Clinical Manifestations of Coronary Heart Disease and the Metabolic Syndrome

A Population-based Study in Middle-aged Men in Uppsala

BY

KRISTINA DUNDER
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Abstract

During the past decades the knowledge concerning risk factors and pathophysiology of coronary heart disease (CHD) has substantially increased. However, despite identification of important risk factors CHD remains the leading cause of death in the western world.

The metabolic syndrome is a cluster of metabolic disorders such as hypertension, hypertriglyceridemia, low HDL-cholesterol, and glucose intolerance associated with an increased risk of cardiovascular morbidity and mortality.

The studies in this thesis are epidemiological in their character, and examine the relationships between different aspects of CHD and the metabolic syndrome in a population-based study of middle-aged men (ULSAM).

The findings indicated that serum lipids were important risk factors for the development of both angina pectoris demanding revascularisation and acute myocardial infarction (MI). Proinsulin and blood pressure were independent predictors of MI only, suggesting these factors to be involved in thrombosis and plaque rupture.

It was also found that antihypertensive treatment with beta-blockers and thiazide diuretics resulted in increased fasting blood glucose concentrations in subjects with insulin resistant state with elevated proinsulin concentrations. Both proinsulin concentrations and increase in fasting blood glucose were associated with increased risk of developing future MI.

The finding of a new Q/QS-pattern on the resting ECG, regardless of history of MI, was associated with impaired insulin secretion and was an independent predictor of total and cardiovascular mortality.

A risk prediction score for MI including proinsulin and the ratio between apolipoprotein B and apolipoprotein A1 was developed in middle-aged men. This score was predictive for future fatal and nonfatal MI, and proved to be at least as good as the Framingham and the PROCAM scores, being based on traditional risk factors.

In summary these studies provide further knowledge about the associations between CHD and the metabolic syndrome and the possible importance of new markers of cardiovascular risk such as proinsulin and the apolipoproteins.

Keywords: coronary heart disease, metabolic syndrome, insulin resistance, antihypertensive treatment, risk score

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Abbreviations

AUC  area under the curve
ACE  angiotensin converting enzyme
ANOVA analysis of variance
BMI  body mass index
CABG coronary artery by pass grafting
CRP  C-reactive protein
CVD  cardiovascular disease
DBP  diastolic blood pressure
FPG  fasting plasma glucose
HDL  high-density lipoprotein
ICD  international classification of diseases
LDL  low density lipoprotein
PAI-1 plasminogen activator inhibitor 1
PG  postchallenge glucose
PTCA  percutaneous transluminal coronary angioplasty
SBP  systolic blood pressure
Tg  triglycerides
VLDL  very low density lipoprotein
WHO  world health organisation
WHR  waist hip ratio
ULSAM  Uppsala longitudinal study of adult men
INTRODUCTION

Epidemiology is the quantitative study of the distribution and determinants of disease in human populations. The term epidemiology, if taken literally, means “the study of that which befalls man” (epi=”befalls, upon”, demo=”man, people”, ology=”the study of”).

The purposes and uses of epidemiology are to explain the etiology of diseases and conditions, to determine if epidemiological data are consistent with current scientific knowledge and hypotheses, for planning and evaluation of public health measures and to describe the natural history of diseases. However, absolute proof of causality can only come from intervention studies.

The studies in this thesis are epidemiological in their character, and examine the relationships between different aspects of coronary heart disease (CHD) and the metabolic syndrome.

Coronary heart disease

During the past decades a great deal of knowledge concerning the pathophysiology of CHD has been achieved, and hypertension, smoking, hyperlipidemia and diabetes are some of the abnormalities that are generally accepted as risk factors. However, despite identification of important risk factors CHD remains the leading cause of death in Europe and in the United States and by 2010 cardiovascular disease is estimated to be the leading cause of death also in the developing countries (WHO).

Clinical presentations

The clinical presentation of CHD can vary substantially from acute myocardial infarction (MI) without previous signs of coronary ischemia, to stable angina pectoris that may exist for many years without acute events. When studying coronary angiograms, it has been shown that patients with chronic stable angina generally have more severe stenotic lesions than those with acute unheralded MI, suggesting that these two presentations of coronary atherosclerosis, at least in part, may develop in different ways.

During the past decade some studies have tried to answer the question whether the differences in clinical presentation are due to different risk fac-
tor profiles, but no consistent pattern has emerged\textsuperscript{8-10}. Therefore further studies concerning the different clinical presentations of CHD are needed.

It is not unusual to find Q/QS-patterns on the resting electrocardiogram (ECG) at a standard routine examination in subjects with a normal ECG at the previous examination. In some subjects it is clear that a MI has occurred, but in many other cases the MI is unrecognised, i.e. no history of typical symptoms suggesting a MI is revealed.

In the case of unrecognised MI, the risk factor profile and prognosis appear to be almost the same as for recognised MI\textsuperscript{11-16}. Some studies have indicated an increased prevalence of diabetes mellitus and hypertension in subjects with unrecognised MI\textsuperscript{15 17 18} and it has been proposed that autonomic neuropathy due to diabetes may explain this association \textsuperscript{19 20}. However, others have failed to prove that diabetics are more prone to unrecognised MI than non-diabetics\textsuperscript{21 22}.

The prognostic significance of different ECG abnormalities, such as Q-wave or QS-pattern, has been extensively examined in several epidemiological studies and a strong association with future cardiovascular morbidity and mortality has been found\textsuperscript{23-32}. Several of the studies have adjusted the results for known cardiovascular risk factors, but few have investigated whether the predictive power of the Q/QS-pattern is dependent of a history of MI or not.

Pathophysiology

The common pathophysiological background to CHD is coronary atherosclerosis with subsequent plaque formation\textsuperscript{33-35}. The initial step of atherosclerosis is migration of lipids and inflammatory cells into the intima of the coronary arteries. This leads to a thickening of the intima that subsequently progresses to a plaque with a lipid core and a fibrous cap. These plaques progress slowly and due to a remodelling of the vessel wall leading to an increased diameter\textsuperscript{36}, the lumen of the vessel can be maintained during several years and the patient may be asymptomatic.

The major pathophysiological difference between acute coronary syndromes and stable angina pectoris is rupture of the atherosclerotic plaque with subsequent thrombosis formation that causes the acute events. Plaque rupture occurs independently of lesion size and degree of stenosis\textsuperscript{37}, and some plaques seem to be more vulnerable than others are. The vulnerable plaque is characterized by a large lipid core, a high content of macrophages and a thin fibrous cap\textsuperscript{38}.

Both external forces, such as circumferential wall stress and blood flow characteristics and internal forces may be involved in plaque disruption. Inflammatory cell activity probably has an important role in plaque disruption, as well as in the pathology of atherosclerosis. The role of the macrophages has been studied more in detail, but also T-lymphocytes seem to be involved in the development of atherosclerosis\textsuperscript{39}. Macrophages can secrete
metalloproteinases that can degrade proteins in the fibrous cap and thus make it more prone to rupture. Accordingly, CRP, a sensitive marker of inflammation, has been proven to be a significant predictor of CHD in several studies.

The Metabolic syndrome

Definitions

In 1988 Reaven suggested the existence of a syndrome, syndrome X, including a number of metabolic disorders such as hypertension, hypertriglyceridemia, low HDL-cholesterol, and glucose intolerance. Insulin resistance was suggested as the common antecedent behind these aberrations, and therefore the syndrome is sometimes called the insulin resistance syndrome. However, the WHO has chosen the term metabolic syndrome, since it is considered that current data cannot establish insulin resistance as the cause of all the components.

Over the years several definitions and explanations of the syndrome have emerged, and several other metabolic disturbances such as abdominal obesity, microalbuminuria, chronic subclinical inflammation, impaired fibrinolysis and left ventricular hypertrophy have been suggested to be parts of the syndrome.

The WHO definition is to be applied to both diabetic and non-diabetic subjects, while the EGIR (the European Group for the Study of Insulin Resistance) definition is defined only for a non-diabetic population. A more recent definition has been presented in the Summary of the Third Report of the National Cholesterol Education Program (NCEP) (Table 1).

The prevalence of the syndrome depends on the definition used. In an investigation of eight European studies the frequency of the syndrome increased with age and was more common in men than women. In non-diabetic subjects the frequency of the WHO syndrome was 7-36% for men and 5-22% for women in the ages 40-55 years, and was prevalent in 1-22% of men and 1-14% of women using the EGIR definition. In the United States the prevalence is estimated to 24%, increasing to 44% in adults ≥ 60 years of age.
Table 1. Definitions of the metabolic syndrome

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Defining level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin resistance</td>
<td>Glucose uptake below lowest quartile for background population</td>
</tr>
<tr>
<td>Impaired glucose regulation</td>
<td>FPG≥6.1 mmol/l, ≥5.6 for venous or capillary whole blood, and/or 2-hour PG≥7.8, ≥ 6.7 for venous or capillary whole blood</td>
</tr>
<tr>
<td>Hypertension</td>
<td>SBP≥140 mmHg and/or DBP≥90 mmHg</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>S-Tg≥1.7 mmol/l and/or HDL cholesterol&lt;0.9 (♂), &lt;1.0(♀)</td>
</tr>
<tr>
<td>Central obesity</td>
<td>WHR&gt;0.90(♂), &gt;0.85(♀) and/or BMI&gt;30 kg/m²</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>Urinary albumin≥20µg/min</td>
</tr>
</tbody>
</table>

(At least one of insulin resistance and impaired glucose regulation, and two more of the other components)
### EGIR

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Defining level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperinsulinemia</td>
<td>Fasting insulin concentration above the upper quartile for the non-diabetic subjects</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>FPG≥6.1mmol/l, ≥5.6mmol/l for venous or capillary whole blood</td>
</tr>
<tr>
<td>Hypertension</td>
<td>SBP≥140mmHg and/or DBP≥90 mmHg or treatment for hypertension</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>S-Tg≥2.0 mmol/l and/or HDL cholesterol&lt;1.0 mmol/l or treatment for hyperlipidemia</td>
</tr>
<tr>
<td>Central obesity</td>
<td>Waist circumference≥94cm (♂), ≥80cm (♀)</td>
</tr>
</tbody>
</table>

(Hyperinsulinemia and two more of the other components)

### NCEP

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Defining level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperglycemia</td>
<td>FPG≥6.1 mmol/l</td>
</tr>
<tr>
<td>Hypertension</td>
<td>≥130/≥85 mmHg</td>
</tr>
<tr>
<td>High triglycerides</td>
<td>≥1.7 mmol/l</td>
</tr>
<tr>
<td>Low HDL cholesterol</td>
<td>&lt;1.0 mmol/l (♂), &lt;1.2 mmol/l (♀)</td>
</tr>
<tr>
<td>Abdominal obesity</td>
<td>Waist circumference&gt;102 cm (♂), &gt;88 cm (♀)</td>
</tr>
</tbody>
</table>

(Three or more components)
Insulin resistance

Insulin sensitivity is a term generally used to define the ability of insulin to mediate glucose disposal in skeletal muscle, but it also includes insulin’s ability to suppress lipolysis in the adipose tissue and gluconeogenesis in the liver.

