Hyperinsulinaemia and the Risk of Cardiovascular Disease
The 22-Year Follow-up Results of the Helsinki Policemen Study

Doctoral dissertation

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ABSTRACT
The association between hyperinsulinaemia and cardiovascular disease has been a subject of interest but also of controversy for more than 3 decades. The Helsinki Policemen Study was one of the first prospective epidemiological studies demonstrating that high plasma insulin is associated with increased risk of coronary heart disease (CHD) independently of other cardiovascular risk factors. The aim of the present study was to investigate the predictive value of hyperinsulinaemia with regard to the risk of CHD and stroke and all-cause mortality, as well as its subcategories, in the Helsinki Policemen Study population during a follow-up of 22 years.

The study was based on a cohort of 970 men aged 34-64 who were free of cardiovascular disease and diabetes. Risk factor measurements at baseline examination in 1971-1972 included an oral glucose tolerance test with blood glucose and plasma insulin measurements at 0, 1 and 2 hours. In this study, the area under the insulin response curve (AUC insulin) during oral glucose tolerance test was used as a composite variable to reflect plasma insulin levels.

During the 22-year follow-up, 276 men died, 130 from cardiovascular and 146 from non-cardiovascular causes. 164 men had a major CHD event (CHD death or non-fatal myocardial infarction) and 70 men had a fatal or non-fatal stroke. Hyperinsulinaemia (highest AUC insulin quintile vs. the combined 4 lower quintiles) was associated with an increased risk of a major CHD event during 5-, 10-, 15-, and 22-year follow-up periods. Adjusting for age, the respective hazard ratios (HRs) were 3.29 (95% confidence interval [CI], 1.56-6.91), 2.72 (95% CI, 1.67-4.42), 2.14 (95% CI, 1.43-3.21), and 1.61 (95% CI, 1.14-2.27). Further adjustment for other risk factors attenuated these HRs to 2.36 (95% CI, 1.00-5.57), 2.29 (95% CI, 1.31-4.02), 1.76 (95% CI, 1.09-2.82), and 1.32 (95% CI, 0.89-1.97), respectively. During the 22-year follow-up, hyperinsulinaemia was associated with the risk of stroke (age-adjusted HR 2.12 (95% CI, 1.28-3.49) but not independently of other risk factors, especially indices of obesity (multiple-adjusted HR 1.54 (95% CI, 0.90-2.62). Hyperinsulinaemia was associated with increased all-cause and cardiovascular mortality; age-adjusted HRs for all-cause mortality during 10 and 22 years of follow-up were 1.94 (95% CI, 1.20-3.13) and 1.51 (95% CI,1.15-1.97), and for cardiovascular mortality 2.67 (95% CI, 1.35-5.29) and 1.73 (95% CI, 1.19-2.53), respectively. Multiple adjustment weakened these HRs, for all-cause mortality to 1.88 (95% CI, 1.08-3.30) and 1.37 (95% CI, 1.00-1.87), and for cardiovascular mortality to 2.30 (95% CI, 1.03-5.12) and 1.39 (95% CI, 0.90-2.15), respectively. A U-shaped association was observed between insulin and non-cardiovascular mortality; multiple-adjusted 22-year HRs for lowest and highest vs. middle quintiles of AUC insulin were 1.85 (95% CI, 1.20-2.86), and 1.43(95% CI, 0.91-2.24), respectively. Factor analysis including 10 cardiovascular risk factors yielded an ‘insulin resistance’ factor, with high loadings for AUC insulin, BMI, subscapular skinfold, AUC glucose, triglycerides, and mean blood pressure. During the 22-year follow-up, this insulin resistance factor predicted the risk of both CHD (HR 1.28 [95% CI, 1.10-1.50]) and stroke (HR 1.64 [95% CI, 1.29-2.08]).

In conclusion, in the 22-year follow-up of the Helsinki Policemen Study, hyperinsulinaemia predicted the risk of CHD events and all-cause and cardiovascular mortality, although its predictive value diminished with the lengthening of follow-up time. Hyperinsulinaemia was also associated with the risk of stroke, but this association was not independent of other risk factors, particularly indices of obesity. However, the insulin resistance factor, reflecting the combined effect of insulin and associated risk factors, was a significant predictor of the risk of both CHD and stroke.

National Library of Medicine Classification: WG 120, WK 880
Medical Subject Headings: cardiovascular disease; cerebrovascular accident; coronary disease; epidemiology; factor analysis, statistical; insulin; insulin resistance; mortality; risk factors
To my father
ACKNOWLEDGEMENTS

This thesis work was carried out in the Department of Medicine, University of Kuopio. It is based on long-term follow-up results of the Helsinki Policemen Study, a prospective epidemiological study of cardiovascular diseases and their risk factors, initiated in 1966 and originally conducted under the auspices of the Finnish Heart Association. Kalevi Pyörälä, MD, Pentti Siltanen, MD, and Sven Punsar, MD, were the planners and principal investigators of the study. When Kalevi Pyörälä became in 1975 appointed Professor of Medicine at the University of Kuopio, he took on the responsibility for the organisation of long-term follow-up of the Helsinki Policemen Study. In 1987 the data files of the study were transferred to Kuopio and the study became a part of the research project ‘Insulin resistance. Epidemiology, pathophysiology and association with atherosclerosis’ led by Professor Markku Laakso, MD, and Professor Kalevi Pyörälä.

First of all, I want to thank my father, Emeritus Professor Kalevi Pyörälä, who proposed the topic of this thesis to me and who has been my personal guide, adviser and source of information from the initiation of this study project to its accomplishment. I cannot find the words to thank him enough for all the help and support he has given to me. I admire his wisdom, his profound knowledge and experience in scientific and clinical work, as well as his enthusiasm and persistence in all he sets out to do. Working with him has been one of the finest and most precious experiences of my life.

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April 2003

Marja Pyörälä
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AACE</td>
<td>American Association of Clinical Endocrinologists</td>
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<td>ADA</td>
<td>American Diabetes Association</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>ARIC</td>
<td>Atherosclerosis Risk in Communities</td>
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<tr>
<td>AUC glucose</td>
<td>Area under the glucose response curve</td>
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<td>AUC insulin</td>
<td>Area under the insulin response curve</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
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<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>EGIR</td>
<td>European Group for the Study of Insulin Resistance</td>
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<tr>
<td>FFA</td>
<td>Free fatty acid</td>
</tr>
<tr>
<td>FINMONICA</td>
<td>FINnish contribution to the MONICA project</td>
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<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
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<tr>
<td>HOMA-IR</td>
<td>Homeostasis model assessment of insulin resistance</td>
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<tr>
<td>HR</td>
<td>Hazard ratio</td>
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<tr>
<td>ICD-8</td>
<td>International Classification of Diseases, Eighth Revision</td>
</tr>
<tr>
<td>ICD-9</td>
<td>International Classification of Diseases, Ninth Revision</td>
</tr>
<tr>
<td>IMT</td>
<td>Intima-media thickness</td>
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<tr>
<td>IRAS</td>
<td>Insulin Resistance and Atherosclerosis Study</td>
</tr>
<tr>
<td>IRS</td>
<td>Insulin resistance syndrome</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>MRFIT</td>
<td>Multiple Risk Factor Intervention Trial</td>
</tr>
<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>O₂</td>
<td>Oxygen</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>PPAR-γ2</td>
<td>Peroxisome proliferator-activated receptor-gamma2</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SSPG</td>
<td>Steady state plasma glucose</td>
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<tr>
<td>tPA</td>
<td>Tissue plasminogen activator</td>
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<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<tr>
<td>WHR</td>
<td>Waist to hip ratio</td>
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which will be referred to by their Roman numerals:


Additionally, some unpublished data are presented.
CONTENTS

1  INTRODUCTION  15

2  REVIEW OF LITERATURE  17
  2.1  Early observations  17
  2.1.1  Clinical and epidemiological studies  17
  2.1.2  Animal experiments and studies of arterial wall metabolism and cell biology  19
  2.2  Insulin resistance syndrome  20
  2.2.1  Evolution of the concept  20
  2.2.2  Insulin resistance: measurement and relationship with plasma insulin levels  21
  2.2.3  Recent developments in the definition of the insulin resistance syndrome  23
  2.3  Factors associated with insulin resistance and hyperinsulinaemia  25
  2.3.1  Biological factors  25
    - Obesity and its distribution  25
    - Glucose tolerance  29
    - Plasma lipids and lipoproteins  30
    - Blood pressure  33
    - Thrombogenic factors  35
    - Uric acid  37
    - Inflammation  38
    - Vascular function  39
    - Neuroendocrine factors  42
  2.3.2  Lifestyles and lifestyle-related factors  43
    - Physical activity and physical fitness  43
    - Dietary factors  44
    - Alcohol consumption  46
    - Smoking  46
  2.3.3  Genetic and early-life factors  47
  2.4  Studies of the insulin resistance syndrome by factor analysis  49
  2.5  An update of prospective epidemiological studies  53
  2.5.1  Insulin and the risk of coronary heart disease or cardiovascular disease  53
  2.5.2  Meta-analysis of studies on the association of insulin with cardiovascular risk  67
  2.5.3  Proinsulin-like molecules and the risk of coronary heart disease and cardiovascular disease  69
  2.5.4  Insulin and the risk of stroke  71
  2.5.5  Insulin and all-cause and non-cardiovascular mortality  74
  2.5.6  Insulin resistance syndrome and the risk of cardiovascular disease and all-cause mortality  76
  2.6  Other studies on the association of insulin and insulin resistance with atherosclerosis  78

3  AIMS OF THE STUDY  81
1 INTRODUCTION

The risk factors of chronic diseases such as cardiovascular disease (CVD) have been identified in prospective epidemiological studies which have examined the relationship of characteristics of healthy individuals with the occurrence of the disease during the follow-up. The first prospective studies of CVD epidemiology, among them the Framingham Study (1), were started in the United States in the late 1940’s, and the famous international prospective epidemiological study, the Seven Countries Study led by Ancel Keys (2), was launched 10 years later. Stimulated by these examples, a large number of prospective epidemiological studies on CVD have been conducted in different parts of the world and as the outcome of these studies there is now a wealth of information about factors that are associated with the risk of coronary heart disease (CHD) and stroke and CVD mortality in different populations.

The term ‘risk factor’ for CVD was used for the first time in 1961 in an article on the Framingham Study (3) and since then it became rapidly adopted into common use. Used in a broad sense, risk factors can be divided into modifiable and non-modifiable factors. Modifiable factors include biological factors, such as plasma lipids and their fractions, blood pressure, blood glucose and degree of obesity, and lifestyle and behavioural factors, such as dietary habits, smoking and physical activity. Lifestyle factors exert their effect on the risk in part through biological factors. Age and gender are non-modifiable factors related to the risk. Genetic factors can also be considered as non-modifiable, but they may interact with lifestyle factors.

Already in the 1960’s it became clear that more than one half of the occurrence of CHD within populations can be explained by a small number of ‘major’ risk factors which include elevated plasma cholesterol, elevated blood pressure, smoking, and diabetes. In the 1970’s better understanding of the role of different lipoproteins improved the prediction of CVD risk to some extent. With time a large number of ‘new’ CVD risk factors have been proposed. Already more than 20 years ago, 246 risk factor candidates had been identified (4) and their number continues to grow. Few of these risk factor candidates have, however, stood the test of further research. Hyperinsulinaemia, elevation of plasma insulin levels, was proposed as a risk factor for CHD in 1979-1980
on the basis of results of 3 prospective studies, the Helsinki Policemen Study (5), the Busselton Study (6), and the Paris Prospective Study (7). At the same time, when these epidemiological observations on the association between plasma insulin levels and CHD risk were published, the so-called euglycaemic hyperinsulinaemic clamp technique for clinical studies of insulin sensitivity of the tissues was described (8) and it became known that hyperinsulinaemia is partly a reflection of the underlying insulin resistance.

In 1988 Reaven introduced the concept of the insulin resistance syndrome (IRS) (9) which in its present form is considered as a clustering of insulin resistance and hyperinsulinaemia with the following risk factors: impaired glucose tolerance, high triglycerides, low high-density lipoprotein (HDL) cholesterol, elevated blood pressure, and obesity and its central distribution. Since then, there has been a growing interest in the IRS and its relation with the risk of CVD and this has intensified the interest in the results of prospective epidemiological studies on the association between insulin and CVD risk. The results of such studies have not been uniform, and the debate on the role of insulin as a risk factor for CVD has at times been rather heated as exemplified by some titles of the debate articles: ‘Is insulin atherogenic?’ (10), ‘Why is insulin not a risk factor for coronary heart disease?’ (11), ‘Insulin and atherosclerosis: villain, accomplice, or innocent bystander?’ (12), and ‘The insulin resistance syndrome: the controversy is dead, long live the controversy!’ (13).

The present study extends the follow-up of the Helsinki Policemen Study up to 22 years and its aim is to investigate the predictive value of hyperinsulinaemia with regard to the risk of CHD and stroke and all-cause, cardiovascular and non-cardiovascular mortality, and to set the findings of this study into perspective provided by other relevant research.
2 REVIEW OF LITERATURE

2.1 Early observations

2.1.1 Clinical and epidemiological studies

The radioimmunological method for the measurement of endogenous plasma insulin was introduced by Yalow and Berson in 1960 (14). The in vivo and in vitro bioassays of insulin used earlier were very laborious and had therefore been of limited use, whereas the radioimmunological insulin assay could be used for the measurement of plasma insulin in large series of samples and thus possibilities were opened for studying plasma insulin concentrations in different physiological conditions and metabolic disorders. With improved standardisation of the methods, plasma insulin measurements could also be introduced into epidemiological research.

The first observations of the association between endogenous insulin and CHD came from the field of diabetes research. The high incidence of CHD and other forms of atherosclerotic disease in diabetic patients had been known for long and this had led to clinical studies investigating the possible role of milder abnormalities of glucose metabolism, detected by an oral glucose tolerance test (OGTT), in the pathogenesis of atherosclerotic disease. In 1965 2 research groups, one from Cambridge, United Kingdom (15) and the other from Helsinki, Finland (16), observed that plasma insulin levels measured during OGTT in non-diabetic patients with previous myocardial infarction (MI) were elevated relative to those observed in healthy control subjects. This observation was soon confirmed in other case-control studies, as reviewed by Stout (17). Clinical case-control studies demonstrated elevated plasma insulin levels also in patients with other forms of atherosclerotic disease, such as lower-extremity arterial disease and cerebrovascular disease (17).

The Helsinki Policemen Study (5), the Busselton Study (6) and the Paris Prospective Study (7) were the first prospective epidemiological studies showing in healthy subjects an association of high plasma insulin, fasting or after oral glucose load, with the future risk of CHD. These studies, conducted independently of each other, reported their first
results on the association between insulin and CHD risk in 1979-1980.

The initial survey of the Helsinki Policemen Study was conducted in 1966-1967 and comprised 1326 policemen aged ≥30 (18). In the second survey carried out in 1971-1972 plasma insulin measurements were made from blood samples taken fasting and 1 and 2 hours after oral glucose load (60, 75 or 90 g according to body surface area). The first follow-up report of the Helsinki Policemen Study on the association of plasma insulin levels with CHD risk was based on a 5-year follow-up of 1042 men who were 35-64 years of age and free of CHD and previously diagnosed diabetes at the time of the second survey (5). During the follow-up, 11 men died from CHD and 36 men had either a fatal or non-fatal MI. The incidence of these ‘hard criteria’ CHD events was highest in the top quintiles of all plasma insulin values. In multivariate analyses, high 1- and 2-hour post-glucose insulin levels as well as high total insulin response to glucose load (the sum of insulin values) were independent predictors of both CHD mortality and CHD events after adjustment for age, body mass index (BMI), systolic blood pressure, plasma cholesterol and triglycerides, blood glucose, and smoking. Fasting plasma insulin did not show an independent association with CHD risk.

The first report of the Busselton Study on the association of insulin with CHD and CVD risk was based on a cohort of 3331 men and women aged 21-74, representing the general population of Busselton Shire, Western Australia (6). At the baseline examination in 1966, the participants were given a 50 g oral glucose load without reference to fasting and blood glucose and plasma insulin concentrations were measured from blood samples taken 1 hour later. Results were reported with regard to both the 6-year incidence of CHD (CHD death, non-fatal MI, new angina pectoris or new ischaemic changes in electrocardiogram [ECG]) and 12-year CHD and CVD mortality. The 6-year incidence study excluded participants with CHD at baseline, resulting in a study cohort of 2179 subjects. Participants with diabetes were not excluded from these follow-up studies. In men, elevated insulin levels were associated with both 6-year CHD incidence and 12-year CHD and CVD mortality. The association of hyperinsulinaemia with CHD and CVD mortality proved to be independent, when other high ranking risk factors, including systolic blood pressure, cholesterol and blood glucose, were taken into account. In Busselton women, no association between insulin levels and 6-year CHD
incidence or 12-year CHD or CVD mortality was observed.

The first report from the Paris Prospective Study on the association of insulin with the risk of CHD was based on a 5-year follow-up of a cohort of 7246 policemen aged 43-54, working in the Paris Civil Cervice (7). These men were free of CHD and diabetes at baseline examination conducted in 1968-1973. The study protocol included an OGTT (75 g of glucose) with measurements of plasma glucose and insulin fasting and 2 hours after the glucose load. There were 128 CHD events (CHD death or non-fatal MI) during the follow-up. In univariate analysis by insulin quintiles, the incidence of CHD events was increased at highest fasting and 2-hour insulin levels. In multivariate analysis, however, only fasting insulin was significantly associated with CHD risk when adjustment was made, in addition to age, for BMI, systolic blood pressure, plasma cholesterol and triglycerides, blood glucose, and smoking.

Further reports on the association between insulin and CHD risk from these 3 pioneer studies, published before the present study, include a 9.5-year follow-up report of the Helsinki Policemen Study (19), a 23-year follow-up report of the Busselton Study (20), and 11-, 15- and 23-year follow-up reports of the Paris Prospective Study (21-23). The results presented in these reports will be discussed in detail in Chapter 2.5.1.

2.1.2 Animal experiments and studies of arterial wall metabolism and cell biology

In 1990, Stout reviewed studies on the role of insulin in experimental atherosclerosis, arterial wall metabolism and cell biology related to atherosclerosis (17). A number of experimental studies in chickens, rabbits, rats and dogs were carried out from the 1950’s until the 1980’s to investigate the role of insulin in the development of diet-induced and spontaneously developing atherosclerotic lesions. These studies showed that in animals with alloxan-induced diabetes insulin deficiency inhibited the development of diet-induced atherosclerotic lesions and that insulin treatment restored it. In some studies in non-diabetic animals long-term insulin treatment resulted in the formation of lipid-containing arterial lesions or arterial wall thickening. Insulin treatment was also shown to stimulate the formation of glycosaminoglycans in the arterial wall. In vitro studies of
arterial wall metabolism carried out from the 1960’s until the 1980’s showed that insulin stimulates lipid synthesis in arterial tissue and perfusion experiments demonstrated that this effect of insulin is influenced by haemodynamic factors. Studies on the effects of insulin at cell biology level from the 1970’s and early 1980’s demonstrated that insulin in physiological concentrations stimulates proliferation and migration of arterial smooth muscle cells.

2.2 Insulin resistance syndrome

2.2.1 Evolution of the concept

Clustering of some metabolic and physiological abnormalities was first described in 1923 by Kylin (24) who published a report on a syndrome comprising hypertension, hyperglycaemia and hyperuricaemia.

In 1947 Vague pointed out that the distribution of body fat is of importance in the development of diabetes, gout and atherosclerosis (25). According to his observations, ‘android’ or central obesity is more strongly related to these diseases than the more peripheral ‘gynaecoid’ obesity. This idea was revived in the 1980’s by 2 research groups, one from Milwaukee, Wisconsin (26-28), and the other from Gothenburg, Sweden (29-32). Kissebah and his coworkers demonstrated that central obesity is strongly associated with hyperinsulinaemia and related metabolic abnormalities (26-28). The Gothenburg investigators reported similar observations (29) and were the first to show in prospective epidemiological studies that central obesity is an independent predictor of diabetes (30) and CHD (31,32).

Hanefeld and Leonhardt described in 1981, based on observations from epidemiological studies, a clustering of risk factors including obesity and disturbances in glucose, lipid and purine metabolism and blood pressure regulation (33). They named it ‘metabolic syndrome’ and suggested that it is associated with increased occurrence of atherosclerotic disease.

In 1988 Reaven, in his Banting Lecture entitled ‘Role of insulin resistance in human
disease’, combining evidence from experimental, clinical and epidemiological studies, presented the following statement: "There is a series of related variables - syndrome X - that tends to occur in the same individual and may be of enormous importance in the genesis of CHD. These changes include resistance to insulin-stimulated glucose uptake, hyperglycaemia, hyperinsulinaemia, an increased plasma concentration of VLDL triglyceride, a decreased plasma concentration of HDL cholesterol, and high blood pressure. The common feature of the proposed syndrome is insulin resistance, and all other changes are likely to be secondary to this basic abnormality” (9). He emphasised that the syndrome could exist also in individuals who are not obese and do not have overt glucose intolerance or diabetes. Reaven did not consider obesity as an essential feature of the syndrome X, although he acknowledged that obesity and the degree of habitual physical activity are important environmental determinants of insulin resistance and related abnormalities.

The clustering of risk factors associated with insulin resistance has later become called ‘insulin resistance syndrome’ (IRS), ‘metabolic syndrome’ or ‘multiple metabolic syndrome’.

2.2.2 Insulin resistance: measurement and relationship with plasma insulin levels

Insulin sensitivity of the target tissues is a quantitative characteristic indicating the ability of insulin to stimulate glucose uptake at a given insulin concentration. The effects of insulin on glucose metabolism are in quantitative terms predominantly directed to liver, muscle and fat tissue. The effects of insulin in the liver comprise promotion of glycogen formation, inhibition of gluconeogenesis and glycogenolysis, thereby reducing hepatic glucose production. In muscle and fat tissue, insulin stimulates the uptake, storage and use of glucose. The largest proportion of the insulin-mediated whole-body glucose disposal occurs in skeletal muscle (34).

In the IRS the focus is on those individuals who are insulin-resistant, that is, who are at the lower end of the insulin sensitivity distribution. Therefore, in this context the term insulin resistance, the counterpart of insulin sensitivity, is usually used.

The euglycaemic hyperinsulinaemic clamp technique (8) is the most precise method
for the measurement of insulin sensitivity of the whole body and, therefore, it has been generally accepted as the ‘gold standard’ for different measures of insulin sensitivity. The method is, however, rather complex and time-consuming and, therefore, not suitable for epidemiological studies. Another method for the assessment of insulin sensitivity is the insulin suppression test (35) in which a steady state plasma glucose (SSPG) concentration in response to a continuous infusion of somatostatin, insulin and glucose provides a direct estimate of insulin-mediated glucose disposal. The so-called minimal model method (36,37) is based on frequently sampled intravenous glucose tolerance test and mathematical modelling. Compared to the euglycaemic clamp, this method provides a relatively accurate measure of insulin sensitivity but is less laborious and, thus applicable also to epidemiological studies.

Fasting plasma insulin concentrations in individuals with normal glucose tolerance are set to a level needed to maintain normal blood glucose concentration and are determined by insulin secretion from pancreatic β-cells and insulin sensitivity. Postprandially or after oral glucose load in OGTT, the influence of insulin secretion becomes important but insulin sensitivity still remains an important determinant.

The applicability of fasting and post-glucose insulin concentrations as surrogate estimates for insulin resistance has been investigated by correlating them with a direct measure of insulin-mediated glucose disposal. In a study evaluating the correlation of insulin sensitivity assessed by the euglycaemic clamp with plasma insulin levels during OGTT, Pearson's correlation coefficients between whole-body glucose uptake and log-transformed insulin variables in 50 subjects with normal glucose tolerance were: -0.68 for fasting insulin, -0.58 for 1-hour insulin, -0.74 for 2-hour insulin, and -0.67 for the area under the insulin response curve (38). In another study of 490 healthy non-diabetic subjects assessing insulin sensitivity by a modification of insulin suppression test, Pearson's correlation coefficients between SSPG concentration, which is directly related to insulin resistance, and log-transformed insulin variables were: 0.61 for fasting insulin, 0.71 for 2-hour insulin and 0.77 for the area under the insulin response curve (39). Thus, fasting or post-glucose plasma insulin concentrations or composite variables derived from them provide only an approximation of insulin resistance, accounting for 40-60% of the variation in insulin resistance.
The so-called homeostasis model assessment of insulin resistance (HOMA-IR), based on fasting plasma insulin and glucose concentrations (fasting insulin [$\mu$U/ml] × fasting glucose [mmol/l]/22.5) (40), has been used in several clinical and cross-sectional epidemiological studies. Although it was originally claimed to show strong correlations with direct measures of insulin sensitivity (40), more recent studies indicate that the correlation between HOMA-IR and insulin-mediated glucose disposal is not better than that for fasting insulin alone (39,41).

### 2.2.3 Recent developments in the definition of the insulin resistance syndrome

Following the introduction of the concept of the IRS by Reaven (9), a wealth of research on this subject has identified, in addition to the original core components of the syndrome, other factors that are closely linked with hyperinsulinaemia and insulin resistance, as will be reviewed in Chapter 2.3. These factors include thrombogenic factors, uric acid, microalbuminuria, inflammation markers, and neuroendocrine factors. Furthermore, insulin resistance and plasma insulin levels are influenced and modified by lifestyles and lifestyle-related factors (Chapter 2.3.2), as well as by genetic and early-life factors (Chapter 2.3.3).

As will be reviewed in detail in Chapter 2.5, the evidence accumulating from prospective studies strongly suggests that hyperinsulinaemia and underlying insulin resistance with a cluster of associated risk factors predict the risk of atherosclerotic vascular disease. Therefore attempts have recently been made by 4 international or national bodies to define the IRS on the basis of the risk factor characteristics of the syndrome. These definitions are presented in Table 1. Two of them, one given by the World Health Organisation (WHO) Consultation (42) and the other by the European Group for the Study of Insulin Resistance (EGIR) (43) have been proposed to improve uniformity in the research, whereas 2 other definitions, one given by the National Cholesterol Education Program (NCEP) (44) and the other by the American Association of Clinical Endocrinologists (AACE) (45), have been proposed for clinical use, for the identification of high-risk individuals. All these definitions include risk factors originally proposed by Reaven as the core components of the IRS, but there are distinct
Table 1. Definitions for the insulin resistance syndrome

**World Health Organisation Consultation (42)**
Presence of diabetes* or impaired glucose regulation* and/or insulin resistance (below lowest quartile of glucose uptake in the euglycaemic clamp) and, in addition, 2 or more of the following:
- Central obesity (WHR >0.9 in men, >0.85 in women) and/or obesity (BMI >30 kg/m²)
- Elevated blood pressure (SBP ≥140 mmHg and/or DBP ≥90 mmHg)
- Dyslipidaemia (triglycerides >1.7 mmol/l and/or HDL cholesterol <0.9 mmol/l in men and <1.0 in women)
- Microalbuminuria (urinary albumin excretion rate ≥20 μg/min or albumin/creatinine ratio ≥30 mg/g)
*By World Health Organisation Consultation criteria

**European Group for the Study of Insulin Resistance (43), for non-diabetic individuals**
Presence of fasting hyperinsulinaemia (above the highest quartile) and 2 or more of the following:
- Hyperglycaemia (fasting plasma glucose ≥6.1 mmol/l but <7.0 mmol/l)
- Central obesity (waist circumference ≥94 cm in men, ≥80 cm in women)
- Elevated blood pressure (SBP ≥140 mmHg and/or DBP ≥90 mmHg or current use of antihypertensive drugs)
- Dyslipidaemia (triglycerides >2.0 mmol/l and/or HDL cholesterol <1.0 mmol/l or current use of hypolipidaemic drugs)

**National Cholesterol Education Program (44)**
3 or more of the following:
- Central obesity (waist circumference >102 cm in men, >88 cm in women)
- High triglycerides (≥1.7 mmol/l)
- Low HDL cholesterol (<1.0 mmol/l in men, <1.3 mmol/l in women)
- Elevated blood pressure (SBP ≥130 mmHg and/or DBP ≥85 mmHg or current use of antihypertensive drugs)
- High fasting plasma glucose (≥6.1 mmol/l)

**American Association of Clinical Endocrinologists (45), for non-diabetic individuals**
Characteristic abnormalities belonging to the syndrome (number requirement not defined)†:
- High 2-h post-load plasma glucose (≥7.8-11.0 mmol/l)
- High triglycerides (≥1.7 mmol/l)
- Low HDL cholesterol (<1.0 mmol/l in men, <1.3 mmol/l in women)
- Elevated blood pressure (SBP ≥130 mmHg and/or DBP ≥85 mmHg or current use of antihypertensive drugs)
†Obesity, to be assessed using BMI and waist circumference, was not included among the criteria but was viewed as a physiological variable that increases insulin resistance

BMI indicates body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; SBP, systolic blood pressure; WHR, waist to hip ratio.
differences between the definitions. Following the view of Reaven, the AACE recommendation, deviating from the other 3, does not include obesity among the core components of the syndrome but rather views it as a characteristic that increases insulin resistance. Cut-off levels used for the definition of central obesity, blood pressure and plasma lipid levels differ in some instances substantially. Because assessment of insulin sensitivity by the euglycaemic clamp or other alternative methods is not feasible in clinical practice and the use of plasma insulin measurement as a marker of insulin resistance is problematic due to lack of standardisation of methods and large intra-individual variability, NCEP and AACE define the IRS on the basis of the core cluster of risk factors known to be associated with insulin resistance.

2.3 Factors associated with insulin resistance and hyperinsulinaemia

2.3.1 Biological factors

*Obesity and its distribution*

Studies using the euglycaemic clamp have demonstrated an association between the degree of overall obesity, usually expressed as BMI (weight [kg]/height [m] squared), and insulin sensitivity (46-51). In some of these studies, this association has been non-linear, with a marked decrease in insulin sensitivity when an individual is 20-30% over ideal body weight after which the decline in insulin action becomes attenuated (46,50). However, in a large multi-center study by the EGIIR comprising 1146 non-diabetic, normotensive subjects with euglycaemic clamp measurements, insulin sensitivity decreased linearly with increasing BMI (51). The prevalence of insulin resistance among the obese subjects (BMI ≥25 kg/m²) was surprisingly low: only one of 4 obese subjects was insulin-resistant, defining it as the lowest decile of glucose disposal in lean subjects. Furthermore, insulin hypersecretion was significantly more prevalent than insulin resistance in obese subjects.

During the last 2 decades, special interest has been focused on the metabolic
implications of central obesity in which body fat is predominantly deposited in the abdominal and truncal region (central, abdominal, truncal or upper-body obesity). Several anthropometric variables, such as waist circumference, waist to hip circumference ratio, sagittal abdominal diameter, subscapular skinfold thickness, and subscapular to triceps skinfold ratio have been used in the assessment of regional fat distribution. As discussed before, in the early 1980’s the research groups in Wisconsin and Gothenburg showed that central obesity is strongly associated with hyperinsulinaemia and related metabolic abnormalities, such as glucose intolerance and hypertriglyceridaemia, in both men and women (26-29). Since then, these findings have been confirmed in numerous clinical studies, including studies using direct measurements of insulin sensitivity (52-55). In the EGIR study, however, neither waist circumference nor waist to hip ratio (WHR) was related with insulin sensitivity, assessed by the euglycaemic clamp, after adjustment for age, gender and BMI, suggesting that gross fat distribution may not impair insulin action over and above the effect of BMI itself (51).

More recently, computed tomography and magnetic resonance imaging have provided means for the quantitative assessment of intra-abdominal (visceral) and subcutaneous compartments of truncal adipose tissue in clinical studies. By the use of these techniques several studies have demonstrated that the size of visceral fat depot is a strong correlate of insulin resistance and other metabolic abnormalities associated with central obesity (56-61). However, the singular role of visceral fat in insulin resistance has also been challenged by some recent studies in which subcutaneous fat in truncal region proved to be at least as strong a correlate of insulin resistance as visceral fat (62-64).

In cross-sectional epidemiological studies in adults, covering a wide range of age groups, correlation coefficients between BMI and fasting plasma insulin have been from 0.35 to 0.60, with rather similar results for men and women (19,22,65-77). Correlations between BMI and post-glucose insulin levels have consistently been somewhat lower (19,22,66-68,74,77). Correlations of WHR with fasting or post-glucose insulin levels have varied from 0.20 to 0.45, without any definite gender difference (65-70,73,74). Similar or even stronger correlations with insulin levels have been reported for
subscapular skinfold (65), waist circumference (70,73,74,77-80) and sagittal abdominal diameter (73).

Three population-based studies have investigated the cross-sectional association of obesity and its distribution with insulin sensitivity, estimated by the minimal model method (81-83). In a Finnish study of 138 non-diabetic middle-aged subjects, the following correlations were observed for BMI and WHR with insulin sensitivity index: -0.47 for BMI both in men and women, -0.47 for WHR in men, and -0.56 for WHR in women (82). In a Danish study comprising 380 healthy young adults, the correlation coefficients for different indices of obesity with insulin sensitivity index in men were: -0.54 for BMI, -0.49 for body fat percentage (measured by impedance technique), -0.44 for WHR, and -0.52 for waist circumference. In women, the corresponding correlation coefficients were, in general, somewhat lower: -0.34 for BMI, -0.32 for body fat percentage, -0.28 for WHR, and -0.36 for waist circumference (81). In the Insulin Resistance and Atherosclerosis Study (IRAS) of men and women from 3 ethnic groups, including subjects with normal glucose tolerance, impaired glucose tolerance and diabetes, the association of waist circumference with insulin sensitivity index was inverse and non-linear across the entire spectrum of glucose tolerance, adjusting for age, sex, ethnicity, BMI and other confounding variables (83). In subjects with normoglycaemia, however, the association was clearly stronger than in subjects with abnormal glucose tolerance.

