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PO Box 117  
221 00 Lund  
+46 46-222 00 00

Department of Clinical Sciences in Malmö  
Epidemiological Research Group  
Malmö University Hospital  
Lund University, Sweden

# Obesity and cardiovascular disease

Aspects of methods and susceptibility

Susanna Calling MD



LUND UNIVERSITY  
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*To Stefan and Alva*

Utan tvivel är man inte riktigt klok.

*Tage Danielsson*

CONTENTS

ABSTRACT.....7

LIST OF PAPERS.....8

ABBREVIATIONS.....9

INTRODUCTION.....10

    OBESITY – A GLOBAL HEALTH PROBLEM .....10

    OBESITY AND CARDIOVASCULAR MORBIDITY AND MORTALITY .....10

    SCOPE OF THE PRESENT THESIS .....11

*How to measure obesity*.....11

*Heterogeneity in risk*.....12

PATHOPHYSIOLOGY .....13

    ATHEROSCLEROSIS – AN INFLAMMATORY DISEASE .....13

    ADIPOSE TISSUE – AN ENDOCRINE ORGAN.....13

*Adipose tissue-derived proteins* .....14

    PATHOPHYSIOLOGY OF THE ASSOCIATION BETWEEN OBESITY AND CVD.....15

*Hypertension* .....16

*Dyslipidemia* .....16

*Disturbances in glucose tolerance and insulin sensitivity*.....16

*The metabolic syndrome* .....17

*Weight loss*.....17

AIMS .....19

    SPECIFIC AIMS .....19

MATERIAL, METHODS AND RESULTS .....20

    THE MALMÖ PREVENTIVE PROJECT .....20

    THE MALMÖ DIET AND CANCER STUDY .....20

    CASE RETRIEVAL.....21

    DEFINITION OF ENDPOINTS .....22

    ANTHROPOMETRIC MEASUREMENTS .....23

    LABORATORY ANALYSES.....24

*Inflammation-sensitive proteins (Paper III)* .....24

    CARDIOVASCULAR RISK FACTORS .....24

*Hypertension* .....24

*Diabetes mellitus*.....24

*Hyperlipidemia*.....25

*Alcohol consumption* .....25

*Smoking*.....25

*Socio-economic and marital status (Paper I-II)* .....26

*Leisure time physical activity*.....26

*History of angina and cancer*.....27

    STATISTICS .....27

*Interaction*.....28

    PAPER I: INFLUENCE OF OBESITY ON CARDIOVASCULAR RISK. TWENTY-THREE-YEAR FOLLOW-UP OF 22 025 MEN FROM AN URBAN SWEDISH POPULATION .....29

*Aim* .....29

*Methods*.....29

*Results*.....29

*Conclusions*.....30

PAPER II: OBESITY AND MYOCARDIAL INFARCTION – VULNERABILITY RELATED TO OCCUPATIONAL LEVEL AND MARITAL STATUS. A 23-YEAR FOLLOW-UP OF AN URBAN MALE SWEDISH POPULATION .....	31
<i>Aim</i> .....	31
<i>Methods</i> .....	31
<i>Results</i> .....	31
<i>Conclusions</i> .....	33
PAPER III: INCIDENCE OF OBESITY-ASSOCIATED CARDIOVASCULAR DISEASE IS RELATED TO INFLAMMATION-SENSITIVE PLASMA PROTEINS. A POPULATION-BASED COHORT STUDY.....	33
<i>Aim</i> .....	33
<i>Methods</i> .....	34
<i>Results</i> .....	34
<i>Conclusions</i> .....	34
PAPER IV: EFFECTS OF BODY FATNESS AND PHYSICAL ACTIVITY ON CARDIOVASCULAR RISK. RISK PREDICTION USING THE BIOELECTRICAL IMPEDANCE METHOD. ....	36
<i>Aim</i> .....	36
<i>Methods</i> .....	36
<i>Results</i> .....	36
<i>Conclusions</i> .....	39
PAPER V: SEX DIFFERENCES IN THE RELATIONSHIPS BETWEEN BMI, WHR AND INCIDENCE OF CARDIOVASCULAR DISEASE: A POPULATION-BASED COHORT STUDY .....	39
<i>Aim</i> .....	39
<i>Methods</i> .....	39
<i>Results</i> .....	40
<i>Conclusions</i> .....	40
<b>GENERAL DISCUSSION</b> .....	<b>42</b>
MARKED DIFFERENCES IN INCIDENCE OF AND MORTALITY FROM CVD IN OBESE MEN .....	42
BEING ALONE IS ASSOCIATED WITH AN INCREASED VULNERABILITY TO CVD MORBIDITY AND MORTALITY IN OBESE MEN.....	43
HIGH LEVELS OF ISP IS ASSOCIATED WITH AN INCREASED INCIDENCE OF CVD IN OBESE MEN .	44
BODY FATNESS AS MEASURED BY BIA IS A STRONGER CV RISK FACTOR THAN BMI IN WOMEN	46
WHR ADDS PROGNOSTIC INFORMATION ON CV RISK IN WOMEN AT ALL LEVELS OF BMI AND IN MEN WITH NORMAL WEIGHT.....	47
HETEROGENEITY AND POTENTIAL CAUSAL PATHWAYS.....	48
MEASUREMENTS .....	51
METHODOLOGICAL LIMITATIONS.....	51
<i>Representativity</i> .....	51
<i>Validity of endpoints and risk factors</i> .....	53
<i>Epidemiological and statistical design</i> .....	54
<i>Missing values</i> .....	55
PUBLIC HEALTH ASPECTS .....	55
<b>CONCLUSIONS</b> .....	<b>58</b>
<b>POPULÄRVETENSKAPLIG SAMMANFATTNING (SUMMARY IN SWEDISH)</b> .....	<b>59</b>
<b>ACKNOWLEDGEMENTS</b> .....	<b>62</b>
<b>REFERENCES</b> .....	<b>64</b>

## ABSTRACT

The aim of this thesis was to study the morbidity and mortality of cardiovascular disease (CVD) in obese individuals, as measured by different obesity measurements, and to explore how the CVD risk related to obesity was modified by other biologic and socio-demographic circumstances.

Data from two population-based cohort studies was used. The Malmö Preventive Project included 22 444 middle-aged men, with a mean follow-up of 17.7 years. In a subcohort of 6193 men, information on inflammatory proteins was available. The Malmö Diet and Cancer Study included 28 098 men and women, with a mean follow-up of 7.6 years. National and local registers were used to follow the incidence of coronary events (CE), stroke and mortality.

Body mass index (BMI) was an independent risk factor for CE and mortality in men. However, the risk associated with obesity was increased by exposure to other atherosclerotic risk factors (smoking, hypertension, diabetes mellitus and hyperlipidemia), of which smoking seemed to be the most important. Obesity was more prevalent in men with manual work and in men living alone, than in men with non-manual work and in cohabiting men. Adjusted for lifestyle and biological risk factors, the increased risk of CE and death for obese men with manual jobs was applicable only to those who were single. There was a positive interaction between obesity and living alone for incidence of CE. Increased BMI was related to plasma levels of inflammation-sensitive proteins (ISP) in men. The CVD risk varied widely between obese or overweight men with high and low ISP.

Body fat percentage (BF%), measured by bioelectrical impedance method, was an independent risk factor for cardiovascular morbidity and mortality in men and women. BF% was a stronger CVD risk factor in women than in men. The raised CVD risk associated with high BF% was reduced by physical activity. Body fat distribution as measured by waist hip ratio (WHR) was associated with increased CVD risk. WHR added to the CVD risk in women at all levels of BMI and in men with normal weight. It is concluded that the susceptibility to CVD in obese people differs substantially according to subsets of other biologic and socio-demographic circumstances.



## LIST OF PAPERS

- I. **Jonsson S**, Hedblad B, Engstrom G, Nilsson P, Berglund G, Janzon L. Influence of obesity on cardiovascular risk. Twenty-three-year follow-up of 22,025 men from an urban Swedish population. *Int J Obes Relat Metab Disord* 2002;26:1046-53.
- II. Hedblad B, **Jonsson S**, Nilsson P, Engstrom G, Berglund G, Janzon L. Obesity and myocardial infarction--vulnerability related to occupational level and marital status. A 23-year follow-up of an urban male Swedish population. *J Intern Med* 2002;252:542-50.
- III. Engstrom G, Hedblad B, Stavenow L, **Jonsson S**, Lind P, Janzon L, Lindgarde F. Incidence of obesity-associated cardiovascular disease is related to inflammation-sensitive plasma proteins: a population-based cohort study. *Arterioscler Thromb Vasc Biol* 2004;24:1498-502.
- IV. **Calling S**, Hedblad B, Engström G, Berglund G, Janzon L. Effects of body fatness and physical activity on cardiovascular risk. Risk prediction using the bioelectrical impedance method. *Scand J Public Health* 2006 (In press)
- V. Li C, Engström G, Hedblad B, **Calling S**, Berglund G, Janzon L. Sex differences in the relationships between BMI, WHR and incidence of cardiovascular disease: a population-based cohort study. *Int J Obes Relat Metab Disord* 2006 (In press).

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## ABBREVIATIONS

BIA	Bioelectrical impedance analysis
BMI	Body mass index
BF%	Body fat percentage
CE	Coronary/ cardiac event
CI	Confidence interval
CRP	C-reactive protein
CV(D)	Cardiovascular (disease)
FFA	Free fatty acids
$\gamma$ -GT	$\gamma$ glutamyltransferase
HDL	High-density lipoprotein
HPA	Hypothalamo-pituitary-adrenal
IGT	Impaired glucose tolerance
IL	Interleukin
ISP	Inflammation-sensitive plasma proteins
LDL	Low-density lipoprotein
MDCS	Malmö Diet and Cancer Study
MI	Myocardial infarction
MMIR	Malmö Myocardial Infarction Register
MPP	Malmö Preventive Project
PAI-1	Plasminogen activator inhibitor-1
RR	Relative risk
SEI/SES	Socio-economic index/ status
SI	Synergy index
STROMA	Stroke Register in Malmö
T2DM	Type 2 diabetes mellitus
TNF- $\alpha$	Tumour necrosis factor $\alpha$
VLDL	Very low-density lipoprotein
WHO	World Health Organization
WHR	Waist hip ratio

## INTRODUCTION

### ***Obesity – a global health problem***

Obesity has been recognized as one of the top ten global health problems by the World Health Organization (WHO) and is rapidly increasing in both industrialised and developing countries (1). WHO has estimated that more than 1 billion adults in the world are overweight (body mass index, BMI  $\geq 25.0$  kg/m<sup>2</sup>); out of which at least 300 million are obese (BMI  $\geq 30.0$  kg/m<sup>2</sup>). In the United States around 60% are overweight or obese, and 27% are obese (2). In Sweden, the share of obese people has almost doubled the last 20 years, and is now including around 500 000 people (3). Recent results from the WHO MONICA project and INTERGENE study in Gothenburg show an increased prevalence of both overweight and obesity in middle-aged men and women since 1985 (4). Increasing prevalence has also been documented in Malmö in southern Sweden (5). Obesity is probably caused by genetic influences in combination with an imbalance in energy, with excess energy intake and lack of physical activity, and the rapid increase is mainly regarded to be a result of modern western life style, characterised by a high amount of sedentary time and a high intake of energy (6, 7).