Reduced insulin sensitivity, denoted insulin resistance, is common in type 2 diabetes mellitus, but can also be found in non-diabetic subjects with normal glucose tolerance. In normoglycemic subjects a compensatory increased pancreatic insulin secretion can maintain the fasting blood glucose at a normal level despite of prevailing insulin resistance. However, most often the ability of the beta cells to produce enough insulin subsequently declines over time in insulin resistant subjects. This leads to lower glucose uptake in skeletal muscle, increased levels of free fatty acids, less inhibition of hepatic glucose production, and thus subsequent hyperglycemia.

The gold standard for measuring insulin sensitivity in skeletal muscle is the hyperinsulineamic euglycemic clamp method. Since this is a rather complicated method not used in clinical practice, fasting serum insulin has often been used as a proxy of insulin sensitivity in epidemiological studies. Elevated concentrations of proinsulin have been associated with measurements of insulin resistance, such as obtained by oral and intravenous glucose tolerance tests or the euglycemic hyperinsulinemic clamp, and therefore elevated concentrations of proinsulin could be used as a marker of insulin resistance.

Etiological factors and pathophysiology

It has been debated whether the etiology of insulin resistance and the metabolic syndrome is of genetic or environmental character.

It is known that obesity, a sedentary life style and smoking favor the development of insulin resistance. Dietary factors may induce oxidative stress, a condition known to promote the development of insulin resistance, as well as improve insulin sensitivity by containing compounds with antioxidant activity.

Low birth-weight, as a result of impaired growth in fetal life, has been associated with components of the metabolic syndrome and type 2 diabetes mellitus, and may be considered as an additional etiological explanation. Men with low birth-weight have been shown to have higher plasma cortisol concentrations compared to those with normal birth weight. Since glucocorticoid excess is known to induce hypertension, central obesity and glucose intolerance, it has been hypothesized that enhanced activity of cortisol could be the cause of insulin resistance and the metabolic syndrome. It has also been shown that insulin resistance is associated with increased density of glucocorticoid receptors in skeletal muscle.
Subjects with the metabolic syndrome have increased concentrations of inflammatory markers, such as CRP, fibrinogen and white blood cells, compared to those without the syndrome. There appears to be a relation between insulin resistance and CRP levels, and CRP has been shown to predict the development of type 2 diabetes. Thus, it has been suggested that chronic inflammation may be a triggering factor for insulin resistance.

An increased activity of the sympathetic nervous system may also be of importance for the development of insulin resistance. Low capillary density have been linked to insulin resistance, implying that reduced peripheral blood flow may play a role in the development of insulin resistance.

However, it has also been shown that non-obese, non-diabetic relatives of subjects with type 2 diabetes are more insulin resistant than nondiabetic controls, suggesting a genetic component. It is therefore most likely that the etiology of insulin resistance and the metabolic syndrome is a combination of both genetic and environmental factors.

The pathophysiological background of insulin resistance has been extensively examined. Defects in the actions of the insulin receptor, insulin receptor substrates, and glucose transport proteins have all been reported as potential mechanisms causing a defect in insulin action. Endothelial dysfunction could alter the presentation and function of insulin receptors and has been proposed to be a common factor in many of the features of the metabolic syndrome.

Another pathophysiological component may be the fatty acid composition of the phospholipids of the skeletal muscle membranes that may affect the ability of the cell membrane to bind and transport insulin and glucose. The fatty acid composition is related to insulin sensitivity, and may be influenced by diet and physical activity. Genetic variations in enzyme actions involved in fatty acid metabolism may also be of etiological importance.

In recent years also the triglyceride content in skeletal muscle and liver have been highlighted as drivers of the development of insulin resistance.
Coronary heart disease and the metabolic syndrome

The presence of the metabolic syndrome has been associated with an increased risk of cardiovascular morbidity and mortality and all the different metabolic disorders included in the syndrome have, by themselves, been associated with increased risk of CHD.

Impaired glucose regulation

Insulin resistance

The association between insulin resistance and CHD has most often been examined by using fasting serum insulin as a proxy for insulin resistance. The role of insulin as a risk factor for CHD has been debated, and in a meta-analysis of 12 studies investigating this relationship it was concluded that hyperinsulinemia was only a weak risk indicator for the occurrence of cardiovascular disease. Moreover, only a moderate correlation between plasma insulin and insulin sensitivity measured by the hyperinsulineamic euglycemic clamp method has been found. However, it has been argued that the association between insulin levels and CHD could be confounded by the effects of other diseases resulting in malnutrition and weight loss and thus low insulin concentrations.

There are few studies examining the association between CHD and insulin resistance measured by the hyperinsulineamic euglycemic clamp. One small study showed a significant association between the magnitude of insulin resistance and the degree of coronary artery disease. A recent study from the ULSAM cohort showed that insulin resistance measured by the hyperinsulineamic euglycemic clamp, was a significant predictor of CHD independent of serum cholesterol, smoking and hypertension.

The mechanisms behind the finding that insulin resistance increases the risk of CHD are not completely known. One explanation could be the strong association of hyperinsulinemia and insulin resistance with other metabolic risk factors included in the metabolic syndrome, such as dyslipidemia and increased PAI-1 activity. Insulin resistance could also be causative in itself, for instance by inducing impaired endothelial function, smooth muscle proliferation and subsequent atherosclerosis. Moreover, increased levels of CRP are associated with the development of type 2 diabetes as well as CHD, which may suggest inflammation as a common basis for insulin resistance, the metabolic syndrome and atherosclerotic cardiovascular events.

Proinsulin

Proinsulin is the precursor of insulin, and is processed in the beta cells of the pancreas to form insulin and C-peptide. In non-diabetic subjects proinsulin-like molecules (proinsulin and 31,32-split proinsulin) comprise about 10% of all insulin-like molecules in the peripheral blood, but in type 2 diabetes
mellitus the proportion is higher. The effect of proinsulin on glucose metabolism is about 10% of that of insulin, but the half-life of proinsulin is longer (146 min versus 5 min).

Proinsulin has recently been shown to be an independent predictor of CHD both in diabetic and non-diabetic populations, but the mechanisms for this association remain uncertain. Increased concentration of proinsulin may be a marker of an underlying metabolic disturbance and thus not have a causative relationship with CHD. However, clinical trials using proinsulin therapeutically for Type 2 diabetes resulted in several-fold higher incidence of MI in the treatment groups. Elevated concentrations of proinsulin and insulin are known to be strong determinants of PAI-1 activity, and may thereby cause impaired fibrinolysis and increased risk of thrombosis. Moreover, in diabetic subjects elevated levels of PAI-1 have been found in extracted coronary atheroma and it has been proposed that PAI-1 might cause a reduction of vascular smooth muscle cells in the plaque and thus make it more vulnerable to rupture. PAI-1 has also been associated with components of the metabolic syndrome, such as insulin sensitivity and hypertriglyceridemia. Thus, increased PAI-1 activity may be one potential link between proinsulin and coronary events.

**Hyperglycemia**

Several studies have shown an association between the degree of hyperglycemia and risk of micro-and macrovascular complications. Increased blood glucose concentrations have been proven to impair endothelial function in patients with type 2 diabetes mellitus, as well as in healthy subjects, and may thereby accelerate the atherosclerotic process.

However, in the United Kingdom Prospective Diabetes Study (UKPDS) intensive blood glucose control resulted only in a borderline significant reduction of MI risk. A possible explanation is that postprandial glycemia may be more strongly related to the risk of CHD than fasting plasma glucose, as suggested by the DECODE (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) study group. An alternative explanation is the existence of proatherogenic metabolic disturbances already in the prediabetic state that may contribute to the risk of macrovascular disease as much as clinical diabetes in itself.

**Dyslipidemia**

After the results of the Scandinavian Simvastatin Survival Study (4S) in 1994 hypercholesterolemia, and especially elevated levels of LDL-cholesterol, are generally accepted as strong risk factors for CHD.

During the last years new parameters for measurement of lipid disorders have emerged. In a recent Swedish study apolipoprotein B (apoB), apolipoprotein A1 (apoA1) and the ratio between apoB/apoA1 were highly predic-
tive in the evaluation of cardiac risk. ApoB was a stronger predictor of risk than LDL-cholesterol. The importance of apolipoproteins was also valid in subjects older than 70 years and in subjects with concentrations of LDL-cholesterol below the median. An explanation might be that apoB is present not only in LDL-particles, but also in the atherogenic VLDL and intermediate density lipoproteins. ApoA1, being present in HDL-particles, may play an important role in the atherosclerotic process especially in low-risk populations with normal cholesterol concentrations. ApoA1 has been shown to modify the ability of HDL-particles to remove cholesterol from cells, and it may therefore be the content of apoA1 rather than cholesterol in HDL-particles that represents the atheroprotective capacity. Moreover, it has recently been shown that treatment with a variant of apoA1 resulted in regression of coronary atherosclerosis compared to placebo.

The most common lipid disorders in subjects with the metabolic syndrome are raised serum triglyceride and lowered HDL-cholesterol concentrations while LDL-cholesterol concentrations may be normal. However, these individuals often have increased concentrations of small dense LDL-particles, a condition that has been associated with an increased risk of CHD, as well as an increased ratio between apoB and apoA1 as signs of an atherogenic lipid profile.

The composition of fatty acids in cholesterol esters has been associated with cardiovascular disease and with endothelial function. Palmitic (16:0) and palmitoleic (16:1) acids seem to impair endothelial function and might thereby be risk factors for CHD.

