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SCREENING for CARDIOVASCULAR RISK and DIABETES in PRIMARY HEALTH CARE

The Söderåkra Risk Factor Screening Study

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To think I did all that;
I faced it all and I stood tall;
And did it my way.

Paul Anka

CONTENTS

ABSTRACT

Background. Cardiovascular disease (CVD) has been the predominant cause of morbidity and mortality for many decades in Sweden. Preventive work in primary health care through individual approach and community-based programmes has shown some success. Still, we need better risk assessment tools and health strategies to lessen the burden of CVD in our population.

Methods. This thesis is based on four studies that explore the cardiovascular risk factor pattern and its development to CVD morbidity and mortality in the middle-aged (40-59 years) population in Söderåkra, southern Sweden, 1989-2006. At a single physician consultation in 1989-1990 the participants provided information about lifestyle in a self-administered questionnaire, underwent a physical examination and received medical advice after a laboratory investigation. The laboratory tests consisted mainly of blood glucose, serum lipids and thyroid function tests. Blood samples were also frozen for later analyses. A telephone interview on self-reported lifestyle changes was conducted ten years later. In 2006, primary health care medical records were studied for incident diabetes and also for impaired glucose tolerance (IGT). Finally, national registers were studied for incident fatal or non-fatal cardiovascular disease until 2006. Cardiovascular risk assessments using three separate risk algorithms were applied on the population.

Results. The participation rate was high with 90% attendance. The conclusion of this cross-sectional baseline analysis was that it is meaningful to check for a secondary cause of hyperlipidemia, hypothyroidism, in women with a cholesterol value above 7.0 mmol/L. After 10 years follow-up women reported significantly more lifestyle changes than men, odds ratio (OR) 1.56 (95% CI: 1.11-2.18; p= 0.010). Men with a history of smoking or CVD at baseline and women with treated hypertension at baseline made successful lifestyle changes, OR 4.77 (95% CI: 2.18-10.5; p<0.001 and OR 1.84 (95% CI: 1.12-3.02; p= 0.016), respectively, than those without these characteristics.

Until 2006, 38 participants had developed diabetes and four subjects IGT out of 664 participants, excluding 10 with diabetes at baseline. A low level of IGFBP-1 at baseline was associated with the development of type 2 diabetes/IGT, hazard ratio (HR) 3.54 (95% CI: 1.18-10.6, p=0.024). This was independent of abdominal obesity or inflammation (CRP). After excluding 16 participants with prevalent CVD at baseline, 71 first fatal or nonfatal CVD events in 689 men and women were registered. Several known risk factors and risk markers were applied on this population.

Those that turned out to be significantly associated with development of incident CVD in univariate Cox´s regression proportional hazard analyses where used in three different risk assessment models: the consultation model, SCORE and the extensive model. A non-laboratory-based risk assessment model, including variables easily obtained during one consultation visit to a general practitioner (GP), predicted cardiovascular events as accurately, HR 2.72; (CI 95% 2.18-3.39, p<0.001), as the established SCORE algorithm, HR 2.73; (CI 95% 2.10-3.55, p<0.001), which requires laboratory testing. Furthermore, adding laboratory measurements covering lipids, inflammation and endothelial dysfunction, did not confer any additional value to the prediction of CVD risk, HR 2.72; (CI 95% 2.19-3.37, p<0.001). The c-statistics for the consultation model (0.794; CI 95% 0.762-0.823) was not significantly different from SCORE (0.767; CI 95% 0.733-0.798, p=0.12) or the extended model (0.806; CI 95% 0.774-0.835, p=0.55).

Conclusions. Our study showed that it is worth searching for hypothyroidism, in women with a cholesterol value above 7 mmol/L. The study identified female gender, previous CVD, hypertension and smoking as predictors of positive lifestyle change during follow-up. A low level of IGFBP-1 predicted future diabetes/IGT in this population as did increased waist and CRP. Finally, data on non-laboratory risk factors obtained during one GP visit predicted future cardiovascular risk as accurately as SCORE or a laboratory-based risk algorithm.

LIST OF PAPERS

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

I. Petersson U, Kjellström T. Thyroid function tests, serum lipids and gender interrelations in a middle-aged population. ***Scand J Prim Health Care*** **2001; 19:183–185.**

II. Petersson U, Östgren CJ, Brudin L, Ovhed I, Nilsson PM. Predictors of successful, self-reported lifestyle changes in a defined middle-aged population: The Söderåkra Cardiovascular Risk Factor Study, Sweden. ***Scand J Public Health*** **2008; 36:389- 396.**

III. Petersson U, Östgren CJ, Brudin L, Brismar K, Nilsson PM. Low Levels of Insulin-like Growth Factor Binding Protein-1 (IGFBP-1) is prospectively associated with incidence of type 2 diabetes and impaired glucose tolerance (IGT). The Söderåkra Cardiovascular Risk Factor Study. ***Diabetes Metab*** **2009 (Epub ahead of print).**

IV. Petersson U, Östgren CJ, Brudin L, Nilsson PM. A consultation-based method is equal to SCORE and an extensive laboratory-based method in predicting risk of future cardiovascular disease. ***Eur J Cardiovasc Prev Rehab*** **2009 (accepted manuscript).**

ABBREVIATIONS

ADMA	Asymmetric dimethylarginine
BMI	Body Mass Index
CRP	C-reactive protein
CVD	Cardiovascular disease
EAU	Area under the curve
GP	General Practitioner
HDL	High density lipoprotein
HR	Hazard ratio
IDF	International Diabetes Federation
IGF-I	Insulin-like growth factor-I
IGFBP-1	Insulin-like growth factor binding protein-1
LDL	Low density lipoprotein
MI	Myocardial infarction
OR	Odds ratio
PHC	Primary health care
PROCAM	Prospective Cardiovascular Münster Study
PTCA	Percutaneous transluminal coronary angioplasty
RCT	Randomised clinical trial
RIA	Radio immunoassay
SDMA	Symmetric dimethylarginine
TSH	thyroid stimulating hormone
UNICEF	United Nations Children's Fund (formerly United Nations International Children's Emergency Fund)
WHO	World health organisation

INTRODUCTION

Prologue:

After 12 years as a practicing physician at various positions in Kalmar County Council, I decided to move back to my place of birth and take over the family farm and apply for the available position as a family doctor in Söderåkra. At that time I knew that I would most probably remain at Söderåkra primary health care centre for a long time. This later turned out to become the longest employment of a local doctor there since the start of the health centre in 1864.

At that time in 1989, increased cholesterol was a highly debated topic in medical society but little was done in our county to combat the cardiovascular health threat of deranged lipids. I noted that even patients who had been subjected to coronary artery surgery were left with untreated high cholesterol values. It was then that I became interested in this health problem, associated with cardiovascular disease development.

Fortunately, the county politicians and administrators showed great interest in my proposed programme of surveying CVD risk in Söderåkra. I was also given the opportunity to participate in a research methodology course in Malmö and decided to conduct a CVD risk factor screening in the Söderåkra middle-aged population, which would also become very useful in my future work. I contacted associate professor Thomas Kjellström, Malmö who agreed to be my supervisor. The baseline study was developed together with him. Later the Kalmar County Council collaborated with the Blekinge research unit and could assist a 10-year follow-up study in the same population. Finally, a research unit was established in Kalmar, which made it possible to study cardiovascular disease and diabetes in that same cohort after 17 years. I became a Phd-student at the Department of Health and Sciences (IHS), Linköping in 2004. Peter Nilsson, Malmö, accepted to become my main supervisor with Carl Johan Östgren and Lars Brudin as co-supervisors.

Cardiovascular diseases in perspective

Cardiovascular disease caused by thrombosis due to atherosclerosis of the arteries, is the predominant cause of death in Sweden. The main atherosclerotic diseases manifestations are ischemic heart disease (angina pectoris, myocardial infarction), stroke and atherosclerotic artery disease (carotid stenosis, claudicatio intermittens, aortic sclerosis).

Myocardial infarction (MI)

MI started to increase at the beginning of the 20th century when Sweden began its development towards a welfare state along with the industrial revolution.

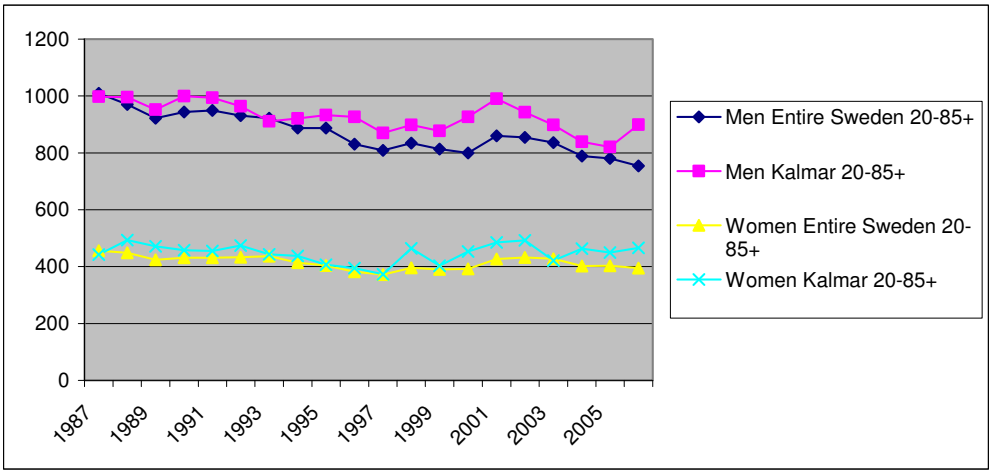
During the years 1987-2006 there occurred about 800,000 cases of acute MI in Sweden (1). However, between 1987 (42 300 MI) and 2000 (38 200 MI) the incidence decreased. As new diagnostic criteria (based on Troponin I levels $\geq$0.1) were introduced in 2001 (2), the incidence of acute MI increased substantially. In 2001 the incidence was 42 000 but decreased again to 39 400 in 2006. More men (60%) than women (40%) received the diagnosis during 1987-2006. Among inhabitants below the age of 60 men accounted for four times as many MIs as women before 1950. However, in this age group men now suffer from threefold more MIs than women.

The incidence of MI increases with age. In 2006, five times more incident MI occurred in men of 70-74 years than those of 50-54. For women, 70-74 years, nine times more incidences occurred than in those, 50-54.

The incidence of MI in Sweden is decreasing (Figure 1), as influenced by advanced medical care but a change in lifestyle could be even more important, e.g. less smoking in the population.

However, in the late 1980´s cardiovascular disease was 15% above the mean for Sweden in Kalmar County Council, the region for this study population. The incidence of MI for men in Kalmar County Council was the highest in Sweden in 2006 with 899 incident cases per 100,000 inhabitants (20 years or older) compared with an average of 754 for Sweden. For women the incidence was third highest, 499 MI cases versus 395 for the entire country. Mortality rates for CVD was also highest in Kalmar for men and second highest for women.

Figure 1. Age standardized incidence of MI per 100 000 persons 1987-2006 in Sweden, age interval 20-85+.



Source: National Board of Health and Welfare, Sweden. Statistics 2009.

Stroke

While Sweden has a 40% higher frequency of MI compared to southern Europe, the proportion of stroke is among the lowest in Europe. Still, stroke affects around 30 000 people in Sweden every year (3). The definition of stroke is a clinical syndrome with "rapidly developing clinical signs of focal or global disturbance of cerebral function", often as hemiparesis or aphasia with symptoms lasting for 24 hours or more (4).

The mean age of stroke patients in Sweden is 75 years (5). The underlying cause of stroke is atherosclerosis in 85%, while 15% is caused by haemorrhage. The traditional risk factors such as hypertension, cigarette smoking, diabetes and hyperlipidemia are estimated to explain about half of the risk of stroke (6). The remaining risk is due mainly to genetic or unknown factors (7).

The atherosclerotic process

The inner monolayer of the arteries, the endothelium, together with inflammatory cells plays an important roll in the development of atherosclerosis. Endothelial dysfunction promotes the atherosclerosis in the vessels through its effects on vasoregulation, platelet and monocyte

adhesion, smooth muscle cell growth and enhanced coagulation (8). Dysfunction of the endothelium is present in cardiovascular risk factor conditions as hypertension (9) and hypercholesterolemia (10).