### ***Obesity and cardiovascular morbidity and mortality***

Obesity is associated with premature death as well as several chronic diseases like cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), osteoarthritis, sleep apnoea, gallbladder disease, reduced fertility and cancer in colon, endometrium, breast and esophagus (8, 9). This thesis will focus on the relationship between obesity and CVD, and closely related conditions like hypertension, dyslipidemia, T2DM, socioeconomic circumstances, physical inactivity and inflammation.

Despite of a declining trend, CVD is still considered the leading cause of death in Sweden and most other developed countries (10, 11). In 2003, CVD was the underlying cause of death in 45% of the women and 44% of the men in Sweden (12). Several cohort studies, such as the Framingham Study, the Nurses' Health Study and the Multifactor primary prevention trial in Gothenburg, have demonstrated that obesity

is associated with an increased risk of CVD and death in both men and women (13-15). Obesity has also been documented to increase the risk of stroke (6, 9, 16). It was long controversial whether there was an association between obesity and mortality and whether the association was linear, U- or J-shaped (17-21). In some studies the U- or J-shaped association turn into a linear shape if studying non-smokers exclusively or if adjusting for pre-existing illness (17, 22, 23). However, others have argued that these confounding factors do not eliminate the higher mortality in lean subjects (23). CVD has also been associated with several lifestyle factors, i.e. smoking, low socioeconomic status (SES), single status and physical inactivity (24-29). These factors are also more common in obese individuals, except for smoking, which is less prevalent in obese (17, 22).

## ***Scope of the present thesis***

### **How to measure obesity**

It has long been controversial how to best measure obesity and BMI (kg/m<sup>2</sup>) has been the most used method (22, 30). However, during the last years it has become evident that adipose tissue, particularly intra-abdominal adipose tissue, is an active endocrine organ with adverse metabolic effects, indicating that body fat per se is crucial for cardiovascular (CV) risk (31). Concurrently with the results of revealed mechanisms linking visceral fat to CVD, measurements that take this parameter into account have become more popular. Increased abdominal adiposity could reflect a higher amount of intra-abdominal fat tissue. Some studies have suggested that waist circumference or waist hip ratio (WHR) are better measures of obesity to assess CV risk than is BMI (22). Body fat percentage (BF%) measured with bioelectrical impedance analysis (BIA) is measuring body fat content per se (32, 33). As “golden standard”, computerized tomography or magnetic resonance imaging have been suggested (30, 34, 35), as they can distinguish between intra-abdominal and subcutaneous fat, however these techniques are too expensive to use in daily clinical practice and computerized tomography also implies a radiation risk.

## **Heterogeneity in risk**

Epidemiology is by definition the study of the distribution and determinants of health-related states or events in specified populations (36). Many diseases, including CVD, have a multifactorial etiology. In spite of a well-known association between obesity and CVD and a plausible biologic pathway between adipose tissue and atherosclerosis, there is a marked heterogeneity of the CV risk between individuals with a similar degree of obesity (37, 38). Many obese individuals never suffer a CV event. From a preventive point of view there is a need of studies in this area so that intervention can be focused on those who most need it. Some CV risk factors tend to interact with others, to increase or reduce the risk of disease, within the concept of the “multifactorial web of causation” (39). To what extent the increased CV risk associated with obesity is modified by exposure to other CV risk factors has received little scientific attention.

There is a well-known association between atherosclerosis and hypertension, dyslipidemia, T2DM and smoking (24, 40). To what extent these risk factors contribute to the heterogeneity in obese individuals is not fully explored (aim I). Socio-economic circumstances are also associated to CV risk. People who are single and people who have a blue-collar job or low income have an increased risk (26, 41). These circumstances are more common in obese individuals (17); however it is not known whether they modify the CV risk related to obesity (aim II). Furthermore, during the last years increasing attention has been turned to the influence of inflammation on the atherosclerotic process (42). How the CV risk related to obesity is associated to inflammation is however not fully known (aim III).

Obesity is also a heterogeneous condition regarding fat distribution (43). BF% measured by BIA is a rather new method of direct measuring body fat content (32). It is not explored whether this method can add any information to identify individuals that are more susceptible to CVD. Moreover, physical activity has been shown to reduce CV risk (27, 44, 45), however it is not fully explored whether the risk is reduced in individuals with high BF% (aim IV). Finally, it is not explored how the CV risk related to overall obesity is modified by abdominal adiposity as measured by WHR (aim V).

## PATHOPHYSIOLOGY

### ***Atherosclerosis – an inflammatory disease***

Atherosclerosis with formation and subsequent rupture of plaques, leading to thrombosis and occlusion of the vessel, is a complex process which has been studied for decades (42). In short, it starts with endothelial dysfunction with increased endothelial permeability leading to migration of lipoproteins and leukocytes into the artery wall. A fatty streak is developed, followed by platelet adhesion and aggregation. As a defending response to the vessel injury, a fibrous cap is formed around this necrotic core and an advanced, fibrous plaque has developed. Continuous influx and activation of macrophages which release proteolytic enzymes result in the final step, an unstable, calcified plaque. This plaque can easily rupture with subsequent thrombosis formation or occlusion of the artery.

During the last years increasing focus has been laid on the inflammatory state that exists in CVD (40, 42). Inflammation is prevalent in the above described process of atherosclerosis, and a range of pro-inflammatory cytokines have been identified, e.g. interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ). These cytokines both increase endothelial damage and are produced in the already damaged vessel and so seem to be part of a vicious circle. Furthermore, they increase circulating concentrations of acute-phase proteins like C-reactive protein (CRP) and fibrinogen, suggesting an effect on the liver to increase the synthesis of these proteins (46). Elevated concentrations of acute-phase proteins have long been used as clinical markers of infections, trauma and cancer, and have also been found to predict future CVD (47). It has been documented that inflammation is associated to traditional CV risk factors such as hypertension, diabetes and dyslipidemia, factors that are also linked to obesity (40, 48-50).

### ***Adipose tissue – an endocrine organ***

Adipocytes were long considered to be inert cells solely storing fat. However, recent research has revealed that adipose tissue is an active endocrine organ that secretes

hormones, cytokines and vasoactive substances (8, 31, 51). Adipose tissue can be divided into subcutaneous and intra-abdominal fat, and it is now clear that intra-abdominal fat is more metabolically active compared to subcutaneous. It has a higher lipolytic activity and is more sensitive to glucocorticoids, as a result of more glucocorticoid receptors. Intra-abdominal fat is drained by the portal vein, and in this way the liver is exposed directly to a high amount of free fatty acids (FFA), resulting in a cascade of metabolic disturbances (52).

### **Adipose tissue-derived proteins**

Adipose tissue is releasing a wide range of proteins involved in several important pathways such as lipid metabolism, complement system and vascular hemostasis (31, 51). This endocrine function is more pronounced in intra-abdominal fat tissue than in subcutaneous fat. The role of each identified protein is not fully explored, but here a few of them are presented shortly:

*Leptin* is increased in obese individuals and decreased by fasting and is therefore thought to be an appetite suppressant, however it is debated whether it has any relevance in humans (51). Others have found that leptin increases sympathetic nervous system activity and plays a role in insulin sensitivity and lipogenesis (53, 54).

*IL-6* and *TNF- $\alpha$*  are inflammatory cytokines which are increased in obese individuals (31, 46). The synthesis of *TNF- $\alpha$*  is stimulated by insulin and is in turn inducing insulin resistance and lipolysis in adipose tissue, and it is possible that it also has systemic effects on insulin sensitivity and the production of acute-phase reactants in the liver. Moreover, *TNF- $\alpha$*  influences the regulation of other adipose tissue-derived factors. Finally, these cytokines act as regulators of the hypothalamus-pituitary-adrenal (HPA) axis, which has been documented to have increased activity in individuals with visceral obesity (55).

*Plasminogen activator inhibitor-1 (PAI-1)* is a vasoactive substance produced in adipose tissue, particularly intra-abdominally. PAI-1 inhibits the activation of plasminogen, which leads to increased coagulation and impaired fibrinolysis, resulting in a prothrombotic state (31, 54).

*Angiotensinogen* plays a central role in blood pressure regulation through the renin-angiotensin-aldosterone system and the release of this peptide from adipose tissue may be a mechanism of hypertension in obese individuals (54).

*Adiponectin* is, in contrast to other adipose-tissue derived products, lower in obese individuals than in normal weight and is increased by weight reduction. It has been suggested to be protective against inflammation and CVD, and is positively correlated to high density lipoprotein (HDL)-cholesterol and negatively correlated to BMI, triglycerides, CRP and PAI-1. (31, 56).

### ***Pathophysiology of the association between obesity and CVD***

It is not fully clear how adiposity is linked to CVD, but several mechanisms have been suggested. The main hypothesis is that intra-abdominal fat tissue is both physiologically and anatomically more disposed to expose the liver to FFA, which results in a variety of metabolic disturbances. Furthermore, the multiple products released from adipose tissue are thought to induce a prothrombotic, proinflammatory and atherogenic state which results in endothelial dysfunction (31, 51, 57). Endothelial dysfunction is considered crucial for subsequent atherogenesis, plaque formation and rupture of plaques (42). Adipose tissue is thought to promote the above described inflammatory process in vessels, by synthesising inflammatory cytokines such as TNF- $\alpha$  and IL-6.



## **Hypertension**

The association between hypertension and obesity is well documented; however the reasons for the association are unclear. Insulin resistance and hyperinsulinemia seem to play an important role, suggested to result in renal retention of sodium and stimulation of the sympathetic nervous system (17, 53, 55, 58). Other possible explanations include endothelial dysfunction, angiotensinogen, leptin and increased catecholamine activity (17, 53-55).

## **Dyslipidemia**

Obese individuals are characterised by a range of abnormalities in lipid metabolism, such as increased triglycerides, increased low (LDL) and very low density lipoprotein (VLDL)-cholesterol, reduced HDL-cholesterol, and a higher amount of small, dense LDL-cholesterol particles, which are especially atherogenic (17, 31, 51).

## **Disturbances in glucose tolerance and insulin sensitivity**

The relationship between obesity and insulin resistance and T2DM is well documented (9, 17, 59). It has been suggested that more than 80% of T2DM can be explained by obesity and the risk is increasing with grade of obesity and with central fat distribution (9). A recent publication on 64-year old women in Gothenburg showed a 9.5% prevalence of diabetes, and 14.4% of impaired glucose tolerance (IGT) (60). Furthermore, half of the diabetic women were previously undiagnosed. IGT and insulin resistance cause hyperinsulinemia, which is associated with dyslipidemia, increased PAI-1 synthesis and hypertension. The mechanisms behind the association between obesity and insulin resistance are not clear, however it is speculated that higher lipolytic activity with increased levels of FFA released to the portal circulation and cytokines released from visceral adipose tissue, are main factors (9, 31, 51).

### **The metabolic syndrome**

Obesity is clustering with several other CV risk factors, a clustering that has been given a number of names, including metabolic syndrome, syndrome X and insulin resistance syndrome, of which the first is the most used. The WHO definition of this syndrome argues that disturbances in insulin sensitivity is the main component, accompanied by two of the components abdominal adiposity, increased triglycerides, hypertension and increased urinary albumin excretion (61). The clamp technique has been suggested to be the “golden standard” for detecting insulin resistance, however this is a complicated and time-consuming method that is not feasible in population-based cohort studies, and is at present not used in daily practice (62). The newer definition by National Cholesterol Education Programme’s Adult Treatment Panel (ATP) III considers five components equally important; abdominal adiposity, serum triglycerides, blood pressure, HDL-cholesterol and serum glucose (61). Recently, the International Diabetes Federation re-defined the syndrome as central obesity (defined by ethnically specific waist circumference) plus two of the factors: increased triglycerides, reduced HDL-cholesterol, hypertension and impaired fasting glucose (63). This new definition argues that abdominal obesity is the main component, accompanied by a clustering of closely related CV risk factors. There is a progressive debate about the definition of the syndrome, which components should be included and the underlying mechanisms. A recent report from the American Heart Association/ National Heart, Lung, and Blood Institute concluded that the ATP III criteria constitute a clinically useful definition and that the syndrome is a complex disorder without a single factor as the cause (64).