Hypertension

It is well known that the incidence of CHD is increased in hypertensive patients compared to normotensive controls, also when hypertension is treated. Hypertension may promote the development of atherosclerosis by causing impaired endothelial function, and may also be a trigger of acute plaque disruption by inducing mechanical stress on the arterial wall. Hypertension has also been associated with impaired fibrinolytic activity, as evaluated by an impaired capacity for stimulated release of tissue plasminogen activator from vascular endothelium, and may thereby promote thrombosis formation.

Hypertension and insulin resistance

During the last decades several studies have shown that a large part of patients with hypertension are resistant to insulin-stimulated glucose uptake and are hyperinsulinemic compared to normotensive controls independently of obesity. It has also been shown that the prevalence of hypertriglyceridemia and low levels of HDL-cholesterol is increased in hypertensive subjects.
The possibility has been raised that insulin resistance with compensatory hyperinsulinemia can cause and/or play a role in regulating hypertension. Insulin is known to promote sodium retention in the kidney, to stimulate sympathetic nervous system activity and may be a stimulant of the growth of smooth muscle cells in the vessel wall. However, not all patients with hypertension are insulin resistant. Insulin resistance has also been found in normotensive first-degree relatives of hypertensive patients, which might imply a genetic predisposition to insulin resistance in subjects prone to a high blood pressure.

**Metabolic effects associated with antihypertensive treatment**

There is evidence that treatment with beta-blockers and thiazide diuretics in hypertensive patients further reduce insulin sensitivity, thereby increasing the risk of developing type 2 diabetes mellitus. The magnitude of the reduction in insulin sensitivity varies between different beta-blockers. Propranolol, atenolol and metoprolol have been shown to reduce insulin sensitivity by 25-32%, while with drugs with intrinsic activity, such as pindolol, the impairment was only 17%. The mechanism by which beta-blockers deteriorates insulin sensitivity is not fully understood, but several possibilities exist. One explanation is a reduced peripheral blood flow due to an increase in total peripheral vascular resistance. It has been shown that vasodilating drugs, such as ACE-inhibitors, alfa-blockers and newer vasodilating beta-blockers, can improve glucose metabolism. Beta-blockers may also lead to reduced insulin clearance resulting in hyperinsulinemia and subsequent down-regulation of insulin receptors and reduced insulin sensitivity. Beta-blockers have also been associated with weight gain, which may further reduce insulin sensitivity.

The mechanism by which thiazide diuretics reduce insulin sensitivity has been debated. One hypothesis is that potassium depletion causing impaired insulin secretion is the primary cause of impaired glucose tolerance seen during thiazide treatment. An alternative explanation is that thiazides may increase circulating catecholamines and thereby decrease insulin sensitivity.

Diabetes mellitus and impaired glucose tolerance are both associated with an increased risk of CHD, but it has been questioned whether these conditions when induced by beta-blockers and/or thiazides are associated with increased risk of CHD.

During the last years beta-blockers and thiazide diuretics have been compared to newer antihypertensive drugs in large-scale studies. In the Captopril Prevention Project (CAPPP) there was no difference between treatments in preventing cardiovascular morbidity and mortality. However, significantly fewer patients developed diabetes in the captopril group compared to the group with beta-blockers/diuretics. In patients with diabetes at baseline there
was also a 66% lower incidence of MI in the captopril group compared to the group with beta-blockers/diuretics.

The Losartan Intervention For Endpoint reduction in hypertension study (LIFE)\textsuperscript{164} concluded that the angiotensin II blocker losartan prevented more cardiovascular morbidity and death than atenolol in hypertensives with left ventricular hypertrophy. There was also a lower rate of new-onset diabetes in the losartan group compared to the atenolol group.

The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT)\textsuperscript{165} showed that thizide diuretics were as good as ACE inhibitors and calcium channel blockers in preventing major forms of CHD even in subjects with diabetes. Also in this study there was a higher rate of new-onset diabetes in patients treated with diuretics compared with those treated with modern antihypertensive agents. However, the ethnic composition of the ALLHAT study population differed from European populations and therefore the results may be difficult to apply in European countries.

The UKPDS\textsuperscript{166} also showed that antihypertensive treatment with atenolol was similarly effective in reducing the incidence of macrovascular complications, such as MI, compared to captopril.

In conclusion, the results are diverging whether the metabolic abnormalities induced by beta-blockers and diuretics are of importance regarding future cardiovascular morbidity and mortality. However, the relatively short follow-up periods (3-5 years) that are common in large intervention studies may not be sufficient to detect effects on morbidity and mortality due to abnormal glucose metabolism induced by treatments with beta-blockers and thiazide diuretics.
Risk scores for coronary heart disease

It is well known that the risk of developing CHD depends on a number of different risk factors\(^2\). Many of these risk factors interact and act synergistically\(^{167,168}\), and it may therefore be difficult to assess the exact impact of each risk factor.

To grasp the overall picture of each patient’s risk of future CHD, several point scoring systems have been developed, and especially the Framingham score\(^{167,169}\) has been extensively used. Several studies have evaluated the performances of scores derived from the Framingham Heart Study in European populations, and an overestimation of absolute risk of future CHD has been found\(^{170-172}\). Recently new schemes based on the Framingham study\(^47\) and the Prospective Cardiovascular Munster (PROCAM) Study\(^{172}\) have been presented, as well as a new European scoring system developed by the Score Project\(^{173}\) for use in clinical management of cardiovascular risk in European clinical practice. These new scoring schemes include traditional risk factors, such as blood pressure, serum cholesterol, smoking and diabetes.

However, during the last years new variables for prediction of CHD, such as the apolipoproteins and proinsulin have emerged. It has not been investigated if these markers of cardiovascular risk can improve the ability of a risk score in predicting future cardiovascular events.
AIMS

The specific aims of the studies were:

**Paper I**
To investigate the impact of variations in fasting blood glucose, blood pressure and BMI occurring between age 50 and 60 on the risk of developing MI during a follow-up of 17 years in a population-based sample of men. In the analysis, special attention was paid to subjects receiving antihypertensive therapy, with the hypothesis that drug-induced increase in fasting blood glucose would increase the risk of MI.

**Paper II**
To examine differences in baseline risk factors between subjects developing stable angina pectoris demanding revascularisation (PTCA or CABG) without previous known MI, and subjects developing MI without previous known CHD during a 22 years follow-up period in a large cohort of middle-aged men.

**Paper III**
To investigate the metabolic risk factors associated with the finding of a new Q/QS-pattern on the resting ECG in a population sample of 70-year-old men, and to investigate if the predictive power of the Q/QS-pattern was dependent of a history of MI or not during a follow-up of almost 10 years.

**Paper IV**
To generate a new risk prediction score (the ULSAM score) including the apoB/apoA1 ratio and serum proinsulin, for the development of fatal and nonfatal MI in a population sample of middle-aged men, and to examine if these new risk factors for CHD could improve the predictive performance compared to schemes including only traditional risk factors, like the Framingham and the PROCAM scoring schemes.
MATERIALS AND METHODS

Subjects

The ULSAM study

The Uppsala Longitudinal Study of Adult Men is a population-based study aimed at identifying risk factors for cardiovascular disease. Between 1970-73 all men born 1920-24 and resident in the municipality of Uppsala, Sweden, were invited to participate in a health survey, and 2,322 out of the 2,841 invited men participated (82 %). All subjects gave informed consent. The local Ethics Committee of the Medical faculty at Uppsala University has approved the studies on several occasions.

Between 1980 and 1984 (at age 60) a re-examination of eligible subjects who had participated in the first survey was performed, in which 1,860 out of 2,130 subjects (87.5%) participated.

All eligible participants investigated in the first survey at age 50, traced by their ten-digit social security number, were invited to reinvestigation at age 70. The survey was carried out between August 1991 and May 1995. Participation rate was 73% (1,221 out of 1,681 invited) (figure1).

Eligible subjects have also been examined at age 77 and age 82, but those results are not included in the present thesis.
Figure 1. The ULSAM study
Study populations

Paper I
The study was based on the 1,860 subjects who participated both in the baseline investigations at age 50 and in the re-examination at age 60.

The population was grouped into subjects with (n=291) and without antihypertensive treatment (n=1,358) at age 60. Seventy-six subjects had monotherapy with non-selective beta-blockers, 55 with selective beta-blockers, 63 with thiazide diuretics and 97 with a combination of beta-blockers and thiazide diuretics. Forty-one subjects had hydralazine added to the treatment.

Subjects that had been hospitalised for MI before the examination at age 60 were excluded (n=75), as were subjects without MI but with angina pectoris (according to in-hospital registers) during the follow-up period (n=115) and subjects lacking information about antihypertensive treatment at age 60 (n=21).

Paper II
The study population was based on the 2,322 subjects who participated in the survey at age 50.

The population was divided into two groups; one group consisting of subjects who during the follow-up developed stable angina pectoris demanding revascularisation with PTCA or CABG without a preceding hospital diagnosis of MI (n=70), and another group consisting of subjects who developed fatal or nonfatal hospital-treated MI during the follow-up, without previous hospital-documented manifestations of CHD (n=372). Also a control group without any known history of CHD during the follow-up period was identified (n=1,701).

Subjects with a history of CHD (questionnaire and hospital files) before age 50 were excluded from the analysis (n=52), as well as subjects without MI, but with other manifestations of CHD (according to hospital files) during the follow-up, that did not require revascularisation (n=127).
**Paper III**

The study was based on subjects that participated in baseline examinations and in the examination at age 70 (n=1,221). The population was divided into four groups according to ECG-status and MI/CHD diagnosis (according to in-hospital registers) at age 70.

The first group had Q/QS pattern (Minnesota code 1.1/1.2/1.3) and MI diagnosis (n=41). The second group had Q/QS but no MI or CHD diagnosis (n=91). The third group had MI diagnosis but no Q/QS (n=45), while the fourth group had normal ECG and no CHD diagnosis (controls, n=545).

Subjects with Q/QS pattern or left bundle branch block at age 50 (n=35), and subjects with pacemaker, left bundle branch block or no ECG registration at age 70 were excluded (n=106). Subjects that did not fulfil the inclusion criteria of the four groups according to ECG-status and MI/CHD diagnosis (ECG pathology other than Q/QS-pattern (Minnesota codes 4.1/4.2, 5.1-5.3 and 7.1) without MI diagnosis, normal ECG with CHD diagnosis other than MI, Q/QS-pattern with CHD diagnosis other than MI) were excluded (n=358).

**Paper IV**

The study was based on the 2,322 subjects who participated in the baseline investigations at age 50.