Some prospective epidemiological studies have examined the temporal aspects of the association between obesity and hyperinsulinaemia. BMI measured in childhood, adolescence or young adulthood, or even in middle-age, has been found to be positively associated with plasma insulin levels measured at a later follow-up (84-87). Also, long-term weight gain from childhood to early adulthood or weight gain from early adulthood to middle-age has been shown to predict future hyperinsulinaemia (84,87). Furthermore, 2 prospective studies of middle-aged men, with repeated measurements of BMI and insulin, demonstrated a strong positive association between changes in BMI and insulin concentrations during a follow-up of over 10 years (86,88). Similarly, an increase in BMI over 7 years was a strong predictor of an increase in insulin levels in a prospective study of young adults (89). Hyperinsulinaemia has been shown to predict weight gain in
Pima Indian children (90), as well as in white and black children and young adults (91). However, in prospective studies comprising middle-aged adults, baseline hyperinsulinaemia has been associated with a decreased rate of weight change (92-94) or no significant weight change (87,95). Moreover, low insulin sensitivity measured by the euglycaemic clamp was associated with lower rates of weight gain in Pima Indian adults (96). To explain the discrepancy between findings in children and young adults and those in adults Odeleye et al (90) have suggested that a common factor could promote both insulin resistance and obesity and that established insulin resistance and attendant hyperinsulinaemia could then protect against further weight gain.

As indicated in the preceding review, obesity and its central distribution are strongly associated with hyperinsulinaemia and insulin resistance. It is however important to note, as Reaven already pointed out in his Banting lecture (9) and re-emphasised recently (97) that obesity is not invariably associated with insulin resistance, and that, on the other hand, a substantial proportion of normal-weight individuals are insulin-resistant. These findings have been confirmed by other investigators (51,98).

The mechanisms explaining the influence of an increase in adipose tissue mass on the development of insulin resistance are complex and not yet fully understood. Investigations on the role of accumulation of intra-abdominal fat tissue have dominated during the last 2 decades the research on the relationship between obesity and insulin resistance. Intra-abdominal adipocytes have high metabolic activity characterised by increased lipolysis and enhanced free fatty acid (FFA) flux into portal and also systemic circulation (99-101). There is evidence indicating that FFAs may interfere with hepatic clearance of insulin thus leading to increased insulin concentrations in systemic circulation (99,100). Randle was the first to propose a link between increased FFA flux and skeletal muscle metabolism, suggesting that excess FFAs would compete with glucose as fuel for muscle cells and other tissues, and as a result of this, insulin resistance might ensue (102).

The most recent hypothesis, as summarised by Frayn (101), proposes that the failure of adipose tissue to act as a buffer for daily lipid flux might play a key role in the development of insulin resistance in obesity. Adipose tissue provides its buffering action by suppressing the FFA release into circulation and by increasing the lipoprotein lipase-
induced clearance of circulating triglycerides. Both of these insulin-mediated mechanisms have been shown to be impaired in obesity (103-107). As a consequence of the impaired buffering action of adipocytes, extra-adipose tissues will be exposed to excessive lipid fluxes, leading to accumulation of triglycerides in these tissues. This ectopic deposition of triglycerides into skeletal muscle and liver is suggested as a causal factor in the development of insulin resistance (101). Supporting this hypothesis, studies in humans have shown that intracellular accumulation of triglycerides in the liver (108-111) and skeletal muscle (64,112,113) is strongly associated with insulin resistance. The mechanisms underlying this association, however, remain so far unknown.

Leptin and adiponectin are adipocyte-derived hormones which participate in the regulation of body weight. Plasma leptin levels have been shown to be positively associated with indices of obesity and plasma insulin levels and inversely with insulin sensitivity measured by the euglycaemic clamp (114-116). The associations observed in these studies between leptin and plasma insulin were in part independent of obesity indices. The role of leptin in the obesity-insulin resistance relationship remains, however, so far unresolved. Plasma adiponectin levels have been shown to be decreased in obesity and insulin resistance (117,118), and the possible participation of this novel hormone in the interplay between obesity and insulin resistance is currently a subject of intensive research.

The possible role of adipocyte derived cytokines, particularly tumor necrosis factor-α, in the obesity-insulin resistance relationship will be briefly reviewed later in this chapter.

**Glucose tolerance**

Because blood glucose is an important physiological determinant of plasma insulin levels, it is not surprising that in non-diabetic individuals plasma insulin levels are correlated with blood glucose levels in cross-sectional studies; correlations ranging from 0.15 to 0.50 have been shown between fasting insulin and fasting glucose (19,22,67,68,70-72,77-80,119,120), and even stronger correlations, ranging from 0.50 to 0.70, between 2-hour post-load insulin and glucose (19,22,67,68,77,78,80).
It is well established that insulin resistance is an important pathogenetic factor in type 2 diabetes. The current concept of the pathogenesis of type 2 diabetes is largely based on prospective studies in Pima Indians, a tribe with the highest incidence of type 2 diabetes in the world (121-124). These studies, applying either OGTT with plasma insulin determinations or the euglycaemic clamp, have demonstrated that insulin resistance and compensatory hyperinsulinaemia exist already at the stage when glucose tolerance is still normal. When the disturbance in glucose homeostasis progresses to impaired glucose tolerance, insulin resistance increases and plasma insulin levels become progressively elevated, and finally, when pancreatic β-cells fail to produce enough insulin, frank diabetic hyperglycaemia ensues. In some subjects, however, the dysfunction of β-cells appears rather early in the evolution towards diabetes (122). Prospective cohort studies of other populations (Nauruans, Mexican Americans, Finns) have confirmed the predictive value of hyperinsulinaemia with regard to progression from normal glucose tolerance to impaired glucose tolerance (125,126) and diabetes (125,127-129). As reviewed by the EGIR investigators (130), several studies have also found evidence of insulin resistance in non-diabetic first degree relatives of type 2 diabetic patients. In many of these studies and also in the EGIR study (130), insulin resistance in the first degree relatives was shown to be independent of weight and body constitution. Furthermore, a 25-year follow-up study of non-diabetic offspring of couples who both had type 2 diabetes showed that the development of type 2 diabetes was preceded and predicted by decreased insulin sensitivity determined by the minimal model method; the defect in insulin action was detectable already at the time when the subjects were normoglycaemic, and in most cases more than a decade before the diagnosis of diabetes (131).

**Plasma lipids and lipoproteins**

Epidemiological and clinical studies have demonstrated that hyperinsulinaemia and underlying insulin resistance are associated with plasma lipids and lipoproteins, particularly with triglycerides and triglyceride-rich very low-density lipoprotein (VLDL) and HDL. In cross-sectional epidemiological studies, correlation coefficients for fasting
plasma insulin with plasma total triglycerides have varied from 0.15 to 0.45 (19,22,67,68,71,72,76-80,119,120,132,133), and for 2-hour plasma insulin levels from 0.15 to 0.50 (19,22,67,68,77,78,80,132,133), respectively. Correlations between insulin concentrations and HDL cholesterol have been slightly weaker, varying from -0.15 to -0.40 for fasting insulin (67,68,72,76-80,119,120,132-134) and from -0.15 to -0.40 for 2-hour insulin (67,68,77,78,80,132,133). In a cross-sectional Finnish study of middle-aged men and women, the correlation coefficient of insulin sensitivity index measured by the minimal model method with plasma total triglycerides was -0.49 in men and -0.40 in women, with VLDL triglycerides -0.46 and -0.42, with VLDL cholesterol -0.41 and -0.36, and with HDL cholesterol 0.28 and 0.43, respectively (132). Three prospective population-based studies have also analysed the longitudinal association of insulin with lipids and lipoproteins. In the 8-year follow-up of the San Antonio Heart Study, hyperinsulinaemia predicted the risk of high triglyceride level (relative risk [RR] 3.5-fold), and low HDL cholesterol level (RR 1.6-fold) (135). In a 3.5-year follow-up of Finnish elderly men and women, hyperinsulinaemia was a predictor of hypertriglyceridaemia (odds ratio [OR] 1.6-fold) but not of low HDL cholesterol (133). In another Finnish study of middle-aged men, hyperinsulinaemia predicted the 4-year risk of dyslipidaemia, defined as coincidence of hypertriglyceridaemia and low HDL cholesterol (RR 2.1-fold) (136).

Correlations of insulin with total cholesterol and low-density lipoprotein (LDL) cholesterol have been weak or absent in cross-sectional epidemiological studies, ranging from -0.20 to 0.25 for total cholesterol (19,22,75,76,119,120), and from 0 to 0.10 for LDL cholesterol (67,76,119,120,132-134). In a Finnish study of elderly age cohorts, total and LDL cholesterol levels were found to be lower in the highest plasma insulin quartile than in the lower quartiles (137). Furthermore, hyperinsulinaemia has not been linked with the risk of developing high total cholesterol or high LDL cholesterol in prospective studies (133,135). There is evidence, however, that hyperinsulinaemia is associated with increased levels of small dense LDL particles that are considered more atherogenic than larger LDL particles (138-141). In cross-sectional epidemiological studies, the correlation coefficients between fasting insulin and LDL particle size have ranged from -0.20 to -0.40 (78,142,143). An inverse association between insulin
sensitivity, estimated by the euglycaemic clamp or the minimal model method, and LDL particle size has also been demonstrated in population-based studies, but this association has not been independent of triglyceride and HDL cholesterol concentrations (142-144).

Clinical studies using the euglycaemic clamp have shown that insulin resistance is a characteristic feature of those forms of hyperlipidaemia, such as isolated hypertriglyceridaemia, non-familial and familial hyperlipidaemia, in which hypertriglyceridaemia is an important component (145). Subjects with these types of hyperlipidaemia usually have also low HDL cholesterol but the correlation of insulin resistance with HDL cholesterol has been found to be weaker than that with triglycerides. However, isolated low HDL cholesterol has been associated with insulin resistance independently of triglyceride levels (146). Insulin resistance has also been linked with post-prandial lipoaemia (147,148). Hyperlipidaemias characterised by high LDL cholesterol concentrations, such as familial hypercholesterolaemia and polygenic hypercholesterolaemia have no direct relationship with insulin resistance (149).

The pathophysiological mechanisms underlying the association of hyperinsulinaemia and insulin resistance with lipid and lipoprotein abnormalities are not fully understood. However, several lines of evidence suggest that insulin-resistant state itself induces elevated VLDL through its link with central obesity. Centrally obese individuals often have increased fatty acid flux through the splanchnic area which is followed by an increased synthesis of triglyceride-rich VLDL in the liver (150,151). In addition to increased synthesis, VLDL clearance from the circulation can be reduced in subjects with insulin resistance, and both increased synthesis and decreased removal rate may coexist (150). VLDL and HDL metabolism are tightly linked and, therefore, factors that elevate VLDL or interfere with its metabolism result in decreased HDL. However, several studies have suggested that the relationship between HDL and insulin is independent of total and VLDL concentrations (150). Reduced adipose tissue lipase activity, which could restrain the removal of triglyceride-rich particles, has been proposed as one possible mechanism for altered HDL metabolism in insulin resistance. In addition, increased activity of hepatic lipase, which is related to enhanced HDL clearance, might also contribute to low HDL cholesterol concentrations in insulin-resistant individuals (150,151). Reduced cholesterol ester transfer protein activity and/or
reduced lecithin cholesterol acyltransferase activity in the insulin-resistant state could also result in decreased HDL cholesterol concentrations (151).

**Blood pressure**

The first suggestion of a relationship between hyperinsulinaemia and hypertension came from a clinical study by Welborn *et al* published in 1966 (152). In this study, hypertensive patients had higher insulin levels in the fasting state and after OGGT than normotensive control subjects. As reviewed by Stout (17), the insulin-hypertension relationship was rediscovered in the 1980’s, when several reports from clinical as well as population-based studies appeared describing an association between high insulin levels and hypertension. In 1987 Ferrannini *et al*, using the euglycaemic clamp to estimate insulin sensitivity, demonstrated a marked impairment in insulin action in relatively young and non-obese study subjects with essential hypertension as compared with age- and weight-matched controls (153). Thereafter, the association between insulin resistance and hypertension has been confirmed in several clinical studies (154-157).

Cross-sectional epidemiological studies of white middle-aged men and women have reported positive correlations, although moderate at most, between insulin and blood pressure; for fasting insulin, raw or age-adjusted correlations with both systolic and diastolic blood pressure have ranged from 0.10 to 0.30 (19,22,68,71,72,75,78,119,158), and those for post-load insulin with systolic blood pressure from 0.10 to 0.25 (19,22, 68,78,158) and with diastolic blood pressure from 0.15 to 0.35 (19,68,158). In elderly subjects, cross-sectional correlations of fasting and post-load insulin with both systolic and diastolic blood pressure have been around zero (67,79,80), implying that the relationship between insulin and blood pressure diminishes with age. In addition, there are racial differences in the relationship between plasma insulin levels and blood pressure, stronger correlations having been observed in white than in non-white populations (159,160). The degree of obesity is known to be an important correlate of blood pressure in a population. Therefore, several studies have addressed the question, whether the correlations between insulin and blood pressure levels are independent of
obesity and many of them have found that after adjustment for BMI these correlations become reduced or even non-significant (159,161-164).

Three cross-sectional epidemiological studies have investigated the association between directly assessed insulin sensitivity and blood pressure. In a study of Pima Indians and whites and blacks, Saad et al found a significant inverse correlation between insulin sensitivity measured by the euglycaemic clamp and mean blood pressure in whites ($r=-0.41$) but not in Pima Indians ($r=-0.06$) and blacks ($r=-0.10$) after adjustment for age, gender and indices of adiposity (159). In the EGIS study using the euglycaemic clamp, after exclusion of subjects aged $\geq 70$, those with morbid obesity and those with abnormal blood pressure, insulin sensitivity was inversely and significantly correlated with both systolic ($r=-0.15$) and diastolic blood pressure ($r=-0.19$) even after adjustment for age, gender, BMI, and fasting insulin (165). However, in a random population sample of Finnish middle-aged normoglycaemic men and women, insulin sensitivity estimated by the minimal model method was not significantly associated with systolic and diastolic blood pressure (166).

Both epidemiological studies and clinical case-control studies imply that the association of insulin levels and insulin resistance with blood pressure is different in non-obese and obese subjects. In the San Luis Valley Study of Hispanic and non-Hispanic white subjects, the correlation between fasting insulin and blood pressure was found to be diminished in subjects with high BMI (167). In the Finnish study by Mykkänen et al, the relationships between insulin levels and blood pressure were weaker in obese than in non-obese subjects (166). In clinical studies, lean hypertensive individuals with normal glucose tolerance have been shown to be more insulin-resistant and hyperinsulinaemic than their lean normotensive controls, whereas obese hypertensive individuals and obese normotensive individuals have been found to be equally insulin-resistant and hyperinsulinaemic (153,154,168,169).

Several prospective epidemiological studies have shown that hyperinsulinaemia is associated with subsequent development of hypertension (135,136,170-173), but the results of these studies have not been in all aspects uniform. In 3 of these studies, hyperinsulinaemia predicted the incidence of hypertension independently of baseline obesity (136,170,172), but in 2 other studies, hyperinsulinaemia was a predictor of
incident hypertension only in lean individuals (135,173). In 3 studies (136,171,173), adjustment was also made for baseline blood pressure, and in only one of these, the association between hyperinsulinaemia and hypertension remained significant (136).

Several patophysiological explanations have been proposed for the association of insulin resistance and hyperinsulinaemia with elevated blood pressure. Acutely, hyperinsulinaemia stimulates sympathetic nervous system (174,175), and increases renal sodium reabsortion (176), but whether these effects persist during chronic hyperinsulinaemia is unclear. Another acute effect of hyperinsulinaemia is vasodilation (177,178) which leads to reduction rather than elevation of blood pressure. However, the vasodilating effect of insulin has been shown to be impaired in insulin-resistant individuals (179). Chronically, hyperinsulinaemia stimulates vascular smooth muscle cell proliferation (180-182), activates sodium-hydrogen exchange leading to elevated cytosolic calcium (183), and could thereby increase vascular tone and blood pressure. It has also been proposed, that insulin resistance and hyperinsulinaemia might contribute to the development of hypertension indirectly by promoting atherosclerosis and vascular remodelling (184). Alternatively, insulin resistance and hyperinsulinaemia could be secondary to the events accompanying hypertension, vasoconstriction due to both functional and structural changes in small vessels and rarefaction of the capillary bed. These may limit the diffusion of glucose and insulin to target cells, particularly in skeletal muscle and thereby lead to an impairment in insulin action and hyperinsulinaemia (185). Finally, it is possible that insulin resistance and hypertension develop in parallel, sharing a common genetic basis (185).

**Thrombogenic factors**

Hypercoagulability and impaired fibrinolysis are potentially important mechanisms of atherothrombosis. As reviewed by Juhan-Vague et al (186), increased levels of several factors involved in coagulation and fibrinolysis, namely, fibrinogen, factor VII, von Willebrand factor, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) have been associated with the risk of CHD in epidemiological studies. It has also been shown, that increased levels of these factors, PAI-1 in particular, are
associated with hyperinsulinaemia and insulin resistance and, furthermore, with other risk factors, such as obesity, hypertriglyceridaemia and hypertension, included in the IRS (186). Therefore, it has been proposed that one or several of these thrombogenic factors might play an intermediary role linking hyperinsulinaemia and insulin resistance with atherosclerotic disease (187-190).

The association of hyperinsulinaemia with impaired fibrinolysis due to increased levels of PAI-1 is well documented (186,187). In a large multicentre study comprising 1500 patients with angina pectoris, correlation coefficient between fasting insulin and PAI-1 was 0.42 (191). In population-based epidemiological studies, correlations reported for fasting or post-load insulin with PAI-1 have ranged from 0.15 to 0.50 (76,80,82,192-196). A relationship between hyperinsulinaemia and increased levels of tPA antigen has also been demonstrated (190,191,193,196). To explain this apparently contradictory finding, it has been suggested that the antigenic mass of tPA reflects predominantly tPA/PAI-1 complexes and thereby tPA acts mainly as a surrogate measure for PAI-1 (186).

Insulin sensitivity, estimated by the euglycaemic clamp or the minimal model method, has been found to be strongly and inversely correlated with PAI-1 activity (correlation coefficients from -0.35 to -0.45) in 4 population-based studies comprising either normoglycaemic subjects (82) or subjects with varying degrees of glucose tolerance (192,194,195). In a study of 70-year-old men applying the euglycemic clamp, insulin sensitivity was a statistically significant determinant of PAI-1 activity independent of obesity indices, triglycerides and other potential confounders (194). In 2 studies using the minimal model method, the inverse relationship between insulin sensitivity and PAI-1 lost its significance after adjustment for triglycerides and indices of obesity (82,192). Instead, triglyceride concentrations and indices of obesity were independent determinants of PAI-1 concentrations in men (82,192) and HDL concentrations and WHR in women (82). However, in the third study also applying the minimal model method, the significant inverse relationship between insulin sensitivity and PAI-1 persisted after adjustment for demographic variables, BMI and glucose tolerance status (195).

The mechanisms linking hyperinsulinaemia and insulin resistance with increased
PAI-1 levels are poorly understood. It has been suggested, that insulin may act directly or indirectly through changes in lipoproteins on hepatocytes and endothelial cells which synthesise PAI-1 (187). In vitro studies have shown that the production of PAI-1 can be affected both by high insulin and triglyceride concentrations (187). In vivo, acute administration of exogenous insulin has not resulted in elevation of PAI-1 levels (197-199), whereas a transient increase in plasma PAI-activity was induced by an acute rise in endogenous insulin after an oral glucose load (200). It has also been proposed that insulin resistance per se at the cellular level could be responsible for increased PAI-1 production (201).

Positive correlations between plasma insulin and fibrinogen levels in population-based studies have been relatively weak, ranging from 0.05 to 0.25 (76,80,193,195,196,202-204), and even weaker correlations have been reported between plasma insulin and factor VII activity (80,202,203) and between plasma insulin and von Willebrand factor (205). Nevertheless, multivariate analyses, adjusting for potential confounders, have shown significant and independent associations of plasma insulin with plasma fibrinogen (189,206), factor VII activity (188,190,206) and von Willebrand factor (190). Furthermore, in the IRAS (195) insulin sensitivity, assessed by the minimal model method, was found to be inversely associated with plasma fibrinogen independently of other risk factors. Since fibrinogen, in addition to its function as a haemostatic factor, is also an acute-phase protein becoming elevated in inflammation, elevated fibrinogen levels in hyperinsulinaemia could be in part a reflection of the association of inflammation markers with hyperinsulinaemia and insulin resistance, as will be described in the following.

**Uric acid**

Cross-sectional epidemiological studies have reported positive correlations of moderate strength (from 0.20 to 0.30) between fasting or post-load plasma insulin and serum uric acid (202,207-210). Furthermore, in a large population study of US whites and African-Americans, the prevalence of hyperuricaemia increased linearly with increasing plasma insulin levels (211). Also insulin resistance per se, assessed by the euglycemic clamp,
has been associated with high serum uric acid concentrations (212-214). In a population-based study using HOMA-IR as an estimate of insulin resistance, the prevalence of hyperuricaemia increased significantly across HOMA-IR quintiles (215). In addition, it has been shown that hyperuricaemia frequently clusters with other conditions included in the IRS (211,216). The causal relationships between hyperinsulinaemia, insulin resistance and hyperuricaemia are incompletely understood. In clinical and experimental studies, a decrease in renal clearance of uric acid has been observed after an acute administration of insulin (217,218) and also in relation with increasing insulin resistance (212) implicating that both hyperinsulinaemia and insulin resistance might play a role in uric acid metabolism.

**Inflammation**

Prospective epidemiological studies have shown that slightly elevated levels of inflammation markers, such as plasma C-reactive protein, some cytokines and blood white cell count, are associated with increased CHD risk (219). This is thought to be a reflection of a low grade inflammation as part of the atherosclerotic process. Interestingly, recent studies have shown, that hyperinsulinaemia and insulin resistance are associated with elevated levels of C-reactive protein (204,220-226), interleukin-6 (204,224,225,227) and blood white cell count (204). Because fibrinogen is an acute-phase protein, some of these studies have also included it among inflammation markers (204,226). Furthermore, both experimental and clinical studies have shown that tumor necrosis factor-α, a cytokine expressed in fat and muscle tissue, may have a relationship with insulin resistance (228). Tumor necrosis factor-α stimulates the synthesis of interleukin-6 in fat tissue and interleukin-6 is the main regulator of the synthesis of C-reactive protein in the liver (229). In a study of lean and obese subjects without diabetes using the minimal model method, the secretion of tumor necrosis factor-α from adipose tissue was found to be 3-fold higher in insulin-resistant than in insulin-sensitive subjects matched for BMI (227). Plasma levels of tumor necrosis factor-α have, however, not been found to be associated with plasma insulin levels, although they have a positive relationship with the degree of obesity (230). A similar positive relationship with
obesity indices has been reported also for other inflammation markers. Therefore, the question, whether the association of insulin and insulin resistance with inflammation markers persists after adjustment for indices of obesity and its distribution, has been examined in several studies but the results have not been uniform; in most studies (204,220,224,225), but not in all (221), this association has remained independent after such adjustment.

**Vascular function**

As briefly reviewed in Chapter 2.1.2, early animal experiments and studies of arterial wall metabolism and cell biology conducted in the 1970’s and 1980’s already showed that insulin has several effects that may relate to the formation of atherosclerotic lesions: stimulation of lipid synthesis in the arterial tissue, proliferation and migration of vascular smooth muscle cells, and stimulation of the formation of matrix glycosaminoglycans. More recently, however, *in vitro* studies of vascular tissue have demonstrated that insulin has direct actions that may contribute to either vascular protection or injury, depending on cell type and circumstances, as reviewed by Hsueh and Law (231) and Mikhail and Tuck (178). As to protective effects, insulin stimulates nitric oxide (NO) production in endothelial cells through phosphatidylinositol 3-kinase pathway, and this may inhibit several of the processes that follow endothelial damage. NO has an inhibitory effect on the growth and migration of vascular smooth cells, an important process in the formation of atherosclerotic lesions. NO attenuates inflammatory reactions by inhibiting the expression of adhesion molecules in endothelial cells and inhibits the activity of inflammatory cytokines, and thereby the binding of inflammatory cells, monocytes and macrophages, to vascular wall. NO decreases lipid peroxidation, a process that increases the atherogenicity of circulating lipoproteins. Furthermore, NO has antithrombogenic effects by attenuating the adhesion and aggregation of platelets and their interaction with vessel wall. Concomitantly with these protective effects of insulin, mediated through the stimulation of NO, insulin has been shown to have a number of deleterious effects through the activation of mitogen-activated protein kinase pathway. Through this pathway insulin may promote vascular
smooth muscle cell proliferation and migration, in part by its potentiating effect on other growth factors, such as platelet-derived growth factor and angiotensin II.

Clinical studies of vascular physiology have demonstrated, as reviewed by Baron (177) and Mikhail and Tuck (178) that the primary haemodynamic effect of insulin is vasodilation mediated through NO synthesis and release from vascular endothelium. Another mechanism for the vasodilatory action of insulin is that insulin decreases the contractile response of vascular smooth muscle cells to other vasoactive agents by decreasing intracellular calcium concentration. Concomitantly with the increase in NO activity, insulin also stimulates the release of endothelin-1, a vasoconstrictor substance, and an imbalance between these 2 effects may result in altered vascular responses (232). It has been shown that in insulin-resistant states, such as obesity (179) and type 2 diabetes (233), insulin-mediated vasodilation in skeletal muscle is blunted. This could be due to a defective NO synthesis associated with insulin resistance, because in healthy male individuals a moderate correlation (r=0.52) has been described between insulin sensitivity and endogenous NO synthesis (234). The results concerning the impairment of insulin-mediated vasodilatory action in insulin resistance have, however, not been completely uniform; in a study of healthy male subjects with varying degrees of insulin sensitivity, no difference in insulin-mediated skeletal muscle blood flow was observed between insulin-sensitive and relatively less insulin-sensitive subjects (235).

In this context it is of interest that in a recent study in healthy non-diabetic subjects plasma levels of an endogenous NO synthase inhibitor, asymmetric dimethylarginine, were found to be inversely correlated with insulin sensitivity and, furthermore, pharmacological intervention with an insulin sensitisier, rosiglitazone, was shown to enhance insulin sensitivity and to reduce plasma levels of asymmetric dimethylarginine (236).

Evidence accumulating from studies of whole forearm or brachial artery blood flow in human volunteers indicates that hyperinsulinaemia and insulin resistance are associated with an impairment of the endothelium-dependent (NO-dependent) dilation capacity of the upper-extremity arteries (237-241). It has also been demonstrated that insulin resistance is associated with impairment of endothelium-dependent dilation of human coronary arteries (242,243). A recent study in healthy subjects using 4-hour
euglycaemic clamp has demonstrated that modest hyperinsulinaemia, mimicking fasting hyperinsulinaemia of insulin-resistant individuals, abrogates endothelium-dependent dilation in brachial and femoral arteries, whereas endothelium-independent dilation remains unaffected (244).

Cardiological syndrome X (microvascular or vasospastic angina pectoris in patients with normal epicardial coronary arteries) is characterized by reduced coronary flow reserve caused by dysfunction of small coronary arteries. Curiously enough, the cardiological syndrome X has been found to share features with the metabolic syndrome X (IRS), because a number of studies measuring plasma insulin levels during OGTT (245-250) or measuring insulin sensitivity either with the euglycaemic clamp (251,252), minimal model method (253,254) or insulin suppression test (255,256) have shown that hyperinsulinaemia and insulin resistance belong to the characteristics of cardiological syndrome X. Blunted NO and altered endothelin-1 responses of the forearm arteries to insulin infusion have been observed in patients with cardiological syndrome X indicating the presence of a generalized endothelial dysfunction (257,258).

Microalbuminuria, mildly increased urinary excretion of albumin, is an expression of microvascular dysfunction in the kidneys (259). Microalbumuria is associated with a general increase in the transcapillary escape of albumin and thus evidently is an expression of a more generalized endothelial dysfunction. Although microalbuminuria originally got attention as an early antecedent of diabetic nephropathy and a marker of increased risk of CVD in diabetic patients, it has been demonstrated that microalbuminuria is associated with increased CVD risk also in non-diabetic subjects (260-264). Population-based studies have shown that microalbuminuria is associated with hyperinsulinaemia (265-267) and with decreased insulin sensitivity assessed by the minimal model method (265). A follow-up study of non-diabetic, normoalbuminuric subjects found that baseline plasma insulin levels were higher in those who progressed to microalbuminuria as compared with plasma insulin levels in non-progressors, suggesting that hyperinsulinaemia precedes the advent of microalbuminuria (268).

Decrease in the compliance of large and medium-sized arteries may be of importance in the precipitation of plaque ruptures in arteries involved with atherosclerotic lesions. Therefore, it is of interest that in a cross-sectional study based on the Atherosclerosis
Risk in Communities (ARIC) Study population, joint effects of hyperinsulinaemia, hyperglycaemia and hypertriglyceridaemia contributed to an increase in arterial stiffness (269). Studies of Westerbacka et al (270,271) on the effects of insulin infusion in physiological concentrations on the aortic pulse wave reflections are of interest in relation with the effects of insulin on large artery compliance; in healthy obese and non-obese men a blunted decrease in arterial compliance occurring in response to insulin infusion was found to be associated with decreased insulin sensitivity assessed by the euglycaemic clamp.

**Neuroendocrine factors**

There is evidence from clinical studies and experiments in animals indicating that neuroendocrine factors and maladaptation to stress are involved in the development of central obesity, insulin resistance and associated cluster of risk factors forming the IRS (272-274). The complex neuroendocrine disturbances involved include stimulation of the hypothalamic-pituitary-adrenal axis leading to discrete elevation of plasma cortisol levels, activation of the sympathoadrenal system, hyperandrogenicity in women and relative hypogonadism in men. Recently, a nested case-control study was performed among men aged 45-63 in the Whitehall II study cohort of London civil servants to investigate the association of markers of neuroendocrine activation and psychosocial factors with the presence of the IRS defined on the basis of a cluster of at least 3 of the component risk factors (275). Relative to controls, men with the IRS were found to have elevated urinary excretion of cortisol metabolites and normetanephrine (a marker of sympathetic activity) and elevated heart rate and lower heart rate variability (signs of vagal withdrawal and cardiac sympathetic activation). In multivariate statistical analyses psychosocial factors related to work (employment grade, assets, job strain) accounted for 13% to 37% of the differences between cases and controls. Other studies have shown a positive association of heart rate with fasting plasma insulin (202,276) and an inverse association with directly measured insulin sensitivity (277,278).
2.3.2 Lifestyles and lifestyle-related factors

Physical activity and physical fitness

Habitual physical activity and engagement in exercise training are important determinants of physical fitness. Physical fitness which can be objectively assessed by maximal oxygen (O₂) uptake or other surrogate measures in an exercise test is, however, also influenced by other factors, such as genetic and constitutional factors, age, gender and concomitant comorbid conditions. Thus, the correlation between these 2 variables is far from perfect.

In epidemiological studies, high level of habitual physical activity has been found to be associated with low plasma insulin levels and enhanced insulin sensitivity in both men and women of various age and ethnicity (19,65,69,279-286). Some of these studies have assessed the level of habitual physical activity by detailed diaries or recall questionnaires, covering physical activity at work, home and leisure time, and have thus been able to give quantitative information on the association between the degree of physical activity and plasma insulin levels or insulin sensitivity. In the San Luis Valley Diabetes Study of a population comprising men and women with impaired glucose tolerance, an increment in activity equivalent to approximately 300 kcal per day (adjusting for confounders, including BMI, WHR and subscapular skinfold) was associated with a 10% decrease in the insulin area during OGTT (281). In the Cross-Cultural Activity Participation Study an increment in activity equivalent to 30 minutes of daily moderate-intensity activity (adjusting for confounders, including BMI and physical fitness) was associated with 7% lower fasting insulin levels (285). In the IRAS, an increment of approximately 200 kcal per day in estimated energy expenditure (adjusting for confounders, including BMI and WHR) was associated with a 2% increase in insulin sensitivity assessed by the minimal model method (284).

Two prospective epidemiological studies assessing cardiovascular risk development in the young have examined the association between changes in physical activity and plasma insulin levels (89,287). In a Finnish study comprising children and adolescents, changes in physical activity over 6 years were inversely and independently associated
with changes in insulin levels in males but not in females (287). However, in a study of young black and white men and women, 7-year changes in physical activity were not significantly associated with changes in insulin levels after adjustment for age, BMI and WHR (89).

Only a few epidemiological studies have assessed the relationship between objectively measured physical fitness and plasma insulin levels or insulin sensitivity. In middle-aged healthy Helsinki policemen, correlation coefficients for maximal O₂ uptake with fasting and 1-hour and 2-hour insulin levels were -0.33, -0.29 and -0.32, respectively (19). In a biracial cohort of young adult men and women, correlations between exercise test duration and fasting insulin ranged from - 0.27 to - 0.33 (65). In a population-based sample of Danish young adults, correlation coefficient for maximal O₂ uptake with insulin sensitivity assessed by the minimal model method was 0.44 in men and 0.32 in women (81).

It has been shown that even a single bout of vigorous exercise causes a temporary improvement in insulin-mediated glucose disposal (288,289). On the other hand, cessation of physical training has been shown to lead to a rapid reversal of enhanced insulin action (290-292), suggesting that a sufficient level of physical activity is needed to maintain the beneficial effect on insulin sensitivity.

Three controlled trials using different study designs have examined the effects of weight reduction by dietary changes, aerobic exercise programme and the combination of these interventions on plasma insulin levels or insulin sensitivity (293-295). Taken as a whole, the results of these trials indicate that aerobic exercise leading to an increase in maximal O₂ uptake improves insulin sensitivity over and above the effect of weight reduction. However, a recent controlled trial showed that a walk training for 6 months, not leading to weight loss and significant increase in maximal O₂ uptake, improved insulin sensitivity in previously sedentary middle-aged subjects (296).

**Dietary factors**

Several epidemiological studies have examined the association of dietary fat intake with plasma insulin or insulin sensitivity (65,74,297-300). In most of these studies, total fat
intake has been positively associated with hyperinsulinaemia (74,297,299,300) and inversely with insulin sensitivity (300). In general, however, these associations have become attenuated with adjustment for other factors, especially when the effects of overall and regional obesity and physical activity have been taken into account. The intake of saturated fat has generally been associated with elevated plasma insulin levels (74,297-299) and with worsening insulin sensitivity (300), and in some studies, even independently of indices of obesity and other life-style related factors (74,298,299). Somewhat surprisingly, also monounsaturated fat intake has been associated with elevated insulin levels (297-300) and decreased insulin sensitivity (300). Two studies have shown a significant inverse association between polyunsaturated fat intake and insulin levels (298,299), but in one of them only in a subpopulation of Hispanic whites, while in non-Hispanic whites the association was near zero (299). However, in 2 other studies, the association of polyunsaturated fat intake with plasma insulin proved to be positive (297,300) and inverse with insulin sensitivity (300).