Type 2 diabetes

Type 2 diabetes is one of the most common chronic diseases on a global scale (11,12). In Sweden the diabetes prevalence is estimated to include 350,000 individuals. Type 2 diabetes is the predominant form of diabetes and constitutes 90 % of all causes of diabetes mellitus.

Figure 2. The diagnostic criteria (mmol/L) according to WHO 1998 are:

	Full blood		Plasma	
	Capillary	Venous	Capillary	Venous
Diabetes mellitus				
Fasting glucose	≥6.1	≥6.1	≥7.0	≥7.0
2 hours after OGTT*	≥11.1	≥10.0	≥12.2	≥11.1
IGT**				
Fasting glucose	<6.1	<6.1	<7.0	<7.0
2 hours after OGTT	7.8-11.0	6.7-9.9	8.9-12.1	7.8-11.0

* oral glucose tolerance test, ** impaired glucose tolerance

Type 2 diabetes is characterised by relative insulin deficiency with increased insulin resistance and is now considered to be at least partly a lifestyle related disease associated with the so called metabolic syndrome. However, insulin resistance is also connected with a higher incidence of CVD and is sometimes considered mainly a metabolic CVD risk factor. People with type 2 diabetes are two to six times more likely to develop cardiovascular disease compared to subjects without diabetes (13). Women with type 2 diabetes have a fourfold elevated risk compared to non diabetic women of developing CVD (14).

Cardiovascular risk factors

Risk factors are factors that increase the probability of developing a disease. Certain risk factors are modifiable, others are not. Male gender, age and genetic factors are non-modifiable risk factors while several modifiable risk factors have been suggested and analysed (15).

Hypertension

The present definition of hypertension is a repeated blood pressure ≥140/90 mmHg (16), or ≥130/80 mm Hg for patients with diabetes or renal disease. In Sweden, 27% of the population above 20 years is hypertensive without major gender differences. The risk for CVD is increased and about the same for men and women. Of the 1.8 million Swedish adults with elevated blood pressure: 60% have mild hypertension (140–159/90–99 mm Hg), 30% have moderate hypertension (160–179/100–109 mm Hg) and 10% have severe hypertension (≥180/≥110 mm Hg). Most hypertension guidelines now recommend total risk assessment, i.e. consideration of all risk factors, target organ damage and any cardiovascular disease that is already present. Paying attention to blood pressure readings alone is usually insufficient in determining the appropriate treatment for mild hypertension or to properly measure the risk of cardiovascular disease.

Lifestyle factors as cardiovascular risk

Many studies have confirmed that poor lifestyle (smoking, sedentary life, unhealthy food, elevated waist/hip ratio, high alcohol consumption and increased psychosocial stress, factors that will increase the risk of elevated waist/hip ratio) are of major importance for the development of future CVD. The global case-control INTERHEART study, conducted in 52 countries on first event MI confirmed that an unhealthy lifestyle was causative of a large proportion of MI (17-19). As much as 90% of CVD was caused by risk markers and poor lifestyle in this large study. One or more risk factors were found in most people and a combination caused more CVD than just one suboptimal lifestyle habit. Smoking, hyperlipidemia, hypertension, diabetes, abdominal obesity and psychosocial factors increased the risk for acute MI ($p<0.0001$; $p=0.03$ for alcohol) while daily consumption of fruits and

vegetables, modest alcohol consumption, and regular physical activity decreased the risk. These associations were noted both in men and women, old and young, and in all regions of the world. This study concluded that prevention can be based on simple and similar principles worldwide and that lifestyle change could reduce the number of MI by 90%.

Biochemical risk markers

A risk factor influences CVD risk directly and when lowered is associated with a decreased risk for disease. A risk marker is a physical trait or a biochemical substance, involved in the CVD risk development but is so far unproven to be directly influenced in a positive way by preventive intervention. There are many biological variables that have been considered as cardiovascular risk markers. In this thesis asymmetric dimetylarginyl (ADMA) and insulin-like growth factor binding protein-1 (IGFBP-1) have been studied along with high sensitive C- reactive protein (CRP) for prediction of CVD risk and risk of new onset diabetes or IGT.

ADMA

The vascular endothelium is the largest organ in the body and its monolayer of cells are located between the lumen and the arterial smooth muscle cells (20). It plays an important role for early changes in the vessel wall which initiate and promote the atherogenic process. Nitric oxide from the endothelial cells, causing vasodilatation, is formed from L-arginine by the enzyme endothelial nitric oxide (NO) synthase. ADMA inhibits NO synthase and thus causes vasoconstriction and hypertension (21). It is a naturally occurring amino acid in human blood plasma and a metabolic by-product of the turnover of proteins and occurs in all cells (22).

Clinical studies have shown that ADMA can serve as a marker of cardiovascular risk with a statistically significant and independent relationship with the incidence of cardiovascular disease (23). ADMA is elevated in hypercholesterolemia, smoking, diabetes mellitus, erectile dysfunction, liver failure, hypertension and chronic renal failure (24).

ADMA was reported to be increased in patients with hypercholesterolemia (25), hypertension (26), diabetes mellitus (27), insulin resistance (28), chronic renal failure (29), and in patients with atherosclerotic disease (30).

Increased levels of ADMA were also associated with coronary heart disease (31). Furthermore, in a study of 52 patients with ischemic stroke from South Korea, plasma levels

of ADMA were elevated (32).

Elevated ADMA levels cause a relative L-arginine deficiency even in the presence of normal plasma L-arginine levels. Studies based on dietary supplementation with L-Arginine have shown, that the inhibitory action of ADMA can be reversed (33). This has also been shown to improve clinical symptoms of cardiovascular disease in some studies (34, 35).

Symmetric dimethylarginine (SDMA) is also present in plasma and is excreted in the urine and related to renal impairment (36). SDMA was formerly considered to be an inactive stereoisomer of ADMA but is now found to inhibit the NO synthesis via competition with L-arginine uptake by endothelial cells. The level of SDMA increases in earlier stages of renal dysfunctions and may contribute to increased CVD (37).

IGFBP-1

Waist circumference (38) and the homeostasis model assessment (HOMA) index are well-known surrogate markers for insulin resistance (39). IGFBP-1 as a part of the insulin-like growth factor IGF system, involved in regulating the glucose metabolism, is associated with insulin resistance and glucose intolerance. IGFBP-1 is one of six binding proteins and is an acute regulator of the IGF-I bioavailability (40). It is produced in the liver, peaking at dawn, and being highly dependent on insulin concentrations. High levels of insulin are in general associated with low IGFBP-1 concentrations.

Low levels of IGFBP-1 are associated with the metabolic syndrome through insulin resistance, obesity and the development of cardiovascular disease (41-45). It has been suggested that IGFBP-1 facilitates the transport of IGF-I from plasma to tissue, thus potentially increasing the activity of IGF-I in the target tissue (46). Many of the processes involved in the formation of the atherosclerotic lesions are IGF-I dependent, promoting macrophage chemotaxis and endothelial cell migration (47) as well as vascular smooth muscle cell proliferation and migration (48). IGFBP-1 can also exert an effect on cellular growth and migration independently of IGFs, by binding to the cell surface via 5ß1 integrins (49,50). IGFBP-1 reflects free IGF-I (51). Serum levels of IGFBP-1 (but not IGF-I), correlated to body mass index and upper arm fat and muscle areas in the elderly (52), but also varied considerably among healthy individuals (53). A monozygotic twin study showed that non-genetic factors explained 64% of the total variation in serum IGFBP-1 levels (54). Insulin and IGF-I explained 28% and 8%, respectively, of the non-genetic variation in IGFBP-1 (55). Hence, approximately 30% of the variation in IGFBP-1 levels remains unexplained, and this

variation might be due to dietary and other lifestyle factors according to a study in healthy men (56). Furthermore, a previous study has shown that high circulating concentrations of IGF-I were associated with reduced risk of development of type 2 diabetes/IGT in normoglycaemic individuals (57). Finally, a recent Swedish study has shown gender differences in levels of IGFBP-1 with higher levels in women (58).

CRP

C-reactive protein is elevated in subjects with infectious diseases but also in subclinical inflammatory conditions as the atherosclerotic process. CRP is stimulated by cytokines and produced in the liver. There is a high-sensitive (hs) laboratory method, which measures low levels of CRP, suitable for assessing atherosclerotic disease risk. A subject with a CRP level of 2mg/l has twice the risk for CVD compared to an individual with 1mg/l (59,60). CRP as a risk marker of cardiovascular disease can be useful when other traditional risk factors are absent in predicting CVD. It can also be used as one of many components in a risk prediction algorithm.

Prevention of cardiovascular diseases

Efforts to prevent CVD have aroused great interest in the developed world during the last century.

Among the most well known early screening projects is the Framingham Study (61,62) in the fifties and the Seven Country Study (63) in USA, the Monica Study (64,65) in several European countries, as well as the North Karelia project (66) in Finland in the seventies and eighties.

In 1978, the WHO-UNICEF Alma-Ata Declaration (67) primary health care (PHC) was seen as the key to achieving an acceptable level of health throughout the world. The declaration affirmed health as a fundamental right and called for community based programmes.

Based on this declaration and the above mentioned early prevention programmes as models, the prevention of CVD was also implemented in Swedish PHC in the three last decades. Among well-known screening programmes are those carried out in Dalby (68), Lyckeby (69), Strömstad (70), Skaraborg (71), Sollentuna (72), and Norsjö (73). In Lyckeby the recruitment for prevention took place through opportunistic screening, which was considered a suitable method of reaching most of the adult population in a cost-effective way (74).

The aim of the prevention programmes was to reduce CVD by diminishing the burden of poor

lifestyle, such as smoking habits, unhealthy foods, overweight, lack of exercise, as well as alcohol over-consumption. The most extensive follow-up evaluations were made in Habo (75,76), Sollentuna (77) and Norsjö (73) and the long-term results were promising. In the "Live for Life" programme in Habo, county of Skaraborg, at the start in 1989, the so called "Health Curve" was used for evaluation of health status in 30 and 35 year olds. Data on lifestyle factors and biological markers were collected. In Habo a decreasing CVD mortality rate was later described (75). Correspondingly, after 10 years, the predicted coronary disease mortality was reduced by 36 percent in Norsjö but only by one percent in the reference area (73).

Hypertensive individuals have shown increased CVD risk factors, both in local (77,78) and national studies (79). A screening study on 40 year old men in the population on Öland described two categories of CVD individuals, one exhibiting the metabolic syndrome, and another with high levels of genetically determined lipoprotein(a), Lp(a) with different outcomes in the two sub-cohorts (80).

Cardiovascular risk score assessment

As CVD occurs frequently, physicians have found it convenient to use simple prognostic tools in order to be able to assess a patient's risk of cardiovascular disease or death, most frequently over a 10- year period (81-86). The Framingham risk score was the first risk model, followed by others, (e.g. PROCAM), but presently the SCORE model is most frequently used. The internet application of SCORE is called HEARTSCORE and is currently the most well-established and used in European countries, however slightly modified for different countries as the total risk differs. The risk algorithm is based on data related to gender, age, present smoking, systolic blood pressure and total cholesterol. What argues against using the algorithm is that it only uses fatal CVD events as endpoint. It would be much easier to talk about risk of first non-fatal CVD event rather than death during an often short consultation in primary health care.

Ethical considerations related to cardiovascular prevention have caused debate (87) and it is important that the score process is accompanied by evidence-based lifestyle advice. Table 1 shows some established score instruments.

In 1989, in Söderåkra, an interest for launching a population based screening for cardiovascular risk factors also emerged, why the Söderåkra Risk Factor Screening Study was started. This thesis presents two new risk score instruments, The Consultation model and the Extended model (Paper IV).

Table 1. Established risk algorithms and scoring instruments for the prediction of cardiovascular risk.