Subjects that had been hospitalised for, or had a history of CHD according to the questionnaire before these investigations, and subjects with baseline ECG showing ischemic signs (Minnesota code 1.1/1.2/1.3/4.1/4.2/5.1-5.3/7.1/9.2), were excluded (n=269). Subjects in the remaining population that lacked observations for any of the variables in the ULSAM score were also excluded (n=945). After this reduction in sample size the total data set consisted of 1,108 subjects. Fatal or non-fatal MI according to registry data was chosen as outcome variable (n=251 over 28.7 years of follow-up).

For cross-validation the total data set was divided into a training data set (subjects born 1921, 1922 and the second half of 1924, n=574, numbers of MI=135) in which the score was generated, and a prediction data set (subjects born 1920, 1923 and the first half of 1924, n=534, MI=116) for validation of the score and for comparisons with the PROCAM and Framingham scores.
Figure 2. Study populations in study I-IV. NK= not known. LBBB=left bundle branch block
Data Collection

Investigations at age 50

Anthropometry
Height was measured to the nearest whole centimetres (cm) and weight (in undershorts) to the nearest whole kilogram (kg). BMI was calculated as weight (in kg) divided by height (in meters) squared.

Blood pressure
Blood pressure was measured on the right arm after 10 minutes' rest in the recumbent position and after another 2 minutes in the sitting position. Mercury manometers (Kifa Ercameter, wall-model) were used. Systolic and diastolic blood pressures were read to the nearest 5 mmHg. The diastolic blood pressure was recorded at the disappearance of the Korotkoff sounds (phase five).

Glucose and Insulin Metabolism
Blood glucose was measured by spectrophotometry using the glucose oxidase method. The intra-individual CV for fasting plasma glucose was 2.9%.

An intravenous glucose tolerance test (IVGTT) was performed by injection of a glucose dose of 0.5 g/kg of a 50% solution given into an antecubital vein during 2.5 minutes. Samples for the determination of blood glucose concentration were drawn before and 6, 20, 30, 40, 50 and 60 minutes after the start of the glucose injection. The serum insulin concentrations during the IVGTT were measured in duplicate of blood samples drawn before and 4, 6, 8 and 60 minutes after the start of the glucose injection. The serum insulin was determined with the Phadebas Insulin Test (Pharmacia AB, Uppsala, Sweden).

Intact proinsulin and 32-33 split proinsulin concentrations were analysed using the two-site immunometric assay technique between 1995 and 1998 at the Department of Clinical Biochemistry, Addenbrooke's hospital, Cambridge, UK, using plasma samples that had been stored in liquid nitrogen for 15 years and thereafter stored frozen in -70°C.

Serum lipids
Determinations of serum cholesterol and Tg concentrations were performed on a Technicon Auto Analyzer type II in 1981-82 on serum samples that had been stored in liquid nitrogen since 1970-73.

HDL-cholesterol was assayed in the supernatant after precipitation with a heparin/manganese-chloride solution. LDL-cholesterol was calculated using Friedewald's formula: LDL= serum cholesterol-HDL-(0.42xserum Tg).
The values presented are "Monarch adjusted", i.e. the values obtained were multiplied with a conversion factor for enabling comparison with the Monarch method used in the survey at age 70.

Apo(a) and apoB were determined by a two-site immuno-radiometric assay and ApoA1 by a competitive radioimmunoassay in 1988, with use of commercial kits from Pharmacia (Uppsala, Sweden), in samples that had been stored in liquid nitrogen since sampling.

The percentage composition of methylated fatty acids (14:0) to (22:6) in serum cholesterol esters was determined by gas chromatography.

**Vitamin E**

In 1986 Alpha tocopherol (vitamin E) was determined by high performance liquid chromatography.

**ECG**

A standard 12-lead resting ECG was recorded at 50 mm/s and 10 mm/mV, including leads I, II, III, aVR, aVL, aVF, and V1-6. The ECG machine was a direct-writing Mingograf 61 (Siemens-Elema Led, Solna, Sweden). The ECGs were classified according to the Minnesota Code by two experienced physicians at the Department of Clinical Physiology.

**Questionnaire**

A self-administered medical questionnaire was used to gather information on medical history. An interview was performed for completion of information regarding smoking habits, cause and age of death of parents and some psychosocial data.

**Investigations at age 60**

**Anthropometry**

Height was measured to the nearest whole cm and weight (in undershorts) to the nearest whole kg. BMI was defined as the weight (kg) divided by height (meter) squared.

**Blood pressure**

The blood pressure was measured on the right arm in the recumbent position after 10 minutes rest by use of a mercury manometer (Kifa Eracometer, wall model) with a cuff width of 12.5 cm and a length of 35 cm. Korotkoff phase 5 was used for identification of diastolic blood pressure.
Glucose
Blood glucose was analysed by a glucose oxidase method (GOD-PERID, Boehringer Mannheim GFR), with use of an LKB 7400 photometer. A fasting blood glucose value was obtained in all participants.

Drugs
Information about medication was taken from the questionnaire and classified according to the, at that time, current list of pharmaceutical specialties available in Sweden (FASS).

Investigations at age 70

Anthropometry
Height was measured to the nearest whole cm, and body weight to the nearest 0.1 kg. BMI was calculated as the ratio of the weight (in kg) to the height (in meters) squared.

Blood pressure
Blood pressure was measured twice on the right arm to the nearest even figure with the subject in the supine position after resting for 10 minutes. The mean of the two values are given. The cuff size was 12x35 cm or 15x45 cm depending on the arm circumference. Systolic and diastolic blood pressures were defined as Korotkoff phases I and V, respectively.

Oral glucose tolerance test (OGTT)
An OGTT was performed where the subjects ingested 75 g glucose dissolved in 300 ml of water, and blood samples for plasma glucose and insulin were drawn immediately before, and 30, 60, 90, and 120 min after ingestion of glucose.

The area under the incremental curves for glucose and insulin were calculated using the following formula: \[ \text{AUC} = 3(M_{30} - M_0 + 2(M_{60} - M_0) + 2(M_{90} - M_0) + M_{120} - M_0) \] where \( M \) is the value of glucose or insulin at the specified time.

Early insulin response (insulinogenic index), as a measure of insulin secretion, was calculated as the ratio of the 30 min increment in insulin concentration to the 30 min increment in glucose concentration.

Euglycaemic hyperinsulinaemic clamp
The euglycaemic hyperinsulinaemic clamp technique was used to estimate in vivo sensitivity to insulin. The technique used was according to DeFronzo\textsuperscript{53}, slightly modified.

Basal samples were taken 40 minutes after cannulation. Semisynthetic regular human insulin was infused in a primary dose for the first 10 minutes
and then as a continuous infusion (at 56 mU/min per body surface area) for 110 minutes to maintain steady state hyperinsulinaemia. The level of plasma glucose during the clamp study was maintained by measuring the plasma glucose every 5 minutes and adjusting the rate of infusion of a 20% glucose solution accordingly. The target plasma glucose level was 5.1 mmol/l. The glucose disposal (M) was calculated as the amount of glucose taken up during the last 60 minutes of the study and is given in mg/kg/min.

**Glucose**
Plasma glucose in samples from the oral glucose tolerance test was measured by the glucose dehydrogenase method (Gluc-DH, Merck, Darmstadt, Germany). The intra-individual CV for fasting plasma glucose was 3.2%.

**Insulin**
Plasma insulin from the oral glucose tolerance test and the clamp study was assayed using an enzymatic-immunological assay (Enzymmun, Boehringer Mannheim, Germany) performed in an ES300 automatic analyser (Boehringer Mannheim) and is given in mU/l.

Intact proinsulin and 32-33 split proinsulin concentrations were analysed using the two-site immunometric assay technique at the Department of Clinical Biochemistry, Addenbrooke's hospital, Cambridge, UK.

**Lipids and apolipoproteins**
Cholesterol and Tg concentrations were analysed in serum and in the isolated lipoprotein fractions by enzymatic techniques using IL Test Cholesterol Trinders's Method and IL Test Enzymatic-colorimetric Method for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, USA). HDL-particles were separated by precipitation with magnesium chloride/phosphotungstate. LDL-cholesterol was calculated using Friedewald's formula.

The apolipoproteins were analysed in a random subsample of 550 men. ApoB and apoA-I concentrations were determined by turbidimetry in a Monarch apparatus (Instrumentation Laboratories, Lexington, USA), using monospecific polyclonal antibodies against apoB and A-I. Apo(a) was measured by the Pharmacia Apo(a) RIA method (Pharmacia (a) RIA, Pharmacia Diagnostics AB, Uppsala, Sweden 1985).
ECG & Minnesota codes
A standard 12-lead ECG was recorded at 50 mm/s and 10 mm/mV and evaluated according to the Minnesota code \(^\text{177}\) by one experienced physician who was unaware of other data of the subjects. ECG status was classified as normal or abnormal on the basis of presence of one or more of the ECG diagnoses listed below.

<table>
<thead>
<tr>
<th>ECG diagnosis</th>
<th>Minnesota code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q or QS pattern</td>
<td>1.1</td>
</tr>
<tr>
<td>Q or QS pattern</td>
<td>1.2 or 1.3</td>
</tr>
<tr>
<td>High amplitude R waves</td>
<td>3.1 or 3.3</td>
</tr>
<tr>
<td>ST junction and segment depression</td>
<td>4.1 or 4.2</td>
</tr>
<tr>
<td>T-wave items</td>
<td>5.1 or 5.2 or 5.3</td>
</tr>
<tr>
<td>AV conduction defects</td>
<td>6.X</td>
</tr>
<tr>
<td>Left bundle branch block</td>
<td>7.1</td>
</tr>
<tr>
<td>Ventricular conduction defects</td>
<td>7.X</td>
</tr>
<tr>
<td>Atrial fibrillation or flutter</td>
<td>8.3</td>
</tr>
<tr>
<td>Arrhythmias</td>
<td>8.X</td>
</tr>
<tr>
<td>ST-segment elevation</td>
<td>9.2</td>
</tr>
<tr>
<td>Artificial pacemaker</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Diseases & drugs
Classification of drugs was performed according to the, at that time, current list of pharmaceutical specialties available in Sweden (FASS 1992/1993). All information about use of drugs was collected from the medical questionnaire.

_Hypertension_ prevalence was defined as a supine DBP of 95 mmHg or greater and/or treatment with anti-hypertensive drugs for hypertension. Men treated with these drugs for other reasons, i.e. cardiac failure, are thus not included in this definition.