Only few epidemiological studies have examined the association of dietary intake of complex carbohydrates, starch and fiber, with plasma insulin levels (65,298,299). Among adolescents, no association with insulin was observed for dietary starch or fiber (65), whereas among adults, significant and inverse associations were demonstrated (298,299).

Two controlled intervention trials have examined the effect of changes of dietary fatty acid composition on directly assessed insulin sensitivity (301,302). The KANWU study (301), a multicentre study conducted in Kuopio, Aarhus, Naples, Wollongong and Uppsala, randomised healthy individuals to receive for 3 months an isoenergetic diet which contained either a high proportion of saturated or monounsaturated fatty acids with an otherwise identical nutrient composition. A change of the proportions of dietary fatty acids, decreasing saturated fatty acids and increasing monounsaturated fatty acids, improved insulin sensitivity. The beneficial effect of the fat quality on insulin sensitivity was, however, not seen in individuals with a high fat intake (>37% of energy intake). These results were corroborated in a study by Pérez-Jiménez et al (302) in young subjects, who after a baseline diet rich in saturated fat where randomly allocated to 2 diets for 28 days each in a cross-over design: a low fat, high carbohydrate diet and a diet
enriched in monounsaturated fatty acids (Mediterranean diet). The isocaloric substitution of carbohydrates or monounsaturated fatty acids for saturated fatty acids was found to improve insulin sensitivity.

**Alcohol consumption**

Subjects consuming moderate amounts of alcohol have been shown to have significantly lower plasma insulin levels and better insulin sensitivity than non-drinkers (303-305). With one exception (283), epidemiological studies with more detailed information about drinking habits have demonstrated either an inverse association between alcohol consumption and insulin (282,306,307) or a U-shaped association, with lowest insulin levels in subjects reporting light-to-moderate alcohol intake (69,282,308). In general, these associations were not markedly altered after adjustment for potential confounders, including obesity indices (69,307,308). In an epidemiological study estimating insulin sensitivity by the minimal model method, a U-shaped association between alcohol consumption and insulin sensitivity was demonstrated, although the association became attenuated with adjustment for BMI, waist circumference and other potential confounders (309). A randomised controlled trial in healthy post-menopausal women, comparing 3 different levels of alcohol consumption (0, 15 or 30 g/day) for 8 weeks each by a cross-over design, showed that 30 g/day of alcohol improved insulin sensitivity (310). However, in a randomised controlled trial in healthy men, with two 4-week cross-over periods, a substantial reduction of alcohol intake from 72 to 8 g/day did not result in any significant change in insulin sensitivity (311).

**Smoking**

Evidence from clinical and epidemiological studies on the association of smoking with insulin resistance is controversial. Acutely, smoking has been shown to impair insulin-mediated glucose disposal (312). Chronic smoking has been found to be associated with hyperinsulinaemia and with a decrease in directly measured insulin sensitivity in some (313-316), but not all (317-319), clinical studies. One cross-sectional epidemiological
study demonstrated slightly but significantly higher fasting insulin levels in current smokers than in non-smokers, adjusting for age, BMI, and other factors influencing insulin levels (320). However, in other epidemiological studies, fasting or post-glucose insulin levels, either with or without adjustment for age, BMI and other potential confounders, have rather been somewhat lower in current smokers than in non-smokers (19,65,67,279,281-283,321). Two large population-based epidemiological studies have examined the relationship between current smoking with insulin sensitivity, estimated by the minimal model method (81,322). Neither of them found current smoking to be associated with alterations in insulin sensitivity in multivariate analyses.

2.3.3 Genetic and early-life factors

Twin studies (323-328) and family studies (329-336) have demonstrated that plasma insulin levels and insulin sensitivity measured either by the euglycaemic clamp or the minimal model method are influenced by genetic factors. In the majority of these studies the contribution of genetic factors has been from 30% to 50% of the variance of plasma insulin or insulin sensitivity measures. By nature the genetic regulation of plasma insulin levels and insulin sensitivity is evidently polygenic and complex. Some studies, however, suggest that there may be major gene effects (329,332,335). Several gene polymorphisms have been reported to show associations with insulin sensitivity, although the results obtained in different study populations have not been consistent. Examples of such gene polymorphisms include: the Ala54Thr polymorphism of the fatty acid binding protein 2 gene with positive results in Pima Indians (337) but not in the Finnish population (338); the polymorphism in the glycogen-associated regulatory subunit of type 1 protein phosphatase gene with positive results in Pima Indians (339) and equivocally positive results in the Swedish population (340); the Trp64Arg polymorphism of the β3-adrenergic receptor gene, with positive results in Japanese-American (341) and mainly Caucasian American (342) populations but not in the Finnish population (343); the K121Q polymorphism of the membrane glycoprotein PC-1 gene, with positive results in the Sicilian population (344,345) but not in Scandinavian populations (346,347). Recently, studies in populations of different ethnic origin have
shown that the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 (PPAR-γ2) gene is associated with insulin sensitivity (348-354). New additions to this list of gene polymorphisms related to insulin sensitivity include preliminary findings on polymorphisms of the genes regulating substances secreted by adipocytes: tumor necrosis factor-α (355,356), adiponectin (357,358), and resistin (359). Studies on the resistin gene expression in mature adipocytes, however, suggest that resistin would not be an important link with insulin resistance in humans (360).

The hypothesis launched by Barker (361) about the influence of factors operating in fetal and infant life on the occurrence of chronic diseases, such as type 2 diabetes, hypertension and CVD, in adult life has become a subject of intensive research during the last decade. Many studies have shown that people with small size at birth are at increased risk of these chronic diseases, particularly, if they become obese during adult life. Findings from a Swedish study (362) suggest that Barker’s hypothesis may have some bearing also on the development of hyperinsulinaemia and insulin resistance in adult age. This study of men examined at the age of 50 showed a weak but significant inverse correlation between birth weight and 60 minute insulin concentration in intravenous glucose tolerance test. The association was stronger in men in the highest tertile of BMI than in the 2 lower tertiles, indicating an interaction between small weight at birth and obesity in adult life. The association of socio-economic position in childhood and adulthood with insulin resistance in late adult life was examined in the British Women’s Heart and Health Study; women who lived in adverse social circumstances in childhood were at increased risk of developing insulin resistance (363). A short leg length, relative to trunk length, in adulthood is known to be an indicator of poor childhood environmental conditions, in particular poor infant feeding (364). The British study of women showed that late adult-life leg length was inversely related to insulin resistance (365). Furthermore, the same study showed that the birth weight of the first offspring of these women was inversely related to the mother’s insulin resistance in late adulthood, suggesting that common genetic factors contribute to the relationship between birth weight and the risk of cardiovascular disease in adults (366). The finding that a low birth weight of the offspring is related to an increased risk of CVD mortality in the parents supports this view (367). In this context it is interesting to note the recent
findings from a Finnish study demonstrating an interaction between the effects of the Pro12Ala polymorphisms of the PPAR-γ2 gene and birth weight in the development of insulin resistance in late adult life; the association between small birth weight and insulin resistance was found only in individuals with Pro12Pro allele (368).

2.4 Studies of the insulin resistance syndrome by factor analysis

The IRS, as originally defined by Reaven (9), and with its subsequent extensions, is composed of biochemical and physiological characteristics that are closely linked with each other. Factor analysis is a method used for decades in psychometry and social sciences to examine relations and structural patterns among variables that show high degrees of intercorrelation. By the use of this method, a larger set of interrelated variables is resolved into a smaller number of underlying factors. Factor analysis has also been introduced into the field of medical research and applied in the identification of underlying patterns of the IRS.

Eighteen population-based studies have applied factor analysis to examine risk factor clustering in the IRS in non-diabetic subjects (68,72,77-80,129,210,369-378). With the exception of one study comprising children, adolescents and young adults (372), these studies were conducted in middle-aged and elderly populations. In 10 studies (68,78,80, 129,369-371,373,376,377) subjects were of Caucasian origin and in 8 studies (72,77,79, 210,372,374,375,378) also other ethnic groups were represented. All studies included in factor analyses the putative components of the IRS, namely, insulin and/or an estimate of insulin sensitivity, glucose, obesity, triglycerides, HDL cholesterol (when available) and blood pressure, but some studies used also other risk factors proposed as components of the IRS, such as thrombogenic factors (80), inflammation markers (80,376) uric acid (210,369-371), leptin (210,370,375,378) and/or a wider set of risk factors and clinical variables (371,373,376).

In different studies, all the factors derived from factor analyses explained cumulatively 41.1% to 78.2% of the total variance within the sets of variables. Two to 4 factors were produced, when only variables proposed as components of the IRS were
included in analyses and, when also different sets of other variables were included, analyses yielded 3 to 7 factors.

The principal factor produced by factor analysis is a combination of variables explaining the largest proportion of the total variance in the data. In the 18 studies, the principal factor explained 15.9% to 43.3% of the total variance, but it has to be taken into account that the proportion of variance explained by the principal factor may become reduced with increasing number of variables included in the analysis. The loadings of the putative variables of the IRS on the principal factor in different studies are presented in Table 2. With only one exception (369), insulin loaded strongly and positively on the principal factor. In all 3 studies including directly measured insulin sensitivity, assessed by the euglycaemic clamp (369) or the minimal model method (77,370), insulin sensitivity showed a strong negative loading on the principal factor, when included either instead of or in addition to insulin. An index of overall obesity was included in factor analyses in all but one study (369) and, with the exception of 6 studies (72,129,371,372,377,378), an index of central obesity was also included. An index of overall obesity loaded on the principal factor in all but one study (370) and an index of central obesity loaded on the principal factor in all but 2 studies (370,374) using these indices. The loadings of both overall and central obesity indices on the principal factor were, in general, even somewhat stronger than those of insulin or insulin sensitivity. The results were less uniform with regard to glucose and the 2 lipid variables associated with insulin, triglycerides and HDL cholesterol: a glucose variable loaded on the principal factor in 11 studies (72,77,78,129,370-376), triglycerides in 12 studies (68,77,129,210,369-375-377) and HDL cholesterol in 9 studies (68,77,129,210,369, 372,373,376,377). Although elevated blood pressure belongs to the original concept of the IRS, blood pressure loaded on the principal factor in only 4 studies (129,369, 371,376).

In addition to the principal factor characterised almost invariably by obesity and insulin and to a differing degree by other putative components of the IRS, 3 other factors emerged in most of these analyses: insulin/glucose (impaired glucose tolerance) factor, lipid (dyslipidaemia) factor and blood pressure (hypertension) factor. An insulin/glucose factor characterised by strong loadings for both insulin and glucose
Table 2. Loadings of the putative components of the insulin resistance syndrome on the principal factor in studies applying factor analysis (18 studies)

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of studies with loading* of the variable/No. of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin; fasting or post-load</td>
<td>17/18</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>3/3</td>
</tr>
<tr>
<td>BMI/ponderal index/body weight</td>
<td>16/17</td>
</tr>
<tr>
<td>Waist/WHR/subscapular to triceps skinfold ratio</td>
<td>10/12</td>
</tr>
<tr>
<td>Glucose; fasting or post-load</td>
<td>11/18</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>12/18</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>9/16</td>
</tr>
<tr>
<td>Blood pressure†</td>
<td>4/18</td>
</tr>
</tbody>
</table>

* Correlation between variable and principal factor ≥0.30.
† Systolic and diastolic blood pressure in 12 studies; systolic blood pressure in 4 studies; mean blood pressure in 1 study; hypertension as a dichotomous variable in 1 study.

variables was derived in 6 studies (68,78-80,210,369) and a separate glucose factor with significant loadings for glucose but not for insulin variables in one study (377); in 5 of these studies, both fasting and post-load insulin and glucose variables were employed (68,78,80,210,377). Nine studies yielded a separate lipid factor with significant loadings for triglycerides and HDL cholesterol (72,78-80,370,371,373,376,378), and in 5 studies, triglycerides loaded with total or LDL cholesterol on a subsidiary lipid factor (129,373-375,377). A separate blood pressure factor was produced in 12 studies (68,72,77,79,80, 210,372-375,377,378); in 11 of these, highly intercorrelated systolic and diastolic blood pressure values were both entered into factor analyses, and in one of them using systolic blood pressure as a single value for blood pressure (373), systolic blood pressure loaded on the same factor with left ventricular hypertrophy on ECG, a parameter with known strong correlation with blood pressure.

Other variables employed in some studies, in addition to the proposed components of the IRS, have varied. In one study, several procoagulation, inflammation and fibrinolysis variables were used (80). Interestingly, PAI-1 loaded on the principal factor together with body mass and waist, as well as fasting insulin, implicating a role for PAI-1 as one of the core components of the IRS. Apart from PAI-1, however, other haemostatic
variables loaded on separate factors, interpreted as procoagulation, vitamin K-dependent proteins and inflammation. In this study, inflammation variables failed to load on the principal factor and the same was found in another study including markers of inflammation (376). Uric acid was included in factor analyses in 4 studies (210,369-371); in 3 of these studies (210,369,371) uric acid loaded on the principal factor. Four studies (210,370,375,378) included leptin, known to be strongly associated with the degree of obesity and plasma insulin levels, and in all of these studies, leptin loaded strongly on the principal factor.

In those studies including both men and women and providing analyses for both genders separately, the factors produced were strikingly similar in both genders (68, 72,77,210,373,374,377). Furthermore, in a study of a biracial (black/white) cohort of children, adolescents and young adults from the age of 5 to the age of 38, the factor loading patterns were very similar in all age- and ethnic groups (372). Also, in a study comprising African-Americans, Hispanics and non-Hispanic whites, the factor pattern was remarkably consistent in the 3 ethnic groups (77).

Four studies have used factor analysis to examine cardiovascular risk factor clustering in subjects with diabetes (72,79,379,380). These studies have produced more discrepant results than those carried out in non-diabetics, especially with regard to the formation of the principal factor. In a Finnish study of elderly type 2 diabetic subjects, BMI, WHR, insulin, triglycerides and HDL cholesterol loaded on the principal factor in men, whereas in women, only insulin and glucose loaded on the principal factor, while the other putative components of the IRS loaded on 3 subsidiary factors (380). In another Finnish study of middle-aged type 2 diabetic subjects (men and women combined), the second factor, instead of the principal factor, was characterised by significant loadings for all the putative components of the IRS (379). In the other 2 studies (72,79), diabetic subjects were not classified according to the type of diabetes, although evidently majority of them had type 2 diabetes. In elderly Japanese-American diabetic men, indices of overall and central obesity loaded on the principal factor, whereas the other putative components of the IRS, including insulin, loaded on separate, subsidiary factors (79). Even more perplexing were the findings in diabetic American Indians of the Strong Heart Study; in both men and women, BMI and insulin failed to
2.5 An update of prospective epidemiological studies

2.5.1 Insulin and the risk of coronary heart disease or cardiovascular disease

Until now, altogether 25 prospective epidemiological studies have published their results on insulin (5-7,67,75,88,134,321,373,381-396) and 3 more studies on HOMA-IR (397-399) as predictors of CHD or CVD. A summary of these studies arranged according to their main results is presented in Table 3. Of those studies with more than one publication, the Helsinki Policemen Study, the Busselton Study, the Paris Prospective Study, the Caerphilly Study, and the Kuopio Study on Elderly, the first publication has been included in the summary (5-7,385) except for the Kuopio Study on Elderly, from which the 7-year follow-up (373) is included instead of the 3.5-year follow-up (262), because the latter did not report data on men and women separately. Three of the studies have reported their results so far only in the form of abstracts, namely, the San Luis Valley Diabetes Study (382), the London Executive Study (388), and the Mauritius Study (389). By study design 25 studies were prospective cohort studies in which insulin measurements at baseline were carried out on all study subjects and 3 studies, the Multiple Risk Factor Intervention Trial (MRFIT) (134), the Quebec Cardiovascular Study (387), and a population-based study conducted in northern Sweden (75), referred to here as the Northern Sweden Study, were nested case-control studies.

Fifteen studies found a positive association between insulin or HOMA-IR and the risk of CHD or CVD (5-7,321,373,385-387,390,393-398). In 10 of these studies, the association proved to be independent of other risk factors in multivariate analyses (5-7, 321,373,386,387,396-398). Five of these studies included men only (5,7,321, 387,396). Of the other 5 studies including both men and women (6,373,386,397,398), 2 provided analyses of men and women separately (6,373) and, in both of them, hyperinsulinaemia
Table 3. Prospective studies of insulin and homeostasis model assessment score for insulin resistance (HOMA-IR) as predictors of coronary heart disease and cardiovascular disease

<table>
<thead>
<tr>
<th>Study</th>
<th>Gender</th>
<th>n</th>
<th>Diabetes excluded</th>
<th>Insulin/ HOMA-IR</th>
<th>Covariates</th>
<th>Follow-up, yrs</th>
<th>Endpoints</th>
<th>n</th>
<th>Association of insulin with endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helsinki Police Study (5)</td>
<td>Men</td>
<td>1042</td>
<td>Yes, known</td>
<td>Insulin, fasting 1-h 2-h</td>
<td>Age, BMI, BP, chol, TG, gluc, smoking</td>
<td>5</td>
<td>CHD death CHD event (CHD death or non-fatal MI)</td>
<td>11</td>
<td>Insulin as continuous variable in multiple regression analyses, independent positive association for 1-h and 2-h insulin with CHD death (2-h insulin, P&lt;0.01) and CHD event (2-h insulin, P&lt;0.01) but not for fasting insulin</td>
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<td>Busselton Study (6)</td>
<td>Men</td>
<td>1634</td>
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<td>Insulin, 1-h</td>
<td>Age, relative body weight, BP, chol, gluc, uric acid</td>
<td>12</td>
<td>CHD death men women CVD death men women</td>
<td>93</td>
<td>Insulin as categorical variable (top quintile vs. lower quintiles), RRs weighing for other variables, 1.66 for CVD death (P&lt;0.05) and 1.70 for CVD death (P&lt;0.01) in men, and 0.94 for CHD death (NS) and 1.14 for CVD death (NS) in women</td>
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<td>Women</td>
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<td>Paris Prospective Study (7)</td>
<td>Men</td>
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<td>Yes, insulin-treated</td>
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<td>Age, BMI, BP, chol, TG, gluc, smoking</td>
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<td>CHD event (CHD death or non-fatal MI)</td>
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<td>Insulin as continuous variable in multivariate analyses using exponential survival model, independent positive association for fasting (P&lt;0.01) but not for 2-h insulin</td>
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<td>Glostrup Study (386)</td>
<td>Men &amp; women</td>
<td>1052</td>
<td>Yes, known</td>
<td>Insulin, fasting 1-h 2-h</td>
<td>Gender, BMI, BP, chol, TG, HDL, gluc, smoking, alcohol, PA</td>
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<td>CHD event (fetal or non-fatal) CVD event (fatal or nonfatal)</td>
<td>54</td>
<td>Insulin as continuous variable in Cox models, independent positive association for fasting insulin with CHD (P=0.002) and CVD (P=0.002). Almost identical results for 1-h and 2-h insulin (detailed data not shown)</td>
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<td>Quebec Cardiovascular Study; nested case-control study (387)</td>
<td>Men</td>
<td>2103</td>
<td>Yes, known</td>
<td>Insulin (intact), fasting</td>
<td>Matching: Age, BMI, smoking, alcohol Adjustment: BP, LDL, HDL, apo-B, chol, HDL, med, CVD in family</td>
<td>5</td>
<td>Any CHD (CHD death, non-fatal MI or new angina)</td>
<td>91</td>
<td>Insulin (intact) as continuous variable in multiple regression analysis, OR for CHD risk per 1 SD increment in insulin 1.6 (95% CI, 1.1-2.3), independent of other covariates</td>
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<td>Insulin Status</td>
<td>Insulin Parameters</td>
<td>Age Parameters</td>
<td>Event Definition</td>
<td>HR</td>
<td>CI</td>
<td>Notes</td>
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<td>British Regional Heart Study (321)</td>
<td>Men 40-59</td>
<td>5550</td>
<td>Yes, known</td>
<td>Insulin (intact), non-fasting</td>
<td>Age, BMI, BP, BP- medic, chol, HDL, heart rate, FEV, smoking, alcoh, PA, social class, prevalent CHD</td>
<td>11.5</td>
<td>521</td>
<td>Insulin as categorical variable (top decile vs. lower deciles) in Cox model, independent positive association with CHD risk (RR 1.6 [95% CI, 1.1-2.3])</td>
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<td>Kuopio Study on Elderly (373)</td>
<td>Women 65-74</td>
<td>396</td>
<td>Yes, known</td>
<td>Insulin, fasting 2-h</td>
<td>Age, WHR, BP, chol, TG, HDL, ACR smoking, LVH on ECG, previous MI, previous stroke</td>
<td>7</td>
<td>92</td>
<td>Insulin as continuous variable in Cox models, an independent positive association with CHD for fasting (HR 4.34 [95% CI, 1.20-15.73]) and 2-h (HR 2.20 [95% CI, 1.10-4.40]) in men, but no independent association in women (HRs 2.03 [95% CI, 0.49-8.42] for fasting, and 2.38 [95% CI, 0.90-6.26] for 2-h insulin)</td>
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<td>San Antonio Heart Study (397)</td>
<td>Men &amp; women 25-64</td>
<td>2569</td>
<td>Yes, known</td>
<td>HOMA-IR fasting</td>
<td>Age, gender, race, waist, BP, LDL, TG, HDL, smoking, alcoh, PA</td>
<td>7.5</td>
<td>187</td>
<td>HOMA-IR as categorical variable (quintiles) in multiple regression analysis, significant increase in CVD events across quintiles (P for trend 0.02; quintile 5 vs. quintile 1, OR 1.94 [95% CI, 1.05-3.59]). Similar results for fasting insulin (detailed data not shown)</td>
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<td>Malmö Study (398)</td>
<td>Men 46-68</td>
<td>4748</td>
<td>Yes, known</td>
<td>HOMA-IR fasting</td>
<td>Age, gender, waist, BP, BP- medic, TG, HDL, gluc, HbA1c, smoking, PA</td>
<td>5</td>
<td>62</td>
<td>HOMA-IR as categorical variable (top quartile vs. lower quartiles) in multiple-adjusted Cox model, positive independent association (RR 2.18 [95% CI, 1.22-3.87])</td>
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<td>Malmö Preventive Project (396)</td>
<td>Men 25-63</td>
<td>6074</td>
<td>Yes, known</td>
<td>Insulin, fasting</td>
<td>Age, BMI, BP, chol, TG, gluc, smoking</td>
<td>16.5</td>
<td>497</td>
<td>Insulin as categorical variable (top decile vs. lower deciles) in Cox model, independent positive association with CHD risk (HR 1.32 [95% CI, 1.00-1.73])</td>
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Table 3 (continued)

<table>
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<tr>
<th>Study</th>
<th>Gender</th>
<th>Age, yrs</th>
<th>n</th>
<th>Diabetes excluded</th>
<th>Insulin/ HOMA-IR</th>
<th>Covariates</th>
<th>Follow-up, yrs</th>
<th>Endpoints</th>
<th>n</th>
<th>Association of insulin with endpoints</th>
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<tbody>
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<td><strong>Positive association not independent of other risk factors</strong></td>
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<td>Caerphilly Study (385)</td>
<td>Men</td>
<td>45-59</td>
<td>2022</td>
<td>Yes, known and new</td>
<td>Insulin, fasting</td>
<td>Age, BMI, TG, prevalent CHD</td>
<td>5</td>
<td>CHD event (CHD death or non-fatal MI)</td>
<td>113</td>
<td>Insulin as categorical variable, adjusting for age, 1.7-fold increase in the CHD risk across insulin quintiles (P for trend 0.04); insulin as continuous variable in regression analyses, age-adjusted OR for 1 SD changes in log insulin 1.20 (P&lt;0.05), but multiple-adjusted OR statistically nonsignificant</td>
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<tr>
<td>ARIC Study (390)</td>
<td>Men</td>
<td>45-64</td>
<td>5408</td>
<td>Yes, known and new</td>
<td>Insulin, fasting</td>
<td>Age, race, centre, education, BMI, WHR, BP, BP-medic, chol, TG, HDL, fibrinogen, smoking, alcoh, PA, hormone repl</td>
<td>4-7</td>
<td>CHD event (fatal or non-fatal MI) men women</td>
<td>209</td>
<td>Insulin as categorical variable, in women, 2.82-fold increase in RR for CHD across insulin quintiles (P for trend 0.02) adjusting for demographic and lifestyle covariates, and 2.06-fold increase (P for trend 0.18) adjusting further for other covariates. In men, no increase in RRs across insulin quintiles</td>
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<tr>
<td>Barilla Factory Study (393)</td>
<td>Men &amp;</td>
<td>42 (mean)</td>
<td>647</td>
<td>Yes, known</td>
<td>Insulin, fasting, 2-h</td>
<td>Age, gender, BMI</td>
<td>12-15</td>
<td>CHD event</td>
<td>23</td>
<td>Insulin as categorical variable, more than 3-fold increase in CHD incidence in highest 2-h insulin quartile vs. lower quartiles (P&lt;0.05); insulin as continuous variable, in regression analysis adjusting for age, gender and BMI, OR for 1 unit of log 2-h insulin 2.01 (95% CI, 1.10-3.67). Detailed data for fasting insulin not shown</td>
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<tr>
<td>Kuopio Ischemic Heart Disease Study (394)</td>
<td>Men</td>
<td>42-60</td>
<td>1521</td>
<td>Yes, known and new</td>
<td>Insulin, fasting</td>
<td>Age, examination yr, BMI, waist, BP, apo-B, HDL, TG, WBC, fibrinogen, maximal O2 uptake, smoking, alcoh</td>
<td>9.5</td>
<td>CVD death (CHD death, non-fatal MI or other acute event) any CVD event</td>
<td>45</td>
<td>Insulin as categorical variable, 2.5-fold increase in RR for CVD death across insulin quintiles (P for trend 0.04) adjusting for age, and 1.4-fold increase in fully adjusted model (P for trend 0.66); insulin as continuous variable in Cox models, adjusting for age, significant positive association with CVD death (P&lt;0.006), CHD event (P&lt;0.04) and any CVD event (P&lt;0.003), but not with full adjustment (P-values 0.59, 0.62 and 0.15)</td>
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<tr>
<td>Study</td>
<td>Gender</td>
<td>Sample Size</td>
<td>Known</td>
<td>Type</td>
<td>Variables</td>
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<td>Observation</td>
<td>P value</td>
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<td>Uppsala Study (395)</td>
<td>Men</td>
<td>50</td>
<td>No</td>
<td>Insulin (intact, immuno-reactive), fasting</td>
<td>BMI, BP, TG, LDL, HDL, smoking</td>
<td>27</td>
<td>Mortality: CVD death, CHD death, fatal MI, Morbidity: MI, CHD, CVD</td>
<td>149, 107, 73, 159, 219, 405</td>
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<td>No association</td>
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<td>12</td>
<td>Clinical CHD (CHD death, MI, CABG, angina)</td>
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<td>Edinburgh Study (88)</td>
<td>Men</td>
<td>40</td>
<td>No</td>
<td>Insulin, fasting 1-h, 2-h</td>
<td>Age, gender, BMI, BP, chol, gluc, smoking</td>
<td>6.7</td>
<td>Abnormal ECG</td>
<td>16</td>
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<tr>
<td>Pima Indian Study (381)</td>
<td>Men &amp; women ≥25</td>
<td>589</td>
<td>Yes, known and new</td>
<td>Fasting, 2-h</td>
<td>Age, gender, race, WHR, BP, TG, HDL, smoking, education, income</td>
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<td>MI</td>
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<td>San Luis Valley Diabetes Study (382)</td>
<td>Men &amp; women</td>
<td>626</td>
<td>Yes, known and new</td>
<td>Insulin, fasting post-glucose</td>
<td>Age, gender, race, WHR, BP, TG, HDL, smoking, education, income</td>
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<td>CHD event (CHD death or non-fatal MI)</td>
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<td>Gothenburg Study (383)</td>
<td>Men</td>
<td>67</td>
<td>Yes, known</td>
<td>Insulin, fasting</td>
<td>chol, TG, gluc</td>
<td>27</td>
<td>Mortality: CVD death, CHD death, fatal MI, Morbidity: MI, CHD, CVD</td>
<td>149, 107, 73, 159, 219, 405</td>
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</tbody>
</table>

Insulin, intact and immunoreactive, as continuous variables, standardised for 1 SD, in Cox models: significant positive association with all endpoints adjusting for age (HRs and 95% CIs for CHD death 1.32 [1.11-1.58] for intact and 1.45 [1.21-1.73] for immuno-reactive insulin), but not in multiple-adjusted models (HRs and 95% CIs for CHD death 0.97 [0.77-1.23] for intact and 1.12 [0.90-1.40] for immunoreactive insulin).

No difference in mean fasting insulin or insulin area during OGTT between men without and with CHD. No multivariate analysis performed.
<table>
<thead>
<tr>
<th>Study</th>
<th>Gender Age, yrs</th>
<th>n</th>
<th>Diabetes excluded</th>
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<th>Covariates</th>
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<th>Endpoints</th>
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<tr>
<td>Nauru Study (384)</td>
<td>Men 85, Women 92</td>
<td>Yes, known</td>
<td>Insulin, fasting 2-h</td>
<td>Age, hypertension, uric acid</td>
<td>5</td>
<td>Abnormal ECG men</td>
<td>6</td>
<td>Insulin as categorical variable, nonsignificant increasing trend in incidence of ECG abnormalities across tertiles of fasting insulin in men, and of fasting and 2-h insulin in women; insulin as continuous variable in multiple regression models, no significant association in men or women</td>
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<tr>
<td>Rancho Bernardo Study (67)</td>
<td>Men 538, Women 705</td>
<td>Yes, known and new</td>
<td>Insulin, fasting 2-h</td>
<td>Age, BMI, BP-medic, HDL, gluc, smoking, alcohol</td>
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<td>CHD death men</td>
<td>11</td>
<td>Insulin as continuous variable, Cox model: in men, adjusting for age, significant inverse association for 2-hr insulin with CHD and CVD mortality (HRs per 1 SD difference in log insulin 0.57 [95% CI, 0.35-0.95] and 0.64 [95% CI, 0.45-0.90] and with multiple adjustment, with CVD mortality (HR. 0.68 [95% CI, 0.47-0.96]. No significant association for fasting insulin in men, and in women, no significant association for neither fasting nor 2-hr insulin</td>
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<td>MRFTI; nested case-control study (134)</td>
<td>Men at high risk 12866, Cases 208, Controls 414</td>
<td>Yes, known</td>
<td>Insulin, fasting</td>
<td>Matching: Age, centre, date, intervention group</td>
<td>7-10</td>
<td>CHD event (CHD death or non-fatal MI)</td>
<td>208</td>
<td>Insulin as categorical variable (highest vs. lowest quartile) in multiple regression analysis, unadjusted OR for CHD 1.05 (NS), multiple-adjusted OR 0.94 (NS). Insulin as continuous variable in multiple regression model, no significant association</td>
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<td>London Executive Study (388)</td>
<td>Men 728</td>
<td>?</td>
<td>Insulin, fasting post-glucose</td>
<td>Age, BP, chol, HDL, gluc, proteinuria, WBC, ESR, PA, CHD in family</td>
<td>11.3</td>
<td>New CHD</td>
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<td>Insulin as categorical variable (quintiles) in Cox models, no significant association for fasting or postglucose insulin with CHD (detailed data not shown)</td>
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<td>Study</td>
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<td>Sample Size</td>
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<td>Age, WHR, BP, chol, TG, HDL, gluc, uric acid, smoking, PA, prevalent DM</td>
<td>CVD death</td>
<td>CHD event</td>
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<td>Mauritius Study (389)*</td>
<td>Men &amp; women 25-74</td>
<td>2166</td>
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<td>Insulin, fasting 2-h</td>
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<td>CVD death</td>
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<td>Southern Finland Elderly Study (391)</td>
<td>Men &amp; women 75, 80, 85</td>
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<td>No</td>
<td>Insulin, fasting</td>
<td>Age, gender</td>
<td>CVD death</td>
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<td>0.86 [95% CI, 0.70-1.28]</td>
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<td>Bhatia Study (392)</td>
<td>Men &amp; women ≥15</td>
<td>137</td>
<td>Yes, known and new</td>
<td>Insulin, fasting</td>
<td>Age, gender, BMI, BP, LDL, TG, HDL, gluc</td>
<td>6.5</td>
<td>New CHD</td>
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<td>0.99 [95% CI, 0.63-1.55]</td>
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<td>Northern Sweden Study; nested case-control study (75)</td>
<td>Men &amp; Women Cases Controls 25-64</td>
<td>36000</td>
<td>Yes, known and new</td>
<td>Insulin (intact), fasting</td>
<td>Matching; Age, gender</td>
<td>Adjustment: BMI, chol, BP, BP-medic, smoking</td>
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<td>MI</td>
<td>67</td>
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<td>The Strong Heart Study (399)</td>
<td>Men &amp; women 45-74</td>
<td>2283</td>
<td>Yes, known and new</td>
<td>HOMA-IR, fasting</td>
<td>Age, centre, gender, BMI, waist, hypertension, LDL, HDL, fibrinogen, smoking, albuminuria</td>
<td>7.6</td>
<td>CVD event (fatal or non-fatal CHD and stroke)</td>
<td>181</td>
<td>HOMA-IR as categorical variable (top tertile vs. lowest tertiles) in Cox models, no significant association with CVD (age- and centre-adjusted HR 0.85 [95% CI, 0.58-1.23], multiple-adjusted HR 1.09 [95% CI, 0.68-1.94])</td>
</tr>
</tbody>
</table>

*Additional data kindly provided by Professor Jaakko Tuomilehto.

Abbreviations: ACR indicates urinary albumine to urinary creatinine ratio; alcoh, alcohol use; apo-B, apolipoprotein B; BP, blood pressure; CAGB; coronary by-pass surgery; chol, cholesterol; DM, diabetes mellitus; ESR, erythrocyte sedimentation rate; FEV1, forced expiratory volume in one second; HbA1c, haemoglobin A1c; gluc, glucose; hormone repl, hormone replacement; medic, medication; PA, physical activity; TG, triglycerides; WBCC, white blood cell count.
was independently associated with CHD or CVD risk in men but not in women. The main results of the San Antonio Heart Study (397) were reported for the combined male and female population, but after stratification for gender and other effect-modifying variables an independent positive association between insulin and CVD risk was observed both in men and women.