### **Weight loss**

Dyslipidemia, hypertension and T2DM are all associated to endothelial dysfunction, by mechanisms that are not fully known (9, 17, 31, 62). Several studies have shown that endothelial function is improved by weight loss (57). It is not fully elucidated whether weight loss in obese people is associated with reduced CV events, however it has been demonstrated that it is associated with improved CV risk factors, i.e.

improved glucose tolerance and lipid profile and reduced blood pressure and inflammation (17, 23, 57, 65). A recent review of the long-term effects of intentional weight loss estimated that a weight loss of 10 kg was associated with a fall in total cholesterol of 0.25 mmol/l and a fall in diastolic blood pressure of 3.6 mmHg (66). A weight loss of 10% was associated with a fall in systolic blood pressure of 6.1 mmHg. Previous studies from the Malmö Preventive Project (MPP) have shown that intervention with increased physical activity and dietary counselling with weight reduction is associated with improvement in glucose tolerance and reduced mortality among IGT patients (67, 68). This has further been proven in randomised clinical trials in Finland and the U.S.A. (69, 70).

## **AIMS**

The general aim of this thesis was to study the morbidity and mortality of CVD in obese individuals, as measured by different obesity measurements, and to explore how the CV risk related to obesity was modified by other biologic and socio-demographic circumstances.

### ***Specific aims***

- To assess to what extent incidence of coronary events (CE) and death related to smoking, hypertension, hyperlipidemia and diabetes is modified by obesity in men.
- To explore whether there are differences of the vulnerability to CE and death associated with overweight and obesity between groups defined in terms of occupation and civil status in men.
- To explore the relationship between BMI and inflammation-sensitive proteins (ISP), and whether these proteins modify the CV risk in obese and overweight men.
- To explore the sex-specific risk of myocardial infarction (MI), stroke and death from CVD, in relation to degree of BF% measured by BIA, and to study the cardio-protective effect of physical activity in relation to the degree of body fatness.
- To explore whether the CV risk for different levels of BMI was modified by the regional fat distribution as measured by WHR in men and in women.

## **MATERIAL, METHODS AND RESULTS**

Malmö is a city in southern Sweden with around 250 000 inhabitants. MPP and Malmö Diet and Cancer Study (MDCS) are two prospective population-based studies in which CVD morbidity and mortality have been followed for several years.

### ***The Malmö Preventive Project***

With the purpose to detect risk factors for CVD, the MPP was performed at the Section of Preventive Medicine, Department of Medicine at Malmö University Hospital between 1974 and 1992 (71). Between 1974 and 1984, 22 444 men were examined. Complete birth cohorts from 1921, 1926-1942, 1944, 1946, 1948 and 1949 were invited by letter to a screening health examination. Participation rate varied between the invited birth cohorts and ranged from 64% to 78%. In the mailed invitation the participants were informed not to change their normal habits but to abstain from food, alcohol and tobacco 12 hours before the examination (72). The age ranged from 27 to 61, and mean age was 44 years old. The health examination included a physical examination, a panel of laboratory tests and a self-administered questionnaire with items relevant for the occurrence of CVD. Determination of five ISP was part of the program for 6193 men, who were randomly selected from birth cohorts examined between 1974 and 1982. Standardised procedures were adopted for the analysis of blood samples and for measurements of height, weight, blood pressure and heart rate after 10 min rest. Around 30% of the attendees were referred to specialised hospital units because of newly detected hypertension, hyperlipidemia, alcohol-related problems or T2DM (71). Smokers were advised to quit, but received no further help to do so. Obesity alone did not lead to any further evaluation or treatment.

### ***The Malmö Diet and Cancer Study***

All men born 1923-1945 and all women born 1923-1950, living in Malmö in 1991, were invited to this prospective cohort study by letter or by advertisement in local

media, in public places and in primary health care centres (73). The baseline examinations took place at the Malmö University Hospital between March 1991 and September 1996. The main objective of the project was to study the impact of diet on cancer incidence, but the individuals were also screened for certain CV risk factors and followed for incidence of MI, stroke and death (74). Participation rate was 41% and 28 449 subjects (60% women) completed the project. The age ranged from 45 to 73 years, and mean age was 59 years in men and 57 years in women. The participants were asked to fill in a detailed questionnaire covering socio-economic, demographic and lifestyle factors and a “menu book”, in which they filled in their meals for seven consecutive days (75). Furthermore, they underwent a health examination including blood samples, blood pressure and anthropometric measurements, i.e. BMI, waist and hip circumference. Body composition was measured with BIA. Participants with severely uncontrolled hypertension or other obviously abnormal findings were referred to their local practitioner.

A random 50% of those who entered the study between November 1991 and February 1994 (n=6103) were invited to take part in a study on the epidemiology of carotid artery disease (76). Those who accepted were re-scheduled for blood samples, i.e. blood lipids, blood glucose and plasma insulin, under standardised circumstances. Because of limited number of individuals in each category of BF% or BMI, these parameters were not used in paper IV or V.

### ***Case retrieval***

Data linkage with the Swedish Hospital Discharge Register (77), the Malmö Myocardial Infarction Register (MMIR), the Swedish Causes of Death Register and the Stroke Registry in Malmö (STROMA) were used for case retrieval (10, 78-80). For patients who had moved out from Malmö, the Swedish Hospital Discharge Register was used for retrieval of stroke events (78). In paper I-III, every participant in the cohort was followed from the baseline examination until death or 31 December 1997. In paper IV and V, every participant was followed from baseline examination until 31 December 2001. In paper II, information on emigration was retrieved by data linkage with the Total Population Register at Statistics Sweden (81).

The Swedish Hospital Discharge Register is a national register of all inpatients at all hospitals in Sweden, kept by the Centre for Epidemiology at the Swedish National Board of Health and Welfare (77, 78). Every patient gets a diagnosis code according to the International Classification of Diseases, Injuries and Causes of Death (ICD) at discharge (82). The MMIR was established in 1972 to monitor incidence and mortality from MI in Malmö and has been described in detail previously (80, 83). It has continuously recorded all cases of MI at Malmö University Hospital, which is the only hospital in the city. Gradually, the Swedish Hospital Discharge Register has replaced the MMIR. STROMA was established in 1989 with the purpose to monitor the incidence of stroke in Malmö (79). A specialised research nurse, with supervision of a senior physician, assesses each case of suspected stroke in both inpatients and outpatients.

### ***Definition of endpoints***

ICD-9 was used for classification and subjects classified according to ICD-10 were transformed into ICD-9 codes (82). A coronary/ cardiac event (CE) was defined as non-fatal MI (ICD-9 code 410, main or secondary diagnosis during hospital care) or death due to ischemic heart disease (ICD-9 codes 412-414, underlying or contributing cause to death). Only the first event was counted. In MMIR, the criteria for a MI were two of the three following circumstances: 1) central chest pain, lung oedema or shock; 2) electrocardiogram signs of acute MI; 3) elevated serum levels of cardiac enzymes (83). The Swedish Hospital Discharge Register used internationally accepted diagnostic criteria for MI (77, 78). Stroke (paper III-V) was defined as cases coded 430 (subarachnoid hemorrhage), 431 (intracerebral hemorrhage), 434 (ischemic stroke) or 436 (unspecified). In STROMA, a stroke was defined as rapid development of clinical signs of local or global loss of cerebral function that lasted for >24 hours or led to death within 24 hours and was classified according to ICD. Computerized tomography scan or autopsy was used for verification of cases coded 434. As obesity has been shown to be a risk factor for both MI and stroke, a composite endpoint, “CVD event”, was used in paper III and V (9). In paper III, a CVD event was defined as non-fatal stroke, non-fatal MI or death from CVD (ICD-9 code 390-448). In paper

V, a first-ever CVD event was defined as fatal or non-fatal CE or ischemic stroke, whichever came first. CVD mortality (paper I and IV) was based on deaths coded 390-448.

## ***Anthropometric measurements***

### *MPP (Paper I-III)*

The examination was performed by trained nurses. Standing height was measured with a fix stadiometer calibrated in centimetres. Weight was measured to the nearest 0.1 kilogram using balance-beam scale with subjects wearing light clothing and no shoes. BMI ( $\text{kg/m}^2$ ) was calculated as  $\text{weight/height}^2$  and categorised according to the WHO classification into normal weight (BMI  $<25.0 \text{ kg/m}^2$ ), overweight (25.0-29.9  $\text{kg/m}^2$ ) and obese ( $\geq 30.0 \text{ kg/m}^2$ ). In paper I, subjects with BMI  $<25.0 \text{ kg/m}^2$  were further divided into underweight (BMI  $<20.0 \text{ kg/m}^2$ ) and normal weight (BMI 20.0-24.9  $\text{kg/m}^2$ ). In paper III, BMI was divided into quartiles.

### *MDCS (Paper IV-V)*

Weight (in kilograms) and height (in centimetres) were measured in the same manner as in MPP and classified according to BMI into normal weight (BMI  $<25.0 \text{ kg/m}^2$ ), overweight (25.0-29.9  $\text{kg/m}^2$ ) and obese ( $\geq 30.0 \text{ kg/m}^2$ ). Waist was measured as the circumference (in centimetres) in the standing position without clothing, midway between the lowest rib margin and iliac crest, and hip circumference (in centimetres) horizontal at the level of the greatest lateral extension of the hips (84). Waist-hip ratio (WHR) was calculated as the ratio of waist to hip circumference.

In paper IV, BIA was used for estimating body composition. The subjects were analysed under non-fasting conditions and BF% was calculated using an algorithm for estimating body fat from BIA, according to procedures provided by the manufacturer (BIA 103, RJL-systems, single-frequency analyser, Detroit, U.S.A.). BF% was categorised into sex-specific quartiles (BF% Q1-4).



## ***Laboratory analyses***

Blood samples were drawn after an overnight fast and analysed according to standard procedures at the Department of Clinical Chemistry at Malmö University Hospital, which is attached to a recurrent standardisation system (85). All analyses were made on venous whole blood.

### **Inflammation-sensitive proteins (Paper III)**

Plasma levels of five ISP, i.e. fibrinogen, orosomucoid,  $\alpha$ 1-antitrypsin, haptoglobin and ceruloplasmin, were determined for 6193 men in MPP. An electroimmuno assay method was used to assess levels of these proteins, which all are commonly used as markers of inflammatory activity in clinical practice (85, 86). It has previously been shown that the correlation coefficients between the individual proteins range between 0.31 and 0.56 and that the CV risk increases with the number of ISP in the top quartile (49, 87).

## ***Cardiovascular risk factors***

### **Hypertension**

Hypertension was defined as use of blood pressure lowering medication or a blood pressure  $\geq 160/95$  mmHg (paper I, II) (88) or  $\geq 140/90$  mmHg (paper III-V) (89), respectively, according to international criteria at the time of baseline examination in respective study.