_Hyperlipidaemia_ prevalence was classified as serum cholesterol >6.5 mmol/l and/or serum triglycerides >2.3 mmol/l and/or lipid-lowering medications.

_Diabetes_ was diagnosed if the 120 min and one or more of the 30-90 min plasma glucose values at the OGTT were greater or equal to 11.1 mmol/l\(^\text{178}\) and/or antidiabetic treatment.

Questionnaire
Two self-administered optically readable questionnaires were used. One was a questionnaire on general and medical background and the other concerned living conditions.
Registry data

The cause of death registry
Mortality data was achieved from the cause of death registry. The register is updated yearly and contains personal identification number, home town, underlying cause of death, nature of the injury, multiple causes of death, date of death, basis for statement of cause of death, sex and age. The international classification code for diseases (ICD-9 and 10) is used for classification of the causes of death.

The hospital discharge registry
Information on hospitalisations was achieved from the hospital discharge registry (HDR). The medical data in the register includes main diagnosis, secondary diagnoses, external causes of injury and poisoning and surgical procedures. The register is updated annually.

Definitions
CHD was defined as ICD-9 codes 410-414, or ICD-10 codes I 20-25, MI as ICD 410/ I 21, angina pectoris as ICD 413/I 20 and cardiovascular diseases as ICD 390-459/I 00-99.

Data concerning subjects who underwent PTCA and CABG during the follow-up according to hospital files was received from the Departments of Cardiology and Thoracic surgery.

During 1970 to 2001 there were 499 registered fatal and nonfatal MI in the cohort, out of which 115 occurred during the first decade (incidence 5%), 195 the second decade (incidence 9%) and 189 during the third decade (incidence 10%).
Statistical analyses

General analysis

The statistical analyses were made using JMP and STATA (paper IV) statistical softwares. A p-value <0.05 was considered as statistically significant.

Non-normally distributed variables tested by Shapiro-Wilk’s test (w<0.95) were log-transformed before being analysed.

Differences between groups regarding metabolic characteristics were evaluated by factorial one-way ANOVA, or the chi squared test for differences in proportions.

The independent power of the variables was evaluated by stepwise multiple Cox proportional hazard analysis (backward selection). Z-transformed standardized variables were used in the Cox proportional hazard analysis in order to make hazard ratios comparable. Hazard ratios are given for a one standard deviation change in the continuous variables with their 95% confidence intervals.

Specific analyses

Paper I

The risk associated with a 10% increase in blood glucose was calculated as follows: Hazard ratio**(log(1.1)/standard deviation).

Paper II

Bootstrapping

Since the group with revascularised angina pectoris and the group with MI without previous known CHD were not independent of each other a bootstrapping method was used to test the significance of the differences in hazard ratios between the groups.

Bootstrapping is a method in which a new reference population is created from the collected data by performing a large number of random samples from the original data. A random sample including an equal amount of observations as the original sample is identified and adequate calculations are performed. The sample is then returned to total dataset and the procedure is repeated 1000-10000 times. In this new reference population the standard errors were estimated and the p-values for the differences between hazard ratios were calculated.

The expression “bootstrap” is derived from the tale of baron von Münchhausen who managed to get himself up from a marsh by lifting himself in his bootstraps.
Paper III
Differences between groups (Q/QS-pattern versus no Q/QS-pattern, and MI diagnosis versus no MI diagnosis) regarding metabolic characteristics were evaluated by factorial two-way ANOVA. A significant interaction term between these two factors was regarded as an evidence for an impact of MI diagnosis on the risk factor profile in subjects with a Q/QS-pattern.

Paper IV
Generation of risk prediction score
For cross-validation the total data set was divided into a training data set in which the score was generated, and a prediction data set for validation of the score and for comparisons with the PROCAM and Framingham scores.

The multiple Cox Proportional Hazard Analysis was used for identifying independent baseline risk factors for fatal or nonfatal MI during the follow-up in the training data set. The continuous variables (serum proinsulin, systolic blood pressure and apoB/apoA1 ratio) were then divided into deciles. The risk of MI during the follow-up was calculated for each decile, and cut-off levels for increasing risk were decided. To calculate the scores between the variables and within each variable category, the decile-groups were dichotomised and entered into a logistic regression model together with family history of MI and smoking. The parameter estimates calculated in this manner were rounded to whole numbers and used as scoring points. The maximal score that could be achieved was 26 points.

Validation of the score
The estimated risk of fatal and nonfatal MI calculated by use of the ULSAM score was compared to the observed event rate over the entire range of deciles of risk, using the Hosmer-Lemeshow goodness of fit statistics. Areas under receiver operating curves were used to measure the abilities of the ULSAM, Framingham and PROCAM scores to separate those who developed fatal and nonfatal MI from those who did not in the prediction data set.
Discussion of Methods

Selection bias; general aspects

Participation
The participation rates were 82%, 87.5% and 73% at the examinations at age 50, 60 and 70. It is known that non-participants in cohort studies generally have more social and alcoholic problems and a higher morbidity in CHD compared with participants\(^{182}\). Thus, estimated risks of CHD may be underestimated rather than overestimated. However, higher participation rates than those in ULSAM are rarely found in population studies.

Mortality and morbidity analyses
No subjects were lost to follow-up due to missing data thanks to the official hospital and cause-of-death registries in Sweden. The cause of death registry includes all deaths of persons registered in Sweden at the time of death; deaths occurring outside Sweden are thus also recorded.

All hospital admissions in the Uppsala health care region have been reported to the HDR since 1965. Since 1984 the reporting to the HDR has been mandatory in Sweden and from 1987, the HDR covers all public, in-patient care in Sweden.

The validity of the HDR concerning MI diagnosis has been investigated by the Center of Epidemiology at the National Board of Health and Welfare. It was found that the MI diagnosis was correct in 95% and missed in 3% of the cases\(^{183}\). The discharge register does not include non-fatal home-treated or unrecognised MI. Unrecognised MI have been estimated to constitute 18-35% of the total number of cases of MI\(^{11-16}\) and was not included as cases in the present study, as in most other cohort studies of MI. To study unrecognised MI was however the scope of paper III.

Proinsulin
Due to a freezer failure proinsulin at age 50 was analysed in a random sample of 1,335 out of the total 2,322 subjects at age 50. Where appropriate, the statistical analyses were also performed only in subjects with proinsulin values. This reduction in sample size did not substantially change the results (paper I, II). In paper IV, in which proinsulin was a focus, the size of the cohort was however substantially reduced.

The proinsulin analyses at age 50 were performed in samples that had been stored in liquid nitrogen for 15 years and thereafter stored frozen in \(-70\)°C until the analyses in 1995-98. It cannot be ruled out that long time storage may have influenced the absolute values of proinsulin. However, it would have affected all samples in the same manner independently of the subjects developing CHD or not.
Smoking
Ex-smokers were classified as non-smokers since there were no differences in risk of fatal and nonfatal MI (p=0.52) or total mortality (p=0.56) between non-smokers and ex-smokers in the ULSAM cohort.

Selection bias; specific aspects

Paper II
The aim of the study was to evaluate differences between subjects developing stable angina pectoris without previous known MI and subjects developing MI without previous known CHD. We chose to study angina patients demanding CABG or PTCA in order to achieve an objective criterion of angina pectoris, as many false positive cases otherwise would be identified by medical history only. By choosing this approach we may have selected an angina pectoris group with a more severe form of arteriosclerosis than the average angina pectoris patient.

On the other hand, subjects with metabolic disorders, such as diabetes, may have been denied surgery because of a high intraoperative risk, and thus the subjects in the revascularised group may have had less metabolic abnormalities compared to the average angina patient and compared to the subjects in the group with MI. However, the prevalence of diabetes (fasting blood glucose >6.1 mmol/l) at the examination closest to the events (examination at age 60) did not differ between the revascularised group and the group with MI (7% versus 11%, p=0.30).

Although the angina pectoris patients did not have hospital diagnosis of MI prior to the revascularisation it cannot be excluded that they had experienced an unrecognised MI. Likewise, it cannot be excluded that the MI cases could have had a history of angina pectoris not rendering a hospital diagnosis. Thus, several types of selection bias might occur in the study population in paper II. It is however very hard to perform a similar population based study with a long follow-up period without selection bias and without risk of diluting an angina group with MI cases and vice versa.

Since 23 subjects in the revascularised group with angina pectoris had unstable angina pectoris according to hospital files, the analyses were also performed without these subjects. However, this exclusion did not substantially change the results.
Paper IV
Proinsulin and apoB/apoA1 were analysed only in 1,108 out of 2,053 eligible subjects, which may have induced a selection bias.

The incidence of MI was significantly higher in those with observations for proinsulin and the apoB/apoA1 ratio compared to those without observations. However, there were no differences concerning traditional atherogenic risk factors between subjects with MI in the analysed group and subjects with MI in the excluded group, except for higher total cholesterol (7.3 versus 6.9 mmol/l, p=0.002) in those with observations. Thus, the subjects with MI in the analysed population were representative of the total MI-population.

The training and the prediction data sets did not differ in traditional cardiovascular risk factors.

The predictive power of CHD risk factors during long follow-ups
The assessment of the ability of different risk factors in predicting CHD morbidity and mortality over a long follow-up was based on single measurements at baseline. It is known that the risk factor status in individuals at baseline may change during the course of follow-up\textsuperscript{184}, and so the length of follow-up may influence the predictive power.

Previous studies have concluded that serum cholesterol is a strong long-term risk factor for CHD, while the long term effect of smoking is likely to be dependant on the rate of giving up smoking during the follow-up. Blood pressure has also been shown to be a strong predictor of CHD even if it tends to loose its predictive power after 15-20 years\textsuperscript{185-187}. HDL-cholesterol, physical activity, diabetes and parental history all remained predictive of CHD over 10-15 years in a British study\textsuperscript{188}, while variables with less reliability, such as serum insulin, were not predictive over a longer follow-up.
RESULTS AND DISCUSSION

Paper I
Results
In the group with antihypertensive medication at age 60 the LDL-to HDL-cholesterol ratio at age 50 and an increase in fasting blood glucose between age 50 and 60 were significant independent risk factors for developing MI after the age of 60 over the 17.4 year follow-up. An increase of 10% in fasting blood glucose in the treatment group was associated with a 21.7% increased risk of MI after age 60. After addition of proinsulin to the model, the impact of increase in blood glucose declined, but was still a significant predictor of future MI (Figure 3a).

