Insulin Resistance and Inflammation as Risk Factors for Congestive Heart Failure

ERIK INGELSSON
Dissertation presented at Uppsala University to be publicly examined in Auditorium Minus, Museum Gustavianum, 753 10 Uppsala, Saturday, October 1, 2005 at 13:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

Congestive heart failure (CHF) is a major cause of morbidity and mortality and the identification of modifiable risk factors is crucial in order to diminish suffering of this common disease.

The primary aim of this thesis was to investigate novel metabolic risk factors for CHF, with a focus on insulin resistance and inflammation. The secondary aim was to examine the validity of the CHF diagnosis in the Swedish hospital discharge register.

This thesis was based on the Uppsala Longitudinal Study of Adult Men (ULSAM) cohort, a community-based prospective study started in 1970. The participants were examined at age 50 and 70 and the data was completed with annual updates on mortality and in-hospital morbidity using national registers.

We showed that insulin resistance predicts CHF incidence independently of established risk factors in both middle-aged and elderly men. The previously described association between obesity and subsequent CHF may be mediated partly by insulin resistance. Moreover, it was established that inflammation, measured as erythrocyte sedimentation rate is a significant predictor of CHF, independent of established risk factors including an interim myocardial infarction. Furthermore, a low beta-carotene level, as well as an increased apolipoprotein B/A-1-ratio was found to predict CHF independently of established risk factors.

We also showed that the validity of the CHF diagnosis in the Swedish hospital discharge register appears less precise than for other recently investigated cardiovascular diagnoses. However, when including only cases from selected clinics or cases with a primary diagnosis of CHF, the validity is comparable to the above diagnoses.

In conclusion, insulin resistance and inflammation are strong independent risk factors for the development of CHF, and seem to be involved in the early process leading to CHF. If confirmed, our observations could have large clinical implications as they may offer new approaches in the prevention of CHF.

Keywords: congestive heart failure, insulin resistance, inflammation, obesity, blood sedimentation, apolipoproteins, antioxidants, beta carotene, oxidative stress, registries, epidemiology, risk factors

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To Maria
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


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<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
</tr>
<tr>
<td>AGE</td>
<td>advanced glycosylation end-product</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CHF</td>
<td>congestive heart failure</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>COX</td>
<td>cyclooxygenase</td>
</tr>
<tr>
<td>CRP</td>
<td>c-reactive protein</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>ECG</td>
<td>electrocardiography</td>
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<tr>
<td>ECG-LVH</td>
<td>electrocardiographic left ventricular hypertrophy</td>
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<tr>
<td>ESC</td>
<td>European Society of Cardiology</td>
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<tr>
<td>ESR</td>
<td>erythrocyte sedimentation rate</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
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<td>HDR</td>
<td>hospital discharge register</td>
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<td>HOMA</td>
<td>homeostasis model assessment</td>
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<tr>
<td>HR</td>
<td>hazard ratio</td>
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<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>IL-6</td>
<td>interleukin-6</td>
</tr>
<tr>
<td>IVGTT</td>
<td>intravenous glucose tolerance test</td>
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<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
</tr>
<tr>
<td>NSAID</td>
<td>nonsteroidal anti-inflammatory drugs</td>
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<tr>
<td>OGTT</td>
<td>oral glucose tolerance test</td>
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<tr>
<td>PPAR</td>
<td>peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>soluble vascular cell adhesion molecule-1</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumor necrosis factor-α</td>
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<tr>
<td>ULSAM</td>
<td>Uppsala Longitudinal Study of Adult Men</td>
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</table>
Introduction

The epidemiology of congestive heart failure

Introduction

Cardiovascular disease (CVD) is a growing cause of morbidity and mortality globally. In the Western countries, this development has been seen for decades, and it is associated with substantial costs and suffering. In less developed countries there is an ongoing dramatic shift from a predominance of nutritional deficiencies and infectious diseases, to chronic diseases such as CVD, diabetes and cancer. This shift has been called “the epidemiological transition” and different countries are at different stages of this transition.23

Congestive heart failure (CHF) is a major public health problem, with considerable suffering of the patients and rising costs for the health care system. CHF is reported to consume 1 to 2% of the total health care costs in a number of industrialized countries.3 About 14 million people suffered from CHF in Europe, US and Japan in 2002, and the incidence is escalating.3 This trend is the result of an aging population and an improving survival after acute myocardial infarction. Furthermore, the incidence of CHF is expected to keep rising the next decades, since the treatment of coronary heart disease is getting better, and the population is getting older.6

Definition of congestive heart failure

A definite and reproducible definition of the disease under study is crucial in all epidemiological studies, but unfortunately there is no such definition for CHF. Heart failure is commonly described as a syndrome, caused by cardiac dysfunction, and recognized by a constellation of signs and symptoms. Several clinical scoring systems have been developed for use in population-based epidemiological studies7 and the most well-known is the Framingham scoring system.8 The systems tend to have high sensitivity, but low specificity, and therefore have problems with a high false positive rate.9

Recent studies, especially clinical drug trials, have used echocardiography to examine the left ventricular systolic function in individuals
with suspected heart failure. However, systolic dysfunction is not the same as heart failure. Individuals can have a systolic dysfunction without symptoms, and therefore should not be classified as cases of heart failure. In fact, recent studies have shown that asymptomatic left ventricular systolic dysfunction is at least as common as systolic heart failure. At the same time, there are individuals with a typical clinical heart failure, without left ventricular dysfunction, and many recent studies have addressed the presence of so-called diastolic heart failure.

The European Society of Cardiology (ESC) has proposed a definition of heart failure for use in clinical practice, clinical trials and epidemiological research. It is based on the two essential features of having symptoms of heart failure and objective evidence of cardiac heart dysfunction at rest. In cases where the diagnosis is in doubt, the response to treatment directed towards heart failure is a non-essential feature, but a useful check on the diagnosis.

**Prevalence, incidence and prognosis**

There is a considerable variation of the prevalence, incidence and prognosis of CHF in different studies, which is caused by different sociodemographic areas, risk factor profiles and research methods.

The crude prevalence ranges from 0.3 to 2% and the prevalence for individuals over 65 years ranges from 3 to 13%. The most well-known population-based studies are based on the Framingham cohorts. They have reported a prevalence of about 1% in those 50 to 59 years of age, with a doubling for each decade of age, to 7% in men and 8% in women in those 80 to 89 years of age.

There is less known about the incidence than the prevalence of CHF. However, there are a number of large studies with crude incidence ranging from 1 to 5 cases per 1000 per year. As with prevalence there is a large increase with higher age. In the Framingham data there is an annual incidence of 3 cases (men) and 2 cases (women) per 1000 in the youngest age group, and an annual incidence of 27 cases (men) and 22 cases (women) per 1000 in those over 80 years.

The prognosis of untreated CHF is not known, since all studies investigating prognosis in CHF are from the era where medication has been in use. There is no doubt about that CHF is a highly lethal condition. The 5-year age-adjusted mortality was 59% in men and 45% in women in a recent Framingham report, and in an earlier report from the Rochester Epidemiology Project, the 5-year mortality was 65%. The mortality ranges from four to eight times that of the general population of the same age, and it is at the same level as the five-year-mortality of all cancers among men and women in US. The improved
treatment over the last decade, due to increased use of angiotensin converting enzyme (ACE) inhibitors and beta blockers, is thought to improve survival and recent reports indeed suggest an improving prognosis for CHF patients.\textsuperscript{17,19}

**Etiology and established risk factors**

The predominant causes of heart failure are hypertension and coronary heart disease. In the Framingham material, over 75\% of the CHF cases had hypertension or received antihypertensive treatment, whereas about 46\% of the men and 27\% of the women had a background of coronary heart disease.\textsuperscript{18} A recent report from Framingham showed that hypertension had a high population-attributable risk for CHF, accounting for 39\% of the cases in men and 59\% in women, compared with myocardial infarction that accounted for 34\% of cases in men and 13\% in women.\textsuperscript{20} In other studies the proportion is different, with less hypertension and more coronary heart disease as causes of CHF. Since the first reports of the heart failure etiology were published, there has been an obvious trend over time that hypertension and valvular heart disease have been less common as primary causes of CHF, while coronary heart disease has become more common.\textsuperscript{6}

Apart from hypertension and coronary heart disease, which are the two most common pre-existing conditions among cases of CHF, several other risk factors for developing CHF have been identified. In a Framingham study from 1993 it was established that left ventricular hypertrophy and diabetes were conditions with high relative risk for CHF, both in men and women. In younger men cigarette smoking increased the likelihood of CHF, but not in women or older men. In this study they did not find any association between a high serum cholesterol level and CHF.\textsuperscript{16} In the Study of men born in 1913 they found that smoking and body weight were the strongest significant risk factors for CHF apart from hypertension.\textsuperscript{21} There was no relationship between a high cholesterol value and CHF in this study either. However, another study has shown an association between the total to high density lipoprotein (HDL) cholesterol ratio and the risk for CHF in both sexes,\textsuperscript{18} suspectedly due to development of coronary heart disease. Diabetes tends to be a more powerful risk factor in women than in men, and only part of this risk is caused by obesity, coronary heart disease, hypertension and dyslipidaemia. Diabetes seems to induce changes of myocardial structure and function, that increase the risk of CHF.\textsuperscript{18,22}
Swedish perspective

The prevalence and incidence for CHF in Sweden is similar to other developed countries, as described above. In a recent national report, it was estimated that more than 160000 Swedes suffer from CHF, which is about 2% of the population. The costs of heart failure have been calculated to 2000 to 2600 millions Swedish kronor, or almost 2% of the total health care budget, and 65 to 75% of this cost is caused by institutional care. Most heart failure patients are cared for by primary care physicians, but hospitalization is common, even the most common cause of hospitalization for patients over 65 years of age. Echocardiography is only performed on a little more than 30% of the patients in primary care, probably because of poor access. In hospitals it is more available and routinely used for diagnosis. There still seems to be an under-prescription of ACE inhibitors and beta-blockers in Sweden.

The validity of the congestive heart failure diagnosis

In epidemiological studies, clinical disease end-points are often assessed using health registers. The reliability of such studies is dependent on the quality of the register data, which varies between different regions and different diagnoses. In many countries, data on hospitalizations are recorded in a national hospital discharge register (HDR). The overall quality of the Swedish HDR is commonly regarded as high, and the validity of the register has been evaluated for some diagnoses, e.g. acute myocardial infarction and acute stroke. The reliability of these cardiovascular diagnoses in the register has been shown to be high.

There are several diagnostic definitions for CHF, which use clinical criteria, with varying sensitivities and specificities, depending on the severity of CHF and the degree of certainty in the diagnosis. These diagnostic schemes usually comprise combinations of clinical signs and symptoms of CHF, laboratory blood and radiological examinations. Assessments of left ventricular filling pressures or systolic function indices may or may not be included in the diagnostic criteria. As left ventricular systolic function is normal in a large proportion of CHF patients, schemes that do not rely primarily on a measurement of systolic function are possibly to be preferred. The previously described definition of CHF proposed by ESC, is intended to be used in clinical practice, clinical trials and epidemiological research.

Studies examining the validity of the CHF diagnosis in different European and American patient samples have been published, but
to date, little is known about the validity of the diagnosis of CHF in the Swedish, or any other, HDR.