In 4 studies, the positive association between insulin and CHD or CVD risk found in age-adjusted analyses became non-significant after adjustment for other risk factors (385,390,394,395). In the Caerphilly Study (385), comprising only men, the association became non-significant when adjustment was made, in addition to age, for BMI, triglycerides and pre-existing CHD. In the female subpopulation of the ARIC Study (390), the positive association of insulin with CHD risk persisted after adjustment for demographic factors and lifestyle factors (smoking, alcohol consumption, sports index, and hormone replacement), but became non-significant when further adjusted for other risk factors (BMI, WHR, blood pressure, total cholesterol, HDL cholesterol, triglycerides, fibrinogen, and antihypertensive medication) which, except total cholesterol, are closely linked with plasma insulin levels. On the other hand, in the male subpopulation of the ARIC Study, no association between insulin and CHD risk was observed. In the Kuopio Ischaemic Heart Disease Study (394), comprising only men, multivariate analyses were performed stepwise: first, adjusting for age and examination years, second, also for other covariates not directly related to the IRS (apolipoprotein B, white blood cell count, fibrinogen, maximal O₂ uptake, smoking, and alcohol consumption), and third, for ‘mediators’, that is, risk factors that are considered to belong to the IRS (BMI, waist circumference, systolic and diastolic blood pressure, HDL cholesterol, and triglycerides). After adjustment for age and examination year, as well as after further adjustment for other covariates, except mediators, the association of insulin with CVD mortality, CHD events and CVD events remained significant but with further adjustment for mediators, these associations lost their statistical significance. Of the mediators, the obesity indices reduced the hazards ratios (HRs) most markedly. In the male population of the Uppsala Study (395), insulin was significantly associated both with fatal and non-fatal CHD and CVD events, when adjusted for age only, but in the fully-adjusted multivariate analyses these associations became non-significant.
In the combined male and female study population of the Barilla Factory Study (393), a positive association between insulin and the risk of CHD was demonstrated in univariate analysis, and after adjustment for age, gender and BMI, this association remained significant. Further adjustment was, however, deliberately not made for triglycerides, HDL cholesterol and blood pressure, because the authors considered that including these highly intercorrelated variables in the same model would make the interpretation of the results difficult.

Thirteen studies found no association or even an inverse association between insulin or HOMA-IR and CHD or CVD risk (67,75,88,134,381-384,388,389,391,392,399). Of these studies, 4 included men only (88,134,383,388) and 9 both men and women (67,75,381,382,384,389,391,392,399), but only 2 studies reported data separately for men and women (67,384). A significant inverse association or a trend towards an inverse association was observed in 2 studies (67,391). In the male subpopulation of the Rancho Bernardo Study (67), 2-hour post-glucose insulin was significantly and inversely associated with CHD and CVD mortality in the age-adjusted analyses, and this inverse association was still significant with regard to CVD mortality after adjustment for other covariates. The Southern Finland Elderly Study (391) found a trend towards an inverse association between insulin and CVD mortality in men and women combined, although this association did not reach statistical significance when adjusted for age and gender. However, in a subanalysis excluding subjects with prevalent CVD at baseline, the association of insulin with CVD mortality proved to be positive and significant ($P=0.021$, adjusting for age and gender). The subjects of both the Rancho Bernardo Study and the Southern Finland Elderly Study were of old age and, in the Rancho Bernardo Study, only 2 of the CVD deaths in men occurred below the age of 70.

Some of the studies summarised in Table 3 have reported results on the association of insulin with the risk of CHD or CVD also for longer follow-up periods. These studies were the Helsinki Policemen Study, the Busselton Study, the Paris Prospective Study, and the Caerphilly Study. In the 9.5-year follow-up of the Helsinki Policemen Study (19), the results were consistent with those of the 5-year follow-up; 1- and 2-hour insulin, but not fasting insulin, were associated with CHD risk independently of other risk factors. In the 23-year follow-up of the Busselton Study (20), a U-shaped relation
was found between insulin and CHD mortality in men, with men both in the lowest and highest insulin quintile at a significantly higher risk for CHD death than men in the middle quintiles, even after adjustment for other risk factors (overall $P=0.024$). No association was observed in women. The Paris Prospective Study has reported results also for 11, 15, and 23 years of follow-up using CHD death as an endpoint (21-23). In accordance with the 5-year follow-up study, the 11-year follow-up study found that fasting insulin, but not 2-hour insulin, predicted CHD risk independently of other risk factors. In the 15-year follow-up, fasting insulin was no longer an independent predictor of CHD risk but, instead, 2-hour insulin was in multivariate analyses significantly associated with CHD risk as a categorical but not as a continuous variable. In the 23-year follow-up, neither fasting nor 2-hour insulin was independently associated with CHD or CVD mortality after adjustment for cardiovascular risk factors. In the Caerphilly Study, the results of the 10-year follow-up (71) were in accordance with those of the 5-year follow-up; adjusting for age, insulin was significantly associated with CHD risk, but with further adjustment for other confounders, it no longer made a significant independent contribution to the risk of CHD. The 10-14-year follow-up report (76), however, found no significant association between insulin and CHD risk in analyses adjusting for age and BMI. The 11.5-year follow-up report of the British Regional Heart Study (321) included also the 5-year follow-up data on the association of insulin and CHD risk. During the first 5 years of follow-up, insulin was actually a somewhat stronger predictor of CHD risk than during the 11.5-year follow-up; RR (the highest decile vs. the lower deciles) were 2.1 (95% confidence interval [CI], 1.4-3.0) for 5 years, and 1.6 (95% CI, 1.1-2.3) for 11.5 years, respectively. Taken as a whole, studies with several reports based on lengthening follow-up times suggest that the predictive value of insulin becomes attenuated over long periods of follow-up.

The results of the 28 studies are, in any case, conflicting and to explain this, at least 4 aspects have to be considered: first, differences in study size and statistical power, second, differences related to insulin measurements, third, differences in the sets of other risk factors included in multivariate analyses, and fourth, differences in the characteristics of study populations.

The studies showing a positive association between insulin or HOMA-IR and CHD
or CVD have, on average, been larger in size than studies showing no association or an
inverse association. In studies that found a positive association, the median number of
subjects was 1860 and the median number of main endpoints 94, whereas in those that
failed to find an association, the respective numbers were 595 and 34 (in these
calculations, studies including both men and women but reporting results for both
genders have been considered as 2 separate studies). Thus, statistical power problems
may in part explain the negative results of some studies.

Fourteen of the 28 studies, including 3 studies using HOMA-IR, based their
observations on fasting insulin only (75,134,383,385,387,390-392,394-399). In 12
studies, both fasting insulin and insulin measured from blood samples drawn at 1 and/or
2 hours during an OGTT were used in the analyses (5,7,67,88,373,381,382,384,386,
388,389,393). In the Busselton Study (6), insulin was measured from blood samples
taken 1 hour after the glucose load without reference to fasting, physical activity or the
time of the day, and in the British Regional Heart Study (321), from non-fasting blood
samples obtained throughout the day. In 12 of the 26 studies with fasting insulin
measurements a positive association between fasting insulin or HOMA-IR and CHD or
CVD risk was demonstrated (7,373,385-387,390,393-398) and in 7 of these 12 studies
the association was independent of other risk factors (7,373,386,387,396-398).
Respectively, 5 of the 13 studies with post-glucose insulin measurements found a
positive association between post-glucose insulin levels and the risk of CHD or CVD
(5,7,373,386) and in all these 5 studies this association proved to be independent of
other risk factors, although in the Paris Prospective Study post-glucose insulin emerged
as a significant independent predictor of CHD risk only during the 15-year follow-up
(22). Moreover, randomly measured non-fasting insulin was an independent predictor of
CHD risk in the British Regional Heart Study (321). Thus, it seemed to have no
influence on the outcome of the studies whether insulin was measured from fasting,
non-fasting or post-glucose samples.

All studies with baseline examinations before the 1990’s have used
radioimmunological assays that measure total immunoreactive insulin, because assays
specific for intact insulin (also called true or specific insulin) became available around
the year 1990 (400-402). Thus, only 4 rather recent studies (75,321,387,395) have
measured intact insulin and 3 of them (321,387,395) demonstrated a positive association between insulin and CHD risk.

The influence of other risk factor variables included or not included in multivariate analyses, especially those with close physiological links with insulin, namely, indices of obesity, blood pressure, HDL cholesterol, triglycerides, and glucose, is one important aspect in interpreting the differences in the study outcomes. The array of risk factors determined at baseline and used as covariates in multivariate analyses varied from study to study. The measurement of HDL cholesterol was not included in the study protocol of the Helsinki Policemen Study and other early studies, because the association between HDL cholesterol and CHD risk was first demonstrated in 1975 (403). More recent studies, however, almost invariably measured HDL cholesterol and 15 of them also included it as a covariate in multivariate analyses; in 5 of these studies, a positive association between insulin or HOMA-IR and CHD or CVD risk remained statistically significant after multivariate adjustment including HDL cholesterol (321,373,386,387,397,398), in 3 studies it lost its statistical significance (390,394,395), and in 7 studies no positive association was observed (67,134,382,388,389,392,399). The degree of obesity, particularly abdominal obesity, is known to be strongly associated with plasma insulin levels. An index of overall obesity (BMI or relative body weight) was included among the covariates in most studies, but only 8 studies included an index of abdominal obesity (WHR or waist circumference) (373,382,389,390,394,397-399).

Diabetes is known to be a strong risk factor for CVD, but several recent studies have demonstrated that there is a continuous relationship between blood glucose levels and CVD risk extending to much lower blood glucose levels than those used as a threshold for the diagnosis of diabetes (404-407). Within the non-diabetic range of blood glucose levels, blood glucose and plasma insulin levels show a strong positive correlation. Therefore, exclusion or inclusion of diabetic subjects and, on the other hand, inclusion of blood glucose among covariates in multivariate adjustment, are of obvious importance. Subjects with prevalent diabetes at baseline examination were reported to have been excluded in all but 6 studies (6,88,388,389,391,395); in 8 studies this was done on the basis of a previous diagnosis of diabetes (5,7,134,321,383,386,387,393), but in 14 studies also subjects with new diabetes diagnosed on the basis of OGTT
(67,75,373,381,382,384,385,390,392,394,397) or fasting glucose values (396,398,399) were excluded. Of the 15 studies demonstrating a positive association between insulin or HOMA-IR and CHD or CVD risk, only 2 studies, the Busselton Study and the Uppsala Study, did not exclude subjects with diabetes at baseline (6,395). In the Uppsala Study, however, a subanalysis excluding subjects with both prevalent and incident diabetes was performed and the results were essentially similar to those in the whole study population (395). Somewhat surprisingly, only 12 of the 28 studies included blood glucose as a covariate in multivariate analyses (5-7,67,381,383,386,388,389,392,396,398).

Differences in the characteristics of study populations probably offer the most likely explanation for the conflicting results of different studies. Characteristics to be considered include gender, ethnic origin, age, and inclusion or exclusion of subjects with chronic illnesses or conditions that might influence plasma insulin levels or insulin sensitivity of peripheral tissues.

As compared to studies of male populations, studies of female populations on the association of insulin with CVD risk are relatively few. Twelve studies comprised only men (5,7,88,134,321,383,385,387,388,394-396). Sixteen studies included both men and women (6,67,75,373,381,382,384,386,389-393,397-399), but only 6 of them reported results for men and women separately (6,67,373,384,390,397). In men, 11 of 18 studies found a positive association (5,7,152,321,373,385,387,394-397) and 8 of these, an independent positive association (5-7,321,373,387,396,397) between insulin and CHD or CVD risk. In contrast, in women a positive association was observed in only 2 (390,397) of 6 studies. In studies of men, the median number of subjects was 1284 and the median number of endpoints 93, and in studies of women, the respective figures were 1035 and 62.

By ethnicity, the study subjects were whites in 22 studies (5-7,67,75,88,134,321,373,382,383,385-388,391,393-398) and non-whites of widely different ethnic origin (North-American Indian, Micronesian, Asian, Creole or Chinese) in 5 studies (381,384,389,392,399). In addition, one study, namely, the ARIC Study, included both whites (74%) and blacks (26%) but did not report results for them separately (390). In 15 of the 23 studies of whites, including the ARIC Study, insulin was positively
associated with the risk of CHD or CVD (5-7,321,373,385-387,390,393-398), whereas none of the 5 studies comprising only non-whites found an association between insulin and CHD or CVD risk (381,384,389,392,399). However, in addition to the fact that the information on non-white populations is far less limited than that on white populations, certain aspects must be taken into account in interpreting the negative findings in non-whites. As compared to studies of white populations, the 5 studies of non-white populations were, on average, much smaller with fewer endpoints; considering subanalyses of men and women as separate studies, the median study size was 1521 and the number of endpoints 91 in the studies of whites, whereas the respective figures were 363 and 20 in the studies including only non-whites. Also, all the 5 non-white study populations comprised both men and women but only one of them, the Nauru Study (384), provided analyses for both genders separately. Moreover, the studies including whites used mainly ‘hard’ CHD or CVD events, such as CHD or CVD death or non-fatal MI as study endpoints, whereas 2 of the 5 studies of non-whites used only ‘softer’ events, namely ECG changes suggestive of CHD, as endpoints (381,384).

Study subjects were middle-aged in the majority of studies (5-7,75,88,134,321,381,382,384-390,392-399). In 4 studies, however, the study population comprised elderly subjects (67,373,383,391). In 14 of the 24 studies of middle-aged subjects a positive association was observed between insulin and CVD risk (5-7,321,385-387,390,393-398), whereas only one of the 4 studies of elderly subjects found a positive association, and then only in men (373). One possible explanation for the weaker association of insulin with CVD risk in the elderly could be survival bias, that is, if hyperinsulinaemia is associated with the risk of cardiovascular death in middle-aged people, those at high risk may have become removed from the population by premature death. On the other hand, old age is associated with increasing prevalence of various chronic diseases which may influence plasma insulin levels and thus confound the association between insulin and CVD risk.

Subjects with prevalent CVD at baseline were not excluded in 4 studies (321,373,385,391), but in all but one (391) of them, the possible confounding effect of prevalent CVD was taken into account in multivariate analyses.
2.5.2 Meta-analysis of studies on the association of insulin with cardiovascular risk

A meta-analysis of prospective studies on the association of hyperinsulinaemia with CVD risk was carried out by Ruige et al in 1997 (408) to estimate the strength of the association and to identify study characteristics that modify it. Data from 11 different study populations were considered eligible for the analysis. When more than one follow-up study had been carried out on the same study population, the study with the follow-up period closest to the mean follow-up of other study populations was selected. Furthermore, studies providing analyses on the association of both fasting and non-fasting insulin levels with CVD risk and/or those examining the association in men and women separately were considered as separate studies instead of one. Thus, the meta-analyses were based on 17 different studies: 10 studies on fasting insulin (the Helsinki Policemen Study (19), the Paris Prospective study (409), the Edinburgh Study (88), the Pima Indian Study (381), the Nauru Study on men and the Nauru Study on women (384), the MRFIT (134), the Glostrup Study (386), the Kuopio Study on Elderly (262), and the Quebec Cardiovascular Study (387)) and 7 studies on non-fasting insulin (the Helsinki Policemen Study (19), the Busselton Study (6), the Paris Prospective Study (22), the Pima Indian Study (381), the Nauru Study on men and the Nauru Study on women (384), and the British Regional Heart Study (321)). From the studies reporting results for different follow-up periods, the follow-up periods included were: the 9.5-year follow-up of the Helsinki Policemen Study, the 6-year follow-up of the Busselton Study, the 11-year follow-up on fasting insulin and the 15-year follow-up on non-fasting insulin of the Paris Prospective Study, and the 11.5-year follow-up of the British Regional Heart Study.

The main results of the meta-analysis are presented in Table 4. Overall, a weak positive association between both fasting and non-fasting insulin levels and the risk of CVD was observed: an increase of 50 pmol/l in fasting insulin resulted in an 18% increase and an increase of 250 pmol/l in non-fasting insulin in a 25% increase in the risk of CVD. However, the meta-analyses revealed heterogeneity across studies on non-
Table 4. Meta-analysis of the relationship between insulin and cardiovascular disease; adapted from Ruige et al (408), with permission

<table>
<thead>
<tr>
<th>Studies</th>
<th>n</th>
<th>Fixed-effects model RR (95% CI)</th>
<th>P value of heterogeneity test</th>
</tr>
</thead>
<tbody>
<tr>
<td>All fasting insulin studies</td>
<td>10</td>
<td>1.17 (1.09-1.26)</td>
<td>0.27</td>
</tr>
<tr>
<td>Studies in non-whites</td>
<td>3</td>
<td>1.16 (1.02-1.33)</td>
<td>0.84</td>
</tr>
<tr>
<td>Studies in whites</td>
<td>7</td>
<td>1.18 (1.08-1.29)</td>
<td>0.09</td>
</tr>
<tr>
<td>Non-specific insulin assay</td>
<td>6</td>
<td>1.16 (1.06-1.27)</td>
<td>0.24</td>
</tr>
<tr>
<td>Specific insulin assay</td>
<td>1</td>
<td>2.31 (1.20-4.46)</td>
<td>...</td>
</tr>
<tr>
<td>All non-fasting insulin studies</td>
<td>7</td>
<td>1.16 (1.06-1.27)</td>
<td>0.007</td>
</tr>
<tr>
<td>Studies in non-whites</td>
<td>3</td>
<td>1.04 (0.93-1.16)</td>
<td>0.87</td>
</tr>
<tr>
<td>Studies in whites</td>
<td>4</td>
<td>1.42 (1.23-1.65)</td>
<td>0.11</td>
</tr>
<tr>
<td>Non-specific insulin assay</td>
<td>3</td>
<td>1.40 (1.21-1.62)</td>
<td>0.34</td>
</tr>
<tr>
<td>Specific insulin assay</td>
<td>1</td>
<td>5.31 (1.43-19.7)</td>
<td>...</td>
</tr>
</tbody>
</table>

n indicates number of studies. Summary relative risks (RRs) are provided for a difference of 50 pmol/l in fasting insulin or 250 pmol/l in non-fasting insulin.

Fasting insulin and the risk of CVD \( (P=0.007, \text{ for heterogeneity}) \). One probable explanation for this heterogeneity was the difference in the association of insulin with CVD risk observed between white and non-white study populations; within the non-fasting insulin studies, the summary RR for hyperinsulinaemia with regard to CVD risk was statistically significant in studies of whites, whereas in studies of non-whites it did not reach the level of statistical significance. However, the authors were not able to identify the study characteristic responsible for this heterogeneity, because ethnic background, mean age of the study population, and the type of outcome studied were highly correlated within studies of whites and non-whites, respectively; studies of white populations included older subjects and most of them used ‘hard’ events, such as CHD death or non-fatal MI as endpoints, as compared to studies of non-white populations including younger subjects and using ‘softer’ events, namely ECG abnormalities, as endpoints. Of all the studies, only 2 used a specific insulin assay, one of them measuring fasting insulin (387) and the other measuring non-fasting insulin (321); in both of these studies the RR was higher than the summary RR of respective studies using non-specific insulin assays. Therefore, in addition to ethnic background, the type of insulin assay used may, according to the authors, modify the association of insulin with CVD risk,
although the probability tests for heterogeneity suggested that its modifying effect was
of limited importance (studies on whites: $P=0.09$ in fasting insulin studies and $P=0.11$
in non-fasting insulin studies). Finally, the authors investigated whether the various
confounding risk factors, for which adjustment was made in different studies, induced
heterogeneity in the relationship of insulin with CVD risk. In meta-analyses, however,
 neither the number of confounding variables, the presence or absence of control for one
specific confounder, the length of the follow-up period, nor gender differences between
the study populations modified the association between insulin and the risk of CVD.

2.5.3 Proinsulin-like molecules and the risk of coronary heart disease and
cardiovascular disease

Radioimmunological methods for the measurement of insulin precursor molecules,
proinsulin and its split-products (collectively called proinsulin-like molecules) became
available around the 1990 at the same time as methods for measurement of intact insulin
(400-402). Concentrations of proinsulin-like molecules have been shown to comprise
only about 10% of all insulin-like molecules in non-diabetic subjects (203,410,411), and
the receptor-binding and biological action of these molecules is only 1-8% of that of
insulin (411). When proinsulin-like molecules were found to show at least as strong
correlations with cardiovascular risk factors as intact insulin (412,413), these
observations stimulated interest in the relationship of proinsulin-like molecules with the
risk of CVD.

Four prospective studies have, in addition to total immunoreactive insulin or intact
insulin, included in their baseline measurements also proinsulin-like molecules (75,76,
392,395). The findings of these studies on the association of insulin with CHD or CVD
risk are shown in Table 3. The Bhatia Study (392), a small prospective study of Asian
Indians in Tanzania, did not show a significant association between proinsulin and
incident CHD (OR for 1 standard deviation [SD] increase in proinsulin 1.13 [95% CI,
0.72-1.78]). In the Northern Sweden Study (75), a nested case-control study, proinsulin
showed a positive association with the risk of MI (unadjusted OR, highest vs. lowest
quartile, 3.5 [95% CI, 1.2-9.9]), and this association persisted after adjustment for blood
pressure, total cholesterol and smoking status (OR 3.5 [95% CI, 1.1-11.6]), but dissappeared after controlling for BMI (OR 3.1 [95% CI, 0.9-11.2]). In the 10-14-year follow-up of the Caerphilly Study (76), no significant association between proinsulin and CHD risk was observed when adjusted for age and BMI (HR for 1 SD increase in proinsulin 1.19 [95% CI, 0.85-1.67]), but des-31-32 proinsulin and the sum of proinsulin-like molecules showed a significant association with the risk of CHD (HR for 1 SD increase in des-31-32 proinsulin 1.38 [95% CI, 1.02-1.85] and in the sum of proinsulin-like molecules 1.54 [95% CI, 1.07-2.20]). The significances of the 2 latter associations were, however, lost in full adjustment for other risk factors. In the 27-year follow-up of the Uppsala Study (395), proinsulin showed a positive association, independent of other risk factors, with CHD mortality and morbidity (HRs for 1 SD increase in proinsulin 1.47 [95% CI, 1.18-1.82], and 1.45 [95% CI, 1.25-1.69], respectively), as well as with CVD mortality and morbidity (HRs 1.37 [95% CI, 1.14-1.64] and 1.25 [95% CI, 1.12-1.39], respectively). An independent association was observed between 32-33 split proinsulin and CHD morbidity (HR for 1 SD increase in 32-33 split proinsulin 1.18 [95% CI, 1.00-1.38]). Because subjects with diabetes are known to have elevated concentrations of proinsulin, the Uppsala Study investigators carried out separate analyses excluding subjects with diabetes at baseline and also those with incident diabetes during the follow-up. In these analyses the associations of proinsulin with CHD and CVD mortality and morbidity, although becoming weaker, remained significant.

One possible explanation for the relationship of the concentrations of proinsulin-like molecules with CVD risk could be that when insulin production in β-cells increases with increasing insulin resistance, the production of proinsulin also increases. In fact, fasting proinsulin has been found to be more strongly correlated with insulin resistance than with acute insulin response to glucose challenge (414,415). Therefore, an increase in fasting proinsulin concentration can also be a marker of insulin resistance, similarly to the increase in fasting insulin concentration. The half-life of proinsulin is much longer than that of insulin and therefore the plasma level of proinsulin shows a smaller intra-individual variation than the plasma level of insulin. This might be an advantage in the classification of individuals according to their insulin resistance on the basis of a
2.5.4 Insulin and the risk of stroke

In comparison with the information available on the longitudinal association of insulin with the risk of CHD or CVD, information on the role of insulin as a risk factor for stroke is still relatively scarce. In addition to the results to be presented from the Helsinki Policemen Study, the association of insulin with the risk of stroke has been investigated in 6 different prospective studies (394,416-420). These studies are summarised in Table 5, in the order of their publication. One of these studies, the Northern Sweden Study, examined also the association of proinsulin, in addition to insulin, with the risk of stroke (420). Results of the Honolulu Heart Program (417) have so far been published as an abstract only. By study design, the Northern Sweden Study was a nested case-control study, whereas all other studies were prospective cohort studies.

Four studies (the Kuopio Study on Elderly, the ARIC Study, the Kuopio Ischaemic Heart Disease Study, and the Northern Sweden Study) demonstrated that high insulin levels were associated with an increased risk of stroke, whereas 2 studies (the Honolulu Heart Program and the British Regional Heart Study) showed a non-linear association between plasma insulin levels and stroke risk. The Kuopio Study on Elderly (416) and the ARIC Study (418) found a significant positive association between insulin and stroke risk even after adjustment for other covariates in multivariate analyses. In the Kuopio Study on Elderly, this positive association was observed for fasting but not for post-glucose insulin. In the Kuopio Ischaemic Heart Disease Study (394) and the Northern Sweden Study (420), the positive association between insulin and stroke risk was slightly diminished and lost its formal statistical significance in fully adjusted multivariate analyses. In the Kuopio Ischaemic Heart Disease Study, the positive association found after adjustment for age and examination year (P=0.02) lost its statistical significance only when blood pressure or, alternatively, indices of obesity were included as covariates in stepwise performed multivariate analyses (P=0.06 and
<table>
<thead>
<tr>
<th>Studies</th>
<th>Gender, Age, yrs</th>
<th>n</th>
<th>Diabetes excluded</th>
<th>Insulin</th>
<th>Covariates</th>
<th>Follow-up</th>
<th>Endpoints</th>
<th>n</th>
<th>Association of insulin with endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuopio Study on Elderly (416)</td>
<td>Men &amp; women 65-74</td>
<td>1069</td>
<td>Yes, known and new</td>
<td>Fasting, 2-h</td>
<td>Hypertension, gluc, FA, previous stroke, AP</td>
<td>3.5</td>
<td>Stroke death or non-fatal stroke</td>
<td>36</td>
<td>Insulin as continuous variables in multiple regression models, independent positive association for fasting insulin ($P&lt;0.05$). No association for 2-hr insulin</td>
</tr>
<tr>
<td>Honolulu Heart Program (417)</td>
<td>Men ?</td>
<td>1068</td>
<td>Yes, known</td>
<td>Fasting</td>
<td>Age and other non-specified covariates</td>
<td>12-14</td>
<td>Definite or probable stroke</td>
<td>59</td>
<td>Insulin as categorical variable (tertiles, second tertile as reference) in Cox model, U-shaped relation between insulin and stroke risk; age-adjusted HRs for lowest tertile 2.3 (95% CI, 1.1-4.7) and for highest tertile 2.1 (95% CI, 1.0-4.4) and multiple-adjusted respective HRs 3.0 (95% CI, 1.4-6.4) and 1.7 (95% CI, 0.8-3.6)</td>
</tr>
<tr>
<td>ARIC Study (418)</td>
<td>Men &amp; women 45-64</td>
<td>12728</td>
<td>Yes, known and new</td>
<td>Fasting</td>
<td>Age, gender, race, centre, education, BMI, WHR, BP, LDL, HDL, vW-factor, smoking, BP-medic</td>
<td>6-8</td>
<td>Definite or probable stroke</td>
<td>191</td>
<td>Insulin as categorical variable (highest vs. lowest quartile) in Cox model, adjusting for demographic and lifestyle variables, significant positive association for insulin; insulin as continuous variable in fully adjusted Cox model, significant positive association (RR for 50 pmol/L increment in insulin 1.14 [95% CI, 1.01-1.31])</td>
</tr>
<tr>
<td>British Regional Heart Study (419)</td>
<td>Men 40-59</td>
<td>5567</td>
<td>Yes, known</td>
<td>Non-fasting (intact)</td>
<td>Age, BMI, BP, smoking, alcoh, PA, previous stroke/CHD, DM, BP-medic</td>
<td>16.8 (mean)</td>
<td>Stroke death or non-fatal stroke</td>
<td>248</td>
<td>Insulin as categorical variable (quintiles) in Cox models, J-shaped relation between insulin and stroke risk, with lowest risk in second quintile; adjusting for age, test for trend from second quintile upwards significant ($P=0.04$), and in fully adjusted model, marginally significant ($P=0.06$)</td>
</tr>
<tr>
<td>Kuopio Ischaemic Heart Disease Study (394)</td>
<td>Men 42-69</td>
<td>5567</td>
<td>Yes, known and new</td>
<td>Fasting</td>
<td>Age, examination yr, BMI, waist, BP, apo-B, HDL, TG, WBCC, fibrinogen, max O2 uptake, smoking, alcoh</td>
<td>9.5</td>
<td>Stroke death or non-fatal stroke</td>
<td>48</td>
<td>Insulin as continuous variable in Cox models, significant positive association (HR 1.006 [95% CI, 1.001-1.011]), adjusting for age and examination yr, and nearly significant positive association in the fully adjusted model (HR 1.006 [95% CI, 0.999-1.012])</td>
</tr>
</tbody>
</table>
### Northern Sweden Study; nested case-control study (420)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cases: men</th>
<th>women</th>
<th>Yes, known and new (intact)</th>
<th>Fasting Matching: age, gender, examination date, region Adjustment: BP, BMI, chol, proinsulin/insulin</th>
<th>0-10 First-ever stroke</th>
<th>men</th>
<th>women</th>
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<tr>
<td></td>
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</table>

Insulin and proinsulin as categorical variables (tertiles, lowest tertile as reference) in multiple regression models:
Insulin: in women, significantly increased stroke risk in the highest tertile (OR 27.4 [95% CI, 1.7-439]) but no more after adjustment for proinsulin.
No association in men
Proinsulin: in women, significantly increased stroke risk in the highest tertile even after adjustment for insulin (OR 13.7 [95% CI, 1.3-146]). No association in men

Abbreviations: AF indicates atrial fibrillation; alcoh, alcohol use; AP, angina pectoris; apo-B, apolipoprotein B; BP, blood pressure; chol, cholesterol; DM, diabetes mellitus; gluc, glucose; medic, medication; PA, physical activity; TG, triglycerides; vW-factor, von Willebrand factor; WBCC, white blood cell count.
adj财 $P=0.11$, respectively); adjustment for other covariates, including HDL cholesterol or triglycerides, had virtually no effect on the age- and examination year-adjusted HR. In the Northern Sweden Study, a positive association between elevated insulin levels and the risk of stroke was observed in women but not in men. This association persisted after adjustment for potential confounders, including BMI, cholesterol, blood pressure and smoking, but became non-significant when proinsulin was also included in the model. Similarly, no association was observed between proinsulin and the risk of stroke in men of the Northern Sweden Study, whereas in women proinsulin was significantly and positively associated with stroke risk before and after adjustment for confounding variables including insulin. In the Honolulu Heart Program (417) the association of insulin with stroke risk was U-shaped, with more than a 2-fold excess in the age-adjusted risk of stroke in both the highest and lowest insulin tertile as compared with the middle tertile. Adjustment for other CVD risk factors reduced the excess risk of stroke in the highest tertile below the level of significance but did not reduce the excess risk in the lowest tertile. In the British Regional Heart Study (419), the relationship between insulin and stroke risk was J-shaped, with the lowest risk in the second quintile. The age-adjusted excess risk in the lowest quintile as compared to the second quintile was 1.42-fold, statistically not significant and not influenced by adjustment for other CVD risk factors or by exclusion of men who developed diabetes during the follow-up. From the second quintile upwards the age-adjusted stroke risk increased progressively and significantly to a 1.61-fold risk in the top 95th percentile. Adjustment for other cardiovascular risk factors reduced the risk in the top 95th percentile to 1.53 and the increasing trend remained near to significance. When those men who developed diabetes during the follow-up were excluded, the excess risk in men with higher insulin levels became further reduced.

2.5.5 Insulin and all-cause and non-cardiovascular mortality

So far, only few studies have investigated the association of insulin with all-cause mortality. Even fewer studies have been published on the association of insulin with non-cardiovascular mortality and these studies have dealt with cancer mortality only.
Five studies have reported observations on the association of insulin with all-cause mortality: the Busselton Study, the Southern Finland Elderly Study, the Paris Prospective Study, the Malmö Preventive Project, and the Uppsala Study. The Uppsala Study examined also the relationship of proinsulin-like molecules with the risk of all-cause mortality, in addition to that of insulin. In the 13-year follow-up of the Busselton Study (421), a significant association was observed in men between 1-hour post-glucose insulin and all-cause mortality, but this association was positive in older men and inverse in younger men. No association between insulin and all-cause mortality was observed in women. In the 5-year follow-up of the combined male and female population of the Southern Finland Elderly Study (391), a significant inverse association between fasting insulin and all-cause mortality was observed in the oldest age group and a trend to a positive association in the youngest age group. In the 23-year follow-up of men participating in the Paris Prospective Study (23), both fasting and 2-hour insulin were associated with all-cause mortality in a U-shaped fashion, with an increased risk at low and high insulin levels, independently of other risk factors. It is noteworthy, that in the Paris Prospective Study the association of insulin with cardiovascular mortality during the 23-year follow-up also followed a U-shaped curve. In the 19-year follow-up of men participating in the Malmö Preventive Project (396), a J-shaped relationship was observed between fasting insulin and all-cause mortality; the relationship between 2-hour insulin and all-cause mortality rather followed a U-shaped pattern. In the 27-year follow-up of the Uppsala Study (395), immunoreactive insulin was positively but not independently associated with all-cause mortality and the same applied to 32-33 split proinsulin. Intact proinsulin, however, showed a positive and significant association with all-cause mortality that persisted after adjustment for other confounding factors.

Two studies, the Busselton Study and the Paris Prospective Study, have reported results on the association of insulin with cancer mortality. In the Busselton Study, a significant positive association between insulin and mortality from all cancers was observed in older men (421). The investigators of the Paris Prospective Study examined the association of insulin with mortality from special forms of cancer, namely, cancers of lip, oral cavity, pharynx, larynx, stomach, and liver (422). Their analyses revealed a positive association between both fasting and 2-hour insulin and liver cancer, whereas
an inverse association was demonstrated between fasting insulin and fatal lip, oral cavity, pharynx and larynx cancers, and between 2-hour insulin and fatal larynx and stomach cancers. These relationships remained stable after adjustment for other risk factors.