Figure 3a. Hazard ratios for MI after age 60 in the group with antihypertensive treatment, with and without proinsulin in the model. Stepwise multivariate CoxProportional Hazard analysis.
In the group without antihypertensive medication the LDL- to HDL-cholesterol ratio and serum proinsulin at age 50, as well as increase in systolic blood pressure between age 50 and 60 were independent predictors of subsequent MI, but increase in fasting blood glucose was not a risk factor in this group (Figure 3b).

Figure 3b. Hazard ratios for MI after age 60 in the group without antihypertensive treatment, with and without proinsulin in the model. Stepwise multivariate Cox Proportional Hazard analysis.
There was a significant interaction between proinsulin and antihypertensive treatment regarding increase in fasting blood glucose. Thus, those with the highest proinsulin concentrations at baseline and antihypertensive treatment at age 60 showed the greatest increases in fasting blood glucose concentrations between the age of 50 and 60 (Figure 4).

![Figure 4](chart.png)

Figure 4. Mean and standard error of the mean for change in blood glucose between age 50 and 60 according to serum proinsulin levels at age 50 in the group with, and the group without antihypertensive treatment at age 60.

There was a significant relation between the change in fasting blood glucose and the average of fasting blood glucose at age 50 and 60, which indicated that there was no regression towards the mean.
Discussion

During the last decade several studies have shown that a large part of patients with hypertension are resistant to insulin-stimulated glucose uptake and are hyperinsulinemic compared to normotensive controls. In previous prospective cohort studies treatment with beta-blockers and/or diuretics was associated with increased risk for diabetes. This is probably due to increased insulin resistance during treatment with beta-blockers and thiazides.

The relation of insulin resistance to CHD has been well-established. However, the influence of the metabolic changes induced by antihypertensive treatment on the risk of MI, has been questioned.

In the present study increase in fasting blood glucose, as well as the LDL-to-HDL-cholesterol ratio and serum proinsulin at baseline, were significant risk factors for MI after age 60 in the group with antihypertensive treatment. In spite of the treatment, the blood pressure at age 60 was not normalised, but neither baseline nor achieved blood pressure at age 60 were related to the incidence of MI.

In the multivariate analysis, the impact of increase in fasting blood glucose on the risk of MI was independent of baseline lipids, fasting insulin and glucose, BMI and blood pressure. However, when proinsulin was added to the models, the predictive power of increase in fasting blood glucose declined, indicating that insulin resistance at baseline, for which proinsulin may be considered as a marker, explains a part of the predictive power of the induced increase in fasting blood glucose in hypertensives.

However, even when the impact of insulin resistance was taken into account, the deleterious effect of the increase in fasting glucose was still significant, suggesting that both insulin resistance at baseline and the induced increase in insulin resistance caused by antihypertensive treatment are risk factors for MI.

An additional explanation of the deleterious effect of the increase blood glucose is the finding that glucose may directly affect the development of arteriosclerosis by impairing endothelial function. It is known that patients with type 2 diabetes mellitus have impaired endothelial function, and increased blood glucose concentrations have been proven to impair endothelial function in healthy subjects as well.
Paper II

Results

Serum lipids, as well as the apolipoproteins, were the most powerful risk factors for later revascularisation because of angina pectoris without previous hospital diagnosis of MI. Neither blood pressure, nor BMI, insulin or glucose parameters at IVGTT were significant risk factors in this group.

Serum lipids and apolipoproteins were also important predictors of MI without previous hospital diagnosis of CHD. However, in this group also smoking, blood pressure, BMI, insulin and glucose parameters at IVGTT, serum proinsulin, as well as palmitic, palmitoleic, stearic and oleic acid were powerful risk predictors.

In stepwise multivariate Cox proportional hazard models baseline values of LDL- and HDL-cholesterol (protective) turned out to be independent risk factors in subjects with angina pectoris. In the group with MI, the same analysis showed that serum proinsulin, SBP, serum apolipoprotein B, smoking and HDL-cholesterol (protective) were independent predictors of MI during the follow-up period. When comparing hazard ratios between the two groups, there were significant differences in hazard ratios for DBP, serum proinsulin and split proinsulin, but not for HDL- or LDL-cholesterol (Figure 5).

![Figure 5. Differences in hazard ratios for a one standard deviation variation in cardiovascular risk factors and their 95% confidence intervals, between subjects revascularised because of angina pectoris without prior MI and subjects with MI without prior CHD.](image)

The hazard ratios for metabolic characteristics at age 50 were also calculated without subjects with diabetes at age 60 (the examination closest to the events), but the results did not change.
Discussion

The main cause of acute coronary syndromes is plaque disruption with superimposed thrombosis\(^ {33-35}\), and it may be the vulnerability of the plaque rather than the extent of coronary atherosclerosis that is the major determinant of acute MI\(^ {38}\). This vulnerability has been extensively examined in several studies and inflammatory cell activity, cigarette smoking, disturbed fibrinolysis and hemodynamic factors\(^ {38} 40 41 112 138\) have been suggested to be potential risk factors for plaque rupture and thrombosis.

It is therefore not surprising that we found both similarities (lipid profile) and differences (serum proinsulin and DBP) between subjects developing acute MI without prior known CHD and subjects with angina pectoris demanding revascularisation without prior known MI, regarding the risk factor profile at baseline.

Insulin resistance\(^ {113 192}\), as well as elevated concentrations of proinsulin and insulin\(^ {108-110}\), are known to be strong determinants of PAI-1 activity, and may thereby cause impaired fibrinolysis and increased risk of thrombosis. In diabetic subjects elevated levels of PAI-1 has been found in extracted coronary atheroma\(^ {111}\) and it has been proposed that PAI-1 might cause a reduction of vascular smooth muscle cells in the plaque and thus make it more vulnerable to rupture. Thus, our finding that proinsulin is a risk factor for MI, but not for angina pectoris may be partly explained by increased PAI-1 activity.

In the present study elevated blood pressure was an independent risk factor for MI. It is well known that the incidence of CHD is increased in hypertensive patients compared to normotensive controls\(^ {133}\), also when the hypertension is under treatment\(^ {89}\). Hypertension may promote the development of atherosclerosis by causing impaired endothelial function\(^ {134-136}\), and may also be a trigger of acute plaque disruption by inducing mechanical stress on the arterial wall\(^ {137 138}\). Hypertension has also been associated with impaired fibrinolytic activity, as evaluated by an impaired capacity for stimulated release of tissue plasminogen activator from vascular endothelium\(^ {139}\), and may thereby promote thrombosis formation.
PAPER III

Results

**Impact of Q/QS-pattern in cross-sectional analysis**

Subjects with Q/QS-pattern at age 70 had lower values for early insulin response and total plasma insulin AUC at the 2-hour OGTT compared to those without Q/QS. This difference seemed to be caused by lower values in the group with Q/QS without MI diagnosis, but the interaction term between Q/QS-pattern and MI diagnosis was not significant. Insulin sensitivity (M-value at the euglycaemic hyperinsulinaemic clamp) and other risk factors analysed did not differ significantly between those with and without Q/QS-pattern.

**Impact of history of MI in cross-sectional analysis**

The subjects with MI diagnosis at age 70 had significantly higher concentrations of serum proinsulin, triglycerides, apolipoproteins and HDL-cholesterol, but lower blood pressure when compared to those without MI diagnosis. There were no differences in total and LDL-cholesterol between the groups.
Prevalence of different risk factors at age 70

The population was divided into 4 groups according to ECG-status and MI/CHD diagnosis at age 70.

While the prevalence of diabetes was elevated to a similar degree in all the groups with Q/QS pattern and/or MI diagnosis compared to the control group with normal ECG and no CHD diagnosis, an increased prevalence of smoking was only seen in those with Q/QS-pattern without MI diagnosis. The prevalence of hyperlipidemia was increased in the two groups with MI diagnosis, but not in the group with Q/QS-pattern without MI diagnosis. There was no significant difference in hypertension prevalence between the groups (Figure 6).

Figure 6. Prevalence of risk factors at age 70.
Follow-up after age 70

Q/QS-pattern was a significant predictor of total and cardiovascular mortality both in the univariate and the multivariate Cox proportional hazard models when the analysis were adjusted for smoking, hypertension, diabetes mellitus, LDL-cholesterol and MI diagnosis (Figure 7a and b).

MI diagnosis was a significant predictor of cardiovascular and total mortality in the univariate analysis, but lost its significance when the variable Q/QS-pattern was included in the model.

Figure 7a. Hazard ratios for total mortality after age 70. Stepwise multivariate Cox Proportional Hazard analysis.

Figure 7b. Hazard ratios for cardiovascular mortality after age 70. Stepwise multivariate Cox Proportional Hazard analysis.
There was a significant difference concerning total and cardiovascular mortality after age 70 between the four groups. In the group with Q/QS without MI diagnosis the incidence of total and cardiovascular mortality was significantly higher compared to the control group in a post-hoc analysis. The highest mortality rates were found in the group with Q/QS and MI diagnosis, but the interaction term between the two variables were not significant in the Cox proportional hazard analysis (Figure 8).

Figure 8. Total and cardiovascular mortality in the population during 9.4 years of follow-up after age 70 divided by the presence or absence of Q/QS pattern and MI diagnosis at age 70.
Discussion

In accordance with previous studies of the prognostic value of ECG abnormalities\textsuperscript{23-32}, the present study showed that the finding of a new Q/QS-pattern on the resting ECG was a significant predictor of cardiovascular and total mortality. The prognostic importance remained significant when the models were adjusted for smoking, diabetes, hypertension, hyperlipidemia and MI diagnosis. When looking at the subgroup with Q/QS-pattern without MI diagnosis, the risk of total and cardiovascular mortality was significantly increased compared to a control group without known CHD, further underlining that an unexpected finding of a new Q/QS on the ECG is not an innocent sign\textsuperscript{11-16}.

The major metabolic disturbances in subjects with Q/QS pattern without MI diagnosis were a reduced early insulin response and reduced plasma insulin AUC at the OGTT, as may be seen in the progressive development of type 2 diabetes mellitus\textsuperscript{193,194}. These findings are in accordance with previous studies that have indicated an association between diabetes mellitus and unrecognised MI\textsuperscript{15,17,18} and it has been proposed that autonomic neuropathy due to diabetes may explain this association\textsuperscript{19,20}. However, others have failed to prove that diabetics are more prone to unrecognised MI than non-diabetics\textsuperscript{21,22}.