	Framingham (6)	**SCORE (10)**	**PRECARD (7)**	**PROCAM(9)**	**BMJ riskscore (8)**
N (men, women)	5 345 m+w	205 178 m+w	11 765 m+w	5 389 m	47 088 m+w
Population	Population-based study	Population-based study	Population-based study	Cohort of men at work	Participants in 8 RCTs on hypertension
Region	USA	Europe	Denmark	Germany	USA + Europe
Risk prediction	10 years	10 years	10 years	10 years	5 years
Endpoints	Cardiovascular death, myocardial infarction (MI), heart failure, angina	Cardiovascular death	Fatal and nonfatal MI, stroke	Fatal and nonfatal MI	Cardiovascular death
Variables (n)	8	5–6	11	8	10
Age					
Gender					
Height					
Weight					
Lipids					
Total cholesterol					
HDL					
Ratio totalchol/ HDL					
LDL					
Triglycerides					
Other					
Systolic blood pressure					
Smoking					
Diabetes					
Present CVD					
CVD in family					
S-kreatinine					
Left ventricular hypertrofi					

Grey colour indicates presence, black colour absence of the variable**.** Source: Medical Products Agency, Sweden.

AIMS

General aim:

To explore the cardiovascular risk factor profile in a defined middle-aged population at baseline and to identify the most important risk factors for predicting the incidence of cardiovascular morbidity and mortality, as well as disturbed glucose metabolism and type 2 diabetes, during long-term follow-up.

Aims for selected papers (I-IV):

Paper I. To study the role of subclinical hypothyroidism on lipid levels in a gender perspective.

Paper II. To explore the long-term determinants of self-reported lifestyle changes in a middle-aged population, following baseline screening and lifestyle counselling.

Paper III. To explore the association between a marker of glucose and insulin metabolism (IGFBP-1) for the long-term development of disturbed glucose metabolism, e.g. impaired glucose tolerance (IGT) and type 2 diabetes in a defined middle-aged population after a 17-year follow-up.

Paper IV. To compare a consultation-based, non-laboratory risk model that uses easily obtained information at a clinical consultation in primary health care, to the established SCORE algorithm as well as an extensive laboratory-based risk model in predicting CVD risk.

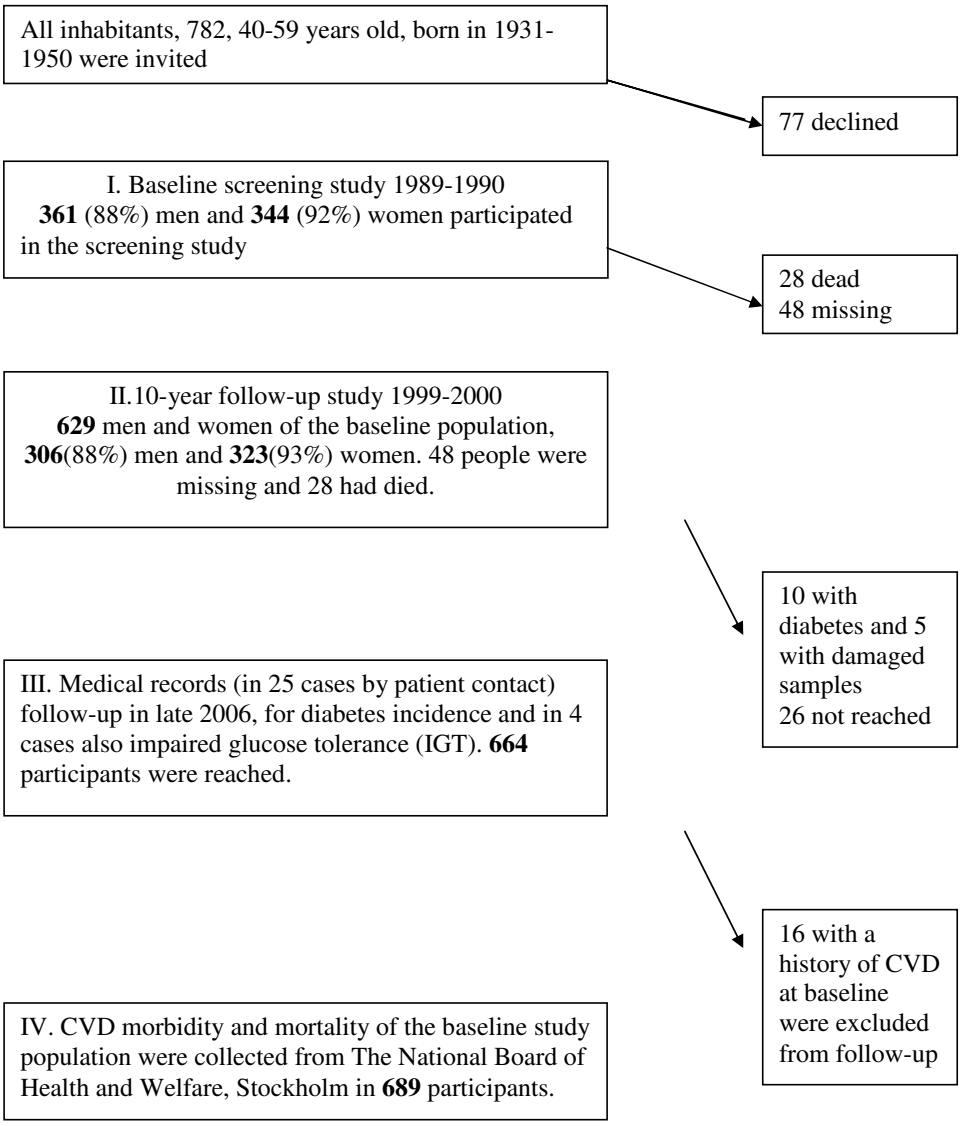
MATERIAL AND METHODS

Population and setting

The Söderåkra Cardiovascular Risk Factor Study was launched in November 1989 and ran until May 1990 as a population-based, cross-sectional, cardiovascular risk factor screening study. All inhabitants aged 40-59 in Söderåkra, southern Sweden, were invited to participate.
Söderåkra is a parish with 3400 inhabitants and part of a small municipality, Torsås, with 7300 inhabitants, situated in Kalmar County on the Baltic Sea. Approximately half of the population lives in the countryside, the other half in three population centres. Farming is the main occupation together with industrial work and social service.
A total of 782 subjects were invited for the study, of whom 705 (90%), 361 males (88%) and 344 females (93%), agreed to participate. Details are shown in Figure 3.

The non-participants, 27 women and 50 men, in the population were not willing to participate because of chronic disease (n=15), fear of syringes and venepunction (n=3), already similarly examined (n=24), but also for unspecified reason (n=35).

Figure 3. The study population of the Söderåkra Risk factor Screening Study 1989-2006.



Methods

Baseline study: The Söderåkra cardiovascular risk factor study 1989-1990. Paper I

All inhabitants born between 1931 and 1950, then 40-59 years old, were invited to a screening for CVD risk factors by an invitational letter. The screening programme received considerable attention from the public and reached a 90% attendance rate. The study was conducted from November 1989 until May 1990

Laboratory tests

Blood samples were drawn after an overnight fast, with participants seated after a 15- minute rest without venous stasis. Blood glucose, serum cholesterol, HDL-cholesterol and serum triglycerides were analysed. LDL-cholesterol was calculated by use of Friedewald´s formula. Thyroid function tests, free T4 and thyroid stimulating hormone (TSH) were also analysed. Routine laboratory methods were used at the Department of Clinical Chemistry, Kalmar County Hospital. Extra serum samples were frozen and stored for future analyses. Anthropometric measurements of height (cm), weight (kg) in light clothing without shoes, waist (cm) and hip circumference (cm) were recorded in the standing position. BMI was calculated (kg/m^2). The subjects were provided with a self-administered questionnaire to be filled in prior to visiting the physician.

Lifestyle questionnaire and blood pressure

After analyses of the blood samples, all but thirteen subjects came for a structured visit and feedback information to the responsible physician, who also performed the clinical examination. Three blood pressure measurements were recorded (mmHg) in the right arm by use of mercury sphygmomanometer with a suitable cuff width. These were taken in the sitting position after five minutes rest. The mean value of the last two recordings was registered.

Following physical examination, the questionnaire containing lifestyle questions on dietary habits focusing on fat intake, physical activity, smoking and alcohol consumption was

completed. Marital status, occupation, medical history, concomitant medication, family history of CVD, diabetes, hypertension and high lipids was noted.

For hyperlipidemia, three teaching sessions in diet adjustment were provided in group meetings, but only half the study participants attended the course, offered by specially trained nurses. The low attendance rate was mainly due to the fact that only day time sessions were offered for this primarily working population. However, no group sessions or individual counselling except for the one at the doctor's visit was provided for alcohol overconsumption, smoking or weight-related problems. Pathological findings were treated.

Tobacco use was expressed in number of cigarettes or packages of tobacco per day. The participants were divided into smokers and non-smokers. Alcohol consumption was expressed in centilitres of beer, wine and hard liquors per week and transformed to gram alcohol per week. Studies (88) have shown that 1-2 drinks of alcohol per day may be associated with reduced risk of cardiovascular events. The agreed definition of one drink contains an average of 12 g alcohol and represents 33 cl beer, 15cl wine or 4cl hard liquor. For women alcohol consumption is recommended to be somewhat less. In this study, two thirds of the recommendation of male consumption was considered healthy. For baseline description, we therefore created a variable of low/moderate alcohol consumption and high consumption, respectively. The cut-off value chosen was 120 g/week for men and 80 g/week for women.

Physical activity (PA) (cycling, walking, swimming, or other exercises involving large muscles) was discussed and clarified at the appointment with the physician. PA was further divided into three groups; (a) daily exercise for half an hour or more, (b) two to three times a week for at least half an hour, or (c) less exercise than at (b). A family history of cardiovascular disease was defined with scores as follows: 0 = no myocardial infarction in first degree relatives, 1 = myocardial infarction in one, and 2 = myocardial infarction in two or more first degree relatives.

10-year follow-up telephone survey. Paper II

In 1999-2000, a follow-up study (Paper II) was carried out as a structured telephone interview, conducted by a specially trained nurse. The questionnaire was previously used in a study on cardiovascular risk factors in Lyckeby, Sweden (69). Questions focused on changes in lifestyle habits since the collection of data at baseline ten years earlier.

Participants were asked if they changed five lifestyle habits or their consequences: (a) overweight, (b) smoking, (c) fat consumption, (d) physical activity, and (e) alcohol use. They answered "less, unchanged or more" in relation to habits present at baseline. Participants received one score-point for each improvement (less body weight, less smoking, less fat consumption, increased physical activity and lower alcohol intake), zero for unchanged conditions and minus one score-point for deteriorated lifestyle. Altogether each participant could thus receive from minus five to plus five score-points. Subjects who scored from plus one point to plus five score-points were considered to belong to the successful group, while subjects receiving from zero to minus five score-points were referred to as unsuccessful in lifestyle change. Individuals with all risk factors and lifestyle habits at optimal level from start could, of course, not further improve their lifestyle and hence scored zero. However, in this study only changes were counted, not manifested healthy lifestyle. Thus, participants who were already leading an excellent lifestyle did not get credit for that according to the study aim and design.

Individual baseline data was linked to information from national censuses collected in 1990 regarding socio-economic data on marital status, occupational status and educational level (Statistics, Sweden). Occupation was categorized as "manual workers", "non-manual workers" or "farmers/employers". Educational level was considered "low" for ninth grade level or less, and "high" when above ninth grade level. Risk conditions for atherosclerotic diseases in study participants were defined as a medical history of coronary heart disease, diabetes mellitus and hypertension.

Follow-up study of incident type 2 diabetes and impaired glucose tolerance (IGT). Paper III

Follow-up procedures

A follow-up survey of the incidence of type 2 diabetes and IGT in the study population was conducted in 2006 by a nurse specialized in diabetes care. Most information was collected from primary health care medical records but in 25 cases of missing medical records, telephone interviews with the study participants were conducted. The vital status of the cohort was obtained through linkage to the Cause of Death Register at the National Board of Health and Welfare, Stockholm. Participants with diabetes at the start of the study (n= 10) were

excluded, as were those with frozen serum samples that had been damaged (n= 5) during storage. We were unable to obtain adequate information in 26 cases, why these individuals were subsequently excluded from the follow-up. Thus, a total of 664 participants (94% of the baseline population) remained for further analyses. Incidence and duration of type 2 diabetes and IGT were registered. The contemporary WHO diagnostic criteria for type 2 diabetes and IGT were applied (89). An oral glucose tolerance test (OGTT) had been conducted in four cases for clinical reasons and revealed IGT, which is why these additional cases were also considered as incident events of abnormal glucose metabolism. In the four IGT cases, capillary blood was drawn after OGTT and diagnosed as IGT, if blood glucose was ≥7.8-11.0 mmol/L.