2.5.6 Insulin resistance syndrome and the risk of cardiovascular disease and all-cause mortality

Three studies from Finland applying factor analysis for investigation of the clustering of hyperinsulinaemia with other CVD risk factors also examined, whether the factors derived in factor analyses predicted the risk of CHD or CVD (373,376,379,380). In one of these studies, the predictive value of factors obtained in factor analyses with regard to all-cause mortality was also examined (376). In the Kuopio Study on Elderly, the insulin resistance factor with significant loadings for all the putative components of the IRS predicted the risk of major CHD events (CHD death or non-fatal MI) during the 7-year follow-up in non-diabetic men, but not in non-diabetic women (373). In diabetic men of the same study population, the components of the IRS also loaded on the same factor with insulin and this factor predicted the risk of major CHD events during the 7-year follow-up (380). In diabetic women, components of the IRS loaded on 2 factors neither of which predicted the risk of CHD events (380). In the 7-year follow-up study of men and women from East and West of Finland with type 2 diabetes, hyperinsulinaemia by itself did not predict CHD mortality independently of other components of the IRS, whereas the ‘hyperinsulinaemia cluster’, a factor having high loadings for BMI, insulin, triglycerides and HDL cholesterol, was a significant predictor of death from CHD (379). In middle-aged non-diabetic men of the Kuopio Ischaemic Heart Disease Study, factor analysis yielded a principal factor characterised by the core components of the IRS and during the 11.4-year follow-up, men with high scores of this factor were at increased risk of mortality from CHD and CVD, as well as mortality from all causes (376).

A different approach to analyse the association of the IRS with the risk of CVD was used in 5 prospective studies (376,399,423-425), one of them examining also the association of the IRS with all-cause mortality (376). These studies based their analyses
on the number of risk factors of the IRS present at baseline. Different definitions for the IRS were used in each of these studies, and in 3 of them, plasma insulin levels or other markers of insulin resistance were not used (399,424,425). A 7-year follow-up study of a large cohort of Italian men and women (424) defined the IRS as a cluster of high blood glucose, high blood pressure, high triglycerides, and low HDL cholesterol. During the follow-up, the risk of death from CVD increased in both genders with increasing number of risk factors belonging to the IRS. In the Framingham Offspring Study (425), the 6 metabolically linked risk factors considered were high BMI, high blood pressure, high glucose, high total cholesterol, high triglycerides (highest quintiles for all) and low HDL cholesterol (lowest quintile). Clusters of 3 or more risk factors were associated with a significantly increased risk of CHD in both men and women over 16 years of follow-up. In the Botnia Study, a large family study of subjects with type 2 diabetes and their relatives (423), the IRS was defined applying the definition by the WHO (42) as the presence of diabetes or impaired glucose regulation and/or insulin resistance and at least 2 of the following risk factors: obesity, hypertension dyslipidaemia, or microalbuminuria. In multivariate analyses, the IRS was a significant predictor of CVD mortality during the 6.9-year follow-up in the whole study population but not in subjects with diabetes. In the Kuopio Ischaemic Heart Disease Study (376), 2 definitions for the IRS were applied, the WHO definition modified for epidemiological studies, in part as proposed by the EGIR (43) (hyperinsulinaemia or hyperglycaemia and at least 2 of the following: abdominal obesity, dyslipidaemia and high blood pressure), and the NCEP definition (44) (at least 3 of the following: hyperglycaemia, abdominal obesity, high triglycerides, low HDL cholesterol, and high blood pressure). During the 11.4-year follow-up the risk of CHD and CVD death, as well as death from all causes, was significantly increased both in men fulfilling the modified WHO definition and in men fulfilling the NCEP definition for the IRS. In contrast, in the 7.6-year follow-up of the Strong Heart Study population of American Indians, the presence of the IRS by the NCEP definition did not predict the risk of CVD independently of other established risk factors (399).
2.6 Other studies on the association of insulin and insulin resistance with atherosclerosis

During the last decade, several studies have examined the association of insulin or insulin resistance with directly assessed atherosclerosis. The intima-media thickness (IMT) of the carotid artery assessed by ultrasound has been the most widely used marker of general atherosclerosis in these studies. The results of cross-sectional epidemiological studies examining the relationship of hyperinsulinaemia with carotid IMT have, however, not been completely uniform. In the large biracial cohort of men and women of the ARIC Study, fasting plasma insulin was positively associated with carotid IMT in subjects without prevalent CVD (426). A positive and independent association between the sum of plasma insulin levels during OGTT and IMT of the carotid artery was also demonstrated in a population-based sample of Japanese middle-aged men without hypertension or diabetes (427). On the other hand, in a population-based study of middle-aged Finnish men and women with and without hypertension, no positive association was found between different insulin parameters and carotid IMT after adjustment for other covariates (428). Furthermore, in the Hoorn Study comprising elderly Dutch men and women, no association could be shown between either fasting or 2-hour post-load intact insulin and carotid artery stenosis in either univariate or multivariate analyses (429). Two prospective studies on the association of insulin with the progression of atherosclerosis in carotid arteries have also been published (430,431). In the 3.5-year follow-up of the Swedish Risk Intervention Study comprising high-risk hypertensive subjects and their controls, fasting insulin at baseline was the only potential risk factor significantly associated with the increase in carotid IMT (430). In the 5-year follow-up of the Bruneck Study comprising middle-aged and elderly non-diabetic men and women, both low and high post-load insulin levels predicted the progression of carotid atherosclerosis estimated by an increase in the atherosclerosis score or by an occurrence of new plaques (431).

Insulin resistance per se, measured by the euglycaemic clamp, has also been shown to be associated with ultrasonographically assessed atherosclerosis. In a Finnish study by Laakso et al, subjects with asymptomatic atherosclerosis in the femoral or carotid
arteries were more insulin-resistant than those without such findings (432). In a Swedish study of men at high and low risk for CHD, a significant inverse relationship between insulin sensitivity index and carotid IMT was observed in both the high-risk and the low-risk group (433). Furthermore, in a cross-sectional study of the IRAS, a large epidemiological study comprising 3 ethnic groups, insulin sensitivity evaluated by the minimal model method was significantly and inversely associated with carotid IMT both in Hispanics and in non-Hispanic whites even after adjustment for traditional CVD risk factors (434). No such association, however, was observed in blacks. In the IRAS, insulin sensitivity was found also to be correlated with a composite score of systemic atherosclerosis based on the presence of one or several of the following findings: intima-media thickening in the common or internal carotid arteries, symptoms and/or signs of CHD, or a low ankle-brachial blood pressure index (435).

A cross-sectional population-based study of middle-aged men in Malmö, Sweden (436), examined the association of insulin resistance, defined by values above the 75th percentile of HOMA-IR, and the presence of the IRS using EGIR criteria (43) with ultrasonographically assessed carotid IMT and carotid stenosis. Carotid IMT was significantly greater and the prevalence of carotid stenosis significantly higher in subjects with the IRS than in those not fulfilling the IRS criteria. Insulin resistance per se estimated by HOMA-IR was positively associated with carotid IMT, but this association disappeared when other variables included in the IRS were taken into account.

Some clinical studies have investigated the relationship of hyperinsulinaemia and insulin resistance with angiographically documented atherosclerosis of coronary arteries. In a study by Bressler et al (437), plasma insulin concentrations were determined during OGTT and a euglycaemic clamp was performed to assess insulin sensitivity in non-obese, normotensive subjects with angiographically documented coronary artery disease (CAD) and in their age- and weight-matched controls without CAD in coronary angiography. Fasting plasma insulin concentration and the area under the plasma insulin curve during OGTT were significantly increased and insulin-mediated glucose disposal was significantly decreased in subjects with CAD as compared with control subjects. Furthermore, in subjects with CAD insulin sensitivity was inversely correlated with the
severity of CAD. A study by Shinozaki et al (438) assessed insulin sensitivity by insulin suppression test in normotensive subjects with normal or impaired glucose tolerance and with or without CAD in coronary angiography. Subjects with CAD were significantly less insulin-sensitive in both groups of glucose tolerance and had higher plasma insulin areas during OGTT than their controls without CAD. In normoglycaemic subjects with CAD, insulin sensitivity correlated inversely and plasma insulin area positively with the degree of CAD estimated by atherosclerosis score. The Framingham Offspring Study examined the association between fasting insulin concentration and the presence of coronary artery calcification assessed using electron beam-computed tomography (439). In age- and gender-adjusted analyses, subjects with high plasma insulin levels (>90\textsuperscript{th} percentile among subjects with normal glucose tolerance) were 2 times more likely to have coronary artery calcification than subjects with lower plasma insulin levels. Hyperinsulinaemia during OGTT has also been shown to be associated with the risk of restenosis after percutaneous transluminal angioplasty (440) and, in a serial intravascular ultrasound study, with increased neointimal tissue proliferation after coronary stent implantation (441).

Only 2 prospective studies have so far been published on the predictive value of insulin resistance \textit{per se} with regard to the risk of CVD (442,443). In a study comprising 147 healthy non-obese volunteers, SSPG concentration during the insulin suppression test was used as an estimate of insulin sensitivity (442). During the 5-year follow-up, there was a marked increase in clinical CVD events in subjects in the highest SSPG tertile and in multivariate analysis SSPG was an independent predictor of CVD events. In a population-based cohort study of 986 men aged 70 in Uppsala, Sweden, insulin sensitivity was measured by the euglycaemic clamp (443). During the 7.5-year follow-up low insulin sensitivity predicted the risk of CHD (fatal or non-fatal) independently of conventional cardiovascular risk factors. In this study, proinsulin was also an independent predictor of CHD risk, and when it was entered into a multivariate model simultaneously with insulin sensitivity index, the latter lost its independent predictive value.
3 AIMS OF THE STUDY

The general aim of this study was to investigate the association of insulin with the risk of atherosclerotic vascular disease in healthy middle-aged Helsinki policemen during 22 years of follow-up. More specifically, the following questions were addressed:

1. Does hyperinsulinaemia predict the risk of major coronary heart disease events (Study I)?

2. Does hyperinsulinaemia predict the risk of stroke (Study II)?

3. What are the associations of insulin with all-cause, cardiovascular and non-cardiovascular mortality (Study III)?

4. Does the risk factor clustering of the insulin resistance syndrome predict the risk of coronary heart disease and stroke (Study IV)?
4 SUBJECTS AND METHODS

4.1 Study population

This study is based on a cohort of 970 men aged 34-64 (median 48 years) who were free of CHD, cerebrovascular disease and diabetes when they participated in the 5-year follow-up examination of the Helsinki Policemen Study in 1971-1972.

The initial examination of the Helsinki Policemen Study took place in 1966-1967 comprising a total of 1326 men aged 30 or over who were employed by the Police Department of Helsinki or by the National Police units which have their headquarters in Helsinki (Central Criminal Police, Mobile Police, Security Police, Police Academy, and the Police Department at the Ministry of the Interior) (18). The participation rate in the initial examination was 98.4%. At the 5-year follow-up in 1971-1972, 1259 men were re-examined, representing 98.5% of surviving men of the initial study cohort.

The study population of the present study was formed as follows: Twenty-nine men who had been 60 years or over at the time of initial examination were excluded, because that age group was already highly selected due to the retirement age of 58 for all but high ranking police officers in the Finnish Police Force. At baseline, 190 men had definite or possible CHD, 8 had a history of hospital-verified stroke, 12 had a clinically significant heart disease other than CHD, 3 had atrial fibrillation, and 47 had diabetes. Altogether 236 men with one or several of these diseases or conditions were excluded. Additionally, 2 men who had moved out of the country and 22 men who had missing values for the variables used in the data analyses were excluded, which led to the final study cohort of 970 men.

In professional hierarchy, the subjects examined in 1971-1972 were positioned as follows: 116 men (12%) were high ranking police officers, 235 (24.2%) were non-commissioned police officers, and 526 (54.2%) were ordinary policemen. In addition, 78 men (8.0%) had retired and 15 (1.5%) had changed their job; for the data analyses, these men were classified into the occupational group they belonged to at the time of leaving the Police Force.
4.2 Study programme and methods at the 1971-1972 examination

The study programme at the 1971-1972 examination included: a questionnaire concerning previously diagnosed diseases, drug therapy, physical activity, and smoking habits; Rose cardiovascular questionnaire (444); measurement of height, weight and other anthropometric measurements, including triceps and subscapular skinfold thickness; clinical examination, including measurement of blood pressure; resting and exercise ECG; assessment of physical fitness by an exercise test on bicycle ergometer; radiological examination of the chest; and laboratory examinations, including determination of plasma total cholesterol and triglycerides, as well as an OGTT with plasma insulin determinations.

Clinical examination was carried out by the same physician throughout the 1971-1972 examination. Resting ECGs were interpreted according to the Minnesota Code (445).

Anthropometric measurements. Weight and height were measured with subjects in light clothing without shoes. BMI was calculated as weight (kilograms)/ height (meters) squared. Subscapular and triceps skinfold thicknesses were measured with the Harpenden caliper (John Bull, British Indicators, St. Albans, Herts., UK). BMI was used as an index of the degree of overall obesity, and subscapular skinfold thickness as an index of upper-body obesity.

Blood pressure. Seated blood pressure on the right arm was measured twice at the interval of 5 minutes with a mercury sphygmomanometer, and the averages of 2 measurements of systolic and diastolic blood pressure were used in data analyses. Mean blood pressure was calculated from the formula [(2 x diastolic) + systolic)/3. Hypertension was considered to be present when systolic blood pressure was ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg or if the subject was receiving drug treatment for hypertension. Only 29 men used antihypertensive drugs at baseline; 23 of them took diuretics.

Smoking habits. The examinees were classified originally into following 7 categories with regard to smoking history: 1=never smoked; 2=ex-smoker, smoked cigars or pipes; 3=ex-smoker, smoked cigarette; 4=current smoker of cigars or pipes; 5=current
cigarette smoker, ≤9 cigarettes/day; 6= current cigarette smoker, 10-19 cigarettes/day; 7= current cigarette smoker, ≥20 cigarettes/day. In the data analyses, a dichotomous variable for smoking was used: current non-smokers (those who never smoked and ex-smokers combined) vs. current smokers (cigar and pipe smokers and all categories of cigarette smokers).

Physical activity and physical fitness. Leisure time physical activity was assessed by the use of a questionnaire modified from that described by Saltin and Grimby (446) and graded into 4 classes: 1=inactive, 2=slightly active, 3=active, and 4= highly active. For the data analyses, leisure time physical activity was recoded into a dichotomous variable: inactive (classes 1 and 2 combined) vs. active (classes 3 and 4 combined). Predicted maximal \( \text{O}_2 \) uptake (millilitres/ minute/ kilogram of body weight) was used as an objective estimate of physical fitness. It was determined by the use of the nomogram of Åstrand and Ryhting (447) on the basis of the heart rate achieved in a bicycle ergometer exercise test in which the subject pedaled at a work load of 150 W for 4 minutes.

Biochemical measurements. The OGTT and collection of blood samples for other biochemical measurements were performed between 8 and 10 a.m. after a minimum of a 12-hour fast. The glucose dose used in OGTT was 75 or 90 g according to the body surface area (847 men received 75 g and 123 received 90 g of glucose). Venous blood samples for blood glucose and plasma insulin determinations were taken before and 1 and 2 hours after the glucose load. Blood glucose was determined by the \( o \)-toluidine method (448) and plasma insulin by the ‘coated charcoal’ radioimmunological assay described by Herbert et al (449). The area under the blood glucose response curve (AUC glucose) was calculated from fasting, 1-hour, and 2-hour blood glucose concentrations with the trapezoid rule. Similarly, the area under the plasma insulin response curve (AUC insulin) was calculated from fasting, 1-hour, and 2-hour insulin concentrations. Plasma total cholesterol was determined by the method of Abell et al (450) and plasma total triglycerides by the method of Björkstén (451).

Diagnosis of prevalent CHD, other heart disease and cerebrovascular disease. Definite or possible CHD was diagnosed if the subject had, either in 1966-1967 or in 1971-1972 examination: 1) a history of MI verified at hospital (hospital records of those
men were checked); or 2) major Q/QS waves in the resting ECG (Minnesota Code 1.1-1.2); or 3) angina pectoris or chest pain attack by the Rose cardiovascular questionnaire.

Clinically significant heart disease other than CHD was diagnosed on the basis of medical history, clinical examination, radiological examination of the chest, and resting ECG. The diagnosis was confirmed by a cardiologist.

History of hospital-verified stroke was based on checking the hospital records of those men who either at the 1966-1967 or 1971-1972 examination gave a history of hospitalisation due to symptoms suggestive of stroke. The diagnosis of stroke was ascertained following the WHO criteria (452) which define stroke as a neurological deficit observed by a physician and persisting for more than 24 hours, without other diseases explaining the symptoms.

Criteria for prevalent diabetes and other categories of impaired glucose regulation. Diabetes was considered to be present if the subject had previously diagnosed diabetes or, in the subjects without previously diagnosed diabetes at either 1961-1966 or at 1971-1972 examination, if the 1985 WHO criteria (453) for diabetes were fulfilled. The 1985 WHO criteria for diabetes based on OGTT are as follows: fasting blood glucose ≥6.7 mmol/l or 2-hour blood glucose ≥10.0 mmol/l. These criteria were applied throughout this study, although in 1997 the American Diabetes Association (ADA) Expert Committee recommended that the fasting blood glucose criterion for diabetes diagnosis should be lowered to ≥6.1 mmol/l (454).

The 1997 ADA Expert Committee introduced a new diagnostic category of milder impairment of glucose regulation, impaired fasting glycaemia (fasting blood glucose ≥5.6 mmol/l but <6.1 mmol/l). The fasting glucose criteria for diabetes and impaired fasting glycaemia of the ADA have been incorporated into the diagnostic classification given in the 1999 Report of a WHO Consultation (42). In this classification the impaired glucose tolerance category of the 1985 WHO criteria, based on 2-hour glucose value in OGTT, was retained but redefined with regard to fasting glucose. Thus, the new definition for impaired glucose tolerance based on OGTT in terms of venous whole blood glucose is: fasting glucose <6.1 mmol/l and 2-hour glucose ≥6.7 mmol/l but <10.0 mmol/l. For some of the analyses of the present study, a strictly normoglycaemic study population was created by excluding men with mild impairments of glucose regulation,
defined by a combination of the ADA criteria for impaired fasting glycaemia and the new WHO criteria for impaired glucose tolerance: fasting blood glucose \( \geq 5.6 \) mmol/l or 2-hour blood glucose \( \geq 6.7 \) mmol/l.

4.3 Collection of follow-up data

The follow-up lasted until January 1, 1994, from the date of the 1971-1972 examination for each study subject. The median follow-up time for those surviving over the whole follow-up period was 22.3 years (range 21.9 to 22.9 years).

Ascertainment of vital status and causes of death. Information on the vital status of all men and copies of death certificates of all deceased men were obtained from the Statistics Finland. In the final classification of causes of death, in addition to the review of death certificates, hospital records and autopsy reports were also used, if available. Autopsy was performed in 142 of the total 276 cases of death (51.4 %). Underlying cause of death was coded by the author using the *International Classification of Diseases, Ninth Revision* (ICD-9). ICD-9 codes 390-459 formed the cardiovascular death category (Study III); within this, ICD-9 codes 410-414 formed the coronary death category (Studies I and III), and ICD-9 codes 431-434 and 436 the cerebrovascular death category (Studies II and III). Death from subarachnoid haemorrhage with ICD-9 code 430 was not regarded as a cerebrovascular disease endpoint and was thus classified into the category of other cardiovascular deaths which included the remaining ICD-9 codes for CVD. Deaths from other causes than CVD formed the non-cardiovascular death category with following subcategories; the category of death from cancer, including ICD-9 codes 140-208; the category of violent death, including ICD-9 codes 800-999; and the category of other non-cardiovascular deaths, including ICD-9 codes not belonging to any of above categories (Study III). Within the category of violent deaths, suicides or fatal accidents with notes on excessive alcohol use or alcohol intoxication in death certificates were considered to be possibly alcohol-related, and within the category of other cardiovascular deaths, deaths from liver cirrhosis and acute pancreatitis without gallstones were considered to be possibly alcohol-related (Study III).
Ascertainment of incident CHD. The combined endpoint category ‘major CHD event’ included CHD death, ascertained as described above, or non-fatal hospital-verified MI as the first CHD event during the follow-up (Study I, IV). For the ascertainment of hospital-verified MIs occurring during the follow-up, information on hospitalisations of all men belonging to the study cohort over the period from 1 January 1971 until 1 January 1994 was obtained from the computerised National Hospital Discharge Register. From this register, hospitalisations with ICD-codes 410-413 as discharge diagnoses (International Classification of Diseases, Eighth Revision [ICD-8] until 1986; ICD-9 since 1987) were identified. Patient records on these hospitalisations were reviewed by the author. The diagnosis of MI was confirmed, if at least 2 of the following criteria were fulfilled: 1) chest pain attack lasting for at least 20 minutes or its equivalent (acute left ventricular failure, syncope); 2) development of ECG changes diagnostic or suggestive of MI; or 3) elevation of serum levels of cardiac enzymes. Thus, it was possible to have a rather complete ascertainment of MIs leading to hospital treatment, including also those non-fatal MIs which occurred in men who later died from CHD or other cause. If the patient died from CHD within 28 days from the hospital admission because of MI, the event was considered as CHD death.

Ascertainment of incident stroke. A combined endpoint for incident stroke, including fatal stroke, ascertained as described above, and non-fatal stroke was used in Studies II and IV. Hospitalisations for acute cerebrovascular events with ICD-codes 431-436 as discharge diagnoses (ICD-8 until 1986; ICD-9 since 1987) were identified from the National Hospital Discharge Register over the follow-up period of all men belonging to the study cohort. The patient records on these hospitalisations were reviewed by the author. The diagnosis of stroke was ascertained, as at baseline, according to the WHO criteria for stroke (452): a neurological deficit observed by a physician and persisting for more than 24 hours, without other diseases explaining the symptoms. Thromboembolic and haemorrhagic strokes, but not subarachnoid haemorrhage, were included in the diagnosis of stroke. Strokes occurring within 28 days after a hospital-verified acute MI were interpreted as secondary complications of MI and excluded. If the patient died from cerebrovascular disease within 28 days from the hospital admission because of stroke, the event was considered as stroke death.
Development of drug-treated diabetes. The Finnish Social Insurance Institution maintains a central register of diabetic subjects receiving reimbursement of hypoglycaemic drugs. The dates of the beginning of such reimbursement during the follow-up for men belonging to the study cohort were obtained from this register.

4.4 Statistical methods

Data analyses were performed with SPSS 6.1.3/8.0 and SAS 6.10/6.12 software. Because of the skewed distribution of blood glucose and plasma insulin variables, as well as plasma triglycerides, these variables were log-transformed for statistical analyses. Age-adjusted Pearson's partial correlation coefficients were calculated to examine the correlations between plasma insulin and other continuous variables. The Student's 2-tailed t test for independent samples, analysis of covariance (ANCOVA), or Mantel-Haenszel test were used in comparisons between groups, as appropriate. In Study III, linear trends of baseline characteristics by quintiles of AUC insulin were tested by ANCOVA for age-adjusted continuous variables and by general linear modelling of the SAS system for categorical variables.

In Study IV, factor analysis was used to investigate intercorrelations between baseline risk factor variables and to reduce them into a smaller set of underlying uncorrelated factors. Principal component analysis was used for the extraction of the initial factors. This analysis transforms the original variables into a new set of uncorrelated components (initial factors) that account for the maximum proportion of the variance in the data, each component being a linear combination of the original observed variables. The first principal component is that linear combination of variables which accounts for the largest proportion of variance in the data, the second accounts for the next largest proportion, and so on. The initial factors (components) obtained in this way were then subjected to Varimax rotation, an orthogonal rotation, to facilitate their interpretation. The orthogonal rotation maintains the independence between the factors, that is, the correlation between the factors is 0. The interpretation of factors is based on factor loadings, equivalent to a Pearson's correlation coefficient between each variable
and each factor, and involves identification of those variables for which the loading is strong on a particular factor. Each factor may then be named descriptively according to variables with strong loadings on it. Conventionally, variables with loadings $\geq 0.40$ on a particular factor (sharing at least 15% of the variance with the factor) are used for its interpretation, although significant correlations ($P<0.01$) between other variables and the factor, corresponding to loadings $\geq 0.30$, are also noted (455).

Factors obtained in the factor analyses of Study IV were retained and used in Kaplan-Meier and Cox model analyses for the prediction of the risk of CHD and stroke, either as continuous variables or as categorical variables, dividing the distribution of factor scores into tertiles. As a result of standardisation, the mean value for each factor is 0 and standard deviation is 1.

Age-adjusted incidences and significances for their trends were calculated by general linear modelling of the SAS system in Studies I and II. In Study III, age-standardised mortality rates per 1000 person-years for AUC insulin quintiles were calculated by the direct method using the age structure of the whole study population as a reference. The 95% CIs were calculated for the age-standardised mortality rates. Kaplan-Meier survival curves were calculated to describe the occurrence of major CHD events (Study I) and strokes (Study II), as well as deaths from all causes and from specific causes (Study III) by quintiles of AUC insulin over the 22-year follow-up period. Differences between and over quintiles were tested by the log-rank test, without and with age adjustment. Similar Kaplan-Meier analyses were performed in Study IV with regard to the occurrence of major CHD events and strokes by tertiles of the ‘insulin resistance factor’.

Cox proportional hazards model was used to estimate the predictive value of AUC insulin with regard to the risk of CHD events (Study I), stroke (Study II), and mortality from all causes and from specific causes (Study III). In Study IV, scores for the factors were used as continuous variables in Cox model analyses for the prediction of the risk of CHD and stroke. In Studies I and IV, one subject became censored from Cox model analyses concerning CHD events because of an early non-coronary death. In Studies II and IV, 3 subjects became censored from Cox model analyses concerning stroke because of an early non-cerebrovascular death. With different endpoints, there were no indications of non-proportional hazards during the 22-year follow-up.
In Study III, missing values for predicted maximal O₂ uptake in 32 men were substituted in 24 men with the measurement made at an earlier examination and in 8 men with the age-specific mean value. In the factor analyses of Study IV, the missing values for maximal O₂ uptake were imputed by using the study population mean value.

4.5 Approval of the Ethics Committee

This follow-up study was approved by the Ethics Committee of the University of Kuopio. All study subjects had given informed consent.
5 RESULTS

5.1 Baseline characteristics

Table 6 shows baseline characteristics of the study population. The age-adjusted Pearson's partial correlation coefficients between plasma insulin variables and other continuous variables at baseline are given in Table 7. Blood glucose variables, all indices of obesity, systolic and diastolic blood pressure, as well as mean blood pressure, and plasma triglycerides were positively and significantly correlated with all insulin variables. Cholesterol had a weak positive correlation with 1-hour and AUC insulin. Maximal \( O_2 \) uptake was inversely and significantly correlated with all insulin variables.

Age- and BMI-adjusted plasma insulin concentrations in smokers and non-smokers did not differ significantly, with the exception of slightly lower 2-hour insulin concentrations in smokers (geometric means: 110 vs. 118 pmol/l, \( P=0.002 \)). Physically active men had significantly lower age- and BMI-adjusted insulin concentrations than physically inactive men (geometric means: fasting, 32 vs. 38 pmol/l, \( P<0.001 \); 1-hour, 261 vs. 330 pmol/l, \( P<0.001 \); 2-hour, 91 vs. 121 pmol/l, \( P<0.001 \); AUC insulin, 337 vs. 427 pmol/l \( \cdot \) h, \( P<0.001 \)). There were no differences in insulin concentrations between police officers and ordinary policemen.

5.2 Mortality and occurrence of coronary heart disease and stroke events during the follow-up

The distribution of causes of death during 5-, 10-, 15-, and 22-year follow-up periods is shown in Table 8. The proportion of cardiovascular deaths as well as non-cardiovascular deaths remained essentially unchanged during the entire follow-up period. Within the category of cardiovascular deaths, the proportion of coronary deaths decreased and, reciprocally, the proportion of cerebrovascular deaths increased towards the end of the follow-up. Within the category of non-cardiovascular deaths, there was an increase in
the proportion of cancer deaths with the lengthening of follow-up time, but this increase was balanced by a decrease in the proportion of violent deaths.

Table 6. Baseline characteristics of the study population (n=970)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD/ Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>47.4 ± 7.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179 ± 5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>84.1 ± 10.8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.2 ± 3.0</td>
</tr>
<tr>
<td>Subscapular skinfold, mm</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>Triceps skinfold, mm</td>
<td>10.3 ± 4.0</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>136 ± 18</td>
</tr>
<tr>
<td>Diastolic blood pressure; mmHg</td>
<td>85 ± 11</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>102 ± 12</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>24.3</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>6.27 ± 1.14</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.74 ± 1.01</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td>1-hour glucose, mmol/l</td>
<td>6.5 ± 1.9</td>
</tr>
<tr>
<td>2-hour glucose, mmol/l</td>
<td>4.4 ± 1.2</td>
</tr>
<tr>
<td>AUC glucose, mmol/l · h</td>
<td>11.2 ± 2.4</td>
</tr>
<tr>
<td>Fasting insulin, pmol/l</td>
<td>44 ± 28</td>
</tr>
<tr>
<td>1-hour insulin, pmol/l</td>
<td>372 ± 260</td>
</tr>
<tr>
<td>2-hour insulin, pmol/l</td>
<td>160 ± 159</td>
</tr>
<tr>
<td>AUC insulin, pmol/l · h</td>
<td>475 ± 321</td>
</tr>
<tr>
<td>Maximal O₂ uptake, ml/min/kg of body weight</td>
<td>35.3 ± 8.3</td>
</tr>
</tbody>
</table>

Smoking status
- Never smoked, %                               | 23.7                 |
- Stopped smoking, %                             | 31.2                 |
- Currently smoking, %                           | 45.1                 |

Physical activity (leisure time)
- Inactive, %                                    | 42.8                 |
- Slightly active, %                             | 23.1                 |
- Active, %                                      | 27.0                 |
- Highly active, %                               | 7.1                  |

Occupational status
- Officers, %                                    | 10.1                 |
- Non-commissioned officers, %                   | 21.4                 |
- Ordinary policemen, %                          | 68.5                 |

AUC glucose indicates the area under the glucose response curve; AUC insulin, the area under the insulin response curve; CHD, coronary heart disease; SD, standard deviation.
Table 7. Age-adjusted Pearson’s partial correlation coefficients between insulin variables and other continuous variables

<table>
<thead>
<tr>
<th></th>
<th>Fasting insulin</th>
<th>1-hour insulin</th>
<th>2-hour insulin</th>
<th>AUC insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose</td>
<td>0.23†</td>
<td>0.21†</td>
<td>0.23†</td>
<td>0.24†</td>
</tr>
<tr>
<td>1-hour glucose</td>
<td>0.20†</td>
<td>0.47†</td>
<td>0.43†</td>
<td>0.49†</td>
</tr>
<tr>
<td>2-hour glucose</td>
<td>0.29†</td>
<td>0.17†</td>
<td>0.67†</td>
<td>0.31†</td>
</tr>
<tr>
<td>AUC glucose</td>
<td>0.26†</td>
<td>0.43†</td>
<td>0.53†</td>
<td>0.49†</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.43†</td>
<td>0.39†</td>
<td>0.35†</td>
<td>0.42†</td>
</tr>
<tr>
<td>Subscapular skinfold</td>
<td>0.40†</td>
<td>0.35†</td>
<td>0.36†</td>
<td>0.39†</td>
</tr>
<tr>
<td>Triceps skinfold</td>
<td>0.24†</td>
<td>0.20†</td>
<td>0.25†</td>
<td>0.23†</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.14†</td>
<td>0.12†</td>
<td>0.17†</td>
<td>0.14†</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.16†</td>
<td>0.15†</td>
<td>0.19†</td>
<td>0.18†</td>
</tr>
<tr>
<td>Mean blood pressure</td>
<td>0.16†</td>
<td>0.14†</td>
<td>0.19†</td>
<td>0.17†</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.002</td>
<td>0.10†</td>
<td>0.02</td>
<td>0.08*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.24†</td>
<td>0.22†</td>
<td>0.18†</td>
<td>0.23†</td>
</tr>
<tr>
<td>Maximal O₂ uptake</td>
<td>-0.29†</td>
<td>-0.30†</td>
<td>-0.33†</td>
<td>-0.33†</td>
</tr>
</tbody>
</table>

AUC glucose indicates the area under the glucose response curve; AUC insulin, the area under the insulin response curve. Glucose and insulin variables and triglycerides log-transformed.

*P < 0.05; †P < 0.01; ‡P < 0.001

Table 8. Distribution of causes of death during 5-, 10-, 15-, and 22-year follow-up periods

<table>
<thead>
<tr>
<th></th>
<th>5 years</th>
<th>10 years</th>
<th>15 years</th>
<th>22 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (% of all deaths)</td>
<td>n (% of all deaths)</td>
<td>n (% of all deaths)</td>
<td>n (% of all deaths)</td>
</tr>
<tr>
<td>All-cause</td>
<td>28 (100.0)</td>
<td>75 (100.0)</td>
<td>141 (100.0)</td>
<td>276 (100.0)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>13 (46.4)</td>
<td>34 (45.3)</td>
<td>61 (43.3)</td>
<td>139 (47.1)</td>
</tr>
<tr>
<td>Coronary</td>
<td>10 (35.7)</td>
<td>25 (33.3)</td>
<td>38 (27.0)</td>
<td>80 (29.0)</td>
</tr>
<tr>
<td>Cerebrovascular</td>
<td>1 (3.6)</td>
<td>5 (6.7)</td>
<td>9 (6.4)</td>
<td>27 (9.8)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (7.1)</td>
<td>4 (5.3)</td>
<td>14 (9.9)</td>
<td>23 (8.3)</td>
</tr>
<tr>
<td>Non-cardiovascular</td>
<td>15 (53.6)</td>
<td>41 (54.7)</td>
<td>80 (56.7)</td>
<td>146 (52.9)</td>
</tr>
<tr>
<td>Cancer</td>
<td>4 (14.3)</td>
<td>19 (25.3)</td>
<td>49 (34.8)</td>
<td>81 (29.3)</td>
</tr>
<tr>
<td>Violent</td>
<td>8 (28.6)</td>
<td>17 (22.7)</td>
<td>20 (14.2)</td>
<td>29 (10.5)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (10.7)</td>
<td>5 (6.7)</td>
<td>11 (7.8)</td>
<td>36 (13.0)</td>
</tr>
</tbody>
</table>
Table 9. The number of major CHD (coronary death or non-fatal MI) and stroke (fatal or non-fatal) events and coronary and stroke deaths as first events occurring during 5-, 10-, 15-, and 22-year follow-up periods

<table>
<thead>
<tr>
<th></th>
<th>5 years</th>
<th>10 years</th>
<th>15 years</th>
<th>22 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Major CHD event</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary death as the</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>first CHD event</td>
<td>28</td>
<td>68</td>
<td>105</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>8 (28.6)</td>
<td>19 (27.9)</td>
<td>28 (26.7)</td>
<td>51 (31.1)</td>
</tr>
<tr>
<td>Stroke (fatal or non-fatal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke death as the</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>first stroke event</td>
<td>7</td>
<td>21</td>
<td>33</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>1 (14.3)</td>
<td>3 (14.3)</td>
<td>7 (21.2)</td>
<td>22 (31.4)</td>
</tr>
</tbody>
</table>

Table 9 shows the number of major CHD and stroke events during different follow-up periods. The number of coronary deaths occurring as first CHD events, as well as the number of fatal strokes occurring as first stroke events is also shown. During the entire 22-year follow-up, almost one third of first major CHD events as well as first stroke events were fatal. The fatality of CHD events remained almost unchanged during the entire follow-up period, whereas there was an increasing trend in the fatality of stroke towards the end of the follow-up.