Even though the Q/QS pattern was associated with indices of diabetes development in the present study, it was a significant predictor of total and cardiovascular mortality even when the effect of diabetes was accounted for in the multiple analyses. Thus, it is not only the diabetic state per se that causes the increased mortality in these subjects. It seems rather that the Q/QS pattern indicates myocardial damage that may lead to heart failure or conductance abnormalities, and thus increased risk of premature death.

Another contributory cause to the increased risk of total and cardiovascular mortality in subjects with Q/QS-pattern may be the high percentage of smokers seen in the group with Q/QS pattern without MI diagnosis compared to the control group. It is well known that smoking accelerates the atherosclerotic process and thus leads to an increased risk of coronary events\textsuperscript{195}. Previous studies have shown that patients with unrecognised MI tend to avoid contacts with physicians\textsuperscript{11} and hence reduce the possibility of getting anti-smoking advices. However, the predictive power of Q/QS pattern was independent of smoking in the multiple analyses.

The high percentage of smokers could also be an explanation to the lowered insulin response seen in this group. Smokers tend to have a lower BMI\textsuperscript{196,197}, and thereby lower insulin concentrations compared to non-smokers. However, when the analyses were adjusted for smoking, subjects with Q/QS-pattern still had significantly lower values for the insulinogenic index and total plasma insulin AUC at the 2-hour OGTT compared to those without Q/QS.
On the contrary, subjects with MI diagnosis did not have an increased percentage of smokers compared to controls, and had significantly lower blood pressure and no difference in LDL-cholesterol compared to those without MI diagnosis. It is likely that subjects with MI diagnosis participated in some form of rehabilitation program after the acute event, and thus may have modified their risk factor patterns. This may also have influenced the fact that MI was not an independent predictor of cardiovascular and total mortality in the multiple analyses.

Paper IV

Results

Generation of a risk prediction score (ULSAM score)
The score was based on 251 cases of fatal and nonfatal MI within 28 years of follow-up in 1,108 men who were free of CHD at baseline. Serum proinsulin, the apoB/apoA1 ratio, SBP, smoking and family history of MI were found to be independent significant predictors of fatal and nonfatal MI in the training data set and were thus included in the score.

Validation of the ULSAM score
There were highly significant differences regarding scoring points of the ULSAM score between subjects who developed MI during the follow-up and subjects who did not in both the training and the prediction datasets.

The estimated risk of fatal and nonfatal MI calculated by use of the ULSAM score was compared to the observed event rate over the entire range of decentiles of risk, using the Hosmer-Lemeshow goodness of fit statistics\(^{179}\). The estimated risk showed a good agreement with the observed incidence.

Figure 9 presents receiver-operating curves\(^{180} 181\) measuring the abilities of the ULSAM, Framingham and PROCAM scores to separate those who developed fatal and nonfatal MI from those who did not in the prediction data set during the follow-up of 28 years. Although the ULSAM score showed the best performance the areas under the curves did not differ significantly between the scores\(^ {198}\).
Figure 9. Receiver operating curves showing performance of ULSAM, PROCAM and Framingham scores in predicting risk of MI within 28 years of follow-up among middle-aged men in the ULSAM cohort. Numbers refers to areas under the curves.

In order to demonstrate the power of the study the 95% confidence intervals for the pairwise differences of areas under receiver-operating curves (expressed as percentages of the mean area) were calculated. These intervals did not exceed 12% of the mean area. These intervals were interpreted as being narrow indicating that the power to detect large differences was high.

Discussion
Serum proinsulin, the ratio apoB/apoA1, smoking, SBP and family history of MI were independent predictors of fatal and non-fatal MI over a period of 28 years in the ULSAM cohort, and were therefore included in the scoring scheme. Traditional risk predictors such as LDL- and HDL- cholesterol, serum triglycerides and diabetes prevalence, were also included in the multiple Cox proportional hazard analysis, but these variables were not independent predictors of MI when apoB/apoA1 and serum proinsulin were added to the analysis.

Hypercholesterolemia and especially high concentrations of LDL-cholesterol are generally accepted as strong risk factors of cardiovascular disease\(^\text{122}\). However, several studies have suggested that apoB and apoA1 also are important risk predictors\(^\text{126} 199-201\). A recent large Swedish study showed that apoB, apoA1 and especially the ratio of apoB/apoA1 were inde-
ependent risk predictors of fatal MI after adjustment for age, total cholesterol and triglycerides. The importance of apolipoproteins was also valid for subjects older than 70 years and for subjects with concentrations of LDL-cholesterol below the median. ApoB is present not only in LDL-particles, but also in VLDL and intermediate density lipoproteins, and may therefore be a better indicator of the total atherogenic content of the lipoproteins. ApoA1 has been shown to modify the ability of HDL-particles to remove cholesterol from cells and may play an important role in the atherosclerotic process especially in low-risk populations with normal cholesterol concentrations, and it may therefore be the content of apoA1 rather than cholesterol in HDL-particles that is of most importance for the development of atherosclerosis.

The role of insulin as a risk factor for CHD has been debated, and in a meta-analysis of 12 studies investigating this relationship, it was concluded that hyperinsulinemia was only a weak risk indicator for the occurrence of cardiovascular disease. Proinsulin, on the other hand has recently been shown to be an independent predictor of CHD, even though the mechanisms for this association remain uncertain. Insulin resistance and elevated concentrations of proinsulin and insulin are known to be strong determinants of PAI-1 activity, and may thereby cause impaired fibrinolysis and increased risk of thrombosis. Thus, increased PAI-1 activity may be one potential link between proinsulin and coronary events.

In the present study, the performance of the ULSAM score, including proinsulin and the apoB/apoA1 ratio, in predicting the absolute risk of fatal and nonfatal MI was good. The ability of the score to separate those who developed fatal and non fatal MI from those who did not during the follow-up (measured as areas under receiver operating curves) was at least as good as the Framingham and PROCAM scores. Thus, newer risk predictors, such as proinsulin and the apoB/apoA1 ratio, seem to contain at least as much predictive power as established risk factors, such as different glucose parameters, HDL- and LDL-cholesterol and serum triglycerides, and might therefore be used more frequently in the future.
GENERAL DISCUSSION

Metabolic consequences of antihypertensive treatment

Antihypertensive treatment reduces the risk of CHD by 14% according to a meta-analysis performed in 1990\textsuperscript{202}. However, in the beginning of the era of antihypertensive treatment an even larger reduction in CHD morbidity and mortality was expected.

One of the reasons for this rather modest effect may be the metabolic disturbances induced by older antihypertensive drugs, such as beta-blockers and thiazide diuretics. During the 1980s it was shown in several studies that individuals with hypertension were insulin resistant compared to normotensive controls\textsuperscript{140-142} and that treatment with beta-blockers and thiazide diuretics induced decreased insulin sensitivity\textsuperscript{146,147} and thus increased risk of developing type 2 diabetes mellitus\textsuperscript{149-152}. This has also been confirmed in a recent study in which treatment with thiazide diuretics and beta-blockers was associated with an aggravated metabolic profile including an increase in the ratio between apolipoprotein B and apolipoprotein A\textsuperscript{148}.

The clinical importance of the induced metabolic abnormalities has been questioned. In a Swedish study\textsuperscript{162} it was found that development of diabetes mellitus related to antihypertensive treatment did not increase the risk of coronary events. However, that study used diabetes mellitus (fasting blood glucose>7.0 mmol/l) as a categorical variable, and since the relationship between blood glucose concentrations and risk of CHD appears to be linear\textsuperscript{203}, a categorical grouping based on fasting blood glucose diminishes the statistical capacity of detecting an effect. Furthermore, the confidence interval of the risk associated with drug related diabetes mellitus was wide. It is therefore hazardous to exclude an effect of drug related diabetes mellitus on the risk of MI based on the data from that study.

In the ULSAM cohort increase in fasting blood glucose during 10 years was an independent risk factor for MI in 291 men with antihypertensive treatment with beta-blockers and thiazide diuretics during a follow-up of 17 years (paper I). There was an interaction between insulin resistance (indicated by the serum proinsulin concentration) at baseline and antihypertensive treatment on the degree of increase in blood glucose. However, increase in fasting blood glucose was an independent risk factor also when the analysis was adjusted for insulin resistance at baseline.
A limitation of the study was that the comparisons were made between one group with metabolic abnormalities at baseline at age 50 (the group with treatment) and one group without such abnormalities (the untreated group). Subjects without antihypertensive treatment at age 60 but with insulin resistance (the highest quartile of serum proinsulin) at baseline also had an increase in blood glucose during 10 years as a sign of an increase in insulin resistance (Figure 4). However, this increase was significantly smaller than in the group with antihypertensive treatment, indicating that insulin resistance at baseline only explained parts of the increase in blood glucose in the treatment group.

To further examine the influence of pre-existing risk factors on increase in blood glucose, a subgroup with hypertension ($\geq$154/93 mmHg) but without antihypertensive treatment at age 60 was identified ($n=166$). This group did not differ from the group with antihypertensive treatment concerning metabolic abnormalities at baseline, but in contrast to the treated group these subjects did not increase in blood glucose during the following 10 years.

During the 1990s several large intervention studies were performed where older antihypertensive drugs were compared to newer agents such as calcium channel blockers, ACE-inhibitors and angiotensin II (AII) blockers. In several of these studies (CAPPP163; LIFE164; ALLHAT165) there was an increased incidence of new-onset diabetes in subjects treated with beta-blockers/diuretics compared with those treated with more modern drugs. In the HOPE trial204 ACE inhibition was even associated with less new-onset diabetes in a population at high cardiovascular risk when compared to placebo. In the LIFE and ANBP2205 studies treatments with ACE-inhibitors/AII-blockers were associated with a lower risk of cardiovascular events compared to older agents, while in the CAPPP, ALLHAT and UKPDS studies older drugs performed at least as well as newer agents in preventing cardiovascular endpoints.

A major limitation of these studies is that the follow-ups are limited to about 5 years. A prolongation of two large intervention studies206 207 showed that a follow-up of at least 8-10 years is necessary to demonstrate the effect of antihypertensive treatment on CHD. In paper I based on the ULSAM population the mean time to the events was at least 8 years from the start of follow-up, which also strengthens the fact that longer follow-ups are needed.