### **Diabetes mellitus**

In MPP (paper I and II), subjects who had a history of the disease or a whole blood glucose  $\geq 6.70$  mmol/l were categorised as diabetic (90). In paper III, men with fasting whole blood glucose  $\geq 6.1$  mmol/L, men with 2-hour glucose values  $\geq 10.0$  mmol/L (glucose load, 30g/m<sup>2</sup> body surface area) on oral glucose tolerance test (91), and men who reported that they had diabetes were considered diabetic patients. As information on fasting glucose or oral glucose tolerance was not available for all participants in

MDCS, diabetes mellitus in paper IV and V was recorded if the participant confirmed that this diagnosis was determined by a physician or if they reported treatment with insulin or oral anti-diabetic medication.

### **Hyperlipidemia**

Hyperlipidemia was defined in MPP as a whole blood cholesterol  $\geq 6.5$  mmol/l or triglycerides  $\geq 2.3$  mmol/l (paper I, II).

### **Alcohol consumption**

In MPP, i.e. paper I-III, the prevalence of problematic drinking behaviour was based on a validated modified version of the Michigan Alcoholism Screening Test (92), where the subjects were asked to answer 9 questions about drinking behaviour. Men with more than 2 affirmative answers were considered to have high alcohol consumption. In MDCS, i.e. paper IV and V, alcohol consumption was based on a “menu book”, in which the subjects filled in their meals for seven consecutive days. Men who reported a daily alcohol intake of  $>40$  g/d and women who reported a daily intake of  $>30$  g/d were categorised as high consumers (93).

### **Smoking**

In both MPP and MDCS, smoking status was based on self-administered questionnaires. Thus, in paper I and II, former smokers were those who had quit smoking at least a year before the examination and current smokers were those who reported a daily consumption of at least 1 g of tobacco. In paper III, subjects were categorised into non-smokers and smokers, the latter were further divided into consumers of  $\leq 9$  cigarettes per day, 10 to 19 cigarettes per day, and daily consumption of  $\geq 20$  cigarettes. In paper IV and V, subjects were categorised into current smokers (daily and occasional), former smokers or non-smokers.

### **Socio-economic and marital status (Paper I-II)**

Information on occupational level and marital status in MPP was obtained by data linkage with the Swedish national population census (“Folk- och Bostadsräkningen”) carried out in the years 1975 (erratum in published article: 1970), 1980, and 1985. To try to reduce the misclassification of people living together without being married, cohabitation status was used instead of marital status in paper II. In paper I, however, marital status (married/ not married) was used (erratum in published article: living alone/ cohabiting). In a re-analysis of the dataset, the use of cohabitation status instead of marital status did not change the results or conclusions.

Occupational status, assessed by answers to questions concerning job titles and work tasks, formed the basis for classification into socio-economic index (SEI) groups, according to methods used by the National Bureau of Statistics Sweden. This classification system considers the educational level required for a particular job, the level of responsibility of the job, and the specific work tasks. In paper II, the SEI groups were further classified into three occupational groups: non-manual workers (i.e. business executives, engineers with university degrees, physicians, college teachers, secondary school teachers, office assistants, sales people), self-employed (i.e. professionals with and without employees, entrepreneurs, farmers), and manual workers (i.e. auto mechanics, metal workers, construction workers, factory workers, waiters, cleaning staff). Unemployed, pensioners, students and men having occupations that did not match any SEI category were excluded from this study. In paper I, subjects were classified into non-manual workers, manual workers and others.

### **Leisure time physical activity**

#### *MPP*

In Paper I-III, leisure time physical activity was assessed by the question “Are you mostly engaged in sedentary activities in spare time, for example watching TV, reading, going to the movies?”

### *MDCS*

In MDCS, physical activity during leisure time was assessed using a modified questionnaire, adapted from the Minnesota Leisure Time Physical Activity Questionnaire (94). The participants were presented a list of 18 different activities and were asked to fill in how many minutes per week they on the average spent on each activity during each of the four seasons. This was multiplied by an activity-specific intensity coefficient and the sum of all the activity products created an overall leisure time physical activity score. The scores were further divided into quartiles in paper IV, i.e. low (Q1), low-moderate (Q2), moderate-high (Q3) and high physical activity (Q4), and further collapsed to low physical activity (Q1) and physically active (Q2-Q4). In paper V the leisure time physical activity score was divided into tertiles, i.e. low (T1), moderate (T2) and high (T3).

### **History of angina and cancer**

In MPP, men who confirmed angina pectoris diagnosed by a physician or reported treatment with nitro-glycerine in the questionnaire were considered to have angina pectoris. History of cancer was based on the question “Have you been treated for cancer?”. Subjects with good health are those who answered yes to the question: “Do you consider yourself to be completely healthy?”.

### **Statistics**

The Statistical Package for the Social Sciences (SPSS) software package was used for all statistical analyses. General linear model and logistic regression were used to study the age-adjusted distribution of risk factors in different categories. Cox’s proportional hazards analysis was used to study incidences of CVD and mortality. This statistical method is a variant of multivariate logistic regression, in which it is possible to calculate the relation between several exposure factors and one dichotome outcome variable in studies with varying length of follow-up (95). It is then possible to evaluate the independent effect of a variable after adjustment for confounding factors, i.e. factors that are associated both with the exposure under investigation and the outcome,

and therefore can bias the association. Cox's analysis is taking into account the follow-up time for each individual case, and is therefore suitable for prospective cohort studies. The result is a hazard ratio (HR), which is the ratio between time to outcome given a particular risk factor, to time to outcome without this risk factor. However, the term relative risk (RR) is mostly used instead of HR. A 95% confidence interval (CI) was calculated around each RR.

### **Interaction**

Interaction (effect modification) occurs when the impact of a risk factor on an outcome is changed by a third variable, and the interdependent operation of these two risk factors produces, prevents or controls disease (36, 95). The interaction is called synergy when the combined effect of two or more risk factors is greater than the sum of their solitary effects. To evaluate potential interactions between risk factors, a synergy index (SI) was calculated by methods described by Hallquist (paper I and II) (96) and Rothman (97). The formula for the SI was:

$$SI = (RR_{AB} - 1) / (RR_A + RR_B - 2),$$

where  $RR_A$  and  $RR_B$  are the adjusted relative risks associated with the risk factors A and B separately, and  $RR_{AB}$  is the relative risk for subjects exposed to both A and B. Values above 1 show a positive synergistic effect between the risk factors. In paper II, IV and V, interaction was evaluated by including interaction terms in Cox's proportional hazards model.

***Paper I: Influence of obesity on cardiovascular risk.  
Twenty-three-year follow-up of 22 025 men from an urban  
Swedish population***

**Aim**

To assess to what extent incidence of CEs and death related to smoking, hypertension, hyperlipidemia and diabetes is modified by obesity in men.

**Methods**

The study cohort consisted of 22 025 men who at baseline were between 27 and 61 years old, without history of MI and stroke. Mean follow-up time was 17.7 years. BMI was divided into underweight (BMI <20.0 kg/m<sup>2</sup>), normal weight (BMI 20.0-24.9 kg/m<sup>2</sup>), overweight (25.0-29.9 kg/m<sup>2</sup>) and obese (≥30.0 kg/m<sup>2</sup>). Incidence of CE, total mortality, CVD mortality and non-CVD mortality was estimated in relation to BMI after adjustment for potential confounding factors. RRs for CE were also studied in subgroups of smokers and non-smokers with normal weight, overweight and obesity. Furthermore, incidence of CE was studied in men without hypertension, hyperlipidemia or diabetes and in men exposed to one and ≥2 of these risk factors, respectively. Potential interactions between obesity and these risk factors were evaluated, calculating a SI.

**Results**

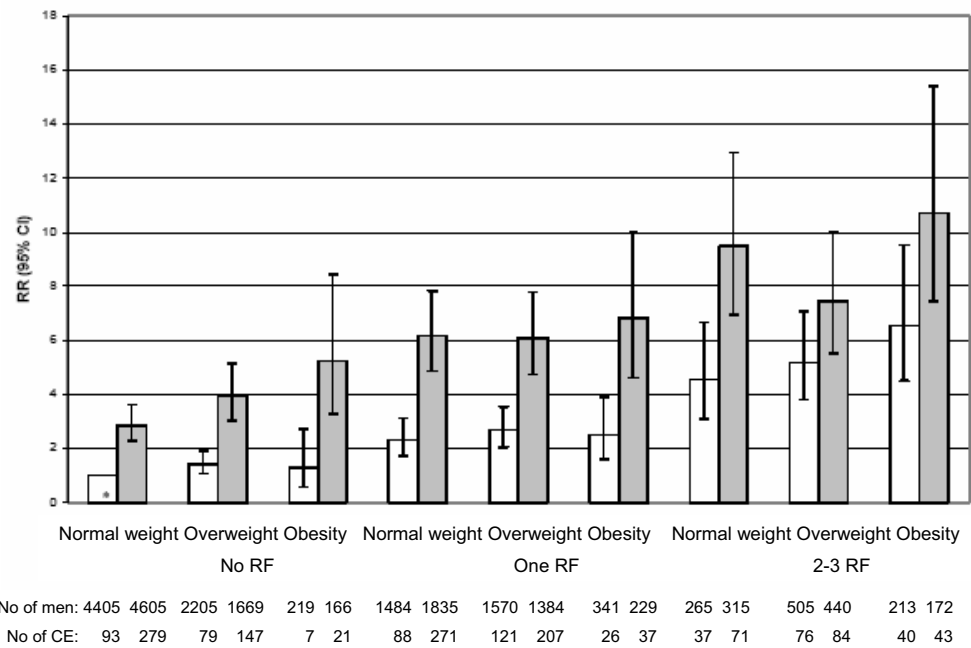
All studied CV risk factors except for smoking increased with BMI. A linear association was found between BMI and incidence of CE and a J-shaped association between BMI and all-cause mortality. The RR for a CE after adjustment for potential confounding factors was 1.18 (95% CI: 1.07 – 1.31) in overweight and 1.39 (95% CI: 1.17 – 1.65) in obese compared to normal weight men. The subgroup analysis showed that only 2 % of the obese men were exposed to both hypertension, hyperlipidemia,

diabetes and smoking, and 16 % of them had none of these risk factors. In the latter group the CV risk was not significantly increased (Fig 1). A positive interaction was found between obesity and smoking for incidence of CE, SI 1.39 (95% CI: 1.02-1.89).

### Conclusions

Obesity is associated with an increased incidence of CE and death in men. The risk associated with obesity is substantially increased by exposure to other atherosclerotic risk factors, of which smoking seems to be the most important.

**Figure 1.** Multivariate adjusted RR of CE by smoking (non-smokers in open bars and smokers in filled bars) and by number (i.e. none, one or 2-3) of other CV risk factors (RF, i.e. diabetes mellitus, hypertension and hyperlipidemia) in 22025 men with normal weight, overweight and obesity. Non-smoking men with normal weight and without diabetes mellitus, hyperlipidemia or hypertension served as the referent group. Covariates included age, heart rate, marital status, socio-economic position, leisure-time physical activity, self-reported health, history of angina pectoris, history of cancer, and history of problematic drinking behaviour.



***Paper II: Obesity and myocardial infarction – vulnerability related to occupational level and marital status. A 23-year follow-up of an urban male Swedish population***

**Aim**

To explore whether there are differences in the vulnerability to CE and death associated with overweight and obesity between groups defined in terms of occupation and civil status in men.