**Promising novel risk factors for congestive heart failure**

*Insulin resistance and congestive heart failure*

Insulin resistance can be defined as a state in which normal levels of insulin produce a subnormal biological response.\(^{37}\) The tissues of the body have a reduced sensitivity to the action of insulin, and therefore greater than normal amounts of insulin are required to elicit a quantitatively normal response. As a result, hyperinsulinaemia develops, which can be accompanied by either normoglycaemia or hyperglycaemia, when the beta cell function eventually subside and diabetes mellitus develops.\(^{38}\) Thus, insulin resistance is an early precursor of diabetes, with sub-clinical dysregulation of the glucose metabolism.

Insulin resistance tends to cluster together with other cardiovascular risk factors, such as hypertension, dyslipidaemia and obesity. The first studies describing these relationships were published almost a century ago.\(^{39-40}\) In 1988, Reaven hypothesized that insulin resistance was the factor linking non-insulin dependent diabetes mellitus, essential hypertension and coronary heart disease.\(^{41}\) Initially he called the clustering of risk factors “Syndrome X”, but currently the terms “insulin resistance syndrome” or “metabolic syndrome” are more commonly used.\(^{42-45}\) The syndrome has been associated with an increased risk for coronary heart disease in different samples.\(^{46-52}\)

Diabetes as a predictor of subsequent CHF was first described in the Framingham Heart Study three decades ago,\(^{22}\) and the disease is frequently cited as a risk factor for CHF.\(^{18,21,53,54}\) In recent years, associations between insulin resistance and altered left ventricular geometry and function have been reported.\(^{55,56}\) Furthermore, in patients with manifest CHF, insulin resistance is associated with a more severe disease and worse prognosis.\(^{57-59}\) Yet, more detailed characterizations of the association between diabetes and subsequent CHF are still lacking, and insulin resistance has not been investigated as a predictor of CHF.

*Inflammation, antioxidants and congestive heart failure*

In recent years, the association between inflammation and CVD has gained considerable interest.\(^{60}\) Several systemic markers of inflammation, including erythrocyte sedimentation rate (ESR), have been found to be predictors of coronary heart disease.\(^{61-63}\)
C-reactive protein (CRP), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6; all markers of cytokine-mediated inflammation) have all been shown to predict incident CHF, but to this date there are no studies of the possible association between ESR and future CHF. ESR is a well-validated and inexpensive tool for evaluating inflammation (including other aspects of inflammation than cytokine-mediated reactions), and is available at every out-patient clinic.

Oxidative stress is a state of excess formation of reactive oxygen species (ROS) or free radicals, independent molecules with at least one unpaired electron. This might be a result of diseases or insufficient antioxidative defense. The unpaired electrons make ROS extremely reactive, and they attack molecules in vivo instantly, e.g. lipids and deoxyribonucleic acid (DNA). It is well recognized that inflammation is one manifestation of oxidative stress, and the pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, are all induced by oxidative stress. In fact, intracellular generation of ROS is a crucial activator of the final common pathway for the prothrombotic and proinflammatory maladaptive cellular behaviors that are associated with vascular injury. Antioxidants prevent and repair the oxidative damage, by different mechanisms. They can both be endogenous and exogenous. There are some prior smaller case-control studies which have pointed out a relationship between antioxidant levels and the severity of CHF and a recent cross-sectional case-control study has found that inflammatory markers and a marker of oxidative stress were significantly correlated in CHF subjects. However, oxidative stress and antioxidants as predictors of subsequent CHF is previously not investigated to our knowledge.
Aims of the thesis

The primary aim of this thesis was to investigate novel metabolic risk factors for congestive heart failure, with a focus on insulin resistance and inflammation. The secondary aim was to examine the validity of the congestive heart failure diagnosis in the Swedish hospital discharge register.

The specific aims were:

I. To determine the validity of a given diagnosis of CHF in the Swedish hospital discharge register against the ESC definition and to investigate if the presence of an echocardiographic examination, the type of hospitalization clinic or the position of the diagnosis code were important for the accuracy of the CHF diagnosis.

II. To examine established and novel metabolic risk factors for development of CHF, and to analyze if the risk factors predicted CHF independently of an interim myocardial infarction during the follow-up.

III. To analyze measures of insulin sensitivity and secretion as predictors of CHF incidence, and to investigate if the previously described association between obesity and CHF may be mediated by insulin resistance.

IV. To investigate ESR, a marker of inflammation, as a possible predictor of CHF and to analyze if it predicted CHF independently of an interim myocardial infarction during the follow-up period.
Methods

Participants

The ULSAM cohort

This thesis is based on the ULSAM (Uppsala Longitudinal Study of Adult Men) cohort. All men born in 1920 to 1924 who were residents of the municipality of Uppsala, Sweden, were invited to participate in a health examination carried out between April 1970 and October 1973, which aimed at identifying risk factors for CVD. Of the 2841 men invited, 2322 participants (82%) participated in the investigation at age 50.\(^\text{72}\)

The men were invited to re-examinations at age 60, age 70, age 77 and at age 82, and the data has been completed with annual updates on mortality and in-hospital morbidity using national registers. The re-examination at age 70 was carried out between August 1991 and May 1995. Between the investigations at age 50 and age 70, 422 participants had died and 219 had moved from Uppsala. Of the 1681 available (i.e. alive and still living in Uppsala) 70-year-old men invited to the follow-up investigation, 1221 men (73%) attended. In this thesis, data from the investigations at age 50 and age 70 are used (Figure 1).

All participants gave written consent to the study and it was approved by the Ethics Committee of Uppsala University.

Study populations

The study populations in the four studies in this thesis are based on the examinations at age 50 and age 70 of the ULSAM cohort.

I. The study population in study I consists of the 321 participants who had a hospital discharge register diagnosis of CHF between entry into the ULSAM cohort at age 50 and the end of 2001.

II. At the age 50 examination, none of the participants had been diagnosed with CHF in the hospital discharge register before baseline. One subject was excluded due to valvular disease, thus 2321 men constitute the study population in study II, free from CHF and valvular disease at
baseline. In a sub-sample, all participants with prior myocardial infarction at baseline or myocardial infarction during follow-up (n=409) were excluded, leaving 1912 participants.

III. Of the 1221 men attending the age 70 examination, 20 participants were excluded due to a previous diagnosis of CHF and 14 due to a diagnosis of valvular disease in the hospital discharge register at baseline. Thus, 1187 men comprise the study population in study III. We examined a sub-sample of 1061 non-diabetic men after exclusion of all participants with diabetes at baseline (n=126). Furthermore, we examined a sub-sample of 1034 non-obese men after exclusion of all men with BMI>30 at baseline (n=153) and another sub-sample of 433 normal-weight men after exclusion of all men with BMI>25 at baseline (n=754).

![Diagram](image_url)

**Figure 1. The Uppsala Longitudinal Study of Adult Men**
IV. The study population of study IV consists of the same participants as in study II, with the additional exclusion of participants with a prior myocardial infarction (n=7). Thus, 2314 men free from CHF, valvular disease and myocardial infarction at baseline comprise the study population. In a secondary analysis, all participants receiving treatment with corticosteroids (n=7) or potentially anti-inflammatory analgesics (n=16) were excluded.

Investigations at age 50
These measurements were used in study II and IV, and they have been described extensively previously.\textsuperscript{72}

**Anthropometry**
Height (without shoes) was measured to the nearest whole cm and weight (in undershorts) to the nearest whole kg. BMI was calculated as weight (in kg) divided by height (in meters) squared.

**Blood pressure**
Blood pressure was measured in the right arm after 10 minutes’ rest in the recumbent position using mercury manometers (Kifa Ércamer, wall-model). Systolic and diastolic blood pressures were read to the nearest 5 mmHg, according to Korotkoff phase I and V, respectively.

**Glucose and insulin**
Blood glucose was measured by spectrophotometry using the glucose oxidase method.

An intravenous glucose tolerance test (IVGTT) was performed by injection of 50% glucose solution at a dose of 0.5 g/kg into an antecubital vein during 2.5 minutes. Samples for the determination of blood glucose and serum insulin concentration were drawn before and 60 minutes after the start of the glucose injection. The serum insulin was determined with the Phadebas Insulin Test (Pharmacia AB, Sweden), based upon a radioimmunosorbent technique.\textsuperscript{73}

Homeostasis model assessment (HOMA)-insulin resistance index was calculated using fasting plasma glucose and insulin concentrations by the formula: fasting insulin*fasting glucose/22.5.\textsuperscript{74}

Intact proinsulin and 32-33 split proinsulin concentrations were analyzed using the two-site immunometric assay technique.\textsuperscript{75} Specific insulin concentrations were determined using the Access Immunoassay
System (Beckman-Coulter), which uses a chemiluminescent immunoenzymatic assay. These analyses were carried out between 1995 and 1998 at the Department of Clinical Biochemistry, Addenbrooke’s hospital, Cambridge, UK, using plasma samples that had been stored frozen in -70°C since sampling.

**Lipids and apolipoproteins**
Determinations of serum cholesterol and triglyceride concentrations were performed on a Technicon Auto Analyzer type II in 1981 to 1982 on serum samples that had been stored in liquid nitrogen since sampling. HDL was assayed in the supernatant after precipitation with a heparin/manganese-chloride solution. Low density lipoprotein (LDL) cholesterol was calculated using Friedewald’s formula: LDL=serum cholesterol-HDL-(0.42*serum triglycerides).

Apo(a) and ApoB were determined by a two-site immunoradiometric assay and ApoA-I by a competitive radioimmunoassay in 1988, with use of commercial kits from Pharmacia AB, Sweden, in samples that had been stored in liquid nitrogen since sampling.

**Chemical lab analyses and vitamins**
The haematocrit was calculated by multiplication of mean corpuscular volume and erythrocytes using a Coulter Counter from Coulter Electronics Lim. USA.

The erythrocyte sedimentation rate was determined by Westergren’s method.

Uric acid in serum was measured by spectrophotometry. Before January 1973, reduction with phosphotungsteic acid was used in a Beckman B spectrophotometer from Beckman Instruments Inc., USA. After January 1973, oxidation with uricase to allantoin and hydrogen peroxide was used in an autoanalyzer from Technicon, USA.

Alpha tocopherol and beta-carotene were simultaneously determined by high-performance liquid chromatography.77 The serum tocopherol concentrations reported were corrected for the sum of serum cholesterol and serum triglycerides (tocopherol/(cholesterol+triglyceride)).78

**Questionnaire**
Information regarding treatment for hypertension and/or diabetes was collected through a self-administered questionnaire. Data on smoking habits (smoker, non-smoker) was based on interview reports.72
Investigations at age 70

These measurements were used in study III, and have been described extensively previously. 56,70,80

Anthropometry

Height was measured to the nearest whole cm, and body weight to the nearest 0.1 kg. BMI was calculated as the ratio of the weight (in kg) to the height (in meters) squared (kg/m²). The waist circumference was measured midway between the lowest rib and the iliac crest in the supine position.

Blood pressure

Blood pressure was measured in the right arm with the subject in the supine position after resting for 10 minutes. The values were recorded twice and to the nearest even figure, and the means of the two values were given. Systolic and diastolic blood pressure was defined as Korotkoff phases I and V, respectively.

Glucose and insulin

Plasma glucose in samples from the oral glucose tolerance test was measured by the glucose dehydrogenase method (Gluc-DH, Merck KGaA, Darmstadt, Germany).

