesis from Eleonor Fransson and Slobodan Zdravkovic for statistical advice, Professor Lars Alfredsson for opening doors (both literally and metaphorically!), Anna Bennet, Anita Berglund and Stefan Lönn for valuable general advice about the final phase of thesis work, Anette Linnersjö for rapid expert help to find data on statistics, and Lena Wärnlöf, Gunilla Bergström and Eva Undsén for administrative assistance. Also, thank you my former room-mates Jenny Sandgren and Anastasia Georgiades, and my current room-mate Mats Halldin, for being such good mates!

Hilary Hocking, thank you for your expert linguistic advice and for taking such great pains to perform a revision although time was very scarce.

Lars Ericsson, Yvonne Tersaeus, Inger Ericsson and Stina Bárány, thank you for all your support. Also, thank you Malin, Lisa, Anna P, Hulda, and Hasse with families for your support and pep-talk.

My beloved Mormor Mia, thank you for your endless love and support, for all your help and for inspiring discussions.

Last, but not least, many special thanks to Anders, Erik and Clas for all your help, love and patience. I love you.

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