Insulin resistance was assessed from fasting blood glucose and serum insulin concentrations by use of the HOMA index (90). The metabolic syndrome was categorized according to criteria defined by the International Diabetes Federation (IDF) in 2005 (91). The main criterion for the metabolic syndrome by IDF, is waist circumference ≥94 cm for men and ≥80 cm for women. If these conditions are fulfilled, two or more of the following conditions must co-exist: triglycerides >1.7 mmol/L, HDL<1.03 mmol/L in men or <1.29 mmol/L in women, systolic blood pressure ≥130 and/or diastolic blood pressure ≥85 mmHg, and plasma glucose ≥5.6 mmol/L. In the present study blood glucose was analyzed and the glucose values were multiplied by 1.1 in order to determine the corresponding plasma glucose levels (92).

Laboratory tests

Serum insulin was analyzed after two years in frozen storage at -20 °C by radioimmunoassay (RIA) technique (Pharmacia Insulin RIA 100) at the Department of Clinical Chemistry, Kalmar, Sweden. In 2005, frozen serum stored at -20 °C from baseline was defrosted, divided into three separate samples for each subject and briefly refrozen at -70 °C, before analyzing serum CRP and serum creatinine by routine methods, with commercially available kits using Cobas Integra 700 (Roche Diagnostics Scandinavia AB). Frozen serum for analyses of serum IGF-I and IGFBP-1 was sent to the Department of Molecular Medicine and Surgery, Karolinska Institutet, Solna, Sweden. Baseline fasting concentrations of IGF-I were determined in serum by RIA after separation of IGFs from IGFBPs by acid ethanol extraction and cry precipitation. To minimize interference of remaining IGFBPs, des (1-3) IGF-I was used as radioligand. The intra- and interassays CV were 4% and 11%, respectively (93). IGFBP-1 concentrations in serum were determined by a RIA method according to Póvoa *et al* (94). The sensitivity of the RIA was 3 µg/L and the intra- and interassays CV were 3% and 10%, respectively.

Follow-up for prediction of first cardiovascular events. Paper IV

Follow-up for cardiovascular events

All participants with a history of prevalent CVD at baseline (n=16) were excluded from follow-up. Hence, cardiovascular mortality and in-hospital care for CVD were followed in 689 individuals (349 men and 340 women). Event data were collected from the National Board of Health and Welfare from study start in 1989 until 2006. The subjects were also asked about previous CVD. The following diagnoses from the International Classifications of Diseases (ICD) versions 8 and 9 (1968-1996) were used: 410-414, 431, 433, 434, 435, 436, 437, 440, and 441 and from ICD 10 (1997-ongoing): I20-I25, I61, I63-I66, and I70-I72.

We compared three (I-III) risk prediction models; (I) the Consultation model, (II) SCORE, and (III) the Extended laboratory-based model. The variables included in the three risk models were all associated with an increased risk of developing CVD and death. The Consultation model (I) comprised data on age, gender, present smoking, present diabetes, treated hypertension, measured blood pressure (systolic ≥140 or diastolic ≥90 or lower), waist/height ratio and family history of CVD (angina, myocardial infarction and stroke).
We compared our Consultation model with the established risk instrument, SCORE (II), which comprises data on age, gender, present smoking, systolic blood pressure and total cholesterol (95,96).

Finally we explored the usefulness of a more elaborate risk prediction tool named the laboratory-based Extended model (III). This model comprised age, gender, smoking, blood pressure at baseline, waist/height ratio, and a family history of CVD together with the following blood analyses: serum triglycerides, serum low density lipoprotein (LDL) cholesterol/ serum high density lipoprotein (HDL) cholesterol, blood glucose, insulin-like growth factor –I (IGF-I), CRP and symmetric dimethyl arginine (SDMA).

All variables except for prevalent diabetes at baseline (borderline significant) included in the Consultation model and the Extended model were associated with CVD morbidity or

mortality in univariate Cox regression analyses. On the contrary, physical activity and alcohol consumption as well as ADMA and IGFBP-1 were not included in the Consultation model or Extended model since these variables did not predict CVD.

Laboratory tests

ADMA and SDMA were analyzed from frozen serum from baseline at the Department of Clinical Chemistry, VU University Medical Centre, Amsterdam, the Netherlands. Serum concentrations of ADMA and SDMA were measured by high-performance liquid chromatography with fluorescence detection with modified chromatographic separation conditions (97,98).

Statistical methods

Paper I: Statistical evaluation was performed by Pearson's coefficient of correlation, while Student's t-test and chi-square analysis was used for differences in means and between group differences. A p-value of <0.05 was considered statistically significant.

***Paper II*:**

Median, lower to upper quartile (Q1 – Q3) range and proportions were presented for baseline variables. Differences between successful and unsuccessful subjects at baseline, stratified for gender, were analysed with non-parametric tests (Mann-Whitney U-test, Fischer's exact test). To study predictors for success, univariate and multivariate analyses were made using logistic regression. All significant variables for success of self-reported lifestyle changes in the univariate analyses were further challenged in the multivariate analyses by stepwise adjustment for the following variables at baseline: serum lipids, anthropometric data, blood pressure and smoking. The analyses also included marital status, socio-economic status, educational level, previous cardiovascular risk conditions and a family history of myocardial infarction. Thus, all statistically significant variables (Table 6) were independent predictors for self-reported lifestyle changes in this study.

OR were calculated and expressed with 95% CI. A p-value less than 0.05 was considered statistically significant.

Paper III:

Participants were divided into five quintiles based on their baseline IGFBP-1 concentrations. The lowest quintile (group 1) had IGFBP-1 values < 24 micrograms/L (µg/L), while the three middle quintiles (group 2) were combined and showed an IGFBP-1 of 25- 59 µg/L. The fifth quintile (group 3) had an IGFBP-1 > 59 µg/L. Data from the three groups were related to baseline characteristics (Table 7). Gender differences between the IGFBP-1 subgroups were analyzed with a Chi2-test, while baseline characteristics of the three subgroups without gender separation were characterized by use of non-parametric analyses of variance for group differences (Kruskal-Wallis test). Differences between the lowest IGFBP-1 quintile and the highest were analyzed by using non-parametric tests (Mann-Whitney U-test). Differences in metabolic, anthropometric and lifestyle characteristics between individuals, with and without the metabolic syndrome at baseline (Table 8), were analyzed by use of Mann-Whitney U-test, except for the numerical differences between genders in both groups, which were analyzed with a Chi2 test.

Waist circumference was categorized into two groups based on the cut-off value for the IDF-definition of the metabolic syndrome. CRP was categorised in three subgroups: lowest quartile, the two middle quartiles, and the highest quartile.

The 38 cases of type 2 diabetes and in addition four cases of IGT were explored by using the Cox regression proportional hazard model in relation to IGFBP-1, waist circumference and CRP, with age, gender, IGF-I and lifestyle factors as covariates. A stepwise deletion method was applied. HR was expressed with a 95% CI. A p-value less than 0.05 was considered statistically significant.

Paper IV:

Descriptive statistics at baseline were given for CRP, ADMA and SDMA. Mann Whitney U-Test was used for gender differences. Furthermore, correlations between ADMA, SDMA, respectively, and several variables were made using Spearman rank order correlations.

Cox proportional hazards regression analyses were used in comparing the three risk predicting models. As dependent variable we used time to first fatal or non-fatal CVD, which included cardiovascular death, angina, myocardial infarction, coronary artery bypass grafting,

percutaneous transluminal coronary angioplasty (PTCA), stroke and peripheral artery disease. The statistically significant variables from the univariate Cox regression analyses were further used in the multivariate analyses, expressed as hazard ratio (HR) with 95% confidence intervals (CI). An exception was diabetes at baseline, which was included although being only borderline significantly (p=0.058) associated with risk of CVD events. We created a risk assessment algorithm for each of the three models based on the beta coefficients in the Cox proportional hazard regression analyses.

We also used receiver operator characteristic (ROC) curves in addition to the Cox regression models. The ROC curve (99) measures the discrimination of a prediction model and represents the graph of the true positive rate (sensitivity) against the false positive rate (1-specificity). The c-statistic (area under the curve=AUC) is a useful single-number summary and represents an estimate of the probability that the model assigns a higher risk to those who experienced CVD events than those who did not (100,101). A ROC curve with 95% CI was constructed for each model and the c-statistic for each model was thereafter calculated using the statistics programme MedCalc Version 6.10, 2001. All statistical analyses were carried out using Statistica (Version 8). A p-value of less than 0.05 was considered significant.

Ethical considerations

The baseline study did not need ethical approval. When consulting Professor Bo Nordenskjöld, chairman of the ethics board, Linköping, he considered the Söderåkra baseline study procedure to be part of regular cardiovascular prevention in primary health care at that time (1989).

The 10-year follow-up telephone survey was approved by the ethics board in Linköping, Sweden, Registration (Dnr. 99256).

The Söderåkra follow-up study for the prediction of incident diabetes and cardiovascular disease, respectively, was approved by the ethics board, Linköping (32/2004).

RESULTS

Paper I:

Hypercholesterolaemia (serum cholesterol > 6.5 mmol/L) was detected in 28 % of the population (112 men and 86 women). The highest value recorded was 10.1 mmol/L. Hypertriglyceridemia (serum triglyceride >2.5 mmol/L) occurred in 5 % of the population (28 men and 10 women) with a highest value of 4.6 mmol/L. TSH was above the reference range (0.30-3.75 mU/L) in 5 % of the population (10 men and 31 women). One patient was intentionally over treated with thyroid hormone replacement (Levaxin) due to thyroid cancer. Another subject had obvious signs and symptoms of thyrotoxicosis, confirmed by free T4 at 46 pmol/L, and a positive scintigram. Two females had very high TSH values (46 and 20 mU/l).

The overall test results, Table 2, were compared for men and women, and significant differences were found in most aspects except for body mass index (BMI). From the analyses of correlations, a number of differences were noted between men and women, Table 3. The correlation between serum cholesterol and thyroid function tests (TSH and free T4) was limited to women. The most apparent gender-related difference in correlations was the inverse relations between serum triglyceride and free T4, and the correlation between LDL cholesterol and TSH, both in women only. BMI had no relation to serum cholesterol levels but a high direct correlation to serum triglyceride levels and inverse correlation with HDL cholesterol in both genders.

Table 2. Baseline characteristics expressed by means with Student's t-test for gender differences.

	All	SD	Men	Women	p
Serum cholesterol (mmol/l)	5.93	1.17	6.03	5.83	<0.050
LDL (mmol/l)	3.84	1.07	3.99	3.69	<0.001
HDL (mmol/l)	1.56	0.38	1.43	1.68	<0.001
Serum triglycerides (mmol/l)	1.20	0.70	1.33	1.06	<0.001
Free T4 (pmol/l)	12.70	3.49	12.99	12.40	<0.050
TSH (mU/l)	1.94	2.31	1.62	2.27	<0.001
BMI (kg/m2)	26.0	4.06	26.0	26.0	ns

TSH=thyroid stimulating hormone, BMI=Body mass index, ns=not significant

Table 3. Correlations between thyroid function tests, serum lipids and BMI stratified for gender by Pearson's coefficient of correlation.

			Men	Women
Serum cholesterol	versus	HDL	0.14**	0.07ns
Serum cholesterol		LDL	0.94***	0.95***
Serum cholesterol		triglycerides	0.35***	0.46***
Serum cholesterol		Free T4	0.07ns	-0.10ns
Serum cholesterol		TSH	0.09ns	0.14***
Serum cholesterol		BMI	0.00ns	0.08ns
HDL cholesterol		triglycerides	-0.37***	-0.40***
HDL cholesterol		TSH	-0.03ns	-0.08ns
HDL cholesterol		Free T4	0.09ns	0.02ns
HDL cholesterol		BMI	-0.24***	-0.27***
LDL cholesterol		TSH	0.05ns	0.15***
LDL cholesterol		Free T4	0.09ns	-0.08ns
LDL cholesterol		BMI	0.02ns	0.12*
Serum triglycerides		TSH	0.18***	0.13*
Serum triglycerides		Free T4	-0.01ns	-0.19***
Serum triglycerides		BMI	0.29***	0.28***
Body Mass Index		TSH	0.07ns	0.09ns
Body Mass Index		Free T4	0.01ns	-0.16**

*, p≤0.05, **, p≤0.01, ***, p≤0.001, and ns, not significant, - negative correlations

Paper II:

Table 4 shows descriptive characteristics at baseline for subjects with success in self-reported lifestyle changes compared to unsuccessful subjects during follow-up. Elevated systolic and diastolic blood pressure for women, higher levels of serum cholesterol for men, lower levels of HDL-cholesterol and higher levels of serum triglycerides for both genders were associated with a higher success rate. On the other hand, LDL-cholesterol and fasting blood glucose

were not associated with subsequent success in lifestyle change. Smokers had a greater success rate than non-smokers, while subjects with low/moderate alcohol intake compared with high intake showed no significant difference in success rate.