Plasma insulin from the oral glucose tolerance test and the clamp study was assayed using an enzymatic-immunological assay (Enzymun, Boehringer Mannheim, Germany) performed in an ES300 automatic analyzer (Boehringer Mannheim).

HOMA-insulin resistance index was calculated using fasting plasma glucose and insulin concentrations by the formula: fasting insulin*fasting glucose/22.5.74

Intact proinsulin and 32-33 split proinsulin concentrations were analyzed using the two-site immunometric assay technique.75 Specific insulin concentrations were determined using the Access Immunoassay System (Beckman-Coulter), which uses a chemiluminescent immunoenzymatic assay. These analyses were carried out at the Department of Clinical Biochemistry, Addenbrooke’s hospital, Cambridge, UK.
Lipids
Cholesterol concentrations were analyzed in serum by enzymatic techniques using II. Test Cholesterol Trinders’ Method for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, USA).

Oral glucose tolerance test
An oral glucose tolerance test (OGTT) was performed where the participants ingested 75 g glucose dissolved in 300 ml of water, and blood samples for plasma glucose and insulin were drawn immediately before and 120 min after ingestion of glucose. The oral glucose tolerance test and the clamp procedure were performed at least one week apart.

Euglycaemic insulin clamp
The euglycaemic insulin clamp technique was developed to estimate in vivo sensitivity to insulin. The technique used was according to DeFronzo et al., with a slight modification; insulin was infused at a constant rate of 56 instead of 40 mU/(min per body surface area (m²)).
Semi synthetic regular human insulin was infused in a primary dose for the first 10 minutes and then as a continuous infusion (at 56 mU/min*m²) for 110 minutes to maintain steady state hyperinsulinaemia. The level of plasma glucose during the clamp study was maintained by measuring the plasma glucose every 5 minutes and adjusting the rate of infusion of a 20% glucose solution accordingly. The target plasma glucose level was 5.1 mmol/l. Plasma was immediately separated in a centrifuge and plasma glucose was assayed in duplicate in a Glucose Analyzer. Steady-state plasma glucose and plasma insulin concentrations were calculated as the mean of all values obtained between the 60th and 120th minute of the clamp study.
Glucose disposal rate, representing insulin sensitivity, was calculated as the amount of glucose taken up during the last 60 minutes of the clamp procedure and was presented in mg/kg bodyweight/min. The calculation of the total body insulin sensitivity was based on the assumption that endogenous hepatic glucose production is entirely suppressed. Under euglycaemic conditions it is known that about 90% of this production is suppressed when the plasma insulin concentration is increased to 56 mU/min*m².

Questionnaire
Information regarding demographic data and treatment for hypertension and/or diabetes was collected through a self-administered ques-
Definition of cardiovascular risk factors

Established risk factors for CHF were used as baseline covariates in studies II to IV. The presence of hypertension at baseline was defined as systolic blood pressure 140 mmHg and/or diastolic blood pressure 90 mmHg and/or anti-hypertensive medication. The presence of diabetes at baseline was defined as fasting blood glucose 6.1 mmol/l (study II and IV) or fasting plasma glucose 7.0 mmol/l (study III) and/or the use of oral hypoglycemic agents or insulin. Electrocardiographic left ventricular hypertrophy (ECG-LVH) was defined as high amplitude R-waves according to the revised Minnesota code together with left ventricular strain pattern. The presence of valvular disease (International Classification of Diseases [ICD]-8 codes 394-396 and 424, ICD-9 codes 394-397 and 424 or ICD-10 codes I05-I08 and I34-I37) and prior myocardial infarction (ICD-8 code 410, ICD-9 code 410 or ICD-10 code I21) were assessed from the hospital discharge register. The diagnosis of acute myocardial infarction was chosen as a proxy for coronary heart disease, as the precision of the myocardial infarction diagnosis in the Swedish hospital discharge register is high. Furthermore, adjusting for, or excluding, interim myocardial infarction is an established method for examining “non-ischemic” CHF.

Follow-up and outcome parameter

Registers

The Swedish HDR is administered by the National Board of Health and Welfare (Socialstyrelsen), which in the 1960’s started to collect data on individual patients who had been treated as in-patients at public hospitals. For the first few years, not all of the county councils reported their hospitalization data to the register since it was not compulsory. However, since 1987, when reporting was made compulsory, the HDR records all in-patient care in Sweden. It contains the dates of hospital admissions and discharges, hospital and clinic codes and up to six coded discharge diagnoses, the first being the principal cause of hospitalization. The register uses the codes of the International Classification of Diseases (ICD), edition eight (ICD-8) until the end of

Selection of possible cases
The ULSAM participants were linked to the Swedish HDR using the unique personal identification number of all citizens of Sweden. The diagnosis of CHF was allowed in any of the six possible diagnosis positions in the HDR. As a diagnosis of CHF, we considered ICD heart failure codes 427.00, 427.10, 428.99 (ICD-8), 428 (ICD-9), I50 (ICD-10) and hypertensive heart disease with heart failure, I11.0 (ICD-10). If a subject had multiple hospital discharge register diagnoses of CHF over the years, only the first occasion was considered in the present analysis.

Data collected for review
Medical records, including referral notes, radiology reports, ECG reports, echocardiography reports if available, other journal records during the hospital stay and the discharge records were collected for each person with a diagnosis of CHF in any diagnosis position in the HDR. Out of these 321 persons, two were excluded because of insufficient hospital and clinic coding in the hospital discharge register and two were excluded as their medical records could not be found, despite extensive searching in the archives.

Diagnostic classification
The HDR cases were classified by a review board consisting of two physicians, who accessed all the journal records described above and classified the cases as either definite, questionable or miscoded. The classification relied on the definition proposed by the ESC.\(^4\) Thus, to be classified as a definite CHF case, there had to be symptoms and signs of CHF and “objective evidence” of cardiac dysfunction at rest. In cases of doubt, the response to CHF treatment was a useful check of the diagnosis. The required “objective evidence” was preferably echocardiography, but since the study commenced prior to the widespread availability of echocardiography, ECG and x-ray were also considered acceptable when an echocardiography report was not available. The HDR cases where the review board could not find supporting evidence of CHF according to the ESC definition were classified as questionable. For example, left ventricular dysfunction classified by echocardiography but without symptoms of CHF, and breathlessness without objective evidence of CHF were both classified as questionable.
questionable cases the review board also looked at any previous and subsequent admissions to clarify the diagnosis. In the few cases where a possible case was coded with an clearly incorrect ICD-code, the case was classified as miscoded.

**Follow-up**

In study I and II, the participants had a median follow-up time of 28.8 years (range 0.04 to 31.7 years), contributing to 58084 person-years at risk. Three hundred and twenty-one men had a hospital discharge register diagnosis of CHF between entry into the ULSAM study and the end of 2001, with the first subject registered with a diagnosis in 1976. After the validation of the possible cases, 259 heart failure cases were defined as definite and were included in study II. The incidence rate for CHF during the follow-up period was 4.5/1000 person-years at risk. None of the participants were lost to follow-up.

In study IV, the follow-up was extended until the end of 2002. The participants had a median follow-up time of 29.6 years (range 0.04 to 32.7 years), contributing to 59122 person-years at risk. Three hundred and forty-six men had a hospital discharge register diagnosis of heart failure between the entry to the ULSAM study and the end of 2002. After the validation of the possible cases, 282 cases of definite CHF were included. The incidence rate for CHF during the follow-up period was 4.8/1000 person-years at risk. None of the participants were lost to follow-up.

In study III, the participants had a median follow-up time of 8.9 years (range 0.01 to 11.4 years), contributing to 9899 person-years at risk. One hundred and thirty-two men had a hospital discharge register diagnosis of heart failure between the age 70 baseline and censor-date at the end of 2002. After the validation of the possible cases, 104 definitive heart failure cases were included in the total cohort. The incidence rate for CHF during the follow-up period was 10.5/1000 person-years at risk. None of the participants were lost to follow-up.

**Statistical analyses**

Data were given as percentages for categorical variables, means ± standard deviation (SD) for normal distributed continuous variables or medians (interquartile range) for skewed variables. Two-tailed 95 % confidence intervals and p-values were given, with p<0.05 regarded as significant. Statistical software package STATA 8.2 (Stata Corporation, College Station, TX, USA) was used in study II, III and IV and JMP 3.2 (SAS Institute, Cary, NC, USA) was used in study I.
**Distribution**

The distributions of the continuous variables were tested using Shapiro-Wilk’s test. Logarithmic transformation was performed to achieve normal distribution for the skewed variables (IVGTT s-insulin 60 min, fasting insulin, fasting proinsulin, fasting split proinsulin, HDL cholesterol, Apo(a), s-triglycerides and beta-carotene at age 50; and fasting p-glucose, OGTT 2-h glucose, fasting insulin, fasting proinsulin, fasting 32-33 split proinsulin and HOMA-insulin resistance index at age 70). The residuals of all regression analyses were examined and found to be normally distributed.

**Descriptive analyses of diagnosis validity**

In study I, the percentage of participants with a register diagnosis of CHF receiving a definite diagnosis in the review process was used to assess the validity of the hospital discharge register (positive predictive value). The hospital discharge register cases were divided into different sub-groups on the basis of which clinic they were hospitalized at (internal medicine, cardiology, lung medicine or other), if they underwent an echocardiographical examination during the hospital stay, and which position the CHF diagnosis code was given (position 1, position 2 and position 3 to 6).

**Basic statistics**

In study II, the persons who developed and did not develop CHF during follow-up were compared at baseline with Kruskal-Wallis tests (skewed continuous variables), t-tests (normal distributed continuous variables) or chi-square analyses (categorical variables). In study III, Pearson’s correlation coefficients were examined to evaluate the correlations between variables reflecting glucose metabolism and those reflecting obesity.

**Cox proportional hazards regression analyses**

The prognostic values for CHF incidence of a 1-SD increase in the continuous variables, or a transfer from one level to another of the dichotomous variables, were investigated with Cox proportional hazards analyses. In accordance with our a priori analysis plan, missing data were handled such that only participants with a missing covariate needed for that particular model were excluded from the analyses in order to maximize the statistical power. Proportional hazards assumptions were confirmed graphically (study II, III and IV) and by
Schoenfeld’s tests (study IV). Non-linear relations were excluded by examining incidence rates in quartiles of the independent variables. Inspecting CHF incidence rates in quartiles of ESR, an apparent threshold level at the median was observed (Figure 2). Based on this ESR was assessed as a nominal variable, both as four groups (quartiles) and as two groups (above versus below or at the median). In the quartiles models, the lowest CHF incidence was observed in the second quartile of ESR (Figure 2), which was used as reference level. In study II and IV, cumulative hazard curves were established by the Nelson-Aalen estimation method.

![Figure 2. Incidence rates of heart failure by quartiles (quartile 1, ESR=1-3 mm/hr; quartile 2, 4-6; quartile 3, 7-10; quartile 4, 11-83) of erythrocyte sedimentation rate (ESR). Lines indicate 95% confidence intervals.](image)

In study II, which was aimed at identifying novel risk factors for CHF, we used four sets of models in a hierarchical fashion: A) Unadjusted analyses; B) Analyses adjusted for the following established risk factors: prior acute myocardial infarction, hypertension, diabetes, ECG-LVH, smoking, BMI and serum cholesterol; C) Novel variables significant in models A and B were included in a multivariable Cox proportional hazards backward stepwise model together with the established risk factors, in order to evaluate the independency between different novel variables. A level of p<0.05 was used for exclusion; D)
As in model C with addition of interim myocardial infarction during the follow-up time as a baseline covariate.