5.3 Area under the insulin response curve (AUC insulin) as a composite plasma insulin variable in analyses of the relationship between insulin and cardiovascular risk

Preliminary analyses were carried out to compare the associations of fasting, 1-hour and 2-hour plasma insulin levels and AUC insulin, a composite variable reflecting the insulin response during OGTT, with the main cardiovascular endpoints of this study: cardiovascular mortality, major CHD events and stroke.
Figure 1. Age-adjusted cardiovascular mortality rates by quintiles of fasting and 1- and 2-hour insulin, and AUC insulin during 5-, 10-, 15-, and 22-year follow-up periods. The quintile cut-off points were as follows: for fasting insulin, 24, 36, 48, and 66 pmol/l; for 1-hour insulin, 180, 264, 342, and 533 pmol/l; for 2-hour insulin, 60, 84, 144, and 234 pmol/l; and for AUC insulin, 237, 337, 437, and 669 pmol/l \cdot h.

Figure 1 shows the age-adjusted cardiovascular mortality rates by quintiles of fasting and 1- and 2-hour insulin, and AUC insulin during different follow-up periods. There was no significant trend in cardiovascular mortality rates across the quintiles of fasting insulin during different periods of follow-up. On the other hand, 1-hour insulin showed a significant positive association with cardiovascular mortality rates during all follow-up periods, except the first 5 years. As to 2-hour and AUC insulin, there was a trend towards an increase in cardiovascular mortality with increasing 2-hour insulin and, more markedly, with increasing AUC insulin during all follow-up periods; for 2-hour insulin, this trend was statistically significant during the first 5 years, whereas for AUC insulin, a significant trend was observed during the 15-year follow-up, and a marginally significant trend during the 22-year follow-up period.
Figure 2. Age-adjusted incidence of major CHD events by quintiles of fasting and 1- and 2-hour insulin, and AUC insulin during 5-, 10-, 15-, and 22-year follow-up periods.

Age-adjusted incidence of major CHD events by quintiles of insulin variables during different follow-up periods is shown in Figure 2. There was no significant association between fasting insulin and CHD incidence during different follow-up periods, whereas 1-hour insulin and AUC insulin were both positively and significantly associated with CHD incidence during all follow-up periods. 2-hour insulin showed a significant positive association with CHD incidence during 5- and 22-year follow-up periods. For 1-hour, 2-hour and AUC insulin the highest incidence of CHD was observed in the highest quintiles during all follow-up periods.

Figure 3 shows the age-adjusted incidence of stroke by quintiles of different insulin variables during the 22-year follow-up. The incidence of stroke tended to increase with increasing fasting, 1-hour and 2-hour insulin, as well as with increasing AUC insulin, with the highest incidence of stroke in the top quintile for all insulin variables, but the trend over the quintiles reached statistical significance only for fasting insulin.
The above analyses demonstrated that the association of fasting, 1-hour and 2-hour insulin levels with cardiovascular mortality, as well as with CHD and stroke incidence was largely similar to the association of AUC insulin with these cardiovascular endpoints. Therefore, AUC insulin was used in subsequent analyses, because it combines the information from all insulin variables measured during OGTT.

5.4 Insulin and the risk of coronary heart disease (Study I)

During the 22-year follow-up, 164 men had a major CHD event. The first CHD event was CHD death in 51 men, and non-fatal MI in 113 men. Kaplan-Meier survival curves for remaining free of major CHD events during the 22-year follow-up by quintiles of AUC insulin are shown in Figure 4. During the entire follow-up period, the proportion
of men having a major CHD event was higher in the top quintile than in the lower quintiles. Moreover, towards the end of the follow-up period the proportion of men remaining free of major CHD events was particularly high among men in the lowest AUC insulin quintile.

![Kaplan-Meier survival curves for remaining free of major CHD events during 22-year follow-up by quintiles of AUC insulin.](image)

**Figure 4.** Kaplan-Meier survival curves for remaining free of major CHD events during 22-year follow-up by quintiles of AUC insulin.

To assess the association of hyperinsulinaemia with the risk of any major CHD event, CHD death as the first event, and non-fatal MI as the first event, hyperinsulinaemia was defined by the cut-off point for the highest AUC insulin quintile (≥669 pmol/l · h) and, comparing the highest AUC insulin quintile with the combined lower quintiles, the age-adjusted and multiple-adjusted HRs for hyperinsulinaemia were calculated by the use of the Cox proportional hazards model (Table 10). Adjusting for age, hyperinsulinaemia was significantly associated with the risk of any major CHD event during all periods of follow-up, although these associations decreased with an increase of follow-up time; with regard to the risk of CHD death as the first event, a statistically significant association was observed during 10- and 15-year follow-up.
Table 10. Hazard ratios and 95% confidence intervals for hyperinsulinaemia (AUC insulin quintile 5 vs. quintiles 1-4) with regard to the risk of CHD events during different follow-up periods

<table>
<thead>
<tr>
<th>Follow-up time</th>
<th>5 year</th>
<th>10 years</th>
<th>15 years</th>
<th>22 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any major CHD event</td>
<td>(n* = 13/28)</td>
<td>(n = 27/68)</td>
<td>(n = 35/105)</td>
<td>(n = 44/164)</td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>3.29 (1.56-6.91)</td>
<td>2.72 (1.67-4.42)</td>
<td>2.14 (1.43-3.21)</td>
<td>1.61 (1.14-2.27)</td>
</tr>
<tr>
<td>Multiple-adjusted†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without AUC glucose</td>
<td>3.07 (1.31-7.20)</td>
<td>2.75 (1.59-4.75)</td>
<td>1.95 (1.24-3.08)</td>
<td>1.50 (1.02-2.20)</td>
</tr>
<tr>
<td>With AUC glucose</td>
<td>2.36 (1.00-5.57)</td>
<td>2.29 (1.31-4.02)</td>
<td>1.76 (1.09-2.82)</td>
<td>1.32 (0.89-1.97)</td>
</tr>
<tr>
<td>CHD death as the first event</td>
<td>(n = 2/8)</td>
<td>(n = 8/19)</td>
<td>(n = 13/28)</td>
<td>(n =15/51)</td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.16 (0.23-5.73)</td>
<td>2.76 (1.11-6.86)</td>
<td>3.42 (1.62-7.18)</td>
<td>1.74 (0.95-3.17)</td>
</tr>
<tr>
<td>Multiple-adjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without AUC glucose</td>
<td>1.43 (0.24-8.48)</td>
<td>3.80 (1.29-11.19)</td>
<td>4.64 (1.96-10.99)</td>
<td>1.83 (0.93-3.59)</td>
</tr>
<tr>
<td>With AUC glucose</td>
<td>1.25 (0.20-7.75)</td>
<td>3.90 (1.25-12.20)</td>
<td>5.46 (2.13-14.00)</td>
<td>1.63 (0.81-3.28)</td>
</tr>
<tr>
<td>Non-fatal MI as the first event</td>
<td>(n = 11/20)</td>
<td>(n = 19/49)</td>
<td>(n = 22/77)</td>
<td>(n = 29/113)</td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>4.75 (1.97-11.50)</td>
<td>2.64 (1.49-4.70)</td>
<td>1.72 (1.05-2.83)</td>
<td>1.51 (0.99-2.31)</td>
</tr>
<tr>
<td>Multiple-adjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without AUC glucose</td>
<td>4.02 (1.46-11.05)</td>
<td>2.44 (1.28-4.66)</td>
<td>1.38 (0.79-2.40)</td>
<td>1.34 (0.84-2.14)</td>
</tr>
<tr>
<td>With AUC glucose</td>
<td>2.93 (1.06-8.07)</td>
<td>1.89 (0.98-3.65)</td>
<td>1.16 (0.66-2.05)</td>
<td>1.17 (0.72-1.91)</td>
</tr>
</tbody>
</table>

*Number of events in quintile 5/total number of events.
†Adjustment for age, BMI, subscapular skinfold, systolic blood pressure, cholesterol, triglycerides (log-transformed), AUC glucose (log-transformed), smoking (yes/no), and physical activity (yes/no).
periods, and with regard to the risk of non-fatal MI as the first event, during 5-, 10-, 15-year follow-up periods, and marginally, also during the 22-year follow-up period. In comparison, the age-adjusted HRs for CHD death as the first event were higher than those for non-fatal MI as the first event, except during the 5-year follow-up.

Variables included in the multiple-adjusted Cox models were: BMI, subscapular skinfold, systolic blood pressure, cholesterol, triglycerides, AUC glucose, smoking, and physical activity. Of these variables, the following, when adjusted for age, were significant predictors over the 22-year follow-up for any major CHD event: systolic blood pressure (P=0.002), cholesterol (P<0.001), triglycerides (P=0.005), AUC glucose (P<0.001), and smoking (P<0.001). When the risk factors included in the multiple-adjusted Cox models were entered into the Cox model separately, in addition to AUC insulin and age, AUC glucose was the only variable leading to a substantial reduction in the predictive value of hyperinsulinaemia. Table 10 shows multiple-adjusted HRs calculated with and without AUC glucose. Without AUC glucose, the age-adjusted HRs for hyperinsulinaemia with regard to the risk of any major CHD event were only slightly reduced. With AUC glucose, the HRs were substantially attenuated, but still remained statistically significant, except for the 22-year follow-up period. With regard to CHD death as the first event, multiple adjustment both with and without AUC glucose rather strengthened the age-adjusted HRs. On the other hand, the age-adjusted HRs for non-fatal MI as the first event were reduced in the multiple adjustment even without AUC glucose, and inclusion of AUC glucose in the model further reduced the HRs, only the HR for the 5-year follow-up retaining its statistical significance.

The multiple-adjusted HRs for hyperinsulinaemia with regard to the risk of CHD events were also calculated by replacing the degree of physical activity by predicted maximal $O_2$ uptake (measurement available for 938 men). The HRs for any major CHD event and non-fatal MI as the first event were essentially similar to those shown in Table 10 (data not shown), and for CHD death as the first event, HRs obtained with maximal $O_2$ uptake in the model were even somewhat greater than those with physical activity in the model, especially during 10 and 15 years of follow-up (5.50 [95% CI, 1.42-21.31] and 7.80 [95% CI, 2.64-23.10], respectively).
The association of insulin and the risk of CHD events was examined further by entering AUC insulin as a continuous variable into the Cox model (Table 11). The HRs and their 95% CIs were calculated for 1 SD differences in AUC insulin and other continuous variables to allow a comparison of their predictive power. Smoking and physical activity were entered as dichotomous variables. With regard to the risk of any major CHD event, the age-adjusted HRs for 1 SD difference in AUC insulin (0.26 logarithm) became attenuated with the lengthening of follow-up, but were statistically significant over the entire follow-up period. With adjustment for other risk factors, the HRs became only slightly reduced and remained significant, except for the first 5 years. With regard to the risk of CHD death as the first event, the evolution of HRs for 1 SD difference in AUC insulin over the 22-year follow-up was largely similar to that observed with regard to the risk of any major CHD event, but multiple adjustment did not reduce the HRs at all. With regard to the risk of non-fatal MI as the first event, the age-adjusted HRs diminished with lengthening follow-up time, but remained statistically significant. With multiple adjustment the HRs became clearly reduced; only the HR for 10-year follow-up remained statistically significant.

In these analyses, cholesterol was an independent predictor of the risk of any major CHD event and non-fatal MI as the first event over the entire follow-up period; during the 22-year follow-up, the HR for 1 SD difference in cholesterol (1.1 mmol/l) was 1.30 (95% CI, 1.11-1.54) for any major CHD event, and 1.37 (95% CI, 1.13-1.66) for non-fatal MI as the first event. Cholesterol was, however, not significantly associated with the risk of CHD death as the first event over the entire 22-year follow-up; for 22 years the HR was 1.12 (95% CI, 0.84-1.51). Systolic blood pressure became a statistically significant predictor of the risk of any major CHD event and CHD death as the first event towards the end of the follow-up; during the 22-year follow-up the HR for 1 SD difference in systolic blood pressure (18 mmHg) was 1.19 (95% CI, 1.03-1.38) for any major CHD event and 1.37 (95% CI, 1.13-1.66) for CHD death as the first event. On the other hand, the association between systolic blood pressure and the risk of non-fatal MI as the first event did not reach statistical significance during the 22-year follow-up; for 22 years the HR was 1.11 (95% CI, 0.92-1.33). AUC glucose predicted the risk of any
Table 11. Hazard ratios and 95% confidence intervals for AUC insulin as a continuous variable* with regard to the risk of CHD events during different follow-up periods

<table>
<thead>
<tr>
<th></th>
<th>Follow-up time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 year</td>
</tr>
<tr>
<td>Any major CHD event</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.77 (1.22-2.57)</td>
</tr>
<tr>
<td>Multiple-adjusted</td>
<td>1.51 (0.96-2.37)</td>
</tr>
<tr>
<td>CHD death as the first event</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.53 (0.73-3.23)</td>
</tr>
<tr>
<td>Multiple-adjusted</td>
<td>1.91 (0.75-4.85)</td>
</tr>
<tr>
<td>Non-fatal MI as the first event</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.86 (1.20-2.88)</td>
</tr>
<tr>
<td>Multiple-adjusted</td>
<td>1.41 (0.83-2.39)</td>
</tr>
</tbody>
</table>

*Hazard ratios were calculated for 1 SD difference in AUC insulin (0.26 logarithm). Other continuous variables entered into the Cox models were also standardised with regard to their 1 SD differences: age (7.5 years), BMI (3.0 kg/m²), subscapular skinfold (7 mm), systolic blood pressure (18 mmHg), cholesterol (1.1 mmol/l), triglycerides (0.20 logarithm), AUC glucose (0.09 logarithm). Smoking and physical activity were entered as dichotomous variables into the multiple-adjusted model.
major CHD event only during the first 5 years (HR for 1 SD difference in AUC glucose [0.09 logarithm] 1.58 [95% CI, 1.04-2.41]), and the risk of non-fatal MI as the first event during the 5-year follow-up (HR 1.86 [95% CI, 1.13-3.08] and the 10-year follow-up (HR 1.48 [95% CI, 1.08-2.04]). Smoking, as a dichotomous variable, predicted the risk of any major CHD event and the risk of non-fatal MI as the first event from 10 years onward; during the 22-year follow-up, the respective HRs were 1.95 (95% CI, 1.42-2.68) and 1.82 (95% CI, 1.24-2.66). With regard to the risk of CHD death as the first event, smoking became an independent predictor only during the 22-year follow-up (HR 2.18 [95% CI, 1.23-3.85]).

As stated above, 63 men developed drug-treated diabetes during the follow-up. This information was used to examine the possibility that the association of hyperinsulinaemia with CHD risk would be explained by the development of diabetes. Diabetes requiring drug treatment developed more frequently among men in the highest AUC insulin quintile as compared to men in the combined 4 lower quintiles (12.8% vs. 4.9%; P=0.001). In the whole study cohort, there was no significant difference in the development of drug-treated diabetes between men who had and those who did not have a major CHD event during the follow-up (9.8% vs. 5.8%; P=0.115). Among men in the combined 4 lower AUC insulin quintiles, however, drug-treated diabetes developed more frequently in those with a major CHD event than in those remaining free of such an event (9.2% vs. 4.1%; P=0.031), whereas among men in the highest AUC insulin quintile, drug-treated diabetes developed equally often in those who had and those who did not have a major CHD event (11.4% vs. 13.2%; P=0.456). Multiple-adjusted Cox model analyses comparing the highest AUC insulin with the combined 4 lower quintiles with regard to the risk of any major CHD event were also performed by excluding those 63 men who developed drug-treated diabetes during the follow-up; the age-adjusted and multiple-adjusted 22-year HRs with regard to the risk of any major CHD event were 1.73 (95% CI, 1.20-2.50) and 1.44 (95% CI, 0.95-2.21), respectively. Furthermore, when Cox model analyses were carried out excluding subjects with mild impairments in glucose regulation at baseline, using the combined WHO and ADA criteria, the corresponding HRs were 1.65 (95% CI, 1.13-2.42) and 1.46 (95% CI, 0.94-2.27).
5.5 Insulin and the risk of stroke (Study II)

During the 22-year follow-up 70 men had a stroke. The first stroke event was fatal in 22 men and non-fatal in 48 men. Of all strokes, 55 (78.6%) were thromboembolic, 7 (10.0%) haemorrhagic, and 8 (11.4%) non-classifiable. Kaplan-Meier survival curves for remaining free of stroke during the 22-year follow-up by quintiles of AUC insulin are shown in Figure 5. The proportion of men without stroke was lowest in the highest AUC insulin quintile; comparison of this proportion in the highest quintile with that in the lowest quintile, however, did not quite reach statistical significance.

![Figure 5. Kaplan-Meier survival curves for remaining free of stroke during 22-year follow-up by quintiles of AUC insulin.]

Cox proportional hazards model was used to calculate HRs and their 95% CIs for hyperinsulinaemia (the highest AUC insulin quintile vs. the combined 4 lower quintiles) with regard to the risk of any stroke event, fatal stroke as the first event, and non-fatal stroke as the first event during different follow-up periods (Table 12). Adjusting for age, the HR for hyperinsulinaemia with regard to any stroke event was not markedly altered.
<table>
<thead>
<tr>
<th>Table 12. Hazard ratios and 95% confidence intervals for hyperinsulinaemia (AUC insulin quintile 5 vs. quintiles 1-4) with regard to the risk of stroke events during different follow-up periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up time</td>
</tr>
<tr>
<td>5 year</td>
</tr>
<tr>
<td><strong>Any stroke event</strong></td>
</tr>
<tr>
<td>(n* = 3/7)</td>
</tr>
<tr>
<td>Age-adjusted</td>
</tr>
<tr>
<td>Multiple-adjusted†</td>
</tr>
<tr>
<td><strong>Fatal stroke as the first event</strong></td>
</tr>
<tr>
<td>(n = 1/1)</td>
</tr>
<tr>
<td>Age-adjusted</td>
</tr>
<tr>
<td>Multiple-adjusted</td>
</tr>
<tr>
<td><strong>Non-fatal stroke as the first event</strong></td>
</tr>
<tr>
<td>(n = 2/6)</td>
</tr>
<tr>
<td>Age-adjusted</td>
</tr>
<tr>
<td>Multiple-adjusted</td>
</tr>
</tbody>
</table>

*Number of events in quintile 5/total number of events.
†Adjustment for age, subscapular skinfold, systolic blood pressure, and smoking (yes/no).
with the lengthening of follow-up time, but became statistically significant only during the 22-year follow-up. Corresponding age-adjusted HRs with regard to fatal stroke as the first event could be calculated only for 15- and 22-year follow-up periods. These HRs were somewhat higher than those for any stroke event, but only the HR for 22 years was statistically significant. The age-adjusted HRs for non-fatal stroke as the first event were somewhat lower than for any stroke event or fatal stroke as the first event, and again, the HR reached statistical significance only during the 22-year follow-up.

For multiple adjustment, only those risk factors were chosen which, in addition to age, proved to be independently associated with the risk of any stroke event in multivariate Cox models during the 22-year follow-up period. These risk factors were subscapular skinfold (\(P=0.008\)), systolic blood pressure (\(P=0.003\)), and smoking (\(P=0.009\)). BMI was a statistically significant independent predictor (\(P=0.016\)) only if subscapular skinfold was omitted from the model. If diastolic instead of systolic blood pressure was entered into the model, it was also a statistically significant predictor of the risk of any stroke (\(P=0.003\)) with predictive power similar to that of systolic blood pressure.

In multiple adjustment including, in addition to age, subscapular skinfold, systolic blood pressure and smoking, the HRs for hyperinsulinaemia with regard to any stroke event, fatal stroke as the first event, and non-fatal stroke as the first event became markedly reduced and the HRs for 22 years lost their statistical significance (Table 12).

The individual impact of other risk factors on the association of hyperinsulinaemia with the risk of stroke events during the 22-year follow-up was examined by entering, in addition to AUC insulin and age, BMI, subscapular skinfold, systolic blood pressure, and smoking separately into the Cox model. Adjustment for BMI or subscapular skinfold had the greatest effect, reducing the age-adjusted HR for hyperinsulinaemia to non-significant 1.47 (95% CI, 0.84-2.58) and 1.53 (95% CI, 0.90-2.61), respectively. Adjustment for systolic blood pressure reduced the HR only slightly, to 1.97 (95% CI, 1.19-3.25), and adjustment for smoking had no effect, resulting in a HR of 2.26 (95% CI, 1.37-3.74). Because of the strong correlation of glucose with insulin, adjustment was also made for AUC glucose, but this resulted in a virtually unaltered HR of 2.05 (95% CI, 1.18-3.55). Similar analyses with regard to the risk of fatal stroke and non-fatal
Table 13. Hazard ratios and 95% confidence intervals for AUC insulin as continuous variable* with regard to the risk of stroke events during the 22-year follow-up period

<table>
<thead>
<tr>
<th></th>
<th>HR (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age-adjusted</td>
</tr>
<tr>
<td>Any stroke event</td>
<td>1.31 (1.02-1.67)</td>
</tr>
<tr>
<td>Fatal stroke as the first event</td>
<td>1.29 (0.82-2.02)</td>
</tr>
<tr>
<td>Non-fatal stroke as the first event</td>
<td>1.31 (0.98-1.75)</td>
</tr>
</tbody>
</table>

*HRs were calculated for 1 SD difference in AUC insulin (0.26 logarithm). Other continuous variables entered into Cox models were also standardised with regard to their 1 SD differences: age (7.5 years), subcapular skinfold (7 mm), and systolic blood pressure (18 mmHg). Smoking was entered as a dichotomous variable.

Stroke as the first events gave comparable results (data not shown).

AUC insulin was also entered as a continuous variable into the Cox models. In addition to age, other continuous variables included in the multiple-adjusted model were systolic blood pressure and subcapular skinfold. HRs for the 22-year follow-up period were calculated for 1 SD differences in AUC insulin and other continuous variables to allow a comparison of their predictive power. Smoking was entered as a dichotomous variable. As shown in Table 13, the age-adjusted HR for 1 SD difference in AUC insulin (0.26 logarithm) with regard to the risk of any stroke event was statistically significant, but became non-significant with multiple adjustment. Although the age-adjusted HRs for fatal stroke as the first event and non-fatal stroke as the first event were of the same magnitude as that for any stroke event, they did not reach statistical significance. In the multiple-adjusted model, the HR for 1 SD difference in subcapular skinfold (7 mm) was 1.59 (95% CI, 1.26-2.00) for any stroke event, 1.87 (95% CI, 1.23-2.85) for fatal stroke as the first event, and 1.46 (95% CI, 1.10-1.93) for non-fatal stroke as the first event. The corresponding HRs for 1 SD difference in BMI (3.0 kg/m²), when it was entered into the model instead of subcapular skinfold, were 1.46 (95% CI, 1.13-1.88), 1.59 (95% CI, 1.02-2.47), and 1.39 (95% CI, 1.02-1.88). When subcapular skinfold and BMI were simultaneously entered into the model, the respective HRs for subcapular skinfold were 1.47 (95% CI, 1.11-1.96), 1.73 (95% CI, 1.05-2.85) and 1.35
(95% CI, 0.95-1.91) and for BMI 1.16 (95% CI, 0.85-1.58), 1.18 (95% CI, 0.70-1.99), and 1.16 (95% CI, 0.79-1.68). The HR for 1 SD difference in systolic blood pressure (18 mmHg) was 1.36 (95% CI, 1.11-1.66) for any stroke event, 1.42 (95% CI, 0.98-2.96) for fatal stroke as the first event, and 1.33 (95% CI, 1.04-1.69) for non-fatal stroke as the first event. For smoking, entered as a dichotomous variable, the HR was 1.90 (95% CI, 1.18-3.06) for any stroke event, 1.58 (95% CI, 0.66-3.81) for fatal stroke as the first event, and 2.10 (95% CI, 1.18-3.74) for non-fatal stroke as the first event.

There was a trend, although statistically non-significant, to a more frequent development of drug-treated diabetes among men who had a stroke during the follow-up than among those who did not have a stroke (11.4% vs. 6.1%, $P=0.116$). This trend was also observed in men in the highest AUC insulin quintile (21.7% vs. 11.6%, $P=0.102$) but not in men in the combined lower AUC insulin quintiles (6.4% vs. 4.8%, $P=0.787$). Cox model analyses similar to those shown in Table 12, in which those 63 men who developed drug-treated diabetes were excluded, resulted in a slight reduction in the HRs for hyperinsulinaemia with regard to the risk of any stroke event during the 22-year follow-up; the age-adjusted HR became 1.92 (95% CI, 1.11-3.32) and the multiple-adjusted HR 1.44 (95% CI, 0.80-2.57). On the other hand, when subjects with mild impairments in glucose regulation at baseline using the combined WHO and ADA criteria were excluded, the HRs for hyperinsulinaemia with regard to the risk of any stroke event during the 22-year follow-up became strengthened; in the age-adjusted model the HR was 2.56 (95% CI, 1.52-4.34), and in the multiple-adjusted model the HR remained statistically significant 1.83 (95% CI, 1.05-3.19).

5.6 Insulin and all-cause, cardiovascular and non-cardiovascular mortality (Study III)

Altogether 276 men (28.5%) died during the 22-year follow-up period. The age-standardised cause-specific mortality rates per 1000 person-years and their 95% CIs by quintiles of AUC insulin during the 22-year follow-up period are shown in Table 14. There was a significant excess of all-cause mortality in the top AUC insulin quintile as
Table 14. Age-standardised cause-specific mortality rates by quintiles of AUC insulin during the 22-year follow-up (276 deaths among 970 men)

<table>
<thead>
<tr>
<th></th>
<th>Quintile 1</th>
<th>Quintile 2</th>
<th>Quintile 3</th>
<th>Quintile 4</th>
<th>Quintile 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>4.18 (3.18-5.18)</td>
<td>4.71 (3.73-5.68)</td>
<td>7.06 (6.08-8.04)</td>
<td>7.50 (6.50-8.50)</td>
<td>10.35 (9.33-11.37)</td>
</tr>
<tr>
<td>Coronary</td>
<td>1.57 (0.57-2.57)</td>
<td>3.47 (2.49-4.44)</td>
<td>4.29 (3.30-5.27)</td>
<td>5.43 (4.44-6.43)</td>
<td>5.99 (4.97-7.02)</td>
</tr>
<tr>
<td>Cerebrovascular*</td>
<td>1.31 (-0.02-2.64)</td>
<td>0.74 (-0.51-1.99)</td>
<td>0.76 (-0.52-2.03)</td>
<td>1.29 (0.36-2.23)</td>
<td>3.00 (1.97-4.02)</td>
</tr>
<tr>
<td>Other*</td>
<td>1.31 (0.24-2.37)</td>
<td>0.50 (-2.40-3.39)</td>
<td>2.02 (1.03-3.00)</td>
<td>0.78 (-1.18-2.73)</td>
<td>1.36 (0.34-2.38)</td>
</tr>
<tr>
<td>Cancer</td>
<td>3.40 (2.39-4.40)</td>
<td>3.96 (2.99-4.94)</td>
<td>3.78 (2.80-4.77)</td>
<td>3.88 (2.88-4.88)</td>
<td>5.99 (4.97-7.02)</td>
</tr>
<tr>
<td>Violent*</td>
<td>2.87 (1.87-3.88)</td>
<td>0.99 (-0.05-2.03)</td>
<td>1.01 (0.02-1.99)</td>
<td>0.78 (-0.83-2.38)</td>
<td>1.91 (0.88-2.93)</td>
</tr>
<tr>
<td>Other*</td>
<td>2.87 (1.87-3.88)</td>
<td>0.74 (-0.62-2.10)</td>
<td>2.77 (1.79-3.76)</td>
<td>1.03 (-0.23-2.30)</td>
<td>1.63 (0.61-2.66)</td>
</tr>
</tbody>
</table>

The cut-off points for AUC insulin were 237, 337, 437, and 669 pmol/l/h.

*In some quintiles, the 95% CI includes 0 because the small number of deaths.
compared with lower AUC insulin quintiles. This was mainly due to a significant excess in cardiovascular mortality in the top quintile of AUC insulin which in turn resulted from an excess in coronary and cerebrovascular mortality. The relationship of AUC insulin with non-cardiovascular mortality was U-shaped with higher mortality rates in the lowest and highest quintiles than in the middle quintiles.

Figure 6 shows the Kaplan-Meier survival curves with regard to all-cause, cardiovascular, coronary, cerebrovascular, non-cardiovascular, and cancer mortality by quintiles of AUC insulin during the 22-year follow-up. During the entire follow-up, the overall survival was poorest in men in the highest AUC insulin quintile. A similar worsening of prognosis in men in the highest AUC insulin quintile was also observed with regard to cardiovascular and coronary mortality, and there was a similar trend for cerebrovascular mortality. With regard to non-cardiovascular mortality, during the first years of follow-up the survival appeared to be poorest in men in the highest AUC insulin quintile. Thereafter, however, a deteriorating trend ensued in the lowest insulin quintile and, during the last half of the follow-up period, the prognosis of men in both the highest and the lowest insulin quintile was poorer than the prognosis of men in the middle quintiles. Towards the end of the follow-up period, the proportion of men dying from cancer was higher in the highest quintile than in the lower quintiles.

To examine the association of hyperinsulinaemia with all-cause, cardiovascular, coronary and cerebrovascular mortality, age- and multiple-adjusted HRs for hyperinsulinaemia (highest AUC insulin quintile vs. the 4 lower quintiles) were calculated using the Cox model. In addition to age, multiple adjustment included BMI, subscapular skinfold, systolic blood pressure, cholesterol, triglycerides, AUC glucose, maximal O₂ uptake, physical activity, smoking, and occupational status. Adjusting for age, hyperinsulinaemia showed a positive and statistically significant association with all-cause, cardiovascular, coronary, and cerebrovascular mortality during the first 10 years of follow-up; HR was 1.94 (95% CI, 1.20-3.13) for all-cause, 2.67 (95% CI, 1.35-5.29) for cardiovascular, 2.54 (95% CI, 1.14-5.65) for coronary, and 6.12 (95% CI, 1.02-36.80) for cerebrovascular mortality. These associations weakened but remained statistically significant during the entire 22-year follow-up period, except for coronary mortality; HR was 1.51 (95% CI, 1.15-1.97) for all-cause, 1.73 (95% CI, 1.19-2.53) for
Figure 6. Kaplan-Meier survival curves for all-cause, cardiovascular, cerebrovascular, non-cardiovascular and cancer mortality by quintiles of AUC insulin.
cardiovascular, 1.58 (95% CI, 0.97-2.59) for coronary, and 3.01 (95% CI, 1.40-6.50) for cerebrovascular mortality. Adjusting for other risk factors, hyperinsulinaemia was significantly associated with all-cause mortality during both follow-up periods; HR was 1.88 (95% CI, 1.08-3.30) during 10 years, and 1.37 (95% CI, 1.00-1.87) during 22 years, respectively. During the 10-year follow-up, the multiple-adjusted HRs for hyperinsulinaemia with regard to cardiovascular and coronary mortality were also statistically significant, 2.30 (95% CI, 1.03-5.1) for cardiovascular and 2.77 (95% CI, 1.06-7.23) for coronary mortality, but during the 22-year follow-up, they were reduced to non-significant level, 1.39 (95% CI, 0.90-2.15) for cardiovascular, and 1.35 (95% CI, 0.77-2.37) for coronary mortality. The association of hyperinsulinaemia with cerebrovascular mortality became non-significant with multiple adjustment; HR was 4.38 (95% CI, 0.44-44.00) during 10 years, and 1.76 (95% CI, 0.68-4.57) during 22 years.

Of other risk factors, systolic blood pressure was independently associated with all-cause, cardiovascular and coronary mortality over the 22-year follow-up period, with respective $P$-values of 0.004, <0.001 and <0.001. Smoking was independently associated with all-cause ($P<$0.001), cardiovascular ($P<$0.001) and coronary mortality ($P$=0.002). During the 22 years, AUC glucose was independently associated with coronary mortality ($P$=0.049) and subscapular skinfold with cerebrovascular mortality ($P$=0.012).

The impact of other risk factors on the association of hyperinsulinaemia with all-cause, cardiovascular, coronary, and cerebrovascular mortality was examined by entering them separately into the Cox model, in addition to AUC insulin (highest quintile vs. combined lower quintiles) and age. AUC glucose caused the most marked reduction in the HRs with regard to all-cause, cardiovascular and coronary mortality during the 22-year follow-up period, whereas the impact of other risk factors, such as systolic blood pressure, cholesterol and smoking, was only slight or moderate. BMI or subscapular skinfold had the most substantial effects on the age-adjusted HRs for cerebrovascular mortality, reducing them to non-significant level, while other risk factors had only a slight (systolic blood pressure) or virtually no effect.