Figure 4 shows the distribution of scores for lifestyle change in men and women, composed of the following, five lifestyle variables: smoking, fat consumption, weight change, physical activity, and alcohol consumption. In general, women reported more positive lifestyle changes than men.

Table 5 shows baseline marital status, educational level, occupation and prevalence of diabetes, treated hypertension, angina pectoris and myocardial infarction. Manual versus non-manual occupation, marital status and educational level showed no significant association with the success rate of lifestyle change. A history of hypertension and myocardial infarction significantly predicted success at the 10-year follow-up for men, but not for women.

Table 6 presents statistically significant predictors for success in lifestyle changes using multivariate analyses. In a multivariate logistic regression model, using serum lipids, anthropometric measurements, blood pressure and tobacco use, women were generally more successful than men, OR 1.56 (95% CI: 1.11-2.18; p= 0.010). When stratified for gender, women with elevated blood pressure at the baseline visit were more likely to report success in lifestyle change than normotensive women, OR 1.84 (95% CI: 1.12-3.02; p= 0.016). Men with established cardiovascular risk factors and conditions at baseline, reported higher success rate, OR 4.77 (95% CI: 2.18-10.5; p<0.001) than men without these conditions. Most improvements were reported if there was a history of myocardial infarction among men, OR 22.8 (95% CI: 4.73-110; p<0.001). Smoking at baseline was associated with significant success, OR 3.36 (95% CI: 2.05-5.51; p<0.001), and OR 1.81 (95% CI: 1.11-2.95; p=0.017), for men and women respectively and was used as the correction variable. If smoking cessation was omitted from the success variable, smoking among men was still statistically significant for successful lifestyle change similar to what is shown in Table 6. However, smoking among women was no longer a significant predictor, indicating that the predominant factor for success in life style change in smoking women emerged from quitting smoking.
Age was dichotomized into two age groups at baseline, 40-49 and 50-59 years, but showed no significance in the step-wise multivariate analyses and logistic regression models.

Table 4. Baseline characteristics for success and non-success in self-reported lifestyle change for men and women respectively. The Söderåkra Cardiovascular Risk Factor Study 1989-1990.

	Men					Women					
	Non-success		Success		Diff	Non-success		Success		Diff	Diff M_F
N	189		134			155		151			
	median	Q1-Q3	median	Q1-Q3	p	median	Q1-Q3	median	Q1-Q3	p	p
Age (years)	46	43-53	48	44-52	0.203	47	43-52	46	43-52	0.895	0.145
Height (cm)	177	173-180	177	173-181	0.930	164	160-168	163	159-167	0.243	<0.001
Weight (kg)	80	72-87	82	73-89	0.171	67	60-75	69	61-78	0.409	<0.001
BMI (kg/m2)	26	24-28	26	24-28	0.225	25	22-28	26	23-29	0.147	0.178
Waist (cm)	92	86-97	93	89-98	0.062	78	73-87	81	74-88	0.130	0.178
Syst BP (mmHg)*	128	120-140	130	120-140	0.070	122	112-135	130	116-140	0.023	0.019
Diast BP (mmHg)*	86	80-90	88	80-90	0.146	82	78-89	86	78-90	0.016	0.002
Cholesterol (mmol/l)	5.8	5.2-6.7	6.2	5.3-6.9	0.044	5.7	4.9-6.5	5.6	4.9-6.6	0.666	0.008
LDL-chol. (mmol/l)	3.8	3.2-4.5	4.0	3.3-4.8	0.173	3.5	2.9-4.2	3.6	3.0-4.5	0.218	<0.001
HDL-chol. (mmol/l)	1.4	1.2-1.7	1.3	1.2-1.6	0.051	1.7	1.5-1.9	1.6	1.4-1.8	0.004	<0.001
Triglycerides (mmol/l)	0.9	0.8-1.5	1.3	0.9-1.9	0.001	0.8	0.6-1.1	0.9	0.7-1.3	0.011	<0.001
Blood glucose(mmol/l)	4.5	4.2-4.8	4.6	4.2-5.0	0.132	4.3	4.1-4.7	4.4	4.1-4.7	0.512	<0.001
Alcohol cons§ (n (%)											
Low/moderate	172	93.0%	122	92.4%	>0.90	148	96.7%	145	96.7%	>0.90	0.044
High	13	7.0%	10	7.6%		5	3.3%	5	3.3%		
Smoking (n (%))											
No	139	75.1%	65	49.2%	<0.001	110	71.9%	88	58.7%	0.016	0.861
Yes	46	24.9%	67	50.8%		43	28.1%	62	41.3%		

Footnotes to table: * Systolic and diastolic blood pressure, § alcohol consumption. Q1 and Q3 are first and third quartile. Diff is difference between non-success and success and diff M-F is difference between males and females as analysed with Mann-Whitney U-test.

Table 5. Socio-economic data and risk factors for cardiovascular-related conditions (diabetes, hypertension and angina) and a history of myocardial infarction at baseline 1989-1990 with respect to success or non-success in self-reported lifestyle change by sex respectively.

	Men					Women				
	Non-success		Success		Diff	Non-success		Success		Diff
N	185		134			153		150		
	n	per cent	n	per cent	p	n	per cent	n	per cent	p
Marital status										
Married/cohabiting	157	84.9	111	84.1	0.876	139	90.8	133	88.7	0.573
Non-cohabiting	28	15.1	21	15.9		14	9.2	17	11.3	
Education										
9 years or less	111	60.0	68	51.5	0.137	74	48.4	88	58.7	0.084
More than 9 years	74	40.0	64	48.5		79	51.6	62	41.3	
Occupation										
Manual	85	45.9	67	50.0	0.265	74	48.4	75	50.0	0.953
Non-manual	40	21.6	34	25.4		40	26.1	41	27.3	
Farmers/employers	44	23.8	20	14.9		14	9.2	12	8.0	
Non-classifiable	16	8.6	13	9.7		25	16.3	22	14.7	
Diabetes										
No	184	99.5	128	97.0	0.16	152	99.3	145	96.7	0.12
Yes	1	0.5	4	3.0		1	0.7	5	3.3	
Hypertension										
No	179	96.8	117	88.6	**0.005**	147	96.1	144	96.0	1.00
Yes	6	3.2	15	11.4		6	3.9	6	4.0	
Angina										
No	183	98.9	129	97.7	0.65	153	100.0	150	100.0	1.00
Yes	2	1.1	3	2.3		0	0.0	0	0.0	
Myocardial infarction										
No	185	100.0	128	97.0	**0.029**	153	100.0	147	98.0	0.12
Yes	0	0.0	4	3.0		0	0.0	3	2.0	

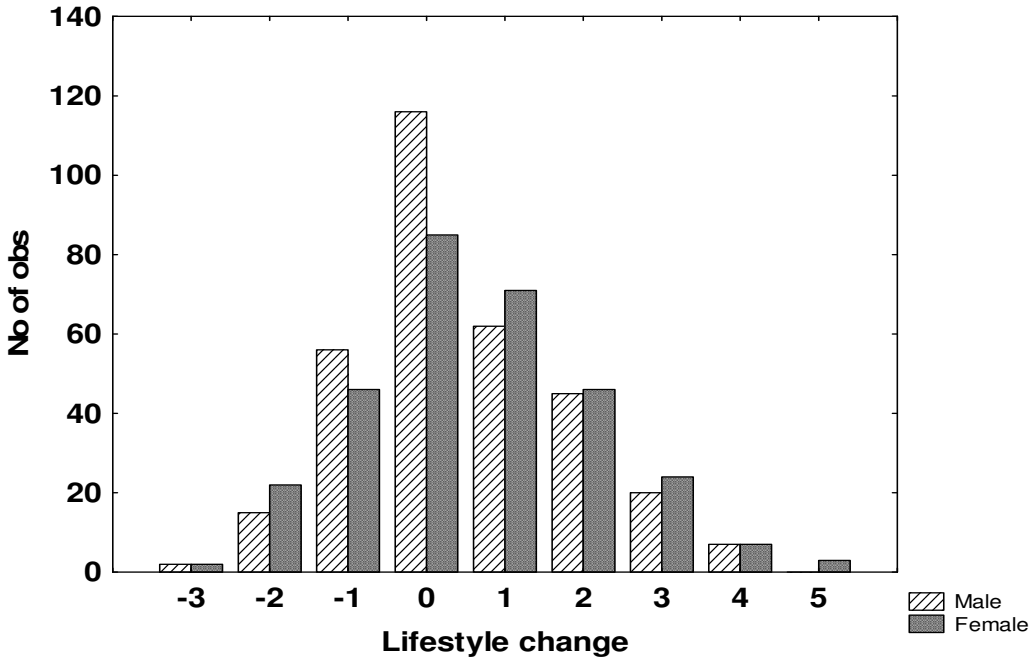
Footnotes to table. Diff is difference between non-success and success and was analysed by Fischer´s exact test if possible, otherwise with Chi^2-test.

Table 6. Statistically significant variables correlated to success of changing life style habits (Success) calculated as odds ratios using logistic regression for men and women separately. Age was not correlated to success and was deleted in the model together with other non-significant variables.

	Men				Women			
	Success		Multivariate analysis		Success		Multivariate analysis	
Variable	N	%	OR (95% CI)	p	n	%	OR (95% CI)	p
Smoking[#]								
Non-smokers	65	32	1.00		88	44	1.00	
Smokers	67	59	3.36 (2.05-5.51)	<0.001	62	59	1.81 (1.11-2.95)	0.017
Disease*								
Not ASR	107	38	1.00		-	-		
ASR	21	70	4.77 (2.18-10.47)		-	-		
MI	4	100	22.8 (4.73-109.7)	<0.001	-	-		
Hypertension[$]								
<160/90	-	-			91	45	1.00	
≥160 or ≥ 90	-	-			59	60	1.84 (1.12-3.02)	0.016

Footnotes to table.
Although smoking at baseline is coupled to the success variable itself it has been kept in the table and is regarded as a variable for adjustment of ASR and hypertension.
Disease from the questionnaire was only significant in men. ASR= any atherosclerotic disease or disease coupled to risk of developing atherosclerosis, like hypertension, diabetes or angina. MI=myocardial infarction.
$ Hypertension (measured at baseline appointment) was only significant in women.

Figure 4. Lifestyle changes (- 5 scores to + 5) by gender in a 10-year follow-up telephone survey 1999-2000 in the Söderåkra study cohort.



Paper III:

A total of 664 consecutively examined individuals (338 males and 326 females) at baseline were included during the 17-year follow-up. Lifestyle factors, such as physical activity, smoking habits and alcohol intake may influence levels of IGFBP-1. In this study, however, we found no association between PA, smoking habits and IGFBP-1 levels. Contrary to that, a higher alcohol intake was associated with lower IGFBP-1 levels. We also corrected for these life style factors in the Cox regression analyses on risk of incident type 2 diabetes/IGT, but found no association. In all, 389 persons did not drink alcohol at all and 265 had a weekly intake of less than 260grams. There were 236 smokers and 418 were non-smokers or ex-smokers at baseline. We lacked data for 10 persons on lifestyle questions.

Table 7 shows baseline characteristics according to the three subgroups of IGFBP-1 levels. Group 1 with the lowest values represents the first quintile of IGFBP-1 (51 females and 92 males). Group 2 was composed of the second, third and fourth quintiles (205 females and 181 males) and group 3 represented the fifth quintile (70 females and 65 males). Women had significantly higher values of IGFBP-1 than men. However, there were no significant age differences between subgroups. Weight, waist, BMI, systolic and diastolic blood pressure declined from the first to the fifth quintile and were significantly lower in the fifth than in the first quintile.