In study III, examining measures of glucose dysregulation as predictors of CHF, we investigated the independent variables in five sets of models: A) Unadjusted models; B) Models adjusted for diabetes at baseline; C) Models adjusted for diabetes and other established risk factors for CHF (prior acute myocardial infarction, hypertension, ECG-LVH, smoking and serum cholesterol) determined at baseline; D) Covariates as in model C, and interim myocardial infarction; and E) Covariates as in models C, and clamp glucose disposal rate (to examine whether the obesity measures remained predictors of CHF independent of the gold standard measure of insulin sensitivity).

In the study of ESR as a predictor for CHF (study IV), three sets of models were investigated in a hierarchical fashion: A) Unadjusted analyses; B) Multivariable-adjusted analyses using the following baseline covariates: hypertension, diabetes, ECG-LVH, smoking, BMI, serum cholesterol and haematocrit; and C) Covariates as in model B, with the addition of interim myocardial infarction during follow-up. Haematocrit was included as a covariate in models B and C, together with the established risk factors for CHF, to adjust for the red blood cells characteristics, leaving ESR to reflect mainly systemic inflammation.

**Secondary and sub-group analyses**

In study II, all analyses were repeated in a sub-sample without participants with prior myocardial infarction at baseline or myocardial infarction during follow-up. Furthermore, since fasting insulin, proinsulin and split proinsulin were analyzed in only 55% of the cohort due to a freezer failure, we excluded these variables in secondary analyses of models C and D, in order to produce a larger sample.

The models in study III were repeated in a sub-sample with the exclusion of all participants with diabetes at baseline. Furthermore, we examined a sub-sample of non-obese men after exclusion of all men with BMI>30 at baseline and another sub-sample of normal-weight men after exclusion of all men with BMI>25 at baseline.

In study IV, the models were repeated in a secondary analysis after exclusion of all participants receiving treatment with corticosteroids or potentially anti-inflammatory analgesics.
Results

Study I

A total of 317 possible CHF cases were enrolled in the study, based on the hospital discharge register diagnosis. Using the ESC definition, 259 (82%) of the possible hospital discharge register cases were classified as having definite CHF by the review board. The presence of an echocardiographical examination increased the validity to 88%, and the absence of an echocardiographical examination gave a diagnostic validity of 76%. For patients treated at an internal medicine or cardiology clinic the validity was 86% and 91%, respectively.

Figure 3. Validity of the congestive heart failure diagnosis in the hospital discharge register using the European Society of Cardiology definition as gold standard. HDR=hospital discharge register, CHF=congestive heart failure.

Furthermore, if the diagnosis was in the first position in the hospital discharge register (primary diagnosis) the validity was 95%. For pa-
tients treated at an internal medicine or cardiology clinic with a primary diagnosis of CHF, the validity was 96%. The percentages of cases that were classified as definite, questionable or miscoded in different sub-groups are listed in Figure 3.

When examining if the validity had changed over time, we could see that the highest validity was found in the earliest diagnosis dates, and that it had fallen from 88% to 78% during the last decade (Figure 4). This trend was not altered by the presence or absence of echocardiography.

Figure 4. Validity of the congestive heart failure diagnosis in the hospital discharge register over time using the European Society of Cardiology definition as gold standard.
Study II

In unadjusted Cox proportional hazards analyses, all established risk factors for CHF, as well as most examined novel variables (IVGTT b-glucose 60 min, IVGTT s-insulin 60 min, fasting insulin, fasting proinsulin, fasting split proinsulin, HOMA-insulin resistance, HDL cholesterol, LDL cholesterol, apolipoprotein B/A-I-ratio, s-triglycerides, beta-carotene and s-uric acid) were significant predictors of heart failure incidence. When adjusting for established risk factors for CHF (prior acute myocardial infarction, hypertension, diabetes, ECG-LVH, smoking, BMI and serum cholesterol), fasting intact proinsulin, fasting 32-33 split proinsulin, HOMA-insulin resistance, HDL cholesterol, apolipoprotein B/A-I-ratio and beta-carotene remained significant predictors of CHF (Figure 5).

![Graph showing significant predictors of congestive heart failure](image)

*Figure 5.* Significant predictors of congestive heart failure after adjustment for established risk factors (prior acute myocardial infarction, hypertension, diabetes, electrocardiographic left ventricular hypertrophy, smoking, body mass index and serum cholesterol). Boxes are point estimates of multivariable Cox proportional hazards ratios (lines indicate 95% confidence intervals) for a 1-standard deviation increase. HOMA=homeostasis model assessment, HDL=high density lipoprotein.

These variables were included in a multivariable Cox proportional hazards backward stepwise model together with the established risk
factors. In this analysis, fasting proinsulin, apolipoprotein B/A-I-ratio, beta-carotene (protective), hypertension, BMI and ECG-LVH were independent predictors of CHF. When adjusting for the incidence of acute myocardial infarction during follow-up, all these six variables remained independent predictors of CHF (Figure 6, Panel A). Cumulative incidence plots for values above and below the median value of the first three of these variables are presented in Figure 7. Evidence of myocardial infarction during the follow-up was present in 409 of the participants in the total cohort, and in 98 of the 259 CHF cases (38%). In an unadjusted Cox proportional hazards analysis, an interim myocardial infarction was a significant predictor of CHF (hazard ratio [HR] 3.50, 95% confidence interval [CI] 2.72-4.50, p<0.001).

Figure 6. Boxes are point estimates of multivariable Cox proportional hazards ratios (lines indicate 95% confidence intervals) for a 1-standard deviation increase of the continuous variables (fasting proinsulin, apolipoprotein B/A-I-ratio [APOB/APOA-I], beta-carotene and body mass index) and for occurrence versus non-occurrence of dichotomous variables (hypertension, electrocardiographic left ventricular hypertrophy and interim myocardial infarction) as predictors of heart failure incidence in middle-aged men free from heart failure and valvular disease at baseline. Variables shown in Panel A are those who remained independently significant in the multivariable Cox proportional hazards backward stepwise model, including interim myocardial infarction. Panel B represent a sub-sample (n=1912) of participants free from myocardial infarction at baseline and myocardial infarction during follow-up.
As there was a significant interaction between ECG-LVH and interim myocardial infarction, and a borderline significant interaction between hypertension and interim myocardial infarction, we performed a secondary analysis in a sub-sample, excluding persons with myocardial infarction before baseline or during follow-up. In a multivariate Cox proportional hazards analysis in this subgroup using the six independently significant predictors of CHF defined in the total cohort, the point estimates remained essentially the same except for ECG-LVH, but with somewhat wider confidence intervals (Figure 6, Panel B).

In secondary analyses, excluding the insulin-like molecule variables, we obtained essentially the same results as in the previous analyses which included these variables. However, when fasting proinsulin was removed from the multivariable model, HOMA-insulin resistance and smoking were included as significant predictors of CHF in the final model (HR 1.29, 95% CI 1.09-1.53, p=0.004 for a 1-SD increase and HR 1.55, 95% CI 1.10-2.18, p=0.012, for smokers versus non-smokers).

![Graph A. Fasting proinsulin](image)

![Graph B. APOB/APOA-I](image)

![Graph C. Beta-carotene](image)

Figure 7. Nelson-Aalen plots of cumulative incidence of heart failure in the cohort, free from heart failure and valvular disease at baseline, by two groups (above versus below median) of fasting proinsulin (Panel A), apolipoprotein B/A-I-ratio (APOB/APOA-I; Panel B) and beta-carotene (Panel C).
Study III

In unadjusted Cox proportional hazards analyses (models A), all examined variables reflecting impaired glucose regulation and obesity were significant predictors of heart failure incidence (Figure 8).

![Graph showing relationships between heart failure incidence and various variables]

Figure 8. Heart failure incidence in relation to glucometabolic and anthropometric variables in the total cohort of elderly men (n=1187). Boxes are point estimates of Cox proportional hazards ratios (lines indicate 95% confidence intervals) for a 1-standard deviation increase. Data are from the unadjusted models (models A). OGTT=oral glucose tolerance test, HOMA=homeostasis model assessment.

Incidence rates by quartiles of clamp glucose disposal rate are shown in Figure 9. When adjusting for the presence of diabetes (models B), clamp glucose disposal rate, OGTT 2-h glucose, fasting insulin, fasting proinsulin, fasting 32-33 split proinsulin, BMI and waist circumference remained significant. When adjusting also for other established baseline risk factors for CHF (prior acute myocardial infarction, hypertension, diabetes, ECG-LVH, smoking and serum cholesterol; models C), clamp glucose disposal rate, OGTT 2-h glucose, fasting proinsulin, BMI and waist circumference were each significant independent predictors of CHF in separate models (Figure 10). These five variables each remained significant predictors of subsequent CHF, with essentially the same point estimates and confidence intervals, when adding
interim myocardial infarction during follow-up to the covariates (models D).

![Graph showing CHF incidence rate by quartiles of clamp glucose disposal rate](image)

**Figure 9.** Incidence rates of congestive heart failure (CHF) by quartiles of clamp glucose disposal rate, reflecting insulin sensitivity (quartile 1, clamp glucose disposal rate=0.70-3.67 mg/kg body weight/min; quartile 2, 3.68-5.06; quartile 3, 5.07-6.59; quartile 4, 6.60-11.64). Lines indicate 95% confidence intervals.

In unadjusted Cox proportional hazards analyses (models A) in the sub-sample excluding participants with diabetes, clamp glucose disposal rate, OGTT 2-h glucose, fasting proinsulin, fasting 32-33 split proinsulin, BMI and waist circumference were significant predictors of CHF incidence. When adjusting for established risk factors for CHF (models C), clamp glucose disposal rate, fasting proinsulin, fasting 32-33 split proinsulin, BMI and waist circumference remained significant predictors of CHF in separate models. These variables, except for fasting 32-33 split proinsulin, remained significant predictors of subsequent CHF, with essentially the same confidence intervals and point estimates, when adding interim myocardial infarction to the covariates (models D).
Figure 10. Heart failure incidence in relation to glucometabolic and anthropometric variables in the total cohort of elderly men (n=1187). Boxes are point estimates of multivariable Cox proportional hazards ratios (lines indicate 95% confidence intervals) for a 1-standard deviation increase. Data are adjusted for established risk factors (prior acute myocardial infarction, hypertension, diabetes, electrocardiographic left ventricular hypertrophy, smoking and serum cholesterol) at baseline (models C). OGTT=oral glucose tolerance test.