**Methods**

The study cohort consisted of 20 099 men who at baseline were between 27 and 61 years old, without history of MI and stroke. Mean follow-up time was 17.7 years. BMI was divided into normal weight (BMI <25.0 kg/m<sup>2</sup>), overweight (25.0-29.9 kg/m<sup>2</sup>) and obese (≥30.0 kg/m<sup>2</sup>). Age-adjusted prevalence of obesity was determined in each category of cohabitation status and occupational level. RRs for all-cause mortality and incidence of CE were calculated in relation to BMI, cohabitation status and occupational level, and in subgroups of these three parameters, with three different models of adjustments. Potential interactions between obesity and cohabitation status and between obesity and occupational level were evaluated, using both SI and interaction term in the Cox model.

**Results**

Obesity was more prevalent in manual workers, self-employed and men living alone. Manual work and living alone were factors associated with increased mortality and CVD risk. Obesity was associated with an increased risk for CE and death in each occupational group. Being single increased the risk associated with obesity. In stratified analyses, after adjustment for biological and lifestyle factors, the risk



**Table 1.** Adjusted incidence of coronary events in relation to body weight, level of occupation and civil status.

		CIVIL STATUS									
		Cohabiting					Living alone				
Occupational level	BMI category #	No. of events (No. of men)	CORONARY EVENTS								
			Events/1000 person-years	RR (95% CI)	Model 2 ‡	No. of events (No. of men)	Events/1000 person-years	RR (95% CI)	Model 1 †	RR (95% CI)	Model 2 ‡
NMW	NW	270 (4,700)	3.17	Referent	Referent	48 (1,080)	2.59			1.1 (0.8 - 1.4)	1.1 (0.8 - 1.5)
	OW	228 (2,578)	5.90	1.4 (1.2 - 1.7)	1.1 (0.9 - 1.4)	38 (447)	5.04			1.7 (1.2 - 2.4)	1.4 (1.01 - 2.0)
	OB	32 (310)	6.06	1.7 (1.2 - 2.4)	1.2 (0.8 - 1.7)	9 (75)	6.98			2.8 (1.4 - 5.5)	1.7 (0.9 - 3.4)
SE	NW	58 (784)	4.15	Referent	Referent	13 (178)	4.06			1.1 (0.6 - 2.1)	1.2 (0.7 - 2.2)
	OW	37 (545)	3.82	0.9 (0.6 - 1.4)	0.8 (0.5 - 1.2)	14 (115)	6.89			1.9 (1.04 - 3.4)	1.6 (0.9 - 2.8)
	OB	10 (118)	4.57	1.3 (0.7 - 2.6)	0.9 (0.5 - 1.9)	6 (19)	21.55			5.6 (2.4 - 13.3)	4.7 (1.9 - 11.4)
MW	NW	270 (3,780)	4.04	Referent	Referent	97 (1,292)	4.47			1.2 (0.9 - 1.5)	1.2 (0.9 - 1.5)
	OW	239 (2,724)	5.00	1.3 (1.07 - 1.5)	1.1 (0.9 - 1.3)	71 (714)	5.78			1.6 (1.2 - 2.0)	1.3 (0.9 - 1.7)
	OB	56 (479)	6.80	1.7 (1.3 - 2.3)	1.1 (0.8 - 1.5)	30 (161)	12.01			3.0 (2.1 - 4.4) <sup>a</sup>	1.9 (1.3 - 2.8) <sup>a</sup>

BMI, body mass index; RR, relative risk; CI, confidence interval; NMW, non-manual worker; SE, self-employed; MW, manual worker; NW, normal weight; OW, overweight; OB, obesity . † Covariates in model 1 included age, smoking habits, sedentary leisure-time physical activity and history of problematic drinking behaviour. ‡ Covariates in model 2 included age, hypertension, diabetes, serum total cholesterol, triglycerides, smoking habits, sedentary leisure-time physical activity and history of problematic drinking behaviour. Cohabiting men with normal weight (NW) served as the referent group for each analysis. # Normal weight is defined as a BMI less than 25; overweight 25.0 to 29.9; and obesity, at least 30.0 kg/m<sup>2</sup>. <sup>a</sup> indicates significantly different from all other groups in respectively occupational level.

associated with obesity was limited to those who were single and who either had a blue-collar job or were self-employed (Table 1). The multivariate-adjusted RR for CE and death in obese manual workers who were single was 1.91 (95% CI: 1.21–3.02) and 2.54 (95% CI: 1.74–3.69), respectively, compared to those who were cohabiting. A positive interaction was found between obesity and living alone for incidence of CE (SI 3.33 [95% CI: 1.18-9.40]) and for mortality (SI 1.85 [95% CI: 1.13-3.20]). In the published paper, p-values for the statistical interaction term in the Cox model between obesity and being single after stratification for occupational level, were erroneously presented as blue-collar workers:  $p=0.033$  and  $0.057$ , respectively for CE and all-cause mortality (page 546 line 13), and for self-employed:  $p=0.017$  and  $p=0.063$ , respectively for CE and all-cause mortality (page 546, line 14). The correct p-values were reversed, i.e.  $p=0.057$  and  $p=0.063$  for CE, and  $p=0.033$  and  $p=0.017$  for all-cause mortality.

## **Conclusions**

Obesity is associated with single status and manual job in men. Adjusted for lifestyle and biological risk factors, the increased risk of CE and death for obese men with manual jobs was applicable only to those who were single. Being single significantly increases the CV risk associated with obesity.

## ***Paper III: Incidence of obesity-associated cardiovascular disease is related to inflammation-sensitive plasma proteins. A population-based cohort study***

### **Aim**

To explore the relationship between BMI and ISP, and whether these proteins modify the CV risk in obese and overweight men.

## Methods

This study cohort consisted of 6075 men who at baseline were between 28 and 61 years old, without history of MI, stroke or cancer. Mean follow-up time was 18.7 years. BMI was divided into quartiles and plasma levels of each ISP, i.e. fibrinogen, orosomucoid,  $\alpha$ 1-antitrypsin, haptoglobin and ceruloplasmin, were estimated in each quartile. The analyses were made in all men, and in men with low levels of other risk factors, i.e. non-diabetic non-smokers without hypertension, dyslipidemia and angina pectoris. Plasma levels of all five ISP were divided into quartiles and subjects were categorised according to number of ISP in the top quartile, i.e. low (0-1 ISP in the upper quartile) or high (2-5 ISP in the upper quartile). Incidence of CVD was calculated in groups of BMI and ISP.

## Results

Obesity was associated with increased levels of ISP, even in men with low levels of other CV risk factors. High levels of ISP were associated with an increased CV risk in all categories of BMI (Table 2). The age-adjusted RRs for CVD events in obese men were 2.1 (95% CI: 1.4-3.4), 2.4 (95% CI: 1.5-3.7), 3.7 (95% CI: 2.3-6.0), and 4.5 (95% CI: 3.0-6.6), respectively, for those with 0, 1, 2, and  $\geq 3$  ISP in the top quartile (trend  $p=0.002$ , reference: BMI  $<25.0$  kg/m<sup>2</sup> and no elevated ISP). This trend persisted after adjustments for several potential confounding factors ( $p=0.02$ ). Incidence of CE showed similar relations with the number of elevated ISP in obese men.

## Conclusions

The CV risk varies widely between obese or overweight men with high and low ISP. Relationships with ISP contribute to, but cannot fully explain, the increased CV risk in obese men.

**Table 2.** Incidence of CVD in relation to ISPs and quartiles of BMI.

	BMI							
	Q1 (<22.7)		Q2 (22.7-24.6)		Q3 (24.6-26.0)		Q4 (>26.0)	
	Low ISPs	High ISPs	Low ISPs	High ISPs	Low ISPs	High ISPs	Low ISPs	High ISPs
N	998	522	1019	501	1038	482	957	558
Age	46.3±4.1	46.6±3.7	46.5±3.7	47.3±3.8	46.9±3.3	47.1±3.9	47.1±3.5	47.6±3.8
Cardiac events %	5.8	11.9	6.5	12.6	8.3	15.1	9.7	20.1
RR*	Reference	2.2 (1.6-3.2) †	1.1 (0.8-1.5)	2.2 (1.6-3.2) †	1.4 (0.99-1.9)	2.8 (2.0-4.0) †	1.6 (1.2-2.3)	3.7 (2.7-5.1) †
RR**	Reference	1.7 (1.2-2.5) †	1.0 (0.7-1.5)	1.5 (1.1-2.2) †	1.3 (0.90-1.8)	2.0 (1.4-2.8) †	1.5 (1.04-2.0)	2.4 (1.7-3.3) †
Stroke (%)	3.0	4.6	2.0	5.0	3.8	2.9	4.3	8.1
RR*	Reference	1.7 (1.0-2.9) †	0.6 (0.4-1.1)	1.7 (1.02-2.9) †	1.2 (0.7-1.9)	1.1 (0.6-2.0)	1.4 (0.9-2.3)	2.9 (1.8-4.6) †
RR**	Reference	1.5 (0.9-2.5)	0.6 (0.3-1.03)	1.4 (0.8-2.4) †	1.1 (0.6-1.7)	0.81 (0.4-1.5)	1.1 (0.7-1.8)	1.7 (1.1-2.9) †
CV events (%)	9.0	16.1	8.7	17.8	12.5	18.0	14.3	28.3
RR*	Reference	2.0 (1.5-2.7) †	0.94 (0.7-1.3)	2.1 (1.5-2.7) †	1.3 (1.02-1.8)	2.2 (1.6-2.9) †	1.6 (1.2-2.1)	3.4 (2.7-4.5) †
RR**	Reference	1.6 (1.2-2.1) †	0.89 (0.7-1.2)	1.5 (1.1-2.0) †	1.2 (0.9-1.6)	1.6 (1.2-2.1)	1.4 (1.02-1.8)	2.2 (1.7-2.9) †

CV, cardiovascular. ISP, inflammation sensitive plasma proteins. RR, relative risk.

\*Age-adjusted relative risk (95% CI)

\*\*Relative risk (95% CI) adjusted for age, smoking, tobacco consumption, systolic and diastolic blood pressure, blood pressure medication, high alcohol

consumption, cholesterol, triglycerides, physical inactivity, diabetes, angina, γ-GT

† p<0.05 vs men with low ISPs within in the same quartile of BMI.

## ***Paper IV: Effects of body fatness and physical activity on cardiovascular risk. Risk prediction using the bioelectrical impedance method.***

### **Aim**

To explore the sex-specific risk of MI, stroke and death from CVD, in relation to degree of body fatness measured by BIA, and to study the cardio-protective effect of physical activity in relation to the degree of body fatness.

### **Methods**

The study cohort consisted of 26 942 men and women, aged 45-73 years, without history of MI and stroke. BF% was assessed through BIA and the subjects were followed for incidence of CE, ischemic stroke and CVD mortality over 7.6 years in relation to sex-specific quartiles (Q1-Q4) of BF%. Potential interactions were evaluated between BF% and sex and between BF% and age, respectively, by introducing an interaction term in the Cox model. Leisure time physical activity was assessed through a modified version of the Minnesota Leisure Time Physical Activity Questionnaire (94) and the effects of leisure time physical activity was studied in groups of low (Q1-Q2) and high BF% (Q3-Q4).

### **Results**

In men, the RR for CE and CVD mortality increased progressively with BF%. RR for CE in BF% Q4 was 1.37 (95% CI: 1.07-1.74), after adjustments for age, height, smoking status, high alcohol intake and physical activity, compared to BF% Q1 (Table 3). Corresponding RR for CVD mortality was 1.97 (95% CI: 1.40-2.77). In women, BF% was significantly associated with incidence of CE and stroke. When comparing the different obesity measurements, waist circumference was associated with higher RRs than BF% and BMI in men. In women, waist circumference and BF% were

**Table 3.** Cox proportional hazards analysis of coronary event, ischemic stroke and CVD death rate in relation to quartiles of body fat percentage in men and women, respectively.