Table 8 shows baseline characteristics of individuals with and without the metabolic syndrome, respectively. There was a slight gender difference ($p<0.05$), with women having the metabolic syndrome less often (14%), in comparison to men (20%). Age, serum insulin, LDL-cholesterol, CRP, but also IGFBP-1 showed significant differences in subjects with and without the metabolic syndrome. Contrary to that, IGF-I did not correlate to the metabolic syndrome, nor did the lifestyle factors in our study.

During follow-up, 38 individuals developed type 2 diabetes and an additional, 4 cases were found with IGT based on OGTT data. These cases were also considered as events of abnormal glucose metabolism. A multivariate Cox proportional hazard regression analysis (Table 9) was used to explore risk factors for the development of diabetes/IGT during the 17-years of follow-up. The regression analysis (age- and gender-corrected) included IGFBP-1, waist and CRP, with lifestyle factors, IGF-I and oestrogen medication as covariates. As seen in Table 9 the lowest quintile of IGFBP-1 was significantly associated with the development of type 2 diabetes/ IGT, HR 3.54 (95% CI: 1.18-10.6, $p=0.024$). Furthermore, both CRP, HR 6.81 (95% CI: 2.50-18.6, $p<0.001$), and waist, HR 3.33 (95% CI: 1.47-7.6, $p=0.004$) remained as independent predictors. The other covariates did not show significant relation to incident diabetes/IGT.

If insulin, IGF-I and IGFBP-1 were all jointly included in a multivariate Cox regression analysis, both insulin ($p=0.0007$) and IGFBP-1 ($p=0.017$) but not IGF-I, remained as statistically significant predictors for the development of diabetes mellitus/IGT.

Table 7. Baseline characteristics of study subjects by IGFBP-1 quintiles (Q1-Q5). The Söderåkra Cardiovascular Risk factor Study 1989-1990.

	IGFBP-1 quintile 1			IGFBP-1 quintile 2-4			IGFBP-1 quintile 5				
	Median	Range		Median	Range		Median	Range		p*	p**
Gender (women/men)	51 / 92			205 / 181			70 / 65			0.001***	
Age (years)	48	44	53	47	44	52	45	42	52	0,062	
Weight (kg)	84	78	92	72	64	83	68	60	77	<0.001	<0.001
Waist (cm)	95	89	100	86	78	93	81	73	88	<0.001	<0.001
BMI (kg/m$^{2)}$	28	26	31	25	23	28	23	22	25	<0.001	<0.001
Systolic BP (mmHg)	130	120	140	129	118	140	120	112	131	<0.001	<0.001
Diastolic BP (mmHg)	90	82	90	86	78	90	82	76	88	<0.001	<0.001
Cigarettes/day	0	0	5	0	0	10	0	0	10	0.127	
Physical activity (group 1-3)	2	1	2	2	1	2	2	1	2	0.499	
Alcohol intake (g/week)	0	0	49	0	0	30	0	0	20	0.009	0,005
Serum cholesterol (mmol/l)	5,9	5,2	6,9	5,8	5,1	6,7	5,8	5,0	6,5	0.310	
LDL (mmol/l)	3,9	3,1	4,6	3,7	3,1	4,5	3,7	2,9	4,5	0.597	
HDL (mmol/l)	1,4	1,2	1,7	1,5	1,3	1,8	1,7	1,3	1,9	<0.001	<0.001
Serum triglycerides (mmol/l)	1,3	0,9	2,1	0,9	0,7	1,4	0,8	0,6	1,1	<0.001	<0.001
Blood glucose (mmol/l)	4,6	4,2	5,0	4,4	4,1	4,8	4,3	4,0	4,7	<0.001	<0.001
Insulin (µU/l)	9,8	7,1	13,3	7,3	5,7	9,6	6,0	4,6	7,5	<0.001	<0.001
HOMA §	2,0	1,4	2,8	1,5	1,1	1,9	1,1	0,8	1,4	<0.001	<0.001
IGF-I (µg/l)	155	132	179	146	121	176	135	111	161	<0.001	<0.001
IGFBP-1 (µg/l)	19	15	21	39	30	48	73	65	88		
CRP (mg/l)	1,6	1,0	3,0	1,5	0,9	2,7	1,3	0,9	2,5	0.533	
Serum creatinine (µmol/l)	72	64	82	68	60	78	71	62	79	0.305	

Range=interquartile range, § Fasting glucose (mmol/L) x fasting insulin (µU/L) / 22.5

* Non-parametric analyses of variance for group differences (Kruskal-Wallis) if not otherwise stated.

** Lower IGFBP-1 quintile versus upper quintile using non-parametric test (Mann-Whitney's U-test) in case of Kruskal-Wallis significance.

*** Chi2-test. Lower IGFBP-1 quintile comprises significantly more men (p<0.0004)

Table 8. Characteristics of individuals with and without the metabolic syndrome (IDF definition, 2005).

	Metabolic syndrome			No metabolic syndrome			
	Median	Q1	Q3	Median	Q1	Q3	p*
Gender (women/men)	45 / 67			281 / 270			0.047†
Age (years)	50	45	54	46	43	52	0.002
Weight (kg)	86	80	93	71	64	81	<0.001
Waist (cm)	97	94	103	85	77	92	<0.001
BMI (kg/m$^{2)}$	29	27	31	25	23	27	<0.001
Systolic BP (mmHg)	134	129	150	126	116	139	<0.001
Diastolic BP (mmHg)	90	86	96	84	78	90	<0.001
Cigarettes/day (n)	0	0	13	0	0	10	0.277
Physical activity(group1-3)	2	1	2	2	1	2	0.380
Alcohol intake (g/week)	0	0	32	0	0	32	0.772
Total cholesterol (mmol/L)	6.2	5.5	7.3	5.7	5.0	6.5	<0.001
LDL (mmol/l)	4.2	3.5	4.9	3.7	3.0	4.4	<0.001
HDL (mmol/L)	1.2	1.1	1.4	1.6	1.3	1.8	<0.001
Triglycerides (mmol/L)	1.9	1.4	2.4	0.9	0.7	1.3	<0.001
Blood glucose (mmol/L)	4.9	4.5	5.3	4.4	4.1	4.7	<0.001
Insulin (µU/l)	10.7	8.0	15.7	6.9	5.3	9.0	<0.001
HOMA	2.4	1.7	3.4	1.3	1.0	1.8	<0.001
IGF-I (µg/L)	149	126	175	146	120	173	0.204
IGFBP-1 (µg/L)	26	19	36	42	28	57	<0.001
CRP (mg/L)	2.1	1.2	4.7	1.4	0.9	2.6	<0.001
Serum creatinine (µmol/L)	74	64	87	69	61	78	0.002

* Non-parametric test (Mann-Whitney U-test) if not otherwise stated
† Chi2-test

Table 9. Results from Cox proportional hazard regression analysis for risk of diabetes/IGT adjusted for age, gender, alcohol consumption, smoking, physical activity, IGF-I and oestrogen medication, all of which were statistically non-significant.

					Univariate Cox regression*		Multivariate Cox regression	
Parameter		Total	Diabetes	Diab%	HR (95% conf int)	p	HR (95% conf int)	p
Waist (cm)**	<80/94	358	8	2.2%	1.00		1.00	
	≥80/94	305	34	11.1%	5.25 (2.43 - 11.3)	<0.001	3.33 (1.47 - 7.6)	0.004
IGFBP-1 (µg/l)	≥59	135	2	1.5%	1.00		1.00	
	24-59	386	22	5.7%	2.60 (1.58 - 4.28)		1.88 (1.09 - 3.25)	
	≤24	143	18	12.6%	6.76 (2.50 - 18.3)	<0.001	3.54 (1.18 - 10.6)	0.024
CRP (mg/l)	≤0.9	174	2	1.1%	1.00		1.00	
	0.9-2.8	328	19	5.8%	2.83 (1.75 - 4.56)		2.61 (1.58 - 4.31)	
	>2.8	161	21	13.0%	8.00 (3.07 - 20.8)	<0.001	6.81 (2.50 - 18.6)	<0.001

* Not adjusted
** Limits for women/men
HR is hazard rate ratio

Paper IV:

The study cohort comprised 689 individuals at baseline. Study participants were followed for 17 years. In all, 69 participants died during the period. An autopsy was performed on 21 subjects and 9 of these were found to have died from MI or stroke. A first fatal or non-fatal event of CVD occurred in 71 subjects. Table 10 shows descriptive statistics for men and women concerning CRP, ADMA and SDMA. Men had significantly higher levels of SDMA ($p<0.001$). Table 11 shows correlations between ADMA and SDMA, respectively, and various other variables. There were significant correlations for ADMA and SDMA and several variables as seen in the table. Table 12 shows baseline characteristics of the study population with and without CVD during follow-up. There were more females in the non-CVD group, and females represented only 25% of the events. The mean age in the study population was an additional two years in the event group, compared with the event-free group. A medical history of treated hypertension but not diabetes was significantly associated with CVD events ($p<0.001$). Smoking was more common in the CVD event group ($p=0.016$).

Table 13 shows the crude estimates of hazard ratios (HR) with 95% confidence intervals for the development of fatal or non-fatal CVD in relation to the parameters used in the three risk models, Consultation (I), SCORE (II) and Extended (III). Age, gender, treated hypertension, family history of CVD, office blood pressure, waist/height ratio, serum cholesterol, serum triglycerides, LDL/HDL ratio and blood glucose showed the highest levels of significance in univariate analyses. However, CRP, SDMA, IGF-I and smoking at baseline were also significantly associated with the risk of developing CVD, whereas prevalent diabetes at baseline was of only borderline significance ($p=0.058$).

Table 14 shows HR for CVD in the three models, respectively, with almost identical values. The three models are further illustrated with ROC curves that are included in Figure 5.
AUC for the Consultation model was 0.794; (CI 95% 0.762-0.823), for SCORE 0.767; (CI 95% 0.733-0.798, $p=0.122$) and for the Extended model 0.806; (CI 95%: 0.774-0.835, $p=0.550$). Finally, the Extended model was significantly better than SCORE, $p=0.033$, in predicting CVD.

Table 10. Mean, median, SD, quartile range (Q1-Q3) and range (min-max) for ADMA, SDMA and CRP, calculated separately for gender.

Variable				p*
		Men	**Women**	
CRP	Mean	2.69	2.67	
mg/l	SD	4.07	4.08	
	Median	1.60	1.50	0.519
	Q1-Q3	0.90 - 2.80	1.00 - 2.80	
	Range	0.05 - 52	0.20 - 36	
ADMA	Mean	0.49	0.48	
µmol/l	SD	0.07	0.08	
	Median	0.48	0.48	0.062
	Q1-Q3	0.44 - 0.52	0.43 - 0.52	
	Range	0.32 - 1	0.32 - 0.87	
SDMA	Mean	0.48	0.45	
µmol/l	SD	0.10	0.10	
	Median	0.47	0.43	**0.001**
	Q1-Q3	0.43 - 0.52	0.39 - 0.49	
	Range	0.30 - 2	0.26 - 1.53	

Differences between men and women by Mann-Whitney U-test.

Table 11. Correlations between ADMA, SDMA, respectively, and various variables using Spearman rank order correlations. Significant correlations are bolded.

ADMA versus	N	R	p
Cholesterol	697	0.07	0.065
LDL-cholesterol	696	0.09	**0.015**
HDL-cholesterol	696	-0.12	**<0.001**
Triglycerides	697	0.12	**<0.001**
Blood glucose	697	0.01	0.767
C-reactive protein	696	0.10	**0.009**
Creatinine	696	0.17	**<0.001**
Insulin	697	0.08	**0.043**
Waist	696	0.18	**<0.001**
IGF-I	697	-0.03	0.465
IGFBP-1	697	-0.01	0.706
Systolic blood pressure	683	0.04	0.342
Diastolic blood pressure	681	0.10	**0.008**
SDMA versus			
Cholesterol	697	0.13	**<0.001**
LDL-cholesterol	696	0.16	**<0.001**
HDL-cholesterol	696	-0.01	0.730
Triglycerides	697	0.03	0.401
Blood glucose	697	-0.06	0.127
C-reactive protein	683	0.03	0.410
Creatinine	681	0.60	**<0.001**
Insulin	696	-0.09	**0.017**
Waist	697	0.15	**<0.001**
IGF-I	697	-0.06	0.130
IGFBP-1	697	0.14	**<0.001**
Systolic blood pressure	696	0.07	0.077
Diastolic blood pressure	696	0.09	**0.014**

R= correlation coefficient

Table 12. Baseline characteristics of participants in the Söderåkra Cardiovascular Risk Factor Study 1989-1990.