When repeating the unadjusted Cox proportional hazards analyses (models A) in the sub-sample of non-obese men, clamp glucose disposal rate, fasting glucose, OGTT 2-h glucose, fasting proinsulin, BMI and waist circumference were significant predictors of CHF incidence. When adjusting for the presence of diabetes (models B), clamp glucose disposal rate, OGTT 2-h glucose, BMI and waist circumference remained significant. When adjusting also for other established risk factors for CHF (models C), only clamp glucose disposal rate remained a significant predictor of CHF (HR 0.74, 95% CI 0.56-0.98, for a 1-SD increase). Clamp glucose disposal rate remained a significant predictor of subsequent CHF in this sub-sample also when adding interim myocardial infarction to the covariates (models D; HR 0.73, 95% CI 0.55-0.97). We also examined a sub-sample of normal weight men (excluding all participants with BMI>25, n=754), but this left us with a too small sample (433 participants, 23 cases) to draw any firm conclusions. Nevertheless, the point estimates for clamp glucose disposal rate re-
mained similar, but with wider confidence intervals due to low statistical power (HR 0.78, 95% CI 0.51-1.18, in the unadjusted model [model A] and HR 0.75, 95% CI 0.45-1.24, in the model adjusted for established risk factors [model C]). The variables describing impaired glucose regulation and obesity, were highly correlated (Pearson's correlation coefficients r=-0.60, p<0.001 for clamp glucose disposal rate versus both BMI and waist circumference). In models including obesity variables, established risk factors and clamp glucose disposal rate (models E), the obesity variables were no longer significant (HR 1.17, 95% CI 0.92-1.50, p=0.20 and HR 1.18, 95% CI 0.91-1.53, p=0.21, for a 1-SD increase in BMI and waist circumference, respectively). When performing the same analyses (models E) in the sub-sample excluding participants with diabetes at baseline (HR 1.17, 95% CI 0.90-1.53, p=0.25 and HR 1.18, 95% CI 0.88-1.57, p=0.26, for a 1-SD increase in BMI and waist circumference, respectively) and in the sub-sample with non-obese men (HR 1.09, 95% CI 0.81-1.47, p=0.55 and HR 1.16, 95% CI 0.86-1.57, p=0.34, for a 1-SD increase in BMI and waist circumference, respectively) the same patterns were observed.
Study IV

In unadjusted Cox proportional hazards analyses, ESR was significantly associated with CHF incidence, with the highest hazard ratio observed in the highest quartile of ESR compared to the reference level (Figure 2 and Table 1, middle column). Also an ESR above median was a predictor of future CHF, compared to an ESR below or at the median (Table 1, middle column). A cumulative CHF incidence plot for ESR split by the median is presented in Figure 11. When evaluating ESR as a diagnostic test for future CHF (ESR above median [>6 mm/hr] considered to be a positive test), the sensitivity was 48%, specificity 57%, positive predictive value 13% and the negative predictive value was 89%. As a comparison, we calculated the corresponding values for hypertension (sensitivity 57%, specificity 59%, positive predictive value 16%, and negative predictive value 91%).

Adjusting for established risk factors for CHF (hypertension, diabetes, electrocardiographic left ventricular hypertrophy, smoking, BMI, serum cholesterol) and haematocrit, ESR remained a significant predictor of CHF in both the dichotomous and the quartile Cox proportional hazards models (Table 1, right column).

Table 1. Heart failure incidence in relation to erythrocyte sedimentation rate in the total sample (n=2314) free from heart failure, myocardial infarction and valvular disease at baseline, unadjusted and adjusted for established risk factors.

<table>
<thead>
<tr>
<th>Quartiles of ESR</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First quartile (ESR=1-3 mm/hr)</td>
<td>1.09 (0.79-1.51)</td>
<td>1.14 (0.82-1.60)</td>
</tr>
<tr>
<td>Second quartile (ESR=4-6 mm/hr)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Third quartile (ESR=7-10 mm/hr)</td>
<td>1.28 (0.93-1.77)</td>
<td>1.34 (0.97-1.86)</td>
</tr>
<tr>
<td>Fourth quartile (ESR=11-83 mm/hr)</td>
<td>1.49 (1.06-2.08) *</td>
<td>1.46 (1.04-2.06) *</td>
</tr>
</tbody>
</table>

Split by the median of ESR

Above median (ESR=7-83 mm/hr) 1.31 (1.04-1.66) * 1.31 (1.03-1.67) *

Cox proportional hazards ratios for a transfer from one level to another of ESR in ordinal groups. The level with the lowest incidence rate was used as the reference level. Data are hazard ratios (95% confidence intervals), unadjusted or adjusted for established risk factors (hypertension, diabetes, electrocardiographic left ventricular hypertrophy, smoking, BMI and serum cholesterol) and haematocrit. P-values <0.05 were considered significant. * p<0.05, † p<0.01, ‡ p<0.001. HR=hazard ratio, CI=confidence interval, ESR=erythrocyte sedimentation rate.
Evidence of myocardial infarction during the follow-up was present in 411 of the participants in the total cohort, and in 107 of the 282 CHF cases (38%). When adjusting for interim myocardial infarction in addition to the established baseline risk factors for CHF, an ESR in the highest quartile (compared to quartile 2; HR 1.46, 95% CI 1.04-2.05) and above median (compared to below or at the median; HR 1.35, 95% CI 1.06-1.72) remained significant predictors of future CHF.

In the sub-sample free from corticosteroids and anti-inflammatory analgesics, the results were essentially the same in all models.

Figure 11. Nelson-Aalen plot of cumulative incidence of congestive heart failure in the cohort, free from heart failure, myocardial infarction and valvular disease at baseline, by two groups (above versus below or at the median [ESR<=6 mm/hr]) of erythrocyte sedimentation rate (ESR).
Discussion

In this longitudinal, community-based cohort study, we first investigated the validity of the diagnosis of CHF in the Swedish hospital discharge register. The CHF diagnosis appeared slightly less precise than for other cardiovascular diagnoses. Moreover, we identified several novel variables at age 50, reflecting insulin resistance together with an increased apolipoprotein B/A-1-ratio and a low beta-carotene level to be independent predictors of CHF. To further examine the possible relation between oxidative stress/inflammation and CHF, we continued with a study in which we found ESR, a marker of inflammation, to be a significant predictor of CHF. Finally, in the study from age 70, we found that insulin resistance predicted CHF incidence independently of established risk factors, and that the previously described association between obesity and subsequent CHF may be mediated partly by insulin resistance.

The validity of a diagnosis of congestive heart failure in the Swedish hospital discharge register

In the first study, we examined the validity of the diagnosis of CHF in the Swedish hospital discharge register. Eighty-two percent of the CHF cases in the hospital discharge register were classified as having definite CHF according to the ESC definition. The validity of the hospital discharge register CHF diagnosis was markedly higher in patients treated at an internal medicine or cardiology clinic, or when it was the primary diagnosis. We also found a tendency of decreasing diagnosis validity over time, and that the presence of an echocardiographical examination increased the validity only slightly. To our knowledge, there is no published original study concerning the validity of the CHF diagnosis in any national hospital discharge register, but in a recent preliminary report, the validity of the Scottish hospital discharge codes for CHF was similar to our results. In that report it was also concluded that the ICD codes alone failed to capture many CHF admissions, and that using the hospital codes alone would underestimate the total burden of CHF. Previous validation studies of other CVDs, such
as acute myocardial infarction and stroke have shown a validity of approximately 95% in the hospital discharge registers in different countries, including Sweden. Since CHF is a more complex disease to diagnose, with symptoms and signs that can easily be misinterpreted, this lower validity is not unexpected.

The presence of an echocardiographic examination increased the validity only slightly. This illustrates that CHF is a disease defined by a constellation of clinical symptoms and signs, not just a measurement of left ventricular dysfunction. It may also reflect the clinician’s uncertainty when facing the fact that echocardiographic left ventricular systolic function is normal in one out of two to three elderly patients with definite CHF, in whom diastolic dysfunction may be the primary cause. The CHF diagnosis in patients with a normal systolic function relies more heavily on clinical symptoms and signs, laboratory and x-ray-findings and response to treatment.

Interestingly, diagnostic validity appeared to decrease over the last two decades. The trend was not dependent of the presence of echocardiography. This pattern might be a result of changed routines for examination and investigation of patients in later years, with a greater reliance on echocardiography and lesser use of pulmonary x-ray and clinical picture, which could have diminished the doctors’ ability to correctly diagnose CHF. More likely though, it is a result of less extensive medical records in later years, i.e. not so carefully described signs and symptoms and shorter discharge records. This makes it harder to validate later hospital discharge register cases as definite, since this is a retrospective study. Another possible explanation of the decreasing validity over time is that the study population has become 25 years older during the observation period and that diagnosing CHF is more difficult in elderly people with a higher degree of co-morbidity. However, it should be noted that a longitudinal cohort study may not be the best study design to examine CHF diagnosis validity at different time points. This observation therefore needs to be confirmed using cross-sectional studies at different time points.

Of course, some of the hospital discharge register cases that were classified as questionable could in fact be true CHF cases, if the evidence in the medical records was insufficient. However, in future utilization of this cohort for CHF studies, as in retrospective epidemiological population studies examining etiology and risk factors for CHF generally, it is better to have some false negative cases in the large referent group, than false positive cases in the smaller case group, in order to minimize the effects of misclassification bias. However, in other types of studies, e.g. assessment of the economic burden of CHF or studies of total incidence and prevalence in the community, such high
specificity at the expense of some decrease in sensitivity, may not be preferable.

**Previously established risk factors for CHF**

Previous studies have shown that the predominant causes of CHF are hypertension and coronary heart disease, and this was confirmed in the present study. In our sample, 61% of the participants who developed CHF were hypertensive at baseline and 38% of the cases had a history of myocardial infarction. This corresponds to the observations of other community-based studies. The risk for CHF associated with hypertension is further captured in the ECG-LVH variable, as previously described. Along with hypertension, left ventricular hypertrophy and myocardial infarction, all investigated established risk factors for CHF were significant predictors of CHF also in ULSAM.

**Insulin resistance and the risk of congestive heart failure**

Several previous longitudinal studies have shown an association between diabetes and CHF. In study II, proinsulin, rather than diabetes prevalence, was included in the multivariable models implying that this measure of glucose metabolism carries important risk information beyond the diabetes diagnosis. Likewise, in study III, clamp glucose disposal rate and fasting proinsulin, mainly reflecting insulin resistance, were the strongest glucometabolic predictors of CHF, both when adjusting for diabetes and in a sub-sample free from diabetes. To our knowledge, these are the first studies to demonstrate a relation between milder states of impaired glucose regulation and CHF incidence. Our observations may indicate that the risk for CHF increases already in the sub-clinical phase of impaired glucose regulation that forges clinical manifest diabetes.

In previous studies, signs of impaired glucose regulation have been related to both left ventricular systolic and diastolic dysfunction and left ventricular remodeling. There are numerous possible explanations of the observed relation of insulin resistance to CHF incidence:

1. The formation of advanced glycosylation end-products (AGEs) is greatly accelerated in patients with diabetes, which in the myocardium leads to increased collagen cross-linking and myocardial stiffness. It has been demonstrated that ventricular function can be improved and myocardial stiffness can be reversed when treating diabetic
dogs with a collagen cross-link breaker such as metformin. Moreover, a recent small clinical trial of patients with diastolic heart failure demonstrated decreased left ventricular mass and improvements in left ventricular diastolic filling after treatment with a cross-link breaker.  

2. Insulin may act as a growth factor in the myocardium, which is supported by the experimental observation that sustained hyperinsulinaemia leads to increased myocardial mass and decreased cardiac output in rats.  

3. Hyperinsulinaemia leads to sodium retention, which may decompensate persons with otherwise sub-clinical myocardial dysfunction due to volume expansion. 

4. Hyperinsulinaemia also leads to sympathetic nervous system activation, which is a presumed causal factor for CHF. 

5. Insulin resistance is related to an increased pressor response to angiotensin II and has recently been demonstrated to increase the stimulating effects of angiotensin II on cellular hypertrophy and collagen production in hypertensives, leading to myocardial hypertrophy and fibrosis and likely subsequent CHF. 