Endpoints	Category of body fat percentage, RR (95% CI)				p for trend
	BF% Q1	BF% Q2	BF% Q3	BF% Q4	
<i>Men</i>					
Events, n (CE/Ischemic stroke/CVD death)	117/72/51	125/71/61	138/76/67	153/88/98	
CE					
RR*	Referent	1.13 (0.88-1.45)	1.38 (1.08-1.77)	1.37 (1.07-1.74)	0.004
<i>Ischemic stroke</i>					
RR*	Referent	1.03 (0.74-1.43)	1.19 (0.86-1.65)	1.20 (0.88-1.65)	0.17
CVD death					
RR*	Referent	1.27 (0.88-1.85)	1.52 (1.06-2.20)	1.97 (1.40-2.77)	<0.001
<i>Women</i>					
Events, n (CE/Ischemic stroke/CVD death)	31/17/25	42/44/27	79/80/47	92/90/49	
CE					
RR*	Referent	1.41 (0.89-2.25)	1.64 (1.08-2.50)	2.28 (1.50-3.46)	<0.001
<i>Ischemic stroke</i>					
RR*	Referent	2.65 (1.51-4.65)	2.94 (1.74-4.98)	3.88 (2.29-6.57)	<0.001
CVD death					
RR*	Referent	1.06 (0.61-1.83)	1.09 (0.67-1.77)	1.28 (0.78-2.10)	0.32

RR, relative risk. CI, confidence interval, CE, coronary event. CVD, cardiovascular disease. BF% Q1-4, quartiles of body fat percentage.

\* Adjusted for age, height, smoking status, high alcohol intake and physical activity.

**Table 4.** Adjusted relative risks for a coronary event, ischemic stroke and CVD death in relation to physical activity in men and women with high and low body fat percentage, respectively.

Endpoints	Men			Women		
	Low BF%, RR (95% CI)	High BF%, RR (95% CI)		Low BF%, RR (95% CI)	High BF%, RR (95% CI)	
Events, n (CE/Ischemic stroke/CVD death)	242/143/112	291/164/165		73/61/52		171/170/96
CE						
Model 1	0.85 (0.63-1.16)	0.68 (0.54-0.87)**		0.60 (0.36-1.00)		0.57 (0.42-0.77)***
Model 2	0.93 (0.68-1.26)	0.73 (0.57-0.93)*		0.68 (0.41-1.13)		0.60 (0.44-0.82)**
Model 3	0.93 (0.68-1.26)	0.75 (0.59-0.96)*		0.74 (0.44-1.24)		0.66 (0.49-0.91)**
Ischemic stroke						
Model 1	0.67 (0.46-0.98)*	0.61 (0.44-0.84)**		0.58 (0.33-1.00)		0.61 (0.45-0.83)**
Model 2	0.70 (0.48-1.02)	0.65 (0.47-0.90)**		0.62 (0.36-1.08)		0.65 (0.48-0.88)**
Model 3	0.69 (0.48-1.01)	0.67 (0.48-0.92)*		0.65 (0.37-1.13)		0.68 (0.50-0.93)*
CVD death						
Model 1	0.73 (0.47-1.13)	0.62 (0.45-0.85)**		0.38 (0.22-0.66)**		0.69 (0.45-1.04)
Model 2	0.78 (0.50-1.21)	0.67 (0.48-0.92)*		0.42 (0.24-0.74)**		0.74 (0.49-1.12)
Model 3	0.78 (0.50-1.20)	0.72 (0.52-0.998)*		0.46 (0.26-0.83)**		0.78 (0.51-1.19)

RR, relative risk. CE, coronary event. CVD, cardiovascular disease. Relative risks comparing physical activity (Q2-Q4) to low physical activity (Q1).  
Model 1: Adjusted for age.  
Model 2: Adjusted for age, height, smoking status and high alcohol intake.  
Model 3: Adjusted for age, height, smoking status, high alcohol intake, body fat percentage, diabetes mellitus, systolic blood pressure, use of blood pressure lowering drugs and use of lipid lowering drugs.  
\* p<0.05, \*\* p<0.01, \*\*\* p<0.001

associated with similar increased risks. BF% was more strongly correlated to BMI ( $r=0.83$ ) and waist circumference ( $r=0.76$ ) in women than in men ( $r=0.59$  and  $r=0.66$ , respectively). A significant positive interaction ( $p=0.013$  for incidence of CE and  $p=0.026$  for stroke) was found between BF% and sex, however not between BF% and age. Furthermore, it was shown that the raised CV risk was reduced by physical activity in both men and women with high BF% (Table 4).

## **Conclusions**

Body fatness is a risk factor for CE and CVD mortality in men, and for CE and ischemic stroke in women. Adjusting for BMI, BF% is an independent risk factor for CE only in women, and a significant interaction between BF% and sex was found for incidence of CE and stroke, suggesting a sex-specific effect where BF% is a stronger CV risk factor in women than in men. The raised CV risk associated with high BF% is reduced by physical activity.

## ***Paper V: Sex differences in the relationships between BMI, WHR and incidence of cardiovascular disease: a population-based cohort study***

### **Aim**

To explore whether the CV risk for different levels of BMI was modified by the regional fat distribution as measured by WHR in men and in women.

### **Methods**

The study cohort consisted of 10 369 men and 16 638 women, aged 45-73 years, without history of MI and stroke. Total body weight was grouped according to BMI category into normal weight (BMI  $<25.0$  kg/m<sup>2</sup>), overweight (25.0-29.9 kg/m<sup>2</sup>) and



obese ( $\geq 30.0$  kg/m<sup>2</sup>). Body fat distribution was classified by sex-specific tertiles of WHR. Cut-off points for tertiles of WHR were as follows: tertile-1 (men  $<0.917$ , women  $<0.768$ ), tertile-2 (men  $0.917$ - $0.962$ , women  $0.868$ - $0.811$ ) and tertile-3 (men  $>0.962$ , women  $>0.811$ ). Incidences and RRs of first-ever ischemic stroke or CE were estimated during a mean follow-up of 7.6 years in relation to BMI or WHR, and in relation to combined patterns of BMI and WHR. Potential interactions were evaluated between WHR and sex, or WHR and age, on the risk of CVD events, by introducing interaction terms in the multivariate model.

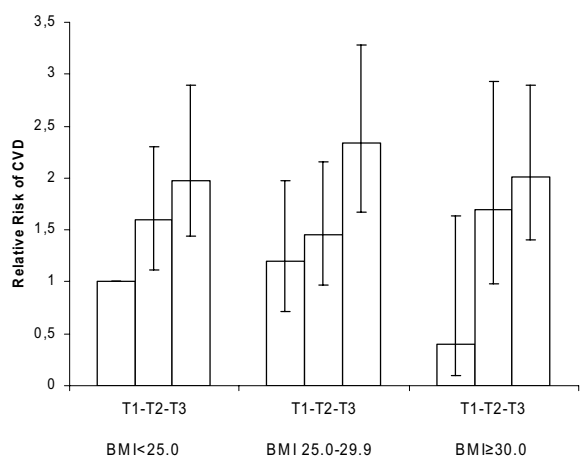
## Results

In each BMI category the prevalence of smoking, physical inactivity, diabetes and use of blood pressure-lowering drugs increased linearly from the lowest to the highest sex-specific tertile of WHR. During follow-up 1280 subjects suffered a CVD event. The risk of CVD in women increased with increasing levels of WHR, irrespective of BMI. In men, WHR (per 1 SD increase) was associated with increased incidence of CVD in those with normal weight, after adjustment for confounding factors. WHR was not related to CVD in overweight or obese men (Fig 3). A significant interaction was observed between sex and WHR on the CVD risk.

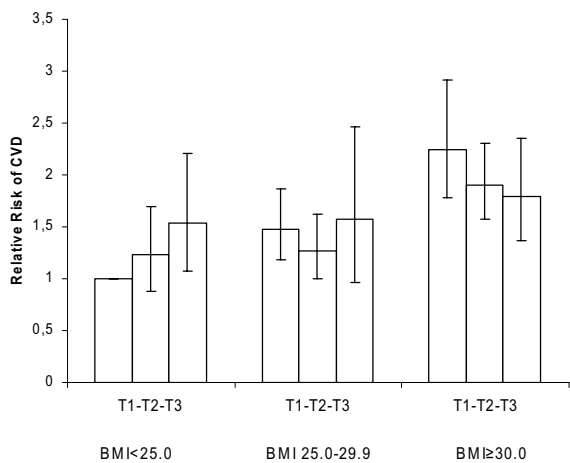
## Conclusions

The effect of body fat distribution as measured by WHR on incidence of CVD is modified by the overall body weight and by gender. WHR adds to the prognostic information on the CV risk in women at all levels of BMI and in men with normal weight.

**Figure 2.** Age-adjusted relative risk (RR) of CVD event in relation to tertiles of WHR in normal (BMI <25 kg/m²), overweight (BMI 25.0-29.9 kg/m²) and obese (BMI ≥30.0 kg/m²) **women**. Normal weight with bottom tertile of WHR was used as the reference group.



**Figure 3.** Age-adjusted relative risk (RR) of CVD event in relation to tertiles of WHR in normal (BMI <25 kg/m²), overweight (BMI 25.0-29.9 kg/m²) and obese (BMI ≥30.0 kg/m²) **men**. Normal weight with bottom tertile of WHR was used as the reference group.



## GENERAL DISCUSSION

Since long, obesity has been associated with increased CV risk. However, many obese individuals never develop the metabolic disturbances associated with the metabolic syndrome and many never suffer a CE or a stroke. Within the concept of the multifactorial web of causation lies the interaction between risk factors that may increase or reduce the CV risk. Differences in CV morbidity and mortality may be related to circumstances modifying the individual susceptibility. The results of this thesis show that obese individuals constitute a heterogeneous group, and it is concluded that the CV risk associated with obesity is modified by several other biologic and socio-economic circumstances.

### ***Marked differences in incidence of and mortality from CVD in obese men***

In paper I it was concluded that there is a marked difference in incidence of and mortality from CVD between subgroups of obese men. These differences were related to exposure of smoking, diabetes, hypertension and hyperlipidemia, the risk increased with number of concomitant risk factors. As much as 16% of the obese middle-aged men were not exposed to any of these risk factors. These men had in comparison to normal weight men, during the average of 18 years of follow-up, no significantly increased incidence of CE. Only 2% of all obese men were exposed to all four risk factors. The age-adjusted incidence of CE in these two groups was 1.8 and 28.4 per 1000 person-years, respectively. Most prominent was the risk increase associated with smoking, and a positive synergistic interaction was found between obesity and smoking for the risk of CE. Although smoking is less common in obese subjects, the results indicate that male obese smokers constitute a particularly vulnerable group.

How smoking and obesity interact with each other is not fully explored. Both have been demonstrated to be related to other CV risk factors like hypertension, dyslipidemia and endothelial dysfunction (24, 31). Smoking triggers the mobilisation of FFA from adipose tissue, resulting in further metabolic disturbances (24, 52) and

activates the HPA-axis (55). Inflammation seems to be an important common feature in adiposity and smoking in the causation of atherosclerosis, both are associated with increased levels of inflammatory markers (24, 40, 42, 98). Thus, a person who is already under increased risk because of a high volume of adipose tissue will be further affected if he is exposed to smoking.