	Patients without CVD events		Patients with CVD events		
Variables*	**n**		**n**		p§
Age years, mean(SD), range	618	48 (6) (40 - 59)	71	50 (6) (40 - 59)	<0.001
Sex (female/male)	618	322 / 296	71	18 / 53	<0.001
Medical history					
Diabetes (%)	618	1.3	71	4.2	0.111
Hypertension,treated(%)	618	3.6	71	21.1	<0.001
Lifestyle history					
Smoking (%)	618	34	71	49	0.016
Family history of CVD					
(1/ ≥2 relatives; %) **		37 / 3.0		49 / 8.6	0.007
Physical examination					
Systolic blood pressure mmHg,mean(SD), range	605	128 (18) (90 - 205)	70	138 (18) (106 - 181)	<0.001
Hypertension*** (yes/no)		254 / 350		45 / 24	<0.001
Waist/height-ratio ****	617	51 (6) (37 - 81)	71	54 (6) (44 - 74)	<0.001
Laboratory measurements					
Total cholesterol, mmol/l	618	5.86 (1.1) (2.2 - 9.9)	71	6.44 (1.3) (3.9 - 10.1)	<0.001
LDL/HDL-ratio,	617	2.6 (1.0) (0.3 - 6.4)	71	3.2 (1.2) (0.7 - 6.7)	<0.001
Triglycerides, mmol/l	618	1.1 (0.7) (0.3 - 4.6)	71	1.6 (0.8) (0.6 - 4.0)	<0.001
Blood glucose, mmol/l	618	4.5 (0.7) (2.7 - 12.9)	71	4.9 (1.1) (3.4 - 9.5)	0.002
IGF-I, µg/l	612	151 (39) (70 - 305)	70	141.5 (39.4) (60 - 254)	0.063
CRP, mg/l	613	2.6 (4.1) (0.1 - 52)	70	3.5 (3.8) (0.1 - 20)	0.009
SDMA, µmol/l	611	0.5 (0.1) (0.3 - 1.7)	70	0.5 (0.1) (0.3 - 0.7)	0.058

* Mean, SD, range for continuous variables and frequencies for categorical, §= MannWhitney U test for continuous variables and Chi2 test for categorical, ** Myocardial infarction or stroke in parents or first-degree siblings, ***Measured blood pressure ≥ 140 and/or ≥ 90 mmHg, **** Waist circumference (cm) divided by height (m).

Table 13. HR with 95% CIs using univariate Cox´s proportional Hazard regression analyses for first major non-fatal or fatal cardiovascular event for the variables included in the three risk models; Consultation (I), SCORE (II) and Extended (III).

Variables at baseline		n	HR (95% CI)	p-value	Included in model
Age		689	1.09 (1.05-1.13)	<0.001	I, II, III
Sex		689	3.07 (1.80-5.24)	<0.001	I, II, III
Medical history					
	Diabetes	689	3.06 (0.96-9.72)	0.058	I, III
	Hypertension, treated	676	6.0 (3.4-10.7)	<0.001	I, III
Lifestyle history					
	Smoking	676	1.82 (1.14-2.90)	0.013	I, II, III
Family history					
	CVD *	676	1.81 (1.24-2.63)	0.002	I, III
Physical	**examination**				
	Systolic blood pressure	675	1.03 (1.01-1.04)	<0.001	II
	Hypertension **	673	2.47 (1.50-4.05)	<0.001	I, III
	Logwaist/height-ratio ***	688	59.7 (9.5-376)	<0.001	I, III
Laboratory	**measurements**				
	Log (total cholesterol)	689	11.1 (3.3-37.1)	<0.001	II
	LDL/HDL-ratio	688	1.62 (1.35-1.95)	<0.001	III
	Log (triglycerides)	689	3.35 (2.16-5.20)	<0.001	III
	Log (blood glucose)	689	8.88 (2.94-26.8)	<0.001	III
	Log (C-reactive protein)	683	1.37 (1.08-1.75)	0.010	III
	Log (IGF-I)	682	0.35 (0.14-0.87)	0.024	III
	SDMA	681	7.43 (1.01-54)	0.048	III

CI, confidence interval, HR, hazard ratio

* Myocardial infarction or stroke in parents or first-degree siblings

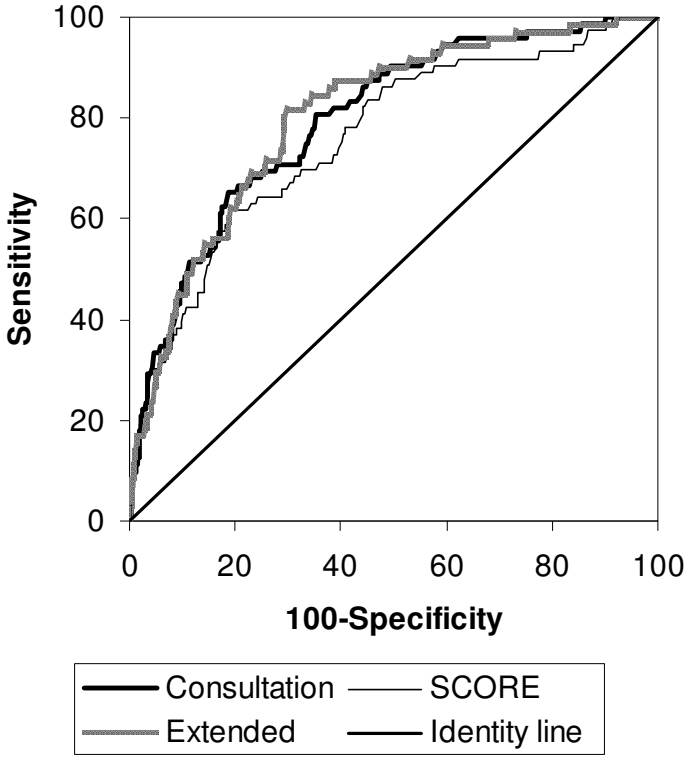
** Measured blood pressure ≥ 140 and/or ≥ 90 mmHg

*** Waist circumference (cm) divided by height (m)

Table 14. Univariate Cox proportional Hazard regression analyses with Hazard ratios (HR) with 95% confidence intervals for first major non-fatal or fatal cardiovascular event calculated for the three risk models: Consultation (I), SCORE (II) and Extended (III).

Model	n	HR (95% CI)	p-value
Consultation (I)	672	2.72 (2.18-3.39)	<0.001
SCORE (II)	675	2.73 (2.10-3.55)	<0.001
Extended (III)	663	2.72 (2.19-3.37)	<0.001

Figure 5. ROC curves for the Consultation model, SCORE and the Extended model for prediction of non-fatal and fatal cardiovascular disease events.



DISCUSSION

A secondary cause of hypercholesterolemia

The results of our investigation (Paper I) must be regarded as highly representative of a rural population in Sweden. Hypercholesterolemia is one of the laboratory signs characterising hypothyroidism. In a consecutive study of 200 referrals to a lipid clinic, 7.5% (3 men and 12 women) had raised TSH levels (102). No correlation was found between cholesterol and TSH levels, however.

In a study by Ball *et al* (103) of 272 individuals, 6% of those with a plasma cholesterol concentration above 7mmol/L also had biochemical evidence of hypothyroidism. In our population-based study with a much a larger sample size (705 individuals), we found 0.57% males and 1.13% females to have evidence of hypothyroidism as defined by a TSH value greater than 3.75mU/L of those with a serum cholesterol above 7mmol/L. In a screening study of 1210 people above 60 years 0.05% had subclinical hypothyroidism, as defined by serum TSH >4.45 mU/L, and normal free T4. These individuals showed no significant differences in circulating lipid levels (104).

The overall prevalence of asymptomatic hypothyroidism (TSH>3.75 mU/L) in the population is unclear. In our population it could be estimated at 1.4% in males and 4.4% in females. In the metabolic work-up of a patient presenting with hyperlipidemia it is essential to identify the secondary causes. Our study indicates that it is beneficial to look for hypothyroidism as a secondary cause in female subjects with a serum cholesterol >7.0mmol/l and important to recognise the correlation with serum triglycerides.

Predictors for lifestyle changes among participants after 10 years

In this population-based study (Paper II) an independent predictor for success in self-reported lifestyle changes in a 10-year perspective was female gender. Previous studies have also

shown female gender to be an independent predictor for success in lifestyle change (105), which our results confirm. In our study, smokers changed their lifestyle in a more positive way than non-smokers. Other authors (106) have found, for example, that with the "Quit and Win contest" model, known in many countries, the most significant determinants for successful quitting were sex, age, marital status, level of withdrawal symptoms, previous quitting attempts, and support received.

Participants with a medical history of diabetes, hypertension, angina pectoris or myocardial infarction at baseline were also more likely to be successful in our study. These findings are in accordance with the observation of a chronic disease being a "teachable moment" for increased motivation towards achieving positive lifestyle changes (107,108).

Contrary to what one may expect, differences in educational level and socio-economic status were not predictors for success in lifestyle change in the Söderåkra cardiovascular risk factor study. These findings are in accordance with a previously reported community intervention, the PHC-based programme in Norsjö, Sweden (109), where all socio-economic groups benefited equally well from health screenings and activities directed to support lifestyle change. In contrast, the Sollentuna Prevention Programme, Sweden (110), showed that younger age and higher educational level were factors associated with a successful reduction of cholesterol levels, but that was in an urban setting – not a rural as in Norsjö and Söderåkra.

Predicting diabetes/IGT by blood testing for CRP, IGFBP-1 as well as the measurement of waist circumference

The main finding of this observational, follow-up cohort study (Paper III) was the statistically significant association between low levels of IGFBP-1 (quintile 1) at baseline and the development of type 2 diabetes/IGT in a middle-aged population. In accordance with our findings other authors (111) have shown that low IGFBP-1 is a marker of hyperinsulinemia in obese, menopausal women. Saitoh *et al* (112), studied pre-pubertal obese children and found that low IGFBP-1 was a predictor of glucose-stimulated hyperinsulinemia. Fasting IGFBP-1

in healthy men is a better predictor of insulin response to hyperglycaemia than plasma glucose and serum insulin (113).

The finding that low IGFBP-1 concentrations predicted increased future risk of diabetes/IGT was still present in the multivariate regression analyses independent of waist circumference and CRP. Age and gender were not significantly related to the development of diabetes/IGT in the same model. Not surprisingly, an increased waist circumference at baseline independently predicted a higher incidence of diabetes/IGT. CRP is well known as a predictor of cardiovascular disease (114), but also for risk of type 2 diabetes (115). If insulin, IGF-I and IGFBP-1 were all included in a multivariate Cox regression analysis, both insulin and IGFBP-1, but not IGF-I, remained statistically significant predictors for the development of diabetes mellitus/IGT. This suggests that the association between IGFBP-1 and the development of diabetes mellitus/IGT might be independent of the actual levels of insulin at baseline.

To the best of our knowledge, the results are the first to show a significant, independent association between low serum concentrations of IGFBP-1 and the development of type 2 diabetes/IGT in a population-based study including both men and women. In a population study of Swedish normoglycaemic men, low levels of IGFBP-1 predicted the development of abnormal glucose regulation (116). Sandhu *et al* (57) conducted a prospective (4.5 year), observational cohort study of 615 middle-aged normoglycemic men and women from a random population-based sample. The odds ratio (OR) for risk of IGT or type 2 diabetes, with IGF-I above median compared to IGF-I below median, was 0.5 and this inverse association was independently modified by IGFBP-1. We could not however, show similar associations for IGF-I in the present study.
In all, 38 (5.7%) subjects developed type 2 diabetes in our study, but the diagnosis was based on primary care records and not on OGTT in general. Furthermore, in only four cases (0.6%) IGT was diagnosed. It is obvious that additional cases of type 2 diabetes as well as IGT could have been detected if OGTT had been performed in all subjects during follow-up. The underestimation of case ascertainment might have resulted in a weaker association than would otherwise have been the case with addition of OGTT on all subjects.