Because the association of AUC insulin with non-cardiovascular mortality appeared
to be U-shaped (Table 14), the Cox model HRs for the prediction of non-cardiovascular mortality and its subcategories during the 22-year follow-up were calculated by comparing the lowest and the highest AUC insulin quintiles with the combined middle quintiles (quintiles 2-4). Low insulin concentrations were significantly associated with increased non-cardiovascular mortality, as well as with increased mortality from violence and causes other than cancer. Age-adjusted and multiple-adjusted HRs (quintile 1 vs. quintiles 2-4) were 1.59 (95% CI, 1.07-2.38) and 1.85 (95% CI, 1.20-2.86) for non-cardiovascular mortality, 3.27 (95% CI, 1.41-7.56) and 3.13 (95% CI, 1.24-7.91) for mortality from violence, and 2.14 (95% CI, 1.01-4.52) and 2.31 (95% CI, 1.01-5.28) for mortality from non-cardiovascular causes other than cancer or violence, respectively. There was no association between low insulin concentrations and cancer mortality; age-adjusted and multiple-adjusted HRs (quintile 1 vs. quintiles 2-4) were 0.97 (95% CI, 0.53-1.81) and 1.26 (95% CI, 0.65-2.44), respectively. High insulin concentrations were significantly associated with increased non-cardiovascular mortality adjusting for age, but this association lost its significance with multiple adjustment; the respective HRs (quintile 5 vs. quintiles 2-4) were 1.50 (95% CI, 1.00-2.24) and 1.40 (95% CI, 0.91-2.24). There was also a trend towards a positive association between high insulin concentrations and cancer mortality, as well as with mortality from violence, although these associations did not reach statistical significance; age-adjusted HR (quintile 5 vs. quintiles 2-4) was 1.57 (95% CI, 0.91-2.61) for cancer mortality, and 2.01 (95% CI, 0.78-5.18) for mortality from violence.

Of other risk factors, smoking was independently associated with non-cardiovascular mortality \((P<0.001)\), mortality from cancer \((P<0.001)\) and mortality from non-cardiovascular causes other than cancer or violence \((P=0.027)\). Maximal \(O_2\) uptake was independently and inversely associated with non-cardiovascular mortality \((P=0.030)\).

Of the 146 non-cardiovascular deaths, 19 (13.0%) were possibly alcohol-related (7 suicides, 6 deaths from accidents, 4 from liver cirrhosis, and 2 from acute pancreatitis). In the lowest AUC insulin quintile, 7 of 35 non-cardiovascular deaths (20.0%) were possibly alcohol-related; in the middle quintiles 2-4, the corresponding proportion was 8 of 76 (10.5%), and in the highest quintile, 4 of 35 (11.4%).

The association of insulin with all-cause, cardiovascular, coronary, and non-
cardiovascular mortality during the 22-year follow-up period was further analyzed by entering base 10-log-transformed AUC insulin as a continuous variable into the age-adjusted and multiple-adjusted Cox model. Adjusting for age alone, log AUC insulin showed a positive, statistically significant linear association with all-cause, cardiovascular and coronary mortality. The respective HRs for 1 base 10-log-unit increase in AUC insulin were 1.65 (95% CI, 1.04-2.61), 3.05 (95% CI, 1.56-5.96), and 3.95 (95% CI, 1.69-9.21). Adjusting for other risk factors, the association of log AUC insulin became non-significant with regard to all-cause mortality (HR 1.16 [95% CI, 0.65-2.07]) and cardiovascular mortality (HR 2.10 [95% CI, 0.91-4.85]), whereas with regard to coronary mortality, it remained virtually unchanged and significant (HR 3.72 [95% CI, 1.32-10.51]). Including also a squared term for log AUC insulin, similar analyses with age adjustment revealed a statistically significant curvilinear relationship between AUC insulin and non-cardiovascular mortality ($\chi^2=5.35$, df=1, $P=0.021$), but with multiple adjustment, this relationship ceased to be significant ($\chi^2=2.82$, df=1, $P=0.093$).

Cox model analyses, similar to those described above, were carried out excluding 63 men who developed drug-treated diabetes during the follow-up, and also excluding 79 men with mild impairments of glucose regulation at baseline according to the combined WHO and ADA criteria. The results obtained in these analyses were similar to those obtained in the whole study population (data not shown).

5.7 Insulin resistance syndrome and the risk of coronary heart disease and stroke (Study IV)

As stated above, during the 22-year follow-up period, 164 men had a CHD event (CHD death or non-fatal MI) and 70 men had a fatal or non-fatal stroke. Nineteen men had both a CHD event and a stroke during the follow-up. The CHD event preceded stroke in 13 of these men and in 6 stroke was the first event. As compared with men remaining free of CHD event or stroke, men with CHD event only were older, had higher systolic and diastolic blood pressures, were more often smokers, had higher cholesterol and
triglyceride levels, higher post-load glucose and AUC glucose levels, and higher fasting and post-load insulin and AUC insulin levels (Study IV, Table 1). Relative to men without incident CHD event or stroke, men with stroke only were older, had higher body weight and BMI, thicker subscapular skinfold, higher blood pressure, higher triglyceride levels, and higher fasting, 2-hour post-load and AUC insulin levels. The baseline characteristics of men with both CHD event and stroke were more closely similar to those of men with stroke only than to those of men with CHD event only.

Different sets of baseline risk factor variables were included in factor analyses. The largest set of 10 variables comprised BMI, subscapular skinfold, mean blood pressure, cholesterol, triglycerides (log-transformed), AUC glucose (log-transformed), AUC insulin (log-transformed), and maximal $O_2$ uptake as continuous variables and current smoking and leisure time physical activity as dichotomous variables. Subscapular skinfold was included as an index of upper-body obesity. Mean blood pressure was included because it combines information involving both the systolic and diastolic pressures. The univariate intercorrelations between these 10 baseline risk factor variables are shown in Table 15. BMI and subscapular skinfold were strongly correlated with each other. They were somewhat less strongly correlated with AUC insulin and maximal $O_2$ uptake (inversely) and also correlated with AUC glucose, triglycerides and mean blood pressure. AUC glucose and AUC insulin were strongly intercorrelated, and positively correlated with triglycerides and mean blood pressure and inversely correlated with maximal $O_2$ uptake. AUC insulin was also inversely correlated with physical activity. As expected, cholesterol was positively correlated with triglycerides.

Of the 10 risk factor variables, the following 4 variables showed statistically significant correlations ($P<0.01$) with age: mean blood pressure ($r=0.27$), AUC glucose ($r=0.17$), AUC insulin ($r=0.11$), and maximal $O_2$ uptake ($r=-0.29$). Therefore, factor analyses were carried out in 2 different ways: 1) by using risk factor variable data adjusted to the median age of the study cohort with the use of the univariate regression coefficient for each variable and 2) by using the data without age adjustment. Because the results obtained by these 2 approaches were virtually similar, results of factor analyses reported in Study IV are based on the original, actually measured data.
<table>
<thead>
<tr>
<th></th>
<th>BMI</th>
<th>Subsc</th>
<th>MeanBP</th>
<th>Chol</th>
<th>TG*</th>
<th>AUCglu*</th>
<th>AUCins*</th>
<th>Smoking</th>
<th>Phys.act</th>
<th>O₂ uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subsc</td>
<td>0.67†</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean BP</td>
<td>0.29†</td>
<td>0.21†</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chol</td>
<td>0.02</td>
<td>0.06</td>
<td>0.11†</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG*</td>
<td>0.30†</td>
<td>0.21†</td>
<td>0.12†</td>
<td>0.37†</td>
<td>1.00</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AUCglu*</td>
<td>0.28†</td>
<td>0.24†</td>
<td>0.28†</td>
<td>0.05</td>
<td>0.20†</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCins*</td>
<td>0.42†</td>
<td>0.39†</td>
<td>0.20†</td>
<td>0.09†</td>
<td>0.28†</td>
<td>0.50†</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.11†</td>
<td>-0.03</td>
<td>-0.13†</td>
<td>0.07</td>
<td>0.11†</td>
<td>-0.04</td>
<td>-0.04</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phys. act</td>
<td>-0.02</td>
<td>-0.13†</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.08†</td>
<td>-0.05</td>
<td>-0.19†</td>
<td>-0.10†</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>O₂ uptake</td>
<td>-0.37†</td>
<td>-0.41†</td>
<td>-0.31†</td>
<td>-0.11†</td>
<td>-0.09†</td>
<td>-0.28†</td>
<td>-0.34†</td>
<td>0.01</td>
<td>0.18†</td>
<td>1.00</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; Subsc, subscapular skinfold; Mean BP, mean blood pressure; Chol, cholesterol; TG, triglycerides; AUCglu, area under the glucose response curve; AUCins, area under the insulin response curve; Phys. act, physical activity; O₂ uptake, maximal oxygen uptake. Smoking and physical activity entered as dichotomous variables.

*Log-transformed.
†P < 0.01
Factor analysis including all the 10 variables (Table 16) yielded 3 factors, explaining 54.4% of the total variance. Factor 1, explaining 28.7% of the variance, comprised BMI, subscapular skinfold, AUC insulin, maximal \( O_2 \) uptake, AUC glucose, mean blood pressure, and triglycerides. Because AUC insulin demonstrated a strong loading on factor 1, it was named the insulin resistance factor. Factor 2 had strong loadings for cholesterol and triglycerides and was thus named the lipid factor. Smoking and physical activity had inverse loadings on factor 3 and, accordingly, this factor was named the lifestyle factor.

Because in our study population cholesterol and smoking showed very little correlation with AUC insulin, factor analysis was then performed excluding these variables (Table 16). With this set of 8 variables only 2 factors were produced. Factor 1 was very similar to the insulin resistance factor described above but now with a stronger loading for triglycerides, and Factor 2 had a strong loading for physical activity. Maximal \( O_2 \) uptake and physical activity correlated with AUC insulin, but traditionally, they are not considered to belong to the insulin resistance cluster. Exclusion of physical activity, leaving 7 variables, as well as further exclusion of maximal \( O_2 \) uptake, leaving 6 variables considered to belong to the core components of the IRS, resulted in only one factor (Table 16).

To assess the predictive value of the factors with regard to the risk of CHD and stroke, factor scores for the 3 factors obtained from factor analysis with 10 variables were entered into Cox models, in addition to age. The age-adjusted HRs and their 95% CIs for the 3 factors were calculated for 5-, 10-, 15-, and 22-year follow-up periods (Table 17). Factor 1, insulin resistance factor, was a significant predictor of CHD risk during all follow-up periods, with some attenuation of the HR with the lengthening of follow-up time. With regard to stroke risk, the predictive value of insulin resistance factor became significant towards the end of the follow-up. Factor 2, lipid factor, was a significant predictor of CHD risk during the entire follow-up, whereas with regard to stroke risk, none of the HRs for lipid factor during the follow-up reached statistical significance. Factor 3, lifestyle factor, was associated with a reduced CHD risk during 10 and 15 years.
Table 16. Factor analyses with different sets of baseline risk factor variables

<table>
<thead>
<tr>
<th></th>
<th>All 10 variables</th>
<th>8 variables</th>
<th>7 variables</th>
<th>6 variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Factor 1</td>
<td>Factor 2</td>
<td>Factor 3</td>
<td>Factor 1</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.77†</td>
<td>0.05</td>
<td>0.14</td>
<td>0.79†</td>
</tr>
<tr>
<td>Subscapular skinfold</td>
<td>0.76†</td>
<td>0.01</td>
<td>-0.03</td>
<td>0.74†</td>
</tr>
<tr>
<td>AUC insulin*</td>
<td>0.71†</td>
<td>0.11</td>
<td>-0.15</td>
<td>0.68†</td>
</tr>
<tr>
<td>Maximal O₂ uptake</td>
<td>-0.66†</td>
<td>-0.08</td>
<td>0.11</td>
<td>-0.63†</td>
</tr>
<tr>
<td>AUC glucose*</td>
<td>0.60†</td>
<td>0.10</td>
<td>0.04</td>
<td>0.60†</td>
</tr>
<tr>
<td>Mean blood pressure</td>
<td>0.48†</td>
<td>0.13</td>
<td>0.37†</td>
<td>0.53†</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>0.30†</td>
<td>0.73†</td>
<td>-0.12</td>
<td>0.45†</td>
</tr>
<tr>
<td>Physical activity</td>
<td>-0.25</td>
<td>0.14</td>
<td>0.73†</td>
<td>-0.08</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.15</td>
<td>0.26</td>
<td>-0.66†</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.00</td>
<td>0.85†</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

% of variance explained   | 29.9   | 13.2    | 11.3     | 36.9     | 13.0     | 41.7     | 43.7     |

*Log-transformed.

Factor loadings represent the correlation between the individual variable and each factor. Bolding indicates loadings ≥ 0.40 (absolute value).
†P < 0.01
Table 17. Age-adjusted hazard ratios and 95% confidence intervals for 3 factors obtained by using 10 variables with regard to the risk of coronary heart disease (CHD) and stroke during different follow-up periods

<table>
<thead>
<tr>
<th>Follow-up time</th>
<th>5 years</th>
<th>10 years</th>
<th>15 years</th>
<th>22 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD</td>
<td>n = 28</td>
<td>n = 68</td>
<td>n = 105</td>
<td>n = 164</td>
</tr>
<tr>
<td>Factor 1</td>
<td>1.56 (1.08-2.26)</td>
<td>1.45 (1.14-1.84)</td>
<td>1.39 (1.14-1.69)</td>
<td>1.28 (1.10-1.50)</td>
</tr>
<tr>
<td>Factor 2</td>
<td>1.70 (1.19-2.44)</td>
<td>1.66 (1.32-2.10)</td>
<td>1.56 (1.29-1.88)</td>
<td>1.47 (1.26-1.71)</td>
</tr>
<tr>
<td>Factor 3</td>
<td>0.94 (0.64-1.38)</td>
<td>0.76 (0.59-0.98)</td>
<td>0.81 (0.66-0.99)</td>
<td>0.87 (0.74-1.01)</td>
</tr>
<tr>
<td>Stroke</td>
<td>n = 7</td>
<td>n = 21</td>
<td>n = 33</td>
<td>n = 70</td>
</tr>
<tr>
<td>Factor 1</td>
<td>1.62 (0.79-3.35)</td>
<td>1.44 (0.94-2.23)</td>
<td>1.64 (1.16-2.31)</td>
<td>1.64 (1.29-2.08)</td>
</tr>
<tr>
<td>Factor 2</td>
<td>0.89 (0.41-1.93)</td>
<td>1.21 (0.78-1.86)</td>
<td>0.08 (0.77-1.54)</td>
<td>1.18 (0.93-1.49)</td>
</tr>
<tr>
<td>Factor 3</td>
<td>1.01 (0.46-2.22)</td>
<td>0.66 (0.41-1.06)</td>
<td>0.76 (0.52-1.10)</td>
<td>0.85 (0.66-1.09)</td>
</tr>
</tbody>
</table>

*n* indicates number of events.
The association of insulin resistance factor with the risk of CHD and stroke was further examined by calculating Kaplan-Meier survival curves for remaining free of CHD and stroke, respectively, during the 22-year follow-up period by tertiles of insulin resistance factor score (Figure 7). The proportion of men remaining free of CHD and stroke as well was significantly smaller in the highest insulin resistance factor tertile than in the 2 lower tertiles.

Figure 7. Kaplan-Meier survival curves for remaining free of CHD and stroke during 22-year follow-up by tertiles (T1 through T3) of insulin resistance factor score.

The single insulin resistance factor obtained from the factor analysis with only 6 variables predicted the risk of CHD and stroke over the 22-year follow-up period, adjusting for age, cholesterol, smoking, physical activity, and maximal O₂ uptake. The HRs for the 22-year follow-up were 1.48 (95% CI, 1.23-1.77) with regard to CHD risk and 2.02 (95% CI, 1.54-2.66) with regard to stroke risk.

Factor analyses were also performed in the subset of men who were strictly normoglycaemic according to the combined WHO and ADA criteria. The factors obtained were virtually similar to those obtained in the whole study population. For insulin resistance factor derived from factor analysis with 10 variables, the age-adjusted HRs over 22 years of follow-up were 1.24 (95% CI, 1.05-1.46) with regard to CHD risk and 1.75 (95% CI, 1.36-2.26) with regard to stroke risk.
6 DISCUSSION

6.1 Study population and methods

6.1.1 Study population

The Helsinki Policemen Study is a prospective study of an occupational cohort of men employed by the Police Department of the city of Helsinki or by the National Police Force units located in Helsinki. The initial examination of the Helsinki Policemen Study was carried out in 1966-1967. The participation rate in this examination was as high as 98.4%. The study cohort of the present study comprised 970 men aged 34-64 who were free of cardiovascular disease and diabetes at the time of the second examination of the Helsinki Policemen Study in 1971-1972, when plasma insulin determinations were included in the study protocol. In this second examination, the participation rate was also high, up to 98.5% of the surviving men.

This occupational cohort of policemen is not a representative sample of Finnish men of their age group. First, the cohort is primarily selected, because only men who are healthy and physically fit are accepted as policemen; a minimum height of 175 cm is another entry requirement, although shorter men may be accepted for special jobs. Second, during the course of the occupational career of policemen, a further selection occurs, because subjects who develop chronic disease are more likely to retire or change their job than men in many other occupations.

As a consequence of the entry criteria with regard to good health and physical fitness, at the time of their recruitment the policemen of the present study may have been leaner than men of the same age in the general population. Information on body weight at the age of 25 was available in a subgroup of the Helsinki Policemen Study population; in this subgroup, the median BMI at the age of 25 was as low as 22.5 kg/m², with an interquartile range from 21.1 to 23.8 kg/m² (456). In a longitudinal study on the weight development of Helsinki policemen, it was shown, however, that after starting their career in the Police Force, policemen relatively soon started to put on weight, the weight gain on average being actually quite marked, especially in the younger age groups (457).
The mean increase of body weight since the age of 25 until the age of 45-49 was as much as 14.8 kg. Comparison of Helsinki policemen with men of comparable age participating in the Mini-Finland Study, a large population study representing general Finnish population, conducted in 1978-1980, showed that the mean values for BMI in Helsinki policemen and those in the general Finnish male population in corresponding age groups were rather similar, but the prevalence of marked obesity (BMI ≥30 kg/m²) was much lower among Helsinki policemen (456).

Although all men participating in the baseline study in 1966-1967 lived and worked in Helsinki, only 8.4% of them were born there, while 46.9% of them were born in western provinces and 44.7% in eastern provinces of Finland. Thus, their genetic and early-life environmental background evidently did not differ much from that of the general Finnish population.

Because the selection of policemen favours good health at the entrance and thereafter, it may be presumed that the mortality rates in Helsinki policemen would be somewhat lower than in the general Finnish male population. To test this assumption, 10-year mortality data of Helsinki policemen and men (N=4302) aged 34-64 participating in 1971-1972 in the Mobile Clinic Study of the Social Insurance Institution (data provided by Dr Antti Reunanen), representing the general Finnish male population, were compared (Table 17). In the original Helsinki Policemen Study population, without exclusion of men with prevalent CVD or diabetes, the 10-year age-standardised all-cause, CVD and CHD mortality rates were 15-20% lower than in the Mobile Clinic Study population. In the present study cohort, formed using strict exclusion criteria for prevalent CVD and diabetes, the mortality rates were, as could be expected, much lower than in the original study population without these exclusions, particularly with regard to CVD mortality and its subcategories.

6.1.2 Risk factor measurements at baseline

Plasma insulin was a key risk factor with regard to the research questions of the present study. At the baseline examination in 1971-1972 plasma insulin concentrations were measured from venous blood samples drawn at 0, 1 and 2 hours during OGTT. It
Table 18. 10-year all-cause, cardiovascular disease (CVD), coronary heart disease (CHD) and stroke mortality per 100 person-years in men aged 34-64, examined in 1971-1972 in the Mobile Clinic Study of the Social Insurance Institution and in the Helsinki Policemen Study; age-standardisation according to the age structure of the Mobile Clinic Study male population

<table>
<thead>
<tr>
<th></th>
<th>Mobile Clinic Study (n=4303)</th>
<th>Helsinki Policemen Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All men (n=1228)</td>
<td>Men free of CVD and diabetes (n=970)</td>
</tr>
<tr>
<td>All-cause</td>
<td>12.7</td>
<td>10.8</td>
</tr>
<tr>
<td>CVD</td>
<td>7.5</td>
<td>6.2</td>
</tr>
<tr>
<td>CHD</td>
<td>5.8</td>
<td>4.4</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

n indicates number of subjects.

is known that there is a diurnal variation in glucose tolerance and insulin sensitivity (458,459) and, in addition, that the length of preceding fast, physical activity and smoking influence plasma insulin levels. Therefore, OGTT and blood sampling for biochemical measurements were carried out in the morning between 8 and 10 a.m. and in circumstances otherwise standardised as far as feasible in studying freely living subjects.

The so-called ‘coated charcoal’ radioimmunological assay (449) was used in plasma insulin determinations. Along with the development of new radioimmunological assays specific for intact insulin, it has become evident that radioimmunological assays of insulin used in the 1970’s have been to some extent cross-reactive with proinsulin and its split-products (460). However, because concentrations of intact or split proinsulin molecules comprise only about 10% of all insulin-like molecules in non-diabetic subjects (203,410,411), this non-specificity of insulin assay cannot have had any major effect on the results of this study.

AUC insulin, a composite variable reflecting the plasma insulin response to glucose challenge during OGTT, was used in this study to examine the associations between insulin and the endpoint events. The choice of AUC insulin was based on the finding
that, when analysed separately, fasting, 1-hour, and 2-hour plasma insulin were rather similarly associated with the incidence of main cardiovascular endpoints of this study, namely, cardiovascular mortality, major CHD events and stroke.

The protocol of the OGGT of this study differed from that currently used in one respect: the glucose dose administered was either 75 g or 90 g, according to the body surface area of the individual, instead of the standard dose of 75 g irrespective of body size, recommended by the WHO Expert Committee in 1980 (461). In the present study cohort of 970 men, 847 men (87.3%) received 75 g, and the remaining 123 (12.7%) received 90 g of glucose. Thus, the proportion of men who received 90 g of glucose was rather small. Moreover, when associations between AUC insulin and the risk of a major CHD event, the endpoint with the largest number, were analysed including and excluding men who received 90 g of glucose, the results were essentially similar. This reassured that the use of 2 different glucose doses in OGGT had no essential impact on the results of this study.

Subscapular skinfold thickness was used in this study as an index of upper-body obesity. The concept about the role of central obesity in insulin resistance and related metabolic abnormalities was already evolving at the time of the baseline examination of this study, but waist circumference and WHR as indices of central obesity had at that time not yet come into general use in epidemiological studies. Nevertheless, there is epidemiological evidence showing that an increase in truncal skinfolds, such as subscapular skinfold, is associated with metabolic disturbances and morbidity in a similar way as WHR and other indices of central obesity (31,57). Moreover, a recent study in which computed tomography was used to measure cross-sectional abdominal subcutaneous and visceral adipose tissue showed that subcutaneous abdominal fat is as strongly associated with insulin resistance as visceral fat (64).

HDL cholesterol measurement did not belong to the set of risk factors measured at the baseline examination of this study, because in the beginning of the 1970’s the measurement of this lipid variable had not yet been introduced into studies of cardiovascular epidemiology.
The study protocol of the Helsinki Policemen Study did not include a questionnaire on alcohol consumption, because questions on this issue were considered to intrude into a too sensitive area in the life of policemen.

6.1.3 Ascertainment of causes of death and non-fatal coronary heart disease and stroke endpoints

In Finland, the circumstances are in many ways favourable for prospective epidemiological research. Every citizen of Finland has a personal identification number which is used in the patient records of the Finnish health services system and in the data collected by the Statistics Finland, including the National Cause-of-Death Register, and by the National Hospital Discharge Register maintained by the National Research and Development Centre for Welfare and Health (formerly National Board of Health). Using the personal identification number it is possible to link data files of epidemiological studies with the data files of the National Cause-of-Death Register and the National Hospital Discharge Register, with a permission obtained from Statistics of Finland and Ministry of Social Affairs and Health.

The validity of the National Cause-of-Death Register with regard to CHD and stroke as causes of death has been investigated from the 1970’s until the 1990’s (462-467). Although the autopsy rate has declined in Finland over this period, the reliability of the Finnish mortality statistics with regard to CHD and cerebrovascular disease mortality has remained reasonably good.

The National Hospital Discharge Register has been found to cover about 95% of all hospitalisations in the 1970’s and in the beginning of the 1980’s (468,469). A common reason for a non-retrieval of hospitalisations of individual subjects has been an error in the personal identification number, but from 1986 onwards this error rate has been less than 3% due to the introduction of computerised registration systems, including an automatic checking of the correctness of the personal identification number in Finnish hospitals. The transcription of diagnoses from the patient records to the National Hospital Discharge Register has been found to be correct in 98% of the MI diagnoses and in 95% of stroke diagnoses made in hospitals (470).
The validity of the National Hospital Discharge Register diagnoses for MI and stroke has been examined by the FINMONICA (FINnish contribution to the MONICA project) investigators. During the period 1983-1990 the FINMONICA criteria for definite or possible MI were fulfilled in 90% of hospital discharge diagnoses of MI (471), and during 1983-1986 the WHO criteria for stroke, including subarachnoid haemorrhage, were fulfilled in 85% of hospital discharge diagnoses of stroke (472). The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study has used in their data analyses the diagnoses of MI and stroke obtained from the National Hospital Discharge Register, and has validated these diagnoses in subsamples of their study population (473-475). Of the hospital discharge diagnoses of MI 94% fulfilled the FINMONICA criteria for definite or possible MI (473) and of the hospital discharge diagnoses of stroke, including subarachnoid haemorrhage, 90% fulfilled the WHO criteria for stroke (474).

In this study, a complete follow-up with regard to the vital status of the study cohort was achieved on the basis of the data obtained from the National Cause-of-Death register. For all deceased men the underlying cause of death could be ascertained on the basis of death certificates, supplemented in a small number of cases with additional information available from hospital records or autopsy reports.

For the ascertainment of hospital-verified MIs and strokes a list of all hospitalisations of the study participants during the follow-up period was obtained from the National Hospital Discharge Register. The diagnosis of MI and stroke was based on a review of the patient records of relevant hospitalisations by the author using predefined diagnostic criteria, as described in Chapter 4.3, and thus the ascertainment of these study endpoints was uniform throughout the follow-up period.

6.2 Insulin and coronary heart disease (Study I)

In this 22-year follow-up study of Helsinki policemen hyperinsulinaemia, defined as the highest quintile of AUC insulin, predicted the risk of major CHD events, and to a large extent independently of other risk factors, although its predictive value diminished with lengthening follow-up time.
During the first 10 years of follow-up, the association of insulin with the risk of CHD was non-linear; the excess risk of CHD was clearly confined to the top quintile of AUC insulin. During the follow-up, however, the association between insulin and CHD risk became more linear, extending over the whole distribution of AUC insulin. As a continuous variable, AUC insulin remained an independent predictor of CHD during the entire 22-year follow-up period. Earlier reports of the Helsinki Policemen Study, based on 5- and 9.5-follow-up periods, already paid attention to the non-linear nature of the association between insulin and CHD risk (5,19). Similarly, in the Paris Prospective Study until its 15-year follow-up (7,21,22) and in the 11.5-follow-up of the British Regional Heart Study (321), the excess risk of CHD was concentrated largely to the upper end of insulin distribution, to the highest quintile or decile. A possible explanation for the finding of the present study, that the association between insulin and CHD risk changed from non-linear to more linear with lengthening follow-up time, could be that different effects of insulin or insulin resistance operate in early and later observation periods. As pointed out in Chapter 2.3.1, insulin and insulin resistance have links with thrombogenic factors and risk factors promoting atherogenesis. If the association of hyperinsulinaemia with thrombogenic factors were strong, the impact of these factors would lead to early occurrence of major CHD events in hyperinsulinaemic men during the follow-up.

One interesting finding of this study was that over the 22-year follow-up, insulin was more strongly associated with the risk of CHD death than with the risk of non-fatal MI. Other studies have not assessed the association between insulin and CHD risk separately for different CHD endpoints according to their severity. It is possible that insulin may associate less strongly with ‘softer’ CHD endpoints. Already the first report of the Helsinki Policemen Study based on 5 years of follow-up demonstrated that, despite the strong association between hyperinsulinaemia and ‘hard criteria’ CHD (CHD death or non-fatal MI), there was no association between insulin and ‘other CHD’ (new angina pectoris, severe attack of chest pain without confirmation of MI, and unexplained heart failure). Some other studies on the association between insulin and CVD risk have included among their CHD endpoints also ‘softer’ CHD manifestations, such as occurrence of new angina pectoris or development of ischaemic ECG changes
(88,381,384,387). This may have led to a dilution of the association between insulin and CHD risk.

As reviewed in Chapter 2.5.1, 28 prospective epidemiological studies have been published on the association of insulin with the risk of CHD or CVD. In 14 studies of male populations (5-7,321,373,385,387,394-397) or combined male and female populations (386,393,398), a positive association was found between insulin and the risk of CHD or CVD and in 10 of them (5-7,321,373,386,387,396-398), this positive association remained significant after adjustment for other risk factors. In one study, the Kuopio Ischemic Heart Disease Study (394), insulin lost its predictive value with regard to CHD or CVD risk when risk factors known to be correlated with insulin (BMI and waist circumference, blood pressure, triglycerides, and HDL cholesterol) were included in multivariate analyses. In the Caerphilly Study (385) a complete multivariate analysis including all baseline risk factor was not performed, but the positive association between insulin and CHD risk was found to become non-significant after successive adjustment for BMI and triglycerides. In the Barilla Factory Study (393) a significant positive association between insulin and CHD risk was found adjusting for demographic factors and BMI, but, because of problems in the interpretation of multivariate analyses including highly intercorrelated variables, the authors chose not to adjust further for other risk factors, such as blood pressure, triglycerides and HDL cholesterol.

In the present study, as expected, plasma insulin levels at baseline were correlated with several of the baseline risk factor variables included in multivariate models, namely, BMI, subscapular skinfold, systolic blood pressure, triglycerides, blood glucose, physical activity, and maximal \( O_2 \) uptake. With the exception of BMI and physical activity, these variables were also individually associated with the risk of CHD and CVD, at least in univariate analyses. However, when the confounding effect of other baseline risk factors on the association between plasma insulin and CHD or CVD risk was examined by entering them separately into the prediction models, in addition to AUC insulin and age, AUC glucose was the only variable reducing the strength of the association to a substantial degree, the other risk factors having virtually no effect.

Because the baseline study protocol of the Helsinki Policemen Study did not include HDL cholesterol measurement, the question arises, whether the lack of this lipid risk
factor belonging to the IRS would have affected the outcome of multivariate analyses. There is, however, a strong physiological link between plasma triglyceride and HDL cholesterol levels, reflected in their strong inverse correlation, and thus inclusion of triglycerides in multivariate analyses of the present study has at least in part reflected the lipid abnormality characteristic of the IRS. In this context it is important to note, that 9 studies showing a positive association between insulin and CHD or CVD risk included HDL cholesterol in multivariate models (321,373,386,387,390,394,395, 397,398), and in 6 of them, the association remained significant (321,373,386,387, 397,398).

It is noteworthy, that all the 14 studies showing a positive association between insulin and CHD or CVD risk in men or in combined male and female populations were conducted in white populations with high or relatively high rates of CVD. Three of these studies, the Helsinki Policemen Study, the Kuopio Study on Elderly (373) and the Kuopio Ischemic Heart Disease Study (394), were carried out in Finns known of their particularly high CVD rate. This is compatible with the hypothesis that the impact of insulin and underlying insulin resistance on the risk of clinical manifestations of atherosclerotic vascular disease becomes best evident in such populations in which atherosclerosis is prevalent due to adverse levels of major cardiovascular risk factors.

6.3 Insulin and stroke (Study II)

High insulin levels were associated with the risk of stroke in Helsinki policemen during the 22-year follow-up, but this association was not independent of other cardiovascular risk factors.

Of all the 70 strokes occurring in the study cohort during the 22-year follow-up period, 79% were thromboembolic, 10% were haemorrhagic, and 11% remained non-classifiable, in part because computed tomography and magnetic resonance imaging were not available during the early phases of follow-up. However, when statistical analyses were carried out excluding haemorrhagic strokes, the association between hyperinsulinaemia and incident stroke remained unchanged, indicating that the excess of strokes in hyperinsulinaemic subjects observed in this study was mainly due to
thromboembolic stroke events.

It is well known that the peak occurrence of cerebrovascular disease becomes later in life than that of coronary heart disease. In the present study, the median age at the first stroke event was 68 (range 48-82 years) and the median age at the first CHD event was 62 (range 42-82 years). Thus, the number of stroke events occurring in this middle-aged healthy male population remained small even during the 22 years of follow-up, limiting the power of the study.

In addition to the present study, 6 prospective epidemiological studies have shown an association between high insulin levels and the risk of stroke (394,416-420). The outcome of multivariate analyses including the most important cardiovascular risk factors has been reported from 3 of these studies (394,418,419), in addition to the present study. In the ARIC Study, the positive association between insulin and stroke risk remained significant after adjustment for other risk factors (418). In the British Regional Heart Study showing a J-shaped association between insulin and stroke risk, the association of high insulin levels with the risk was reduced to marginally significant in multivariate analyses and this was due to the inclusion of systolic blood pressure in the model (419). In the Kuopio Ischaemic Heart Disease Study, the positive association between insulin and stroke risk was only slightly reduced in multivariate analyses and this was caused by blood pressure and obesity indices, BMI and waist circumference (394).

In the present study, obesity indices, BMI and subscapular skinfold, were the only variables having a substantial effect on the association between hyperinsulinaemia and the risk of stroke, reducing it to statistically non-significant level. Subscapular skinfold, indicating upper-body obesity, was also an independent predictor of stroke risk in this study population, in addition to blood pressure and smoking. BMI was independently associated with the stroke risk only, when subscapular skinfold was omitted from the multivariate model. The role of elevated blood pressure and smoking as risk factors of stroke has been documented in many prospective studies, including also Finnish population-based studies (394,476-478), but research evidence on the role of obesity and its distribution as predictors of stroke risk is less uniform. In 2 Finnish population-based studies BMI was not an independent predictor of stroke risk in men (394,476) and the
same applied to waist circumference in one of these studies (394). Prospective studies conducted in other countries have, however, given support to the view that obesity, and particularly abdominal or upper-body obesity, is associated with the risk of stroke (31,479-484).

Thus, the results of the present study suggest that obesity, and abdominal obesity in particular, contribute to the association of insulin and the risk of stroke. The mechanisms for this are unclear, but in this study population, the impact of obesity on the risk of stroke could not be explained through its known effect on blood pressure, because blood pressure and obesity were both independent predictors of stroke in multivariate analyses. On the other hand, in addition to being strongly correlated with each other, both insulin and obesity are associated with impaired fibrinolysis, reflected in elevated PAI-1 levels, and with other factors involved in thrombogenesis. Thus, it is possible that the association of both hyperinsulinaemia and obesity with the risk of stroke could be, at least in part, mediated through factors promoting thrombus formation.