Why some smokers are obese despite the fact that smoking generally is associated with lower body weight remains to be evaluated. It is possible that those smokers who despite this fact are overweight, are relatively even more “overweight” from a metabolic point of view, i.e. have a higher degree of metabolic disturbances than they would have had if they were non-smokers. Furthermore, the inverse relationship between smoking and obesity tends to reduce the relationship between obesity and CVD.

Why some obese individuals develop hypertension, hyperlipidemia and T2DM and others do not is not clear. It has been speculated that obese individuals without associated risk factors have a lower amount of visceral fat and have an earlier onset of obesity than obese individuals with metabolic risk factors (38). There are certainly other contributing genetic, metabolic or lifestyle factors that are still unknown.

### ***Being alone is associated with an increased vulnerability to CVD morbidity and mortality in obese men***

In paper II it was concluded that between groups defined in terms of cohabitation status and occupation there are significant differences of the CV risk associated with obesity. A significant interaction was found between obesity and living alone for incidence of CE and mortality, identifying a particularly vulnerable group of obese men.

Living alone and low SES are circumstances associated with a range of unhealthy habits, e.g. diet, smoking, alcohol and physical inactivity (29, 41, 99). Psychosocial factors like social network have been linked to healthy lifestyle, and it has been argued that social support reduces psychological stress and that socially isolated people experience increased stress (29). A marital dissolution or death of a spouse can be a stressful event with major health impacts (99). Occupation is a proxy of SES and

differs in a variety of parameters like education, daily work and income. SES has also been linked to traditional CV risk factors such as blood pressure and lipid status (29, 41). However, it has been documented that SES has an independent effect on CVD even after adjustment for these other risk factors. This effect may be related to psychosocial factors like social support, coping style, behaviour, job strain or anger (41).

Psychological stress is increasing the activity of the HPA-axis, which is stimulating cortisol secretion, resulting in increased lipolysis, redistribution of adipose tissue to central depots and hyperglycemia (29). An increased activity of the HPA-axis has been shown in individuals with central adiposity (55). It has been shown that adipose stromal cells from omental fat can generate active cortisol from inactive cortisone and that visceral adipose tissue has more cortisol receptors than subcutaneous adipose tissue, suggesting that central obesity may reflect a “Cushing’s disease of the omentum” (51, 100). A hypersecretion of cortisol has been documented in depression, work stress, hostility and low SES (29, 101). These facts could partly explain the increased obesity seen in individuals with low SES.

Furthermore, it has been shown that environmental stress is increasing the sympathetic nervous system activity with subsequent increased levels of catecholamines (29, 102). Increased catecholamine activity could contribute to CVD through a variety of mechanisms, i.e. IL-6 release from adipose tissue, platelet activation, inflammation, endothelial dysfunction, hypertension and glucose intolerance (29, 102). These data further strengthen the theory that all the obesity-associated risk factors are strongly connected to each other, acting on CV risk in a complex way. Thus, stress due to socio-economic circumstances can aggravate an already existing metabolic imbalance that exists in obese individuals, which could explain our results of a vulnerable group of obese men with blue-collar jobs who are living alone.

### ***High levels of ISP is associated with an increased incidence of CVD in obese men***

In paper III it was concluded that ISP concentrations vary markedly between men with obesity and men with normal weight. This relationship was observed even in those

with low levels of other major risk factors. Furthermore, the CV risk was very different in obese men with high and low ISP. The results show that presence of high ISP further increased the CV risk in obese men.

Obese men had higher ISP, even in absence of other major risk factors associated with obesity and inflammation (smoking, diabetes, hypertension and dyslipidemia). There could be several reasons for this relationship. The production of proinflammatory cytokines in adipose tissue, i.e. TNF- $\alpha$  and IL-6, could increase the hepatic synthesis of ISP. This theory is supported by the findings of reduced inflammation in weight loss (65). Another possibility is that inflammation causes adiposity. It has been shown that a low-grade inflammation predicts future weight gain (103). A third possibility is that other factors, e.g. diet, chronic inflammatory disorders or infections, influence both inflammation and obesity.

Obese subjects with high ISP had a higher CV risk than obese men with low ISP. These data add further evidence to the theory that obese people constitute a heterogeneous group of individuals. Thus, assessing inflammatory markers is a way to identify obese individuals that are under high risk to develop CVD.

Ceruloplasmin showed a U-shaped relationship to BMI, with the lowest plasma level in the second quartile. These results were unexpected and we do not know the underlying reason. Smoking could not explain the results, as the U-shape remained in men with low levels of other risk factors. One theory was that subjects with liver disease would get both low BMI and high levels of ceruloplasmin, however we do not have any support for this. Two recent publications from MPP reported that increased levels of complement C3 are related to large weight gain and development of diabetes, independent of ISP, indicating that other still unknown pathways exist (104, 105).

The studied ISP have other functions except for their inflammatory actions. Fibrinogen aggregates together with other products in thrombogenesis (85) and is moderately strongly associated with CV risk (87, 106).  $\alpha$ 1-antitrypsin inhibits proteolytic enzymes and a lack of this protein causes lung emphysema (85). Haptoglobin binds free hemoglobin, e.g. after hemolysis. The physiologic roles of ceruloplasmin and orosomucoid are more uncertain, ceruloplasmin binds copper in Morbus Wilson, and

may also be a regulator of iron metabolism (107). As we could not control for these other conditions we do not know whether they might have influenced the associations.

### ***Body fatness as measured by BIA is a stronger CV risk factor than BMI in women***

In paper IV it was concluded that BF% is independently associated to CVD morbidity and mortality. As described in the Pathophysiology section, adipose tissue is considered an active endocrine organ with adverse metabolic effects (31, 51). An independent association between body fat per se and CVD, strengthens the hypothesis that the amount of adipose tissue is crucial for CV risk, and that these endocrine and metabolic effects partly describe the biologic pathways. The multiple products released from adipocytes, e.g. FFA, inflammatory cytokines (i.e. TNF- $\alpha$ , IL-6) and PAI-1 may increase insulin resistance, dyslipidemia and promote a prothrombotic and proinflammatory state.

The results further indicate that BF% adds prognostic information beyond BMI, as BF% was independent of BMI in multivariate analyses in women. A significant positive interaction was found between BF% and sex for incidence of CVD, suggesting a sex-specific difference for the effect of BF% on CVD with a stronger vulnerability in women. BF% was associated with higher RRs for CE than BMI in women. Cross-sectional studies have indicated that abdominal fat is associated with a poorer CV risk profile in women than in men (108). However, gender differences in relative risks should always be treated with caution, as women have a lower baseline CV risk, making it easier to find significant associations in women.

Furthermore, it was found that physical activity reduced the increased risk in men and women with high BF%. It has been shown that physical activity improves CV abnormalities like lipid profile, insulin sensitivity, blood pressure, fibrinolytic activity and inflammation (28, 44). These beneficial effects seem to be present also in subjects who stay overweight and obese. Physical activity may also act via pathways that are not yet known. In this study, the effect attenuated after controlling for BF%, diabetes, systolic blood pressure and use of blood pressure and lipid lowering medication, however remained statistically significant. Moreover, people who engage in physical

activity are more prone to live healthy also in other regards, e.g. diet and socio-economic circumstances, a kind of “self selection” effect.

### ***WHR adds prognostic information on CV risk in women at all levels of BMI and in men with normal weight***

In paper V it was concluded that both BMI and WHR were associated with an increased incidence of CVD, which is consistent with results from other studies (37, 109). However the patterns were more complex when combined effects of BMI and WHR were assessed. The impact of body fat distribution on CVD risk was modified by the levels of overall body weight and gender. WHR added prognostic information at all levels of BMI in women, however in men only in those with normal weight. Our results suggest that both BMI and WHR measurements and sex-specific relationship should be taken into account to classify the CVD risk, and add further evidence to the hypothesis that one single weight measure is not enough to evaluate the CV health hazards of obesity. Recently a case-control study with data from the INTERHEART study reported a similar relationship between WHR and MI persisting in subgroups of BMI (110).

In this study, WHR was used as a proxy-estimate of intra-abdominal fatness. Women with low WHR had a rather low CV risk in this cohort and had a significantly lower prevalence of hypertension and diabetes. It has been suggested that the adipocytes in gluteo-femoral region are associated with minimal fatty acid flux, high lipoprotein lipase activity and high insulin-sensitivity (111). These bio-metabolic characteristics are highly efficient in fatty acid storage. These data suggest that women with low WHR constitute a subgroup with low CV risk, even if they are obese according to BMI.

The present results indicate that high WHR is a stronger risk factor in women than in men. The causes of the gender-difference are unclear. Differences in anatomic, physiologic, metabolic and sex hormonal status between genders may provide certain explanations. The volume of visceral fat mass differs by sex. Men, on average, store 21% of total body fat in visceral region in comparison to 10% in women (112, 113). In



addition, women have a wider hip circumference due to wider pelvis and larger gluteo-femoral muscle and fat depots.

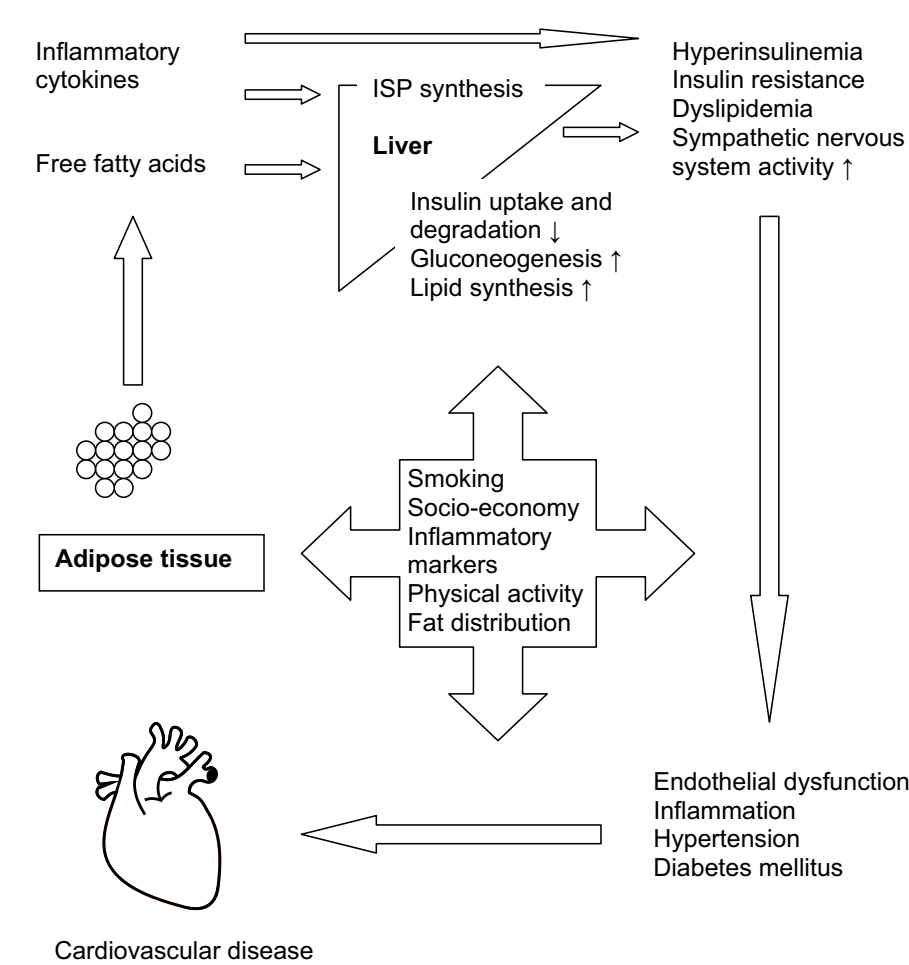
### ***Heterogeneity and potential causal pathways***

CV risk factors may promote the development of atherosclerotic plaques or increase the probability of complications, i.e. rupture of plaque with subsequent thrombus formation (42). It is possible that the influence of obesity on the incidence of CVD is related to the distribution and severity of atherosclerotic lesions (114). Studies on carotid intima-media thickness have shown that both obesity and the metabolic syndrome are associated with progression of carotid atherosclerosis in both men and women (115-117). The biologic mechanisms behind the association between obesity and incidence of CVD are however not fully understood. The present findings in this thesis indicate that obesity could act through a variety of mechanisms, i.e. hypertension, dyslipidemia, T2DM and inflammation, and that the CV risk associated with obesity is modified by smoking, socio-economic circumstances, physical inactivity and fat distribution (Fig 4). Obese people, as measured by BMI, WHR or BIA, constitute a heterogeneous group where the CV risk is very different according to presence or absence of other risk factors.