In a similar study by Sandhu *et al* (57), 44 (7%) of 615 normoglycaemic subjects aged 45-65, developed IGT and 7 (1%) developed type 2 diabetes after 4.5 years of follow-up. Our study

had a 17-year follow-up period that was almost four times longer. It was, therefore, expected that baseline IGT had progressed to type 2 diabetes in most cases in our study.

The Consultation model as a CVD risk score instrument

There was no statistical significance for ADMA in the prediction of CVD. However, other studies have shown that ADMA can serve as a marker of cardiovascular risk with a statistically significant and independent relationship with the incidence of cardiovascular disease (23). One explanation could be that this study was too small. ADMA and SDMA were, however, significantly correlated to several variables connected to cardiovascular disease.

The main finding in this population-based study, involving virtually all subjects in the Söderåkra population aged 40-59 at baseline, is that a non-laboratory-based risk assessment Consultation model, including variables easily obtained during a visit to a general practitioner, predicted cardiovascular events as accurately as the established SCORE algorithm that requires laboratory testing (Paper IV). Furthermore, a combination of sophisticated laboratory measurements covering lipids, inflammation and endothelial dysfunction conferred no additional value to the prediction of the CVD risk, as the values of c-statistics did not differ significantly.

An early instrument for estimating CVD risk was the Framingham risk scoring for coronary heart disease (CHD) over 10 years (81). In Europe, the European Society of Cardiology (ESC) has developed a corresponding risk assessment tool, SCORE (83,84). The variables used are age, gender, systolic blood pressure, total cholesterol and smoking. The SCORE system is based on data from the 1970´s and 1980´s with higher risk factor levels than found today. The risk prediction is however restricted to fatal CHD events and not morbid events that are less dramatic to discuss with the patient during a consultation.

There have been several attempts made to create better risk prediction tools. Recently two risk prediction models were compared on the NHANES follow-up cohort (117). One was based

only on medical history of diabetes or hypertension and physical investigation, the other also laboratory-based. There appeared to be no additional benefit from laboratory testing in risk prediction. Accordingly, our study confirms the results from NHANES although that study comprised follow-up data on 6186 subjects.

We included data on only 689 subjects with 71 cardiovascular first events and thus did not have adequate statistical power to perform further sub-analyses. However, the strength of our analysis lies in the fact that the study involved virtually all subjects in a geographically defined Swedish population. Contrary to our results and that of the NHANES follow-up study, another Swedish study of a cohort of elderly men has recently suggested that the combined addition of biomarkers of cardiovascular and renal abnormalities substantially improves the risk prediction for fatal cardiovascular events beyond that of a model that is based only on established risk factors (118). The biomarkers were: troponin-I, NT-pro BNP, cystatin C, and CRP.

Following the recent JUPITER-trial (119), where healthy subjects without hyperlipidemia but with elevated CRP-levels benefited from treatment with rosuvastatin, the issue of measuring CRP in clinical practice is under debate. However, from our observational study, we can conclude that CRP did not contribute any further information on future CVD risk to the non-laboratory based Consultation model, although CRP did univariately predict the CVD risk by HR 1.37 (1.00-1.75).

General discussion

The Söderåkra study cohort was recruited from the total population of both healthy and diseased subjects in a defined geographic rural area. The cohort was relatively small compared with other larger cohorts with a similar study design. However, our cohort was followed for 17 years, thus comprising almost twelve thousand person years under observation.

An advantage with the study is the high attendance rate. The baseline study (Paper I) recruited 90 percent of the age (40-59 years) group, a satisfactory result. However, at the 10-year-follow-up telephone survey study (paper II) 28 subjects had died and thus information on these subjects was not obtainable. An additional 48 subjects could not be reached with our

method of tracing the baseline population. We could eventually have applied a more effective follow-up method, as the dropouts most likely were individuals either living in other parts of the country or without reachable telephone number, not because of refusing the interview as such.

Much debating has previously focused on ethical values when conducting population based screening studies. Nowadays, the view among many is that it is not only permissible but also highly recommendable to find a cluster of risk factors at an early stage to enable suitable actions. However, this view is currently not supported by official Swedish guideline concerning screening and primary prevention directed to the general population (14). Among different ways for a more targeted recruitment strategy, opportunistic screening at visits to primary health care centres is considered to be the most cost-effective and convenient method compared with inviting subjects to a centre for testing at a specific time.

The study participants benefitted from the screening programme in mainly two ways. The first advantage was that an unhealthy lifestyle could be revealed early and dealt with at first during a short visit to the GP. Advice was given mainly about diet, smoking habits, physical activities and alcohol consumption, but also on overweight and increased waist circumference. Such a procedure with short time-limited counselling is now known as a brief intervention in epidemiology, mainly used for alcohol intervention (120) but lately used also in drug addiction prevention programmes (121) as well as in anxiety management in primary health care (122). However, the pathological findings of the participants could also be dealt with later on, at other consultations following the baseline study.
The second advantage of the intervention was that risk conditions such as hypertension, hypothyroidism, diabetes and hyperlipidemia were detected earlier and could hence be treated according to contemporary guidelines.

One question arising from the study results is, whether the CVD rate has decreased in the investigated population. This cannot easily be evaluated due to lack of a defined control group or control area. However, the remaining inhabitants in the same age group in the community could possibly have served as a control group but information has not been obtainable. We know, however, that the declining rate of CVD in Sweden and Kalmar County Council also applied to the local community, but still remains above the mean.

The most important finding of the study, in our opinion, was the description of a new simple risk prediction tool for cardiovascular risk, the Consultation model, which works as accurately as SCORE or a method comprising elaborate laboratory testing.

It should however be acknowledged that this consultation took place in a rural setting. It therefore remains to be proven if similar results can be obtained in other settings, including other populations.

CONCLUSIONS

General conclusions

Screening for risk of cardiovascular disease and impaired glucose metabolism in primary health care is feasible using simple methods. Long-term benefits for improved self-reported lifestyle changes are independent of social background of the individual, but less effective in men compared to women. This calls for more effective methods in lifestyle counselling tailored to the needs of middle-aged men with elevated cardiovascular risk factors but otherwise healthy.

Specific conclusions

Study I

Screening for subclinical hypothyroidism in females from the general population with serum cholesterol above 6.8-7.0 mmol/L seems appropriate.

Study II

Female gender was associated with significant improvements in self-reported lifestyle changes. Furthermore, in men, smoking, a medical history of diabetes, hypertension, angina pectoris or myocardial infarction at baseline predicted success in lifestyle change during a 10-year follow-up study. For women, treatment for hypertension at baseline was also associated with improvement in lifestyle change.

Study III

Low levels of IGFBP-1 predicted the development of impaired glucose metabolism, e.g. type 2 diabetes or IGT, in a defined, middle-aged population. The association was independent of CRP and abdominal obesity, as markers of inflammation and the influence of abdominal fat tissue.

Study IV

A non-laboratory, consultation based risk prediction model, including data on age, gender, medical history, lifestyle history, family history and findings during a physical examination, yielded similar risk estimates to risk models relying on laboratory testing. Practical implications could be substantial, if replicated, for cardiovascular risk factor screening without laboratory testing, with its reduced costs and greater convenience.

FUTURE PERSPECTIVES

Further studies are needed to confirm our finding that sufficient information for prediction of future cardiovascular risk is obtainable at one single clinical GP consultation, based on medical history and the clinical examination but without laboratory testing.
It is of great importance to implement and test the consultation model in other populations. The risk algorithm is, hopefully, feasible even for developing countries because of its low cost and simple application without laboratory testing in primary health care. One prerequisite is that enough time is allotted to each clinical consultation so that sufficient data can be accumulated from the medical history and clinical examination.

The role of risk markers such as IGF-I, IGFBP-1, CRP, ADMA and SDMA has to be further explored in population-based studies as well as in intervention studies based on lifestyle advice or drug therapy.

SVENSK SAMMANFATTNING

Bakgrund. Hjärtkärlsjukdomar har länge utgjort den vanligaste orsaken till sjuklighet och död i Sverige. Förebyggande arbete i primärvården har på senare år bidragit till minskad sjuklighet och död inom denna sjukdomsgrupp. Det behövs dock bättre metoder både för påvisande och förebyggande för att ytterligare minska eller uppskjuta risken att insjukna i hjärtkärlsjukdom.

Metod. Denna avhandling omfattar fyra delstudier. Forskningen studerar riskfaktorer samt riskskattning av hjärtkärlsjukligheten samt störd sockeromsättning i en medelålders (40-59 år) befolkning mellan åren 1989 och 2006 i Söderåkra i sydöstra Sverige.
Vid ett besök hos distriktsläkaren 1989-1990 uppmättes blodtrycket. Resultatet av mätning av vikt, midjemått, blodsocker, blodfetter och sköldkörtelprover meddelades deltagaren. En enkät avseende ärftlig sjukdom, tidigare egen sjukdom, medicinering samt livsstilsfrågor genomfördes och blev sedan granskad av läkare. Läkaren gav råd avseende anpassade livsstilsförändringar och erbjöd behandling av nyupptäckta sjukdomstillstånd eller förhöjda riskfaktorer.

Via en telefonenkät år 1999-2000 följdes de ursprungliga deltagarna upp angående omfattningen av genomförda livsstilsförändringar efter det att undersökningen påbörjats 1989-1990.

År 2006 studerades patientjournaler från primärvården med avseende på nyinsjuknande i typ 2 diabetes samt i fyra fall även nedsatt glukostolerans, upptäckt via glukostoleranstest (OGTT).

Slutligen inhämtades år 2006 uppgifter om studiedeltagarna i Socialstyrelsens register över vård på sjukhus samt död i hjärtkärlsjukdomar. Totalt 71 personer hade insjuknat för första gången eller dött i hjärtkärlsjukdom.

Därefter jämfördes tre olika metoder (riskmodeller) för att bedöma risken att insjukna i hjärtkärlsjukdom, varav en utan och två med data från laboratorieprover.

Resultat. Totalt 90 % av den inbjudna befolkningen, sammanlagt 705 män och kvinnor undersöktes. Den första delstudien visade bland annat, att kvinnor med ett kolesterolvärde över 7.0mmol/L hade större risk än de med ett lägre kolesterolvärde att också vara drabbade av nedsatt produktion av sköldkörtelhormon, talande för en utveckling mot s.k. hypotyreos. Vid telefonintervjun 10 år efter studiestarten fann man att kvinnor rapporterade genomförda livsstilsförändringar i högre utsträckning än män. Män som haft hjärtkärlsjukdom eller rökte och kvinnor med högt blodtryck rapporterade högre grad av livsstilsförändringar.

Till och med 2006 fick 38 personer diagnosen typ 2 diabetes samt ytterligare fyra personer nedsatt glukostolerans. Ett lågt värde på ett laboratorieprov, som speglar insulinkänslighet, IGFBP-1, visade sig förutsäga framtida insjuknade i diabetes. Dessutom var ett större midjemått samt ett högre mått på inflammation (CRP) i kroppen förenat med ett ökat insjuknande i typ 2 diabetes.

Slutligen jämfördes tre olika riskbedömningar. Vi fann att en enkel metod, kallad Konsultationsmetoden, förutsade framtida hjärtkärlsjukdom lika tillförlitligt som två andra metoder med inslag av blovprovstagning. De ingående variablerna i Konsultationsmetoden var ålder, kön, rökning, hjärtkärlsjukdom i släkten, egen sjukdom i högt blodtryck och diabetes, samt även midjemått och uppmätt blodtryck. Den etablerade metoden SCORE innehåller provtagning (kolesterol). Den tredje metoden som definierades speciellt för denna studie, omfattade dessutom även flera ovanliga blodprover som speglar negativ påverkan på kärl- och njurfunktion. Den var likvärdig med Konsultationsmetoden men bättre än SCORE.

Slutsats. Vi bedömer att en enkel metod för riskskattning som kan erhållas vid ett läkarbesök, och som inte kräver provtagning, är användbar i primärvården, både i Sverige och i länder med begränsade ekonomiska resurser, även om kontrollstudier behövs.

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