6. Proinsulin has previously been shown to be an independent long-term predictor of coronary heart disease. There is some evidence of a direct atherogenic action of the proinsulin-molecule, through coronary microcirculatory changes leading to ischemic injury. Clinical trials with proinsulin in diabetic patients were prematurely terminated due to an increase in myocardial infarctions in participants treated with proinsulin. However, in our study increased proinsulin levels predicted CHF independently of an interim myocardial infarction. Likewise, a prior study of the ULSAM-cohort has demonstrated that high proinsulin levels precede left ventricular systolic dysfunction independently of a myocardial infarction. Thus, non-atherogenic effects of the proinsulin molecule, with direct myocardial effects might also be of importance for development of CHF.

**Inflammation, antioxidants and CHF**

In the last decade, evidence of inflammation as a crucial part of the atherosclerosis process has emerged. Inflammation can be initiated in the vessels as a response to retained and modified low-density-lipoprotein cholesterol, injury and infections. Also other major risk factors for CVD, such as hypertension, diabetes, obesity and smoking, have been associated with a low-grade chronic inflammation. Several markers of inflammation has been found to predict coronary heart disease, and the most studied are IL-6, serum amyloid A protein, and CRP.
In recent years, the role of inflammation in the pathogenesis of CHF has been investigated. Elevated levels of various inflammatory markers have been observed in patients with manifest CHF,\textsuperscript{113,114} and in recent prospective studies raised levels of CRP, TNF-\(\alpha\) and IL-6, markers of cytokine-mediated inflammation, predicted subsequent CHF.\textsuperscript{64,65} Cesari \textit{et al.}\textsuperscript{115} utilized a study sample about as large as ours, whereas Vasan \textit{et al.}\textsuperscript{65} used a smaller sample with slightly more than 700 participants. Both studies had much shorter follow-up (3.6 years and 5.2 years, respectively), and fewer CHF cases (92 and 56 cases) than our study. The participants in these studies were older (mean age 74 and 78 years). The hazard ratios observed for high versus low levels of the studied cytokines in these studies were somewhat higher than for high versus low ESR in our study, but it is difficult to draw any firm conclusions about possible differences in the strengths of associations between the various inflammatory markers and CHF because of the large differences in study design.

CRP, TNF-\(\alpha\) and IL-6 are specific markers of cytokine-mediated inflammation, but provide no information about other (potentially equally or more important) aspects of inflammation. ESR is a less specific marker of systemic inflammation, known to be elevated in many acute and chronic diseases characterized by tissue necrosis and inflammation. ESR is a simple and inexpensive laboratory test, which is in widespread use and easily accessible. In his classic report, Wood observed a low ESR in a small sample of patients with CHF of different origins,\textsuperscript{115} but more recent studies have reported that in patients with manifest CHF a high ESR is associated with a more severe stage of CHF and a worse prognosis.\textsuperscript{113,114} The aforementioned studies have examined the association between ESR and already diagnosed CHF,\textsuperscript{113-115} whereas the present study is the first to examine ESR as a predictor of subsequent incident CHF.

ESR has repeatedly been found to be a predictor of subsequent coronary heart disease in longitudinal studies.\textsuperscript{51-63} Nonetheless, in the present study the association between ESR and CHF remained significant even after adjusting for interim myocardial infarction. This may indicate that inflammation could directly impair myocardial function. Previous studies have shown that proinflammatory cytokines, such as TNF-\(\alpha\), can depress myocardial contractility\textsuperscript{116} and affect left ventricular remodeling through local induction of matrix metalloproteinases.\textsuperscript{117,118} Inflammation can also induce endothelial dysfunction in small vessels,\textsuperscript{119} resulting in an impaired coronary flow reserve and impaired left ventricular function.

Recent studies have shown that chronic low-grade inflammation is associated with insulin resistance.\textsuperscript{120,121} This might be another possible explanation of why inflammation predicts CHF. However, this associa-
tion has to be further confirmed, and the causality between inflammation, insulin resistance and CHF must be established in further studies. Some prior smaller case-control studies have pointed out a relationship between antioxidant levels and the severity of CHF. It is possible that antioxidants, such as beta-carotene, lower the levels of free radicals which are important mediators of oxidative stress and inflammation. However, it is still to be investigated if beta-carotene is an inverse marker of inflammation or possibly itself has anti-inflammatory effects. A recent cross-sectional case-control study has also found that inflammatory markers and a marker of oxidative stress were significantly correlated in CHF participants. Furthermore, a previous report from the ULSAM cohort showed that serum levels of beta-carotene predicted left ventricular diastolic function after 20 years of follow-up. However, no previous longitudinal studies have shown that low serum levels of antioxidants, such as beta-carotene, predict a higher risk of developing CHF, independent of established risk factors. Exactly what beta-carotene is a measure of is uncertain. In addition to being a possible marker of oxidative stress, it might also reflect nutritional intake. The serum level of beta-carotene might be considered a marker for a high vegetable intake, reflecting a healthy life style, which in turn would decrease the risk of CHF regardless of a possible anti-oxidative effect of beta-carotene itself.

Apolipoproteins and future risk for CHF

Several studies have proposed apolipoproteins as a more specific alternative to LDL-cholesterol as a risk marker for CVD. A recent study showed that the APOB/APOA-I-ratio was a powerful independent risk marker of fatal myocardial infarction after adjustment for age, total cholesterol and triglycerides. The reason for this may be that the APOB/APOA-I-ratio also includes information on other atherogenic lipoproteins, such as the APOB containing VLDL, rich in triglycerides, as well as APOA-I containing HDL. As such, the ratio gives comprehensive information on the classical risk of LDL-cholesterol, as well as on other important components of the metabolic syndrome. It is not known how this directly affects cardiac performance independently of myocardial infarction, but recent studies have shown apolipoproteins to be closely linked to endothelial function, a determinant of cardiac afterload. As an increased afterload would influence both left ventricular systolic and diastolic function, effects in the vasculature rather than a direct action on the heart might be the mechanism whereby the APOB/APOA-I-ratio increases the risk for CHF.
Strengths and limitations

The strengths of this study include the large, community-based study sample, the long follow-up period and the few cases lost to follow-up. Furthermore, all CHF cases were validated, limiting the inclusion of false positive cases. Another strength of the study is the detailed metabolic characterization of the cohort. To our knowledge, the ULSAM-cohort is the largest population which has been examined with the gold standard for measurement of insulin resistance, the euglycaemic insulin clamp method. Since the study is based on data derived from the general population, the selection bias is limited. The study population is homogenous, consisting of men of same age and from a well-defined area.

The most obvious limitation of the study is that we only examined men. In the seventies, when the study was initiated, the cardiovascular risk factors were thought to be represented mainly in men, and thus only men were included in the ULSAM-cohort. It is now known, that CVD is the leading cause of mortality in women and a major cause of morbidity. Gender differences in coronary heart disease include a later age of onset for women, a greater prevalence of co-morbid diseases, and differences in the initial manifestations of the disease. As we only examined men of the same age with a similar ethnic background, this study has an unknown generalizability to women or other age- and ethnic groups. On the other hand, we circumvent the powerful effects of age and gender on CHF incidence.

Since the CHF diagnosis was based on a review of medical records, it was not possible to differ between systolic and diastolic heart failure as echocardiography was not available at the time of diagnosis for many of the cases. Thus, in our material it is not possible to examine whether the risk factors are different in systolic and diastolic heart failure, which might be the case.

Ninety percent of the discharges were from the local university hospital; hence the results could be biased by local routines. Moreover, milder non-hospitalized cases of CHF were not included in our endpoint, which would tend to bias the results towards the null hypothesis.

Another possible limitation of the study is that we chose the hospital diagnosis of acute myocardial infarction as a proxy for coronary heart disease. However, as pointed out previously, the precision of the myocardial infarction diagnosis in the Swedish hospital discharge register is high and adjusting for, or excluding, interim myocardial infarction is an established method for examining “non-ischemic” CHF.
As ESR was the only measured inflammatory marker at the time of the study, it was not possible to directly compare ESR and other inflammatory markers as predictors of CHF in this study.

A remark should also be made about the "healthy cohort effect" that usually is associated with population-based cohort studies. It is probable that the subjects that participate in this sort of cohort study are somewhat healthier than those that choose not to attend. This may lead to some underestimation of the associations found in our study, and drive the results towards the null hypothesis.
Future perspectives

This study has examined the validity of the CHF diagnosis and has identified several novel risk factors for CHF, with a focus on insulin resistance and inflammation. Further research is needed for confirmation of our findings and to determine potential gender, age and ethnic differences.

The primary aim of this thesis was to identify potentially modifiable risk factors for CHF, in order to be able to prevent new-onset CHF. Most interventional studies in the area of CHF have compared different treatments against placebo in patients with already diagnosed CHF in order to evaluate the prognosis. There are indeed very few studies examining how a specific intervention affects the risk for incident CHF, especially studies with incident CHF as the primary outcome and with sufficient power to assess this. Thus, there is a call for interventional studies of both established and novel risk factors for CHF, and these should preferably be carried out in the primary preventive setting, aiming at lifestyle intervention.

In order to characterize our novel risk factors further, it would naturally be interesting to examine whether they differ for systolic and diastolic heart failure. Left ventricular systolic function is normal in a large proportion of CHF patients, and diastolic heart failure is more frequent in the elderly than the younger and in women than men. Apart from age and sex, the previously known risk factors for diastolic heart failure are similar to those for systolic heart failure. Hypertension is the most common cause, and diabetes and obesity are also important risk factors for diastolic heart failure. Prior coronary heart disease is less common in diastolic than in systolic heart failure. In our material we could unfortunately not examine whether the risk factors are different in systolic and diastolic heart failure, which is possible, or even probable. To do this, one would have to examine all participants that are diagnosed with heart failure with echocardiography at the time of diagnosis, to assess their systolic function. This was not possible to do in this study, since the cases were validated retrospectively, and all cases had not been examined with echocardiography.

In this thesis, the presence of an interim myocardial infarction during follow-up was used as a proxy for coronary heart disease. Even if
Inhibiting insulin resistance – a new therapeutical possibility for treatment of heart failure?

In this study, we have shown that insulin resistance is a risk factor for CHF, independent of established risk factors, including obesity and diabetes. Consequently, it might be possible to restrain the development of CHF by inhibiting insulin resistance. In order to evaluate this, intervention studies addressing the issue should be performed. As outlined in the previous section regarding suggested mechanisms, there are numerous possible explanations for the observed relation of insulin resistance to CHF incidence and these explanations also offer possible points of attack for intervention.