6.4 Insulin and all-cause, cardiovascular and non-cardiovascular mortality (Study III)

The present study based on the 22-year mortality data of Helsinki policemen demonstrated that hyperinsulinaemia was associated with an increase in all-cause and cardiovascular mortality independently of other risk factors, but these associations weakened with the lengthening of follow-up time. A U-shaped association was observed between insulin and non-cardiovascular mortality.

Information from other prospective epidemiological studies on the association of insulin with all-cause mortality is scarce and conflicting. In accordance with our findings, the risk of death was significantly increased in hyperinsulinaemic men in the 23-year follow-up of the Paris Prospective Study (23) and in the 19-year follow-up of the Malmö Preventive Project (396). In these 2 studies, however, there was an excess of deaths also at the low end of the plasma insulin distribution; the association between
insulin and all-cause mortality was U-shaped in the Paris Prospective Study and J-shaped in the Malmö Preventive Project. In the 13-year follow-up of the Busselton Study (421), insulin was inversely associated with the risk of death in younger men aged 40-59, but the association was positive in older men aged 60-74. In the 5-year mortality follow-up study of the Southern Finland Elderly Study (391), there was a trend towards a positive association in the youngest age cohort of 65 years, whereas in the oldest age cohort of 85 years, an inverse association was demonstrated.

In the present study, the association of hyperinsulinaemia with all-cause mortality was to a large extent explained by an increase in cardiovascular deaths, and among them, coronary deaths in particular, in men with high insulin levels. An increase in cardiovascular and coronary mortality in the upper end of insulin distribution was also demonstrated in the 23-year follow-up of the Paris Prospective Study, although the death rates for cardiovascular and coronary mortality in the Paris Prospective Study were much lower than in the Helsinki Policemen Study. Moreover, in the Paris Prospective Study, there was an excess of cardiovascular deaths, but not of coronary deaths, also in men with low insulin levels. In our study, no curvilinearity in the relationship of insulin with cardiovascular mortality could be demonstrated.

The U-shaped association between insulin and non-cardiovascular mortality observed in the present study resulted from an excess of violent deaths and other non-cardiovascular deaths except cancer deaths in men with lowest insulin levels, and from a less marked excess of deaths from cancer and violence in men with highest insulin levels. There is no obvious explanation for the excess of violent deaths and other non-cardiovascular deaths excluding cancer in men with low insulin. Chronic illnesses are often accompanied with weight loss, and weight loss, in turn, is associated with lowering of insulin levels. The subjects of the present study were, however, healthy at baseline, and therefore it is unlikely, that underlying chronic illnesses would have contributed to the association of low insulin levels with increased non-cardiovascular mortality. Chronic smoking has been associated with lower insulin in several epidemiological studies (67,282,283,321), and in accordance with these findings, in the Helsinki Policemen Study population 2-hour insulin was significantly lower in smokers than non-smokers. However, no marked increase in deaths that could be attributed to the
long-term effects of smoking, such as chronic pulmonary disease or lung cancer, was observed in men with lowest insulin levels. Regular use of alcohol has been associated with low insulin concentrations (69,304,307) and enhanced insulin sensitivity (305). Therefore, the possibility was considered that the increased non-cardiovascular mortality in men with low insulin levels could at least in part be due to excessive use of alcohol. Unfortunately, the baseline study programme of this study did not include a questionnaire on alcohol consumption. However, by identifying possibly alcohol-related deaths on the basis of information available in death certificates, it was shown that the proportion of possibly alcohol-related deaths was almost 2-fold in men in the lowest AUC insulin quintile as compared to those in the higher quintiles. The approach used in the present study probably underestimated the contribution of alcohol to non-cardiovascular mortality, but the findings support the view that excessive alcohol use could be one of the factors explaining the increase in non-cardiovascular mortality in men with low insulin levels.

Only 3 prospective studies have previously examined the association between plasma insulin levels and the risk of cancer (421,422,485). In the 13-year follow-up of the Busselton Study (421) a significant positive association between insulin and cancer mortality was observed in older but not in younger men. In the Paris Prospective Study during the 23.8 years of follow-up hyperinsulinaemia predicted fatal liver cancer, but plasma insulin was inversely associated with fatal lip, oral cavity, and pharynx cancers, stomach cancer, and larynx cancer (422). In the Cardiovascular Health Study cohort (485), comprising both men and women, both fasting and post-glucose insulin levels showed a positive association with incident colorectal cancer over a median follow-up of 6.4 years, but also glucose levels and waist circumference were positively associated with this type of cancer. In the present study, the number of cancer deaths was too small to allow further analyses of the relationship of insulin with mortality from different types of cancer.
6.5 Factor analysis of insulin resistance syndrome for the prediction of the risk of coronary heart disease and stroke (Study IV)

As discussed above, the results of conventional multivariate analyses of the 22-year follow-up data from the Helsinki Policemen Study concerning the association of hyperinsulinaemia with the risk of CHD and stroke were different: hyperinsulinaemia predicted the risk of CHD independently of other risk factors, whereas the association between hyperinsulinaemia and the risk of stroke became non-significant after adjustment for other risk factors, particularly indices of obesity. Close physiological links of hyperinsulinaemia and underlying insulin resistance with several other cardiovascular risk factors make the interpretation of conventional multivariate analyses complex because of problems related to overadjustment and collinearity. Thus, it was not possible to decide with certainty whether the difference in our results with regard to the role of hyperinsulinaemia as a predictor of CHD and stroke was real or only apparent.

Factor analysis provides a method for investigating intercorrelated variables and reducing them to a smaller number of uncorrelated composite variables. Therefore, factor analysis was applied to investigate the clustering of cardiovascular risk factors, particularly those proposed to belong to the IRS, in the baseline data of the Helsinki Policemen Study and to examine, whether these clusterings predicted the risk of CHD and stroke during the 22-year follow-up.

As reviewed in Chapter 2.4, the findings of other studies in non-diabetic subjects applying factor analysis to examine risk factor clustering in the IRS have been in one respect remarkably uniform: the principal factor explaining the largest amount of variance between variables has almost invariably been characterised by loadings for an index of overall or central obesity and insulin. Of the other putative components of the IRS, a glucose variable or lipid variables, triglycerides and HDL cholesterol, have loaded on the principal factor in more than half of these studies, but with regard to blood pressure the findings have been less uniform.

In the present study, factor analyses were performed with different sets of baseline risk factor variables, first by including 10 potential risk factors for CHD and stroke in
the analysis, and then reducing the number of included variables stepwise into the set of 6 putative components of the IRS (BMI, subscapular skinfold, AUC insulin, AUC glucose, mean blood pressure, triglycerides). Regardless of the number of variables included in factor analysis, all the putative components of the IRS loaded on the principal factor, indices of obesity and insulin showing strongest correlations, glucose a relatively strong correlation, and blood pressure and triglycerides somewhat weaker correlations with the principal factor. It is noteworthy that maximal O₂ uptake which was strongly and inversely correlated with AUC insulin in this study population, also loaded with a strong inverse correlation on the principal factor instead of loading on the same factor with physical activity. This finding accords with studies demonstrating a strong relation between maximal O₂ uptake and insulin sensitivity measured by the euglycaemic clamp or the minimal model method (81,486). Interestingly, exclusion or inclusion of maximal O₂ uptake in factor analysis had no substantial impact on the loadings of other variables on the principal factor.

With the full set of 10 risk factor variables, in addition to the principal factor, named insulin resistance factor, 2 subsidiary factors were obtained: lipid factor, with strong loadings for cholesterol and triglycerides, and lifestyle factor, with a strong positive loading for leisure time physical activity and a strong inverse loading for smoking. When only the 6 components of the IRS mentioned above were included, they all clustered into a single factor.

In contrast to our findings, other studies including only variables proposed as components of the IRS in factor analyses have found 2 to 4 factors (68,72,77-79,129,210,369,370,372,374,375,377,378), and those studies including a wider set of risk factor variables 3 to 7 factors (80,371,373,376). However, one of the limitations of factor analysis is its sensitivity to the variables included. Especially, inclusion of variables which are very closely intercorrelated or measure the same characteristic in different ways may have influence on the number of factors produced. Selection of insulin and glucose variables is an example of such influence. A separate insulin/glucose factor or a glucose factor was produced in 5 (68,78,80,210,377) of the 6 previous studies including both fasting and post-load insulin and glucose variables (68,78,80,210,371,377) but in only 2 (79,369) of the 12 studies applying single insulin
and glucose variables (72,77,79,129,369,370,372-376,378). In the present study, a
decision was made to use composite variables (AUC insulin and AUC glucose) to
reflect plasma insulin and blood glucose levels, and these variables loaded on the
principal factor. However, also in the present study, a separate insulin/glucose factor
emerged when both fasting and post-load insulin and glucose variables were entered
into factor analyses. Similar problems apply to the selection of blood pressure variables.
With one exception (369), all the previous studies which applied 2 blood pressure
variables (systolic blood pressure and diastolic blood pressure or left ventricular
hypertrophy) (68,72,77,79,80,210,372-375,377,378) yielded a separate blood pressure
factor. Likewise, a subsidiary blood pressure factor was produced in the present study, if
both systolic and diastolic blood pressure were included in factor analysis. In contrast,
the use of a single blood pressure variable, mean blood pressure, in the main factor
analyses of the present study resulted in the loading of this variable with insulin and
other metabolic components of the IRS on the principal factor. Five other studies have
also used a single blood pressure variable (systolic blood pressure (78,370,376), mean
blood pressure (371) or hypertension (129)) in their factor analyses, and in 3 them,
blood pressure loaded on the principal factor (129,371,376).

In the present study triglycerides, in addition to loading with total cholesterol on a
separate lipid factor, showed also a moderate loading on the principal factor, and the
strength of this loading increased when cholesterol, known to be strongly correlated
with triglycerides, was excluded from the analyses. The same phenomenon was
observed in another study performing factor analyses with different sets of variables
(371). The baseline study protocol of the present study did not include HDL cholesterol
measurement, and therefore the possible contribution of HDL cholesterol to the IRS in
this study population remains unknown. In many of the previous studies, however, HDL
cholesterol clustered with triglycerides on a subsidiary lipid factor (72,78-
80,370,371,373,376,378), even when either one or both of them loaded also on the
principal factor (371,373,376).

The identification of several underlying factors in previous factor analyses of the IRS
has been interpreted to mean that the etiology of the IRS may be heterogenous: that is,
insulin resistance, reflected by hyperinsulinaemia, alone may not underlie all features of
the syndrome (68,78,487). However, as pointed out above, the unifying feature of the results of all factor analyses of the IRS, including ours, is the characterisation of the principal factor by obesity, its central distribution and hyperinsulinaemia, implying that these characteristics belong to the core of the syndrome. The finding of the present study that factor analysis restricted to the proposed components of the IRS yielded only a single factor would even be compatible with the possibility that there would be a single underlying cause for this cluster of risk factors. This underlying cause could be insulin resistance itself.

When scores for the factors were entered into Cox models, adjusting for age, the insulin resistance factor, obtained from factor analysis including 10 potential risk factor variables for CHD and stroke or from factor analysis including only 6 core risk factor variables of the IRS, was a statistically significant predictor of both CHD and stroke. With regard to CHD risk, the findings of the present study are in accordance with those from 2 Finnish studies of non-diabetic subjects, the Kuopio Study on Elderly (373) and the Kuopio Ischaemic Heart Disease Study (376). In both studies, an insulin resistance factor, characterised by all the components of the IRS, was identified by factor analysis and this factor predicted the risk of CHD in men. Furthermore, in the Kuopio Ischaemic Heart Disease Study, insulin resistance factor was also a significant predictor of cardiovascular and all-cause mortality. Interestingly, an insulin resistance factor was also identified by factor analysis in men of the diabetic subpopulation of the Kuopio Study on Elderly (380) and, likewise, in a population of diabetic men and women of another Finnish study (379), and in both of these study populations insulin resistance factor was a predictor of CHD risk.

It was of interest that insulin resistance factor was even a somewhat stronger predictor of stroke than of CHD. In conventional Cox model analyses, adjusting only for age, insulin also predicted CHD and stroke rather similarly, but with adjustment for other risk factors, insulin lost its predictive value for stroke. In these multivariate analyses, obesity and its central distribution were strong independent predictors of the risk of stroke but not of CHD. Strong loading of obesity indices on insulin resistance factor may explain why this factor was strongly related to the risk of stroke.
6.6 Is the association of hyperinsulinaemia with incident coronary heart disease and stroke linked with the development of diabetes?

It is well known that insulin resistance is an antecedent of type 2 diabetes and that, during the long period of time before clinical manifestation of diabetes, other atherogenic risk factors which cluster with hyperinsulinaemia are already present. Therefore, it has been proposed that the association between hyperinsulinaemia with incident atherosclerotic vascular disease might be confined to those individuals who later develop diabetes (321).

In the present study, it was possible to identify those men who during the 22-year follow-up developed diabetes requiring drug treatment, but not those who developed milder forms of diabetes. Although these data underestimate the true incidence of new diabetes in this study population, there was an approximately 2.5-fold excess of drug-treated diabetes in hyperinsulinaemic men in the highest AUC insulin quintile as compared to those with lower insulin levels. However, men having a CHD event during the follow-up did not develop diabetes more often than those not having a CHD event, and this also applied to men with hyperinsulinaemia. Furthermore, in age- and multiple-adjusted analyses, excluding men who developed drug-treated diabetes, the associations of hyperinsulinaemia with CHD risk were even slightly strengthened. As to stroke events, diabetes developed approximately 2 times more frequently in men who had a stroke than in those who did not during the follow-up, and this excess appeared to be mainly confined to men with highest insulin levels. However, in age- and multiple-adjusted analyses excluding subjects who later developed drug-treated diabetes, the associations of hyperinsulinaemia with stroke risk were only marginally reduced.

It is known that subjects with milder abnormalities of glucose tolerance carry a high risk of developing diabetes. Our study population included 79 men fulfilling the WHO criteria for impaired glucose tolerance and/or the ADA criteria for impaired fasting glucose. An important finding was that, after exclusion of these men, the age- and multiple-adjusted associations of hyperinsulinaemia with both CHD and stroke risk became even somewhat stronger than in the whole study population, and the association of hyperinsulinaemia with the risk of stroke remained significant after adjustment for
other risk factors. Also, in this subpopulation of strictly normoglycaemic men, the predictive power of insulin resistance factor obtained in factor analysis with regard to the risk of both CHD and stroke remained essentially unaltered.

Based on these findings, it appears unlikely that the development of diabetes would explain the association observed in the present study between hyperinsulinaemia and the risk of CHD and stroke.

6.7 Why the association of hyperinsulinaemia with coronary heart disease and cardiovascular disease risk decreases with lengthening follow-up time?

This study showed that the association of hyperinsulinaemia with CHD and CVD risk decreased with lengthening follow-up time. Two other prospective studies have systematically assessed the association of hyperinsulinaemia with CHD risk during different periods of follow-up (396,488). In accordance with the findings of the present study, in the 15-year follow-up of the British Regional Heart Study, the predictive value of hyperinsulinaemia, defined as the highest decile of non-fasting insulin, became attenuated after 10 years of follow-up (488). However, in the 19-year follow-up of the Malmö Preventive Project, the predictive value of hyperinsulinaemia, defined by highest deciles of fasting and 2-hour insulin, remained almost unchanged over the whole follow-up period (396).

The present study, like all the other prospective studies examining the association of plasma insulin with the risk of CHD and other forms of CVD, is based on single measurements of plasma insulin levels at baseline. Single measurements of all biological risk factors are, in addition to measurement error, subject to intra-individual variation, and this also applies to plasma insulin levels. The measurement error can be kept at a reasonable minimum by careful standardisation of the laboratory methods. The biological variation in plasma insulin levels includes short-term variations, such as diurnal variation and the effects of different incidental influences (e.g., preceding physical activity, smoking etc.), and long-term variations due to changes in diet, body weight, level of physical activity etc. Short-term intra-individual variation of fasting and
2-hour plasma insulin levels during 75 g OGTT was examined in the Hoorn Study (489), based on 2 OGTTs performed at the interval of 2 to 6 weeks. The intra-individual variation of plasma insulin levels was found to be relatively great, and for 2-hour insulin clearly greater than for fasting insulin. For these reasons, single measurements of plasma insulin levels do not necessarily classify individuals accurately with regard to their 'usual' plasma insulin levels.

Because of fluctuations in the measured values of risk factors, prospective studies based on single risk factor measurements tend to underestimate the real association between the 'usual' level of some risk factor and the disease rate during the follow-up, particularly during long periods of follow-up time (490-492). This phenomenon is called 'regression dilution' effect. Clarke et al (492) have shown, applying appropriate 'regression dilution' corrections, based on repeated blood pressure and cholesterol measurements of the Framingham Study, to the 26-year follow-up data of the Whitehall Study of middle-aged men that the predictive value of systolic and diastolic blood pressure and cholesterol remained almost unchanged over this long follow-up time and was almost similar in all age groups, whereas uncorrected associations diminished with the lengthening of follow-up time and were weaker in older age groups than in younger age groups. Analogously to these observations, it appears likely that the diminishing predictive value of plasma insulin with regard to CHD with lengthening follow-up time could mainly be due to 'regression dilution' phenomenon and that the impact of plasma insulin as a long-term predictor of CHD risk may, in fact, have become underestimated.

Another possible explanation for the decrease in the predictive value of plasma insulin with lengthening follow-up time in the present study could be a selective CHD mortality and morbidity among hyperinsulinaemic men during the first half of the follow-up period. The prerequisite for this explanation, however, is that hyperinsulinaemia should have particularly strong links with mechanisms leading to serious CHD events, CHD death and non-fatal MI. One such link could be the association of hyperinsulinaemia with thrombogenic factors, as reviewed in Chapter 2.3.1.

During the 22 years of follow-up of the Helsinki Policemen Study extending from the beginning of the 1970’s until the middle of the 1990’s, substantial changes in the
population mean levels of the 3 major cardiovascular risk factors have occurred in the Finnish population (493). Serum cholesterol and blood pressure levels, as well as the prevalence of smoking among men, have declined. Concomitantly, mortality from CHD and stroke has declined by about 60% both among men and women (494,495). The incidence of CHD and stroke has also declined, although less markedly than mortality (496,497). These favourable trends in CVD mortality and morbidity are mainly explained by changes in the major cardiovascular risk factors in the population (494,495). During the same time period the prevalence of obesity has, however, increased in the Finnish population, particularly among men (498). It is probable that the development of cardiovascular risk factors and CVD outcome among Helsinki policemen has followed national trends. Declining trends in cholesterol, blood pressure and smoking during the follow-up may have diminished the predictive value of the baseline levels of these risk factors and thereby strengthened the impact of insulin in the prediction of CVD risk. On the other hand, the increase in the prevalence of obesity during the follow-up can be presumed to have led to an increase in plasma insulin levels in a substantial proportion of the study population. Thus, among those gaining weight, the baseline plasma insulin levels may have been lower than the true insulin levels during the follow-up and this may have led to a reduction in the predictive value of insulin.

6.8 Possible explanations for the association of hyperinsulinaemia and insulin resistance with the risk of cardiovascular disease

Two main explanations have been proposed for the association between hyperinsulinaemia and the risk of CVD: 1) a direct effect of insulin on the development of atherosclerotic lesions in the arterial wall and 2) an effect mediated through a clustering of several other risk factors with hyperinsulinaemia and insulin resistance.

There is evidence from animal experiments and studies on cell biology suggesting that insulin may promote atherogenesis (17). In vitro studies have demonstrated that insulin stimulates smooth muscle cell proliferation (180), as well as LDL binding to
fibroblasts (499), smooth muscle cells (500) and monocytes (501), and also stimulates cholesterol synthesis in monocytes (502). Experiments in dogs have shown that insulin may stimulate the synthesis of glycosaminoglycans in the arterial wall (503). The results of studies of the effect of exogenous hyperinsulinaemia in animals have been conflicting. One study in chickens demonstrated more extensive lipid containing lesions in the aortas of insulin-treated chickens than in controls (504), and another study in rats found an increased accumulation of triglycerides and a greater thickening of the intima in the aortas of insulin-treated animals as compared with the controls (505). In contrast, a study in cholesterol-fed rabbits found no significant differences in the extent or severity of atherosclerosis between insulin-treated and placebo-treated rabbits (506).

As reviewed in Chapter 2.3.1, insulin has a number of different effects on vascular function some of which might have a role in the development of atherosclerosis and its complications. On the other hand, as reviewed in Chapter 2.6, many cross-sectional studies have demonstrated an association between hyperinsulinaemia and the presence and extent of atherosclerotic lesions in carotid and coronary arteries, independently of other risk factors. However, the findings of these studies cannot be taken as an evidence for the direct effect of insulin on atherosclerosis, because they could equally well be due to the underlying insulin resistance.

Two clinical trials in diabetic subjects are of some relevance with regard to the effect of hyperinsulinaemia induced by insulin treatment on the risk of CVD in human. In the University Group Diabetes Program subjects with type 2 diabetes were randomly allocated to 3 treatment groups, including 2 insulin treatment groups (insulin standard and insulin variable) and a placebo treatment group (507). During 13 years of follow-up, there were substantial differences in blood glucose levels between the 3 groups, but only minor and non-significant differences in the incidence of CVD and all-cause and cardiovascular mortality. In the United Kingdom Prospective Diabetes Study subjects with newly diagnosed type 2 diabetes were randomly assigned to intensive treatment with insulin or with sulphonylureas or conventional treatment with diet (508). As compared with subjects treated with diet alone, a similar non-significant reduction in CVD incidence and all-cause mortality was observed in subjects treated with insulin and in subjects treated with sulphonylureas over 10 years of follow-up. Thus, these 2 trials
have provided no evidence that hyperinsulinaemia resulting from insulin treatment would be associated with an increased risk of atherosclerotic CVD.

As reviewed in Chapter 2.5.1 (Table 3) and Chapter 6.2, in the majority of those prospective studies which primarily showed a positive association between insulin and CHD or CVD risk, this association remained statistically significant although became attenuated with adjustment for other risk factors. Similarly, the meta-analysis by Ruige et al based on 11 prospective studies demonstrated a statistically significant and independent but weak positive association between insulin and CVD risk (408). Thus, on the basis of these results, the possibility that insulin might have a direct effect on the development of atherosclerosis cannot be excluded.

The view that the association between hyperinsulinaemia and CVD risk is mediated through the effects of risk factors clustering with insulin resistance – obesity and its central distribution, dyslipidaemia, elevated blood pressure, and impaired glucose regulation – is based on the well known physiological links of hyperinsulinaemia and insulin resistance with these risk factors and on the information available on their effects on the development of atherosclerosis and the risk of CVD. Dyslipidaemia associated with insulin resistance, characterised by elevated triglycerides and low HDL cholesterol, enhances atherogenesis in several ways. Elevated triglyceride levels are a reflection of abnormal metabolism of triglyceride-rich lipoproteins resulting in increased concentrations of small VLDLs and intermediate-density lipoproteins which can enter the arterial wall (509). Hypertriglyceridaemia is also associated with increased formation of particularly atherogenic small dense LDL particles. On the other hand, the metabolism of triglyceride-rich lipoproteins is closely linked with the metabolism of HDLs known to have a protective effect against atherosclerosis by at least 2 different mechanisms, participation in reverse cholesterol transport and direct protective effects in the arterial wall (510). Although elevated blood pressure is less strongly associated with insulin resistance than other components of the IRS, its importance as a CVD risk factor is indisputable. The association of mildly elevated blood glucose levels with CVD risk may be explained by accompanying dyslipidaemia and elevated blood pressure, because so far it is not known whether such elevations of blood glucose would have any direct effect on atherogenesis. Obesity and its central distribution are strongly linked
with insulin resistance, but their impact on CVD risk is evidently mainly mediated through dyslipidaemia and elevated blood pressure.

The attenuation of the positive association between insulin and CHD or CVD risk with adjustment for other risk factors in the present study, as well as in all other studies primarily demonstrating this association, and the loss of its statistical significance in some studies, is compatible with the interpretation that the association of insulin with the risk would be mediated through the effect of other risk factors. With regard to those studies in which the statistically significant association still persisted after adjustment for other risk factors, it can be claimed that this presents residual confounding by some unmeasured risk factors which are closely linked with insulin resistance and strongly associated with CVD risk, e.g., thrombogenic factors or inflammation markers.

Complex interrelationships between the risk factors proposed to belong to the IRS have led to the use of factor analysis for the examination of its nature. In the present study, the principal factor produced in factor analyses included all the putative components of the IRS (BMI, subscapular skinfold, AUC insulin, AUC glucose, mean blood pressure, and triglycerides) and this ‘insulin resistance factor’ predicted both the risk of CHD and stroke. This finding evidently reflects the combined effect of the risk factors forming this cluster and suggests that this combined effect is similar with regard to the 2 different manifestations of atherosclerotic disease, CHD and stroke.

A third alternative explanation for the association between hyperinsulinaemia and insulin resistance and CVD risk is that insulin resistance itself or some unidentified factor leading to insulin resistance and hyperinsulinaemia may directly enhance the development of atherosclerosis. As reviewed in Chapter 2.6, there is evidence from cross-sectional studies showing that insulin resistance measured by direct methods is associated with ultrasonographically thickening of arterial walls, angiographically documented coronary atherosclerosis, or a composite score for systemic atherosclerosis. In some of these studies, the association between insulin resistance and atherosclerosis has been, in part, independent of risk factors belonging to the IRS. So far, only 2 prospective studies have assessed the association between directly measured insulin sensitivity and CVD risk (442,443). In one of them (442) the small study population comprised healthy non-obese volunteers and in the other (443) a cohort of men aged 70
was examined. In both of these studies, low insulin sensitivity was found to be associated with increased CVD events. The small study of healthy volunteers was not powered to allow adjustment for other risk factors. In the study of 70-year-old men, low insulin sensitivity was an independent predictor of CVD events after adjustment for conventional risk factors, but lost its predictive value when adjusted for plasma proinsulin.

6.9 Implications for further research and practice of cardiovascular disease prevention

Taken as a whole, epidemiological studies, supported by basic and clinical research, strongly suggest that hyperinsulinaemia and the underlying insulin resistance participate in the causal chain leading to the development of atherosclerosis and clinical complications of atherosclerotic vascular disease. Because of the complex mechanisms involved, the possibilities for further elucidation of causality issues by epidemiological studies are, however, limited. Since it appears likely that the association between hyperinsulinaemia and CVD risk reflects the association between the underlying insulin resistance and CVD, the logical next step would be to conduct prospective epidemiological studies in which insulin sensitivity is measured at baseline using direct methods. Because all the reliable methods for the direct assessment of insulin sensitivity including the euglycaemic clamp, insulin suppression test and minimal model method, are rather complex and time-consuming, very few such studies have been started and, as mentioned above, only 2 of them have reported results concerning the association of insulin sensitivity with CVD risk. In the IRAS (434), comprising almost 1500 participants recruited with the goal to obtain approximately equal numbers according to gender, ethnicity and glucose tolerance status (normal, impaired glucose tolerance, type 2 diabetes), the minimal model method was used for the assessment of insulin sensitivity at baseline, and the follow-up with regard to clinical CVD endpoints is in progress.

With increasing number of prospective studies, in which plasma insulin has been
measured at baseline and participants followed up with regard to CVD endpoints, meta-analyses combining the data from such studies may with their greater statistical power help solve some outstanding issues, such as the question whether or not the association between insulin and CVD risk is similar in men and women, as well as the question of the effect of ethnicity on this association.

Further research on the physiology and molecular genetics of the regulation of insulin resistance will be of central importance in the clarification of those mechanisms by which insulin resistance and hyperinsulinaemia take part in the causation of atherosclerosis and its complications. Such research may also give new clues for epidemiological studies. The introduction of a new class of drugs, thiazolidinediones, the so-called insulin sensitisers, offers new possibilities for clinical research and trials elucidating the effects of changes in insulin resistance on associated risk factors and the risk of CVD.

The current great interest in the IRS as a predictor of increased CVD risk has led to definitions of the syndrome for the purposes of research and also for prevention in clinical practice, as summarised in Chapter 2.2.3 (Table 1). The modification of the WHO definition of the IRS given by the EGIR for research purposes includes hyperinsulinaemia as one of the diagnostic criteria of the syndrome, defining hyperinsulinaemia as fasting plasma insulin levels exceeding the cut-off point for the highest quartile in healthy subjects. It has also been proposed that fasting insulin measurement could be used as a marker of insulin resistance in the screening of individuals at high CVD risk because of the IRS (511). The use of fasting plasma insulin for screening purposes is, however, not without problems. First, the correlation of fasting plasma insulin with directly measured insulin sensitivity is from -0.60 to -0.70 which means that only 40-50% of the variation in insulin sensitivity can be explained by fasting insulin (38,39). This is in part explained by a relatively great intra-individual variation of plasma insulin levels (489). Therefore, in order to get a correct estimate of the usual plasma insulin level of an individual, repeated measurements at separate well standardised occasions would be needed. Second, although precise methods for the measurement of intact plasma insulin are available, problems in the standardisation of the methods within and between laboratories have not yet been solved, although in
principle this should be possible (512). Further research is needed to find out how much incremental information plasma insulin measurement, in addition to measurement of other components of the IRS (obesity and its central distribution, triglycerides, HDL cholesterol, glucose, blood pressure), would give to the assessment of CVD risk.

Although many unsolved questions still remain in the association of hyperinsulinaemia and insulin resistance with the risk of CVD, the information already accumulated has important implications for the development of strategies of CVD prevention. The same lifestyles that have for long been recommended for the maintenance of optimal plasma lipid and blood pressure levels – prevention and control of obesity, regular physical activity, and a diet with a low content of saturated fats – also help in keeping insulin sensitivity of the tissues at optimal level and prevent the development of the risk factor cluster of the IRS. An epidemic increase in the prevalence of obesity is currently a major problem in the industrialised world and a concomitant increase in the prevalence of type 2 diabetes and the IRS will be its inevitable consequence. It appears likely that favourable trends in CVD mortality and morbidity which have been going on in many countries, including Finland, during the last decades may be slowed down or even become reversed by this adverse change in the risk factor pattern. Therefore, population strategies for CVD prevention should pay more attention to the prevention and control of obesity by promotion of prudent dietary habits and regular physical activity. The same lifestyle measures will also be of importance in the clinical practice of CVD prevention in individuals with the IRS. With regard to individual approach for prevention, it is encouraging that 3 clinical trials conducted in China (513), Finland (514) and the United States (515) have demonstrated the effectiveness of lifestyle changes (reduction of obesity, increased physical activity and dietary changes) in the prevention of type 2 diabetes in subjects with impaired glucose tolerance most of whom are insulin-resistant. The logical next step would be to contemplate lifestyle intervention trials of CVD prevention directed to non-diabetic people who are characterised by insulin resistance and associated risk factor cluster, but before such trials can be launched, further research is needed to validate and improve methods for the identification of such high-risk individuals.
7 SUMMARY

This 22-year follow-up of the Helsinki Policemen Study was based on a cohort of 970 men who were 34-64 years of age and free of CVD and diabetes at the time of the baseline examination in 1971-72. Risk factor measurements at baseline included an OGTT with blood glucose and plasma insulin determinations at 0, 1 and 2 hours. A composite variable, AUC insulin, was used to reflect plasma insulin levels. During the follow-up, 276 men died, 130 from cardiovascular and 146 from non-cardiovascular causes. 164 men had a major CHD event (CHD death or non-fatal MI) and 70 had a fatal or non-fatal stroke.

The results of this study can be summarised as follows:

Study I. Hyperinsulinaemia, defined as the highest AUC insulin quintile, was associated with an increased risk of CHD over the 22-year follow-up period and this association was largely independent of other cardiovascular risk factors (obesity and its central distribution, blood pressure, cholesterol, triglycerides, blood glucose, smoking, and physical activity). Also, when AUC insulin was entered as a continuous variable into the prediction models, a significant and positive association between insulin and CHD risk during the 22-year follow-up period was demonstrated. These associations were shown for major CHD events (CHD death or non-fatal MI), and also for CHD deaths and non-fatal MIs, when analysed separately. The association between AUC insulin and CHD risk was strongest during the 5- and 10-year follow-up period and decreased with lengthening follow-up time. Nevertheless, the predictive power of AUC insulin over the 22 years of follow-up was of the same magnitude as that of cholesterol, when conventional risk factors were entered as continuous variables into multivariate analyses.

Study II. A positive association between AUC insulin and the risk of stroke, adjusting for age, was demonstrated during the 22-year follow-up period. This association was shown for all strokes (fatal or non-fatal), and for fatal and non-fatal strokes. However, further adjustment for indices of obesity, blood pressure, and
smoking weakened the association between insulin and stroke to non-significant level. This was mainly due to the impact of obesity, and to upper-body obesity in particular. The strength of the association between AUC insulin and stroke risk remained unchanged over the 22-year follow-up period.

**Study III.** During the 22-year follow-up, hyperinsulinaemia, defined as the highest AUC insulin quintile, was associated with increased all-cause mortality independently of other risk factors, and this resulted mainly from an increase in cardiovascular mortality, particularly coronary mortality. These associations, however, weakened with lengthening follow-up time. The results were largely similar when AUC insulin was entered into Cox models as a continuous variable. The association of AUC insulin with non-cardiovascular mortality was U-shaped.

**Study IV.** Factor analysis of 10 baseline risk factor variables produced 3 underlying factors: insulin resistance factor (comprising body mass index, subscapular skinfold, AUC insulin, AUC glucose, maximal \( \text{O}_2 \) uptake, mean blood pressure, and triglycerides), lipid factor (cholesterol and triglycerides), and lifestyle factor (physical activity and smoking). In multivariate Cox models, adjusting for age, insulin resistance factor predicted the risk of both CHD and stroke during the 22-year follow-up. With increasing length of follow-up, the predictive power of insulin resistance factor with regard to CHD risk decreased but remained unaltered with regard to stroke risk. Lipid factor predicted the risk of CHD but not that of stroke, and lifestyle factor predicted a reduced CHD risk.

**Conclusions.** In the 22-year follow-up of the Helsinki Policemen Study, hyperinsulinaemia predicted the risk of CHD events and all-cause and cardiovascular mortality, although its predictive value diminished with the lengthening of follow-up time. Hyperinsulinaemia was also associated with the risk of stroke, but this association was not independent of other risk factors, particularly indices of obesity. However, the insulin resistance factor derived from factor analysis, reflecting the combined effect of insulin and associated risk factors, was a significant predictor of the risk of both CHD and stroke.
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