In view of an expected steep increase in the incidence of type 2 diabetes it appears important to avoid further deterioration of insulin sensitivity in already insulin resistant subjects. Hence, a better screening of hypertensive patients in order to detect prevailing insulin resistance is needed to individualize the antihypertensive treatment.
Differences in clinical manifestations of coronary heart disease

From a clinical point of view it is clear that CHD can vary substantially in its presentation with manifestations such as stable and unstable angina pectoris, unrecognised MI and diagnosed MI with or without ST-elevation and with or without previous signs of cardiac ischemia.

Incidence

During the last decades the incidence of MI has decreased while hospitalisations due to unstable angina pectoris has increased. These changes are probably to a large extent reflecting changes in coronary risk factors, improvements in coronary care and a possible diagnostic coding drift. Unrecognised myocardial infarctions have been estimated to constitute 18-35% of the total number of cases of MI, but the incidence and prevalence may be underestimated since it is not unusual that typical ECG findings for MI disappear with time.

Prognosis

The prognosis associated with different manifestations of CHD has been examined in several studies. Early investigations of the prognosis associated with uncomplicated angina showed a prognosis just as bad as for MI. However, more recent population-based studies of prevalent CHD have concluded that patients with angina have a better prognosis compared to patients with MI, but still a reduced survival compared with individuals without CHD. In the case of unrecognised MI, the prognosis appears to be almost the same as for diagnosed MI.

The prognostic significance of different ECG abnormalities, such as Q-wave or QS-pattern, has been extensively examined in several epidemiological studies and a strong association with cardiovascular morbidity and mortality has been found. This was confirmed in paper III where a new Q/QS-pattern on the resting ECG was a significant predictor of cardiovascular and total mortality even when the analyses were adjusted for smoking, diabetes, hypertension, hyperlipidemia and MI diagnosis over a time-period of almost 10 years.

What is the pathophysiological background to the increased mortality risk associated with the Q/QS-pattern? In paper III the Q/QS-pattern was associated with impaired insulin secretion and the prevalence of diabetes was increased compared to a control group. However, Q/QS-pattern was a significant predictor of total and cardiovascular mortality even when the effect of diabetes was accounted for in the multiple analyses. Thus, it is not only the diabetic state per se that causes the increased mortality in these subjects. It seems rather that the Q/QS pattern indicates an objective sign of myocardial damage that may lead to heart failure or conductance abnormalities, and thus
increased risk of premature death. Individuals with adverse risk factor profiles should therefore have frequent ECG monitoring and subjects with a new Q/QS-pattern on the routine ECG should be given a high priority to preventive measures against both CHD and diabetes.

The relative risks associated with ECG-findings and CHD diagnosis have been shown to attenuate over long follow-ups probably due to changes in risk factor patterns, selective mortality and interventions, such as CABG and PTCA. However, the excess mortality risk for subjects with CHD compared to those without do persist over follow-ups as long as 15 years.\textsuperscript{212,213}

**Risk Factors**

Can differences in presentation, incidence and prognosis be explained by different pathophysiology and risk factor patterns in subjects with stable and unstable CHD?

The major pathophysiological difference between acute coronary syndromes and stable angina pectoris is the rupture of an atherosclerotic plaque with subsequent thrombosis formation that causes acute coronary events.\textsuperscript{33,35} Hypertension, smoking, hyperlipidemia and diabetes are the major abnormalities that are generally accepted as risk factors for all manifestations of CHD, while inflammatory cell activity, disturbed fibrinolysis and hemodynamic factors also have been suggested to be potential risk factors for plaque rupture and thrombosis.

Unrecognised MI has been associated with increased prevalence of hypertension and diabetes in some studies, while others have failed to show differences in risk factor patterns between diagnosed and unrecognised MI.\textsuperscript{21,22}

Some previous studies have examined differences in risk factor profiles between subjects with stable and unstable CHD. In one study stable CHD was associated with increased levels of HDL cholesterol and increased prevalence of diabetes, while in another study no differences between the different manifestations of CHD was found.\textsuperscript{10}

In paper II serum proinsulin and blood pressure were independent risk factors for MI without previous known CHD, but not for angina pectoris demanding revascularisation with PTCA or CABG. Indeed there is a biological plausibility for these findings in that elevated concentrations of proinsulin are known to be strong determinants of PAI-1 activity, and may thereby cause impaired fibrinolysis and increased risk of thrombosis. Hypertension may promote the development of atherosclerosis by causing impaired endothelial function, and may also be a trigger of acute plaque disruption by inducing mechanical stress on the arterial wall.\textsuperscript{137,138}

Thus, current knowledge on the pathophysiology of plaque rupture may strengthen the hypothesis that there indeed exists a biological ground for the differences in clinical presentation. The result of paper II may implicate that treatment of insulin resistance and thus lowering proinsulin and treatment of
hypertension may be favourable in stabilizing the atherosclerotic plaque. However, the findings in paper II are only valid for the specific groups identified in the study and cannot be transferred to general angina pectoris and MI populations. Further investigations concerning risk factor patterns associated with different clinical manifestations of CHD are therefore needed.

The metabolic syndrome and coronary heart disease; future perspectives and clinical implications

How to find subjects at high risk

The diagnosis of the metabolic syndrome implies a substantial additional risk of cardiovascular morbidity and mortality also above and beyond the individual risk factors. However, it has been shown that proatherogenic metabolic disturbances already in the prediabetic state may contribute to the risk of macrovascular disease as much as clinical diabetes in itself. It is therefore important to identify individuals at high risk even before the development of disturbances in traditional risk markers in order to prevent the development of diabetes and CHD. New risk markers such as CRP, apolipoproteins and serum proinsulin may help to identify these individuals.

Serum proinsulin has been shown to be a strong predictor of both diabetes and CHD also in non-diabetic populations. By using serum proinsulin instead of fasting blood glucose as a marker of increased risk, it may therefore be possible to capture individuals who are at high risk of developing CHD although not yet being diabetic.

Recently apoB and apoA1 and especially the apoB/apoA1 ratio have emerged as powerful predictors of CHD risk. It is plausible that these markers of dyslipidemia contain more information concerning lipid status than conventional markers, such as LDL- and HDL-cholesterol. Moreover, it has been shown that apoB and apoA1 retain their predictive power in patients receiving lipid-lowering agents. There are also methodological advantages since the apolipoproteins are measured directly in blood samples while LDL-cholesterol most often is calculated using the Friedewald formula.

The methods for measuring apoB and apoA1 are internationally standardized and can therefore be used in clinical practice, while standardization for the analysis of serum proinsulin is yet lacking.

In paper IV proinsulin and the apoB/apoA1 ratio were included in a scoring scheme for the risk of fatal and nonfatal MI. This score was highly predictive for future MI and showed better performance in separating those who developed MI from those who did not compared to Framingham and PROCAM scores, although the difference between the areas under the curves was
only borderline significant (p=0.08). Future investigations in larger populations may confirm that these new risk markers indeed do contain more information than traditional risk factors.

**Treatment of the metabolic syndrome**

Life style changes should always be the first treatment of choice for the metabolic syndrome. Intervention trials have concluded that life style changes can reduce body weight, blood pressure and serum triglycerides, increase HDL-cholesterol and lower the risk of progression from impaired fasting glucose to type 2 diabetes by 58%\textsuperscript{218,219}.

However, there most certainly exists a genetic component in the pathophysiology of insulin resistance and the metabolic syndrome, and therefore life style changes may not be enough to reduce cardiovascular risk. Treatment with insulin-sensitising agents, such as the thiazolidinediones, has not only been shown to increase insulin sensitivity, but also to decrease levels of CRP\textsuperscript{220}, and thus these agents may have a potential to reduce cardiovascular events in subjects with insulin resistance and type 2 diabetes. Moreover, matrix metalloproteinase-9, a marker of plaque instability, has also been decreased during treatment with these agents\textsuperscript{220} and hence they may have a plaque-stabilizing effect. Ongoing clinical trials of the effects of insulin-sensitisers on cardiovascular outcomes will bring more information about the potential of these agents in treating insulin resistance and type 2 diabetes.

Since cardiovascular disease is estimated to be the leading cause of death in the world in a not too remote future, there is an ongoing development of new drugs. The so-called “dual PPAR agonists” that activate both PPAR-\(\alpha\) and PPAR-\(\gamma\), may offer a treatment for the major metabolic disturbances of the metabolic syndrome, i.e. lower triglycerides, raise HDL-cholesterol and increase insulin sensitivity\textsuperscript{221}. Another strategy to deal with high risk factors for CHD has been advocated by Wald and Law who in a recent publication suggested that aspirin, blood pressure lowering agents, a statin and folic acid should be merged together in a “polypill”. According to a meta-analysis of several studies, this treatment would prevent more than 80% of CHD events if given to everyone with existing cardiovascular disease and to everyone over the age of 55\textsuperscript{222}.

However, the safety of these kinds of drugs is still to be confirmed in large-scale studies. Furthermore, the risk of less interest in lifestyle changes as a consequence of the introduction of a universal polypharmacy with a “polypill” must be taken under consideration.
CONCLUSIONS

I. Study I emphasized the existence of an insulin resistant state with elevated proinsulin concentrations resulting in increased fasting blood glucose concentrations during antihypertensive treatment with beta-blockers and thiazide diuretics. Both proinsulin concentrations and increase in fasting blood glucose were associated with increased risk of developing future MI in those on antihypertensive treatment with beta-blockers and thiazide diuretics.

II. Serum lipids were important risk factors for the development of both angina pectoris demanding revascularisation and acute MI without previous known CHD. In addition, proinsulin and blood pressure were independent predictors of MI only, suggesting these factors to be involved in thrombosis and plaque rupture.

III. The finding of a new Q/QS-pattern on the resting ECG, regardless of history of MI, was associated with impaired insulin secretion and was an independent predictor of total and cardiovascular mortality. Therefore, subjects with a new Q/QS-pattern on the routine ECG must be given a high priority to preventive measures against both CHD and diabetes.

IV. A risk prediction score for MI including proinsulin and the ratio between apolipoprotein B and apolipoprotein A1 was developed in middle-aged men. This score was predictive for future fatal and nonfatal MI, and proved to be at least as good as the Framingham and the PROCAM scores, being based on traditional risk factors.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to October, 1985, the series was published under the title “Abstracts of Uppsala Dissertations from the Faculty of Medicine”.)