Blocking of neurohormonal systems

As described above, insulin is known to stimulate the renin-angiotensin-aldosterone and sympathetic nervous systems and increased activity in these systems has been suggested to be causal factors for CHF. These neurohormonal systems are central in the pathophysiology of CHF, and are considered to have a fundamental role in the development and subsequent progression of chronic heart failure. Treatment for CHF, according to current guidelines, largely depend on inhibiting these two systems by using ACE inhibitors, angiotensin II inhibitors and beta blockers. These classes of drugs have been extensively investigated in large randomized clinical trials (Table 2). The efficiency of these drugs has been proven to be high, both in patients with presence of CHF – by reducing symptoms, hospitalizations and mortality – and by reducing the risk of development of CHF in a secondary preventive setting.
The inhibitors of the renin–angiotensin-aldosterone and sympathetic systems have also been demonstrated to affect the insulin sensitivity and the risk of new-onset diabetes. Several ACE inhibitors and angiotensin II inhibitors have been found to decrease the risk of diabetes, which have been attributed to improvements in peripheral insulin sensitivity and glucose metabolism. \(^{195}\) Depending on the receptor specificity of the individual agent, beta blockers have varying effects on glucose metabolism, as well as on the risk for developing new-onset diabetes. \(^{196}\)

However, it still remains to be evaluated if reducing the insulin resistance is part of the mechanism by which these drugs exert their positive effects in CHF patients, both in diabetics and non-diabetics, as well as in obese and non-obese. Moreover, it should be examined if these drugs could be used to prevent the development of CHF in insulin resistant participants, with normal ventricular function and no signs of CHF at baseline.
Table 2. Large randomized, placebo controlled trials examining survival when treating congestive heart failure by blocking neurohormonal systems.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>NYHA</th>
<th>Year</th>
<th>Size</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Angiotensin converting enzyme (ACE) inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONSENSUS</td>
<td>Enalapril</td>
<td>IV</td>
<td>1987</td>
<td>n=253</td>
<td>Enalapril significantly reduced mortality and improved symptoms as compared to placebo.131</td>
</tr>
<tr>
<td>V-HeFT II</td>
<td>Enalapril</td>
<td>I-IV</td>
<td>1991</td>
<td>n=804</td>
<td>Better survival in patients randomized to enalapril compared with hydralazine plus isosorbide dinitrate.132</td>
</tr>
<tr>
<td>SOLVD</td>
<td>Enalapril</td>
<td>I-IV/</td>
<td>1991/ n=2569/ I-II</td>
<td>n=4228</td>
<td>Significantly reduced mortality and hospitalizations for CHF as compared to placebo133 and reduced incidence of CHF in patients with asymptomatic systolic dysfunction.134</td>
</tr>
<tr>
<td>SAVE</td>
<td>Captopril</td>
<td>I</td>
<td>1992</td>
<td>n=2231</td>
<td>Improvement in survival and reduced morbidity in patients with asymptomatic left ventricular dysfunction after myocardial infarction.135</td>
</tr>
<tr>
<td>AIRE</td>
<td>Ramipril</td>
<td>I-III</td>
<td>1993</td>
<td>n=1986</td>
<td>Ramipril to patients with acute myocardial infarction and CHF resulted in a substantial reduction in premature death from all causes.136</td>
</tr>
<tr>
<td>TRACE</td>
<td>Trandolapril</td>
<td>I-IV</td>
<td>1995</td>
<td>n=1749</td>
<td>Significantly reduced risk of mortality and morbidity in patients with reduced left ventricular function after myocardial infarction.137</td>
</tr>
<tr>
<td><strong>Angiotensin II inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELITE-II</td>
<td>Losartan</td>
<td>II-IV</td>
<td>2000</td>
<td>n=3152</td>
<td>No significant differences in mortality between the groups receiving losartan and captopril, but less adverse effects for the first group.138</td>
</tr>
<tr>
<td>Val-HeFT</td>
<td>Valsartan</td>
<td>II-IV</td>
<td>2001</td>
<td>n=5010</td>
<td>Valsartan reduced mortality and morbidity when added to prescribed therapy. However, concerns about the safety of a combination of valsartan, an ACE inhibitor, and a beta-blocker were raised.139</td>
</tr>
<tr>
<td>OPTIMAAL</td>
<td>Losartan</td>
<td>II-IV</td>
<td>2002</td>
<td>n=5477</td>
<td>A non-significant difference in total mortality in favor of captopril was found. However, losartan was better tolerated than captopril.140</td>
</tr>
<tr>
<td>VALIANT</td>
<td>Valsartan</td>
<td>I-IV</td>
<td>2003</td>
<td>n=14703</td>
<td>Valsartan was as effective as captopril in CHF subjects after a myocardial infarction, but combining valsartan with captopril increased adverse events without improving survival.141</td>
</tr>
<tr>
<td>Study</td>
<td>Treatment</td>
<td>Stage</td>
<td>Year</td>
<td>n</td>
<td>Notes</td>
</tr>
<tr>
<td>-----------</td>
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<td>---------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>CHARM</td>
<td>Candesartan</td>
<td>II-IV</td>
<td>2003</td>
<td>n=7601</td>
<td>Significantly reduced cardiovascular mortality and morbidity as compared to placebo, also when added to treatment with ACE inhibitors. Well tolerated in subjects with ACE inhibitor intolerance. Moderate impact in preventing hospitalization among subjects with diastolic heart failure, but no effect on mortality.</td>
</tr>
<tr>
<td><strong>Beta blockers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIBIS I</td>
<td>Bisoprolol</td>
<td>III-IV</td>
<td>1994</td>
<td>n=641</td>
<td>A non-significant trend towards improved survival.</td>
</tr>
<tr>
<td>Carvedilol (US)</td>
<td>Carvedilol</td>
<td>II-IV</td>
<td>1996</td>
<td>n=1094</td>
<td>Carvedilol superior to placebo for morbidity and mortality.</td>
</tr>
<tr>
<td>ANZ trial</td>
<td>Carvedilol</td>
<td>I-III</td>
<td>1997</td>
<td>n=415</td>
<td>Reduced mortality and morbidity, and beneficial effects on ventricular function and size in patients with CHF due to ischemic heart disease.</td>
</tr>
<tr>
<td>CIBIS II</td>
<td>Bisoprolol</td>
<td>III-IV</td>
<td>1999</td>
<td>n=2647</td>
<td>Bisoprolol therapy had benefits for survival in stable heart-failure patients, superior to placebo.</td>
</tr>
<tr>
<td>MERIT-HF</td>
<td>Metoprolol</td>
<td>II-IV</td>
<td>1999</td>
<td>n=3991</td>
<td>Metoprolol once daily in addition to optimum standard therapy improved survival.</td>
</tr>
<tr>
<td>COPERNICUS</td>
<td>Carvedilol</td>
<td>IV</td>
<td>2001</td>
<td>n=2289</td>
<td>Carvedilol superior to placebo for morbidity and mortality also in patients with severe chronic CHF.</td>
</tr>
<tr>
<td>CAPRICORN</td>
<td>Carvedilol</td>
<td>I-IV</td>
<td>2001</td>
<td>n=1959</td>
<td>Significantly reduced mortality and morbidity in patients with ventricular dysfunction after myocardial infarction.</td>
</tr>
<tr>
<td>BEST</td>
<td>Bucindolol</td>
<td>III-IV</td>
<td>2001</td>
<td>n=2708</td>
<td>No significant overall survival benefit, but significantly decreased cardiovascular mortality and hospitalization as secondary end-points.</td>
</tr>
<tr>
<td>COMET</td>
<td>Carvedilol-metoprolol</td>
<td>II-IV</td>
<td>2003</td>
<td>n=3029</td>
<td>The results suggested that carvedilol extends survival compared to metoprolol.</td>
</tr>
</tbody>
</table>

Placebo groups received appropriate conventional treatment, according to guidelines at the time of the study. Year refers to publication year for the main results of the study. NYHA=Classification of the New York Heart Association (I=no symptoms, II=mild, III=moderate, IV=severe).
Breaking cross-links formed by AGEs

Another suggested link between insulin resistance and CHF that might be able to affect with drugs is the “AGE:s pathway”. The formation of AGEs is known to be greatly accelerated in patients with diabetes. AGE cross-links generally impair the normal function of proteins, cells and organs. In the cardiovascular system, their presence within the vascular wall and myocardium increase the collagen cross-linking, which leads to the development of vascular and ventricular stiffness. If these cross-links should be broken by drugs, perhaps the stiffness could be reversed.

A recent trial demonstrated decreased cross-linking of collagen and myocardial stiffness after treating diabetic dogs with metformin. Metformin is considered to be contraindicated in patients with chronic heart failure, due to the risk of lactate acidosis. However, recent studies have pointed out that the use of metformin in patients with heart failure and diabetes is common and that it is increasing rapidly, in direct contrast to explicit warnings by health authorities, like the Food and Drug Administration (FDA) in the US. Moreover, a recent study has evaluated the safety of continued use of metformin in patients with CHF, and it concluded that there was no reason for discontinuation of using metformin for these patients. Nevertheless, even though extraordinarily interesting, it would be questionable to perform a randomized clinical trial to evaluate metformin as a treatment in patients with chronic CHF, without performing larger safety studies with a long follow-up time first. Already in a UK Prospective Diabetes Study (UKPDS 34) report from 1998, metformin was shown to be associated with considerable risk reductions for diabetes-related endpoints including CHF, in a sample of overweight diabetic patients, as compared to other anti-diabetic medications. Further studies have confirmed that metformin may offer some protection from incident CHF when compared to treatment with sulphonylurea, insulin and thiazolidinediones.

A more specific approach to decrease the myocardial stiffness would be to treat CHF patients with AGE cross-link breakers. These have repeatedly been demonstrated to reduce collagen and myocardial stiffness, and improve cardiac function in rats, dogs and monkeys. Moreover, a recent small clinical trial including 23 elderly patients with diastolic heart failure has demonstrated decreased left ventricular mass and improved ventricular diastolic filling and quality of life after 16 weeks of treatment with ALT-711. These studies are promising, but there is obviously a need for larger, randomized clinical trials before this could lead to any changes in the therapy of CHF.
Increasing insulin sensitivity with insulin sensitizers

The most established insulin sensitizing drug is probably metformin, for which promising results regarding heart failure prevention in diabetics have been shown, as described in the previous section.

A more recently introduced class of oral anti-diabetic drugs that also enhance the insulin sensitivity are the thiazolidinediones (or glitazones). They increase the insulin sensitivity, induce adipogenesis and suppress the expression of cytokines via activation of peroxisome proliferator-activated receptor (PPAR) gamma. Initially, in animal studies and small human samples, positive effects of glitazones on the ventricular function were reported. Glitazones were demonstrated to have positive inotropic effects in rat hearts and to improve left ventricular remodelling and function in mice with heart failure after a myocardial infarction. Further, troglitazone was shown to reduce left ventricular mass and to improve diastolic function in a small sample of patients with diabetes. On the other hand, another animal study showed that the examined glitazone increased mortality after a myocardial infarction in rats, without positive effects on the remodeling.

Recent studies have set focus on the risk of fluid retention, which has been found to be increased in CHF patients treated with glitazones. This is a potentially dangerous adverse effect of this class of drugs, even if it usually is reversible after drug withdrawal. Moreover, a recent large, retrospective cohort study has shown that glitazones increase the risk of incident CHF in patients free from heart failure at baseline. However, this could be caused by subjects having sub-clinical heart failure at baseline, becoming symptomatic in higher degree in patients treated with glitazones, due to the fluid retention.

In the latest consensus statement from the American Heart Association and American Diabetes Association, it was concluded that glitazones can be used in diabetic patients without symptomatic CHF, and with caution in heart failure patients with NYHA class I and II. Further, it dissuades the use in NYHA III and IV patients. It is still not finally elucidated whether the glitazones do modify the risk of incident CHF and/or worsen the CHF symptoms and prognosis. However, there are several large clinical trials currently underway, and hopefully they will be able to answer some of these questions.

Antioxidant and anti-inflammatory therapy – possible approaches against congestive heart failure

In this study, we have shown that inflammation measured as erythrocyte sedimentation rate and a low beta-carotene level, which might be
considered as a marker of oxidative stress, are significant predictors of CHF, independent of established risk factors including an interim myocardial infarction.

Inflammation is nowadays recognized as an important part of the pathophysiology of the atherosclerosis. Recent years, inflammation as a part of the CHF pathophysiology, possibly independent of arteriosclerosis, has been suggested. As ESR is a simple and inexpensive laboratory test, which is in widespread use and easily accessible, it would be interesting to examine whether it could be used as a risk marker for CHF in clinical practice. To do this, further studies with comparisons of different markers of inflammation should be done.

**Antioxidant supplementation**

Observational studies, including descriptive, case-control and cohort studies, have repeatedly shown that antioxidants beneficially affect the risk for CVD. This is also supported by in vitro studies, showing that oxidative processes are important in the development of the atherosclerotic plaque. As a consequence, in the past years, a large number of interventional studies examining the effects of antioxidant supplements on CVD risk has been performed. In summary, most clinical trials have failed to show any beneficial effect of antioxidant supplements on CVD mortality and morbidity. However, some studies have shown beneficial effects of vitamin E (alpha-tocopherol) and vitamin E and C combined. On the other hand, there are also studies showing adverse effects of treatment with vitamin E, beta-carotene and antioxidant cocktails with increasing CVD mortality and morbidity. In general, studies showing positive or negative effects are smaller than the large clinical trials that consistently have shown no effect at all of antioxidant supplementation on different CVD endpoints.

Almost all clinical trials evaluating antioxidant supplementation have focused on coronary heart disease and stroke as endpoints, but much less is known about antioxidants as a possible prevention for CHF. In fact, the only larger antioxidant study that has examined CHF as an endpoint is the HOPE trial, which evaluated effects of long-term vitamin E supplementation on cancer and CVD. In this study, patients in the vitamin E group had a higher risk of CHF (relative risk 1.13, 95% confidence interval 1.01-1.26) and hospitalization for CHF (relative risk 1.21, 95% confidence interval 1.00-1.47). As CHF was only a secondary study outcome, and the results were borderline significant, this finding may just be due to chance. However, the finding was consistent and present in all pre-specified analyses, and therefore it is disturbing and must be further investigated.
The disappointing results from these clinical trials do not necessarily rule out a role for oxidative stress in the pathophysiology of CVD and antioxidants as a possible treatment for CVD in general and CHF in particular. Antioxidants should not be lumped together, since they differ both quantitatively and qualitatively. We still do know too little about the oxidative mechanisms in vivo to evaluate the best candidate antioxidative compounds. The selection of different forms of antioxidants might be of importance, as the natural forms of antioxidants in food may have different biological activity or potency compared to the synthetic compounds used in supplements. Furthermore, other naturally occurring antioxidants and micronutrients may accompany the intake of antioxidant-rich foods. As dietary intake of vitamin E, vitamin C and beta-carotene – the compounds evaluated in the large interventional studies – only contribute to a small portion of the total intake of dietary antioxidants, it is possible that it is the combination of antioxidants or other unknown factors found in antioxidants rich foods that are protective rather than an effect of a specific antioxidant.

Furthermore, antioxidant supplementation might need to start earlier in life and be used for a longer time period in order to be effective. This could be an explanation of the discrepancy between the longer epidemiological studies and the clinical trials that typically have a five-year follow-up period. That possibility is demonstrated in an illustrative way in our study II (Figure 7, panel C), where the lag-time before the incidence curves start to separate is more that 10 years. Another substantial problem with earlier studies is that there are no studies that have measured the baseline plasma levels of antioxidants, and used this to group the subjects into those with normal levels, and those with low levels. Such an approach could investigate if subjects with low levels of antioxidants at baseline would benefit from antioxidant supplementation, which is possible. One study that did something like this, only used the serum levels in a post-hoc analysis, and therefore it is difficult to draw firm conclusions from it.185

Thus, there is still a call for randomized clinical trials evaluating antioxidants as a preventive treatment for cardiovascular diseases, and especially for CHF, since this is a largely unexplored area. The follow-up time need to be longer than previous studies, and the serum levels of the antioxidant should be measured at baseline and be used to group the subjects.

Anti-inflammatory drugs

Since chronic inflammation seems to be an important part of the pathophysiology of CHF, both for incident CHF and for outcome in CHF patients, anti-inflammatory therapy against CHF has attracted
many investigators. Traditional treatment against CHF such as ACE inhibitors,\textsuperscript{186} angiotensin II inhibitors\textsuperscript{186} and beta blockers\textsuperscript{187} has been shown to have some anti-inflammatory effects. Nevertheless, these effects have been rather modest, stressing the need for more specific immunomodulating treatments.

As TNF-\(\alpha\) seems to be central in the pathophysiology of CHF, therapeutic modulation of this cytokine has been in focus. The first reports of TNF-\(\alpha\) inhibition with a soluble TNF receptor (etanercept) was promising,\textsuperscript{188} but two larger trial programs testing etanercept in CHF patients have been halted due to lack of beneficial effects.\textsuperscript{189,190} However, these trials had several limitations, and this approach of treating CHF is not fully elucidated.\textsuperscript{191}

Glucocorticoids are widely prescribed anti-inflammatory drugs. They have been shown to be associated with fluid retention, which is induced by an enhanced sodium reabsorption and expansion of the extracellular fluid volume, and therefore traditionally is not recommended to CHF patients. One would expect that the anti-inflammatory actions in the vessels possibly could balance these negative effects. However, on the contrary, a recent nested case-control study identified oral glucocorticoid use as a risk factor for CHF.\textsuperscript{192} Still, there are no randomized controlled trials with glucocorticoid treatment in CHF patients (or for any other CVD endpoints), and only such studies could definitely disentangle this.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most common class of anti-inflammatory drugs, but they do not seem to be a possible choice for an anti-inflammatory approach against CHF. On the contrary, they have been associated with an increased rate of hospitalization for CHF in several studies.\textsuperscript{193-195} NSAIDs inhibit the enzyme cyclooxygenase (COX), which results in a decrease in prostaglandin synthesis. Prostaglandins have an important role in renal physiology, and inhibition of their synthesis may give rise to fluid retention. Fluid retention caused by NSAIDs may adversely affect cardiovascular homeostasis, and patients with susceptibility for congestive heart failure seem to be particularly susceptible to the cardiovascular effects of NSAIDs.\textsuperscript{193} Moreover, NSAIDs may interfere with the cardiovascular effects of angiotensin-converting enzyme inhibitors and diuretics. However, in a recent study, the use of NSAIDs was not associated with an increased risk of incident heart failure.\textsuperscript{193}

Even though aspirin is an old and widely used drug in patients with CVD, it has not been evaluated in CHF patients until recently. It could be expected that it would be beneficial, since it has anti-thrombotic properties in addition to the anti-inflammatory effect. However recently, it was suggested that aspirin is neither effective nor safe in patients with CHF, and rather contributed to polypharmacy.\textsuperscript{196}
Recent reports have provided convincing evidence that the use of COX-2 inhibitors increase the risk of serious cardiovascular events, including CHF. In the United States, the FDA recently chose to allow the medications to continue to be sold in the United States, although they recommended placing black-box warnings on their labels and instituting other measures to severely restrict their use. However, there are studies that imply that the risk for incident CHF may differ between different COX-2 inhibitors.

HMG-CoA reductase inhibitors (statins) have been shown to beneficially affect outcomes in chronic heart failure due to anti-inflammatory effects, irrespective of the lipid-lowering statin effects. The positive effects of statins were first shown in observational studies, and they have been further confirmed in small randomized clinical studies. In these, short-term statin therapy in patients with CHF has been showed to improve symptoms and cardiac function, as well as to decrease plasma concentrations of TNF-α, IL-6, soluble vascular cell adhesion molecule-1 (sVCAM-1) and brain (B-type) natriuretic polypeptide. This suggests that statins may have therapeutic benefits in patients with CHF irrespective of serum cholesterol levels, and that the mechanism possibly includes anti-inflammatory effects.

In conclusion, anti-inflammatory therapy against CHF has not been very successful so far, but there are still some approaches that have not been sufficiently investigated. In particular, treatment against specific parts of the inflammation process, like the trials with TNF-α inhibition with a soluble TNF receptor could be successful in the future. Also statin treatment is much promising, and the next step is large randomized controlled trials, evaluating these drugs in CHF patients, and in the future perhaps also as primary preventive therapy against incident CHF.
Conclusions

The validity of the CHF diagnosis in the Swedish hospital discharge register appears less precise than for other recently investigated cardiovascular diagnoses, such as acute myocardial infarction and stroke, at least when including all clinics and all diagnosis positions. However, when including only cases from internal medicine and cardiology clinics or cases with a primary diagnosis of CHF, the validity is comparable to the above diagnoses. Our findings imply that for population-based research, only those with a primary diagnosis of CHF in the hospital discharge register should be regarded as definite CHF cases, or alternatively the cases should be validated individually.

Insulin resistance was found to predict CHF incidence independently of established risk factors in both middle-aged and elderly men. The previously described association between obesity and subsequent CHF may be mediated partly by insulin resistance.

Inflammation, measured as erythrocyte sedimentation rate was demonstrated to be a significant predictor of CHF, independent of established risk factors, including an interim myocardial infarction.

Previously established risk factors for CHF were confirmed as risk factors also in our cohort, and a low beta-carotene level, as well as an increased apolipoprotein B/A-I-ratio, was found to predict CHF independently of these established risk factors.

Further research is needed to confirm our findings and to determine potential gender, age and ethnic differences. If confirmed, our observations could have large clinical implications as they may offer new approaches in the prevention of CHF.
Summary in Swedish
(Sammanfattning på svenska)

Hjärtsvikt är en viktig orsak till sjuklighet och död i världen, och sökandet efter modifierbara riskfaktorer är centrale för att minska lidandet av denna folksjukdom.

Huvudmålet med denna avhandling var att undersöka nya metabola riskfaktorer för hjärtsvikt, med fokus på insulinresistens och inflammation. Det sekundära målet var att undersöka hur hög validitet hjärtsviktsdiagnosen har i svenska slutenvårdsregistret.


Avhandlingen visar också att validiteten av hjärtsviktsdiagnosen i svenska slutenvårdsregistret tycks vara mindre än för andra kardiovasculära sjukdomar, som hjärtinfarkt och stroke. Om man bara inkluderar fall från internmedicinska och kardiologiska kliniker eller bara fall med hjärtsvikt som huvuddiagnos, är validiteten jämförbar med ovan nämnda diagnoser.

Sammanfattningsvis visar denna avhandling att insulinresistens och inflammation är starka och oberoende riskfaktorer för utveckling av hjärtsvikt, och de förefaller vara inblandade i den tidiga processen som leder fram till hjärtsvikt. Om fynden kan bekräftas i andra studier kan de komma att ha stor klinisk betydelse, eftersom de kan erbjuda nya sätt att arbeta preventivt för att förhindra uppkomsten av hjärtsvikt.
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The cover illustration of a failing heart is a picture by the medical illustrator Audra Geras. It was originally created as a cover painting for a magazine called Illustrated Medicine. The particular issue was focused on congestive heart failure and she designed it to represent some of the clinical features seen in patients with this condition. It is used with her very kind permission.
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