In paper I, smoking obese men were shown to be particularly vulnerable to CV risk, in paper II obese men who were living alone had a substantially increased risk. In paper III it was shown that high levels of inflammatory markers increased the CV risk in obese men. In paper IV and V, it was concluded that both BF% and WHR add to the prognostic information beyond BMI in women and that physically active obese individuals reduce their CV risk. These results indicate that there are several different subgroups within the obese population and that it seems possible to identify those obese individuals that are most susceptible to CV morbidity and mortality. It is possible that these subgroups have atherosclerotic lesions that are more severe or more susceptible to rupture and formation of occluding thrombi than other obese individuals.

Risk factor clustering seems to be a central problem in obese individuals and the inter-

**Figure 4.** Schematic illustration of theoretical biological pathways between adiposity and cardiovascular disease. Smoking, socio-economy, inflammatory markers, physical activity and fat distribution may affect these pathways by making certain obese individuals more susceptible.



relationships between different risk factors are complex. Other studies have also confirmed that CV risk varies markedly according to the number of other risk factors (37). There are obese subjects who are “metabolically healthy” and do not have an increased CV risk, and subjects who are categorised into the normal weight group but still are “metabolically unhealthy” (38). These differences may partly be explained by the subgroups of different risk factors in our studies. Similarly to the results reported in Paper I, a recent French study involving >240 000 men and women found that overweight subjects without associated risk factors did not have an increased CV mortality (118). The risk of CVD death increased significantly when overweight was associated with hypertension, indicating that hypertension is a key factor in the association between overweight and CV mortality. A Japanese study reported that Sumo wrestlers could be described as “metabolically healthy” because of normal amounts of visceral fat and blood lipids, despite marked obesity. However, in retired wrestlers who remained heavy eaters, the incidence of diabetes increased markedly, probably because of the decline in physical activity (119).

The underlying mechanisms behind obesity are still unclear. It has been suggested that genetic abnormalities act together with a positive energy imbalance with excess dietary intake and lack of physical activity. The imbalance could often be very small but exist over a long period (7, 55). A study of energy expenditure over 24-hours in a human respiratory chamber, showed that a large portion of the differences in energy expenditure were attributable to variability in the degree of spontaneous physical activity. However, recent research has discussed the possibility of obesity being an infectious disease (120). Two types of viruses have been associated to human obesity, and it is hypothesised that an infection with any of these viruses could start a low-grade inflammation resulting in increased volume of adipose tissue (120). Other theories include changes in intestinal bacterial flora, which could lead to more effective energy uptake in obese individuals. Furthermore, infections intrauterine or in early childhood have been linked to adult disease (121) and it has been speculated whether obesity may be a cause of early events intrauterine or in early childhood (122).

## ***Measurements***

Today there is no simple “golden standard” method for measurements of overall and intra-abdominal fatness, and most of the existing methods have been found to be independently associated to CV risk. Our studies show that BF% and WHR add further prognostic information beyond BMI in women. However, in men with BMI  $\geq 25$  kg/m<sup>2</sup>, WHR did not add further information. A recent study showed that waist circumference was independently associated to all-cause mortality, after adjustment for BF% or BMI (123). Recreating these analyses on our cohort gave similar associations for both CVD morbidity and mortality. The RR for all-cause mortality in relation to waist circumference in men was 1.37 (95% CI: 1.58-2.12), after adjustment for body fat mass/height<sup>2</sup>, fat free mass/height<sup>2</sup> and smoking, and 1.48 (95% CI: 1.28-1.71), after adjustment for BMI and smoking. In women, the associations were even stronger, i.e. RR 1.69 (95% CI: 1.49-1.91) and 1.73 (95% CI: 1.53-1.95), respectively. These results further strengthen the theory that the different measures add different information. While the correlation between BMI and waist circumference is strong ( $r=0.87$  in the MDCS cohort), the correlation between BMI and WHR is less prominent in both sexes ( $r=0.62$  in men and  $r=0.42$  in women), paper V. This implies that WHR can provide more information about the CV risk above the information provided by BMI.

## ***Methodological limitations***

### **Representativity**

The extensive data and long follow-up time of MPP and MDCS provide a unique opportunity to study subgroups of individuals with CV risk. It is a well-known fact that attendance rates in cohort studies have declined during the past decades, internationally and also in Malmö (124). This is reflected by the lower participation rate in MDCS (41%) in comparison to MPP (71%). The lower participation rate in MDCS could also be explained by the relatively time-consuming baseline examinations and diet registration. Another common fact in cohort studies is that non-participation is associated with higher mortality, which has been shown both in MPP

and MDCS (74, 125). Factors related to non-participation that could explain the increased mortality include poorer socio-economic circumstances and lifestyle factors like smoking and alcohol. Moreover, subjects who already have contact with a doctor are under-represented in cohort studies (124).

#### *MPP*

A study comparing invited men in MPP with non-invited men from the birth cohorts of 1925, 1943, 1945 and 1947 showed that total and CVD mortality and incidence of nonfatal MI and stroke did not differ between these two groups (125). Thirty percent of the invited men did not attend the health examination. Analyses on non-participants showed that all-cause and CV mortality was more than twice as high in non-participants (125). Non-participants were characterised by less favourable socio-economic circumstances. These data indicate that the studied health problem is even larger in the background population than in the studied population.

#### *MDCS*

A study on non-participation in MDCS showed that all-cause mortality was 2-3 times higher in non-participants than in participants (74). As there is no information available on risk factors in non-participants, the representativity of the MDCS study population has been evaluated by comparison of subjects living in Malmö who 1994 took part in a mailed health survey with a participation rate of 75% in corresponding age groups (74, 126). In this health survey, life style and BMI were assessed from a self-assessed questionnaire. This comparison showed no significant differences in socio-demographic structure and prevalence of smoking and obesity between the two cohorts, however the proportion of individuals who reported poor health and the proportion of manual workers were somewhat lower in MDCS. A study comparing community versus personal invitation, and subjects responding late versus early to personal invitation, showed that individuals who were recruited by personal invitation and who responded late, had a more unfavourable situation with regard to socio-

demographic and lifestyle factors (127). These results indicate that selection of a “healthy cohort” may have been reduced in MDCS.

## **Validity of endpoints and risk factors**

### *Endpoints*

Vital status at the end of the follow-up was updated on all individuals by data linkage with regional and national registers. The completeness and validity of these registers have been documented in several other studies from the city (80, 128, 129). A validation study from The Swedish Hospital Discharge Register has validated the diagnosis MI, and found that it was false in only 5% of the cases (78). STROMA was used to find cases with stroke. This register continuously searched for and validated stroke cases since 1989. The accuracy of the diagnosis stroke was ensured by a specialised research nurse, with supervision of a senior physician. This is a major strength, because routine hospital discharge registries poorly reflect the incidence of stroke in the population, and among patients discharged alive from hospital almost 30% of the stroke diagnoses could be false-positive and 6% false-negative (130). No such validation has been done for the diagnosis stroke for cases where Swedish Hospital Discharge Register was used. However, only 5% of all incident strokes in the MDCS occurred outside Malmö (paper IV-V).

Autopsy is an important instrument in the validation of causes of death. Traditionally, the frequency in Malmö has been as high as 80%, but has declined dramatically during the last decades (74). However, a misclassification because of this would probably dilute the reported associations.

### *Risk factors*

Some information on participants was drawn from self-reported questionnaires of paper and pencil type. In MDCS, questions that had not been answered were completed orally. It is well known that obese subjects tend to under-report unhealthy

habits, e.g. physical inactivity and alcohol intake (7). However, if this was the case in our studies, it would probably have caused a dilution of the associations.

A difficulty in this kind of studies is the cut-off points for e.g. obesity. These cut-off points are always arbitrary and the risk is increasing for every small increase in weight. However, to be able to study associations it is easier to use categories.

### **Epidemiological and statistical design**

Multivariate proportional hazards analysis is a well established method to estimate incidences of disease. However, some methodological limitations need to be discussed. A common problem in long-term follow-up studies is change of exposure. Without re-examinations, it is impossible to know what happens between baseline examination and outcome. In these studies, subjects with newly detected CV risk factors, i.e. hypertension, T2DM etc, were referred for treatment in other clinics. It is reasonable to assume that they have reduced their risk, which means that the associations we have found would be even stronger in a population which has not received this intervention. Moreover, change in body weight is usually a slow progress, and it has been shown that the variation of body weight and adipose tissue distribution is remarkably stable throughout the life-span (131).

Another problem with multivariate analyses is that many variables are introduced into the model at the same time, and many of them interact in ways that are difficult to estimate. There is always a risk of over-adjustments. An attempt to evaluate this problem was to perform multicollinearity analyses. These analyses did not show any low tolerance values except for the analyses in paper IV, where BMI and BF% were included in the same model. This resulted in a tolerance value of 0.30 in women, which is rather low, however it has been suggested that only values below 0.20 constitute a collinearity problem (132). Moreover, it has been debated whether risk factors that can be explained as factors in the causal chain of events between obesity and CVD, i.e. hypertension, T2DM and dyslipidemia, should be treated as confounding factors or not (22). According to this theory, these factors were only included in the multivariate analyses in a final model in paper II, IV and V. In the

other two papers, it is possible that the risks were under-estimated. Finally, even if several possible confounding factors were included in the multivariate analyses, there is still a risk of rest confounding. Flegal et al 2004 (133) recently reported that the obesity-associated mortality rates estimated in Allison et al 1999 (134) may be dramatically overestimated as a result of inadequate adjustment. In this thesis some CV risk factors, e.g. blood lipids and inflammatory markers, were only present in a few papers, and other risk factors, e.g. cardio-respiratory fitness and disturbances in the coagulation system, were not accessible at all.

To include an interaction term in a Cox model has been criticised to express interaction only in a statistical meaning and does not evaluate causal interaction (96). Therefore, a synergy index (SI) was calculated by methods described by Hallquist and Rothman in paper I and II.

### **Missing values**

Before starting the analyses, subjects with missing values on any of the main variables were excluded from the study cohorts. In paper I and II, there were as many as 2174 and 1995 men, respectively, with missing values on physical activity. To avoid losing power in stratified analyses, the variable was coded as a dummy variable into the models, with missing values as one category. In paper II, 1177 men were excluded because of missing values on BMI, civil status or SEI (in the published paper it was erroneously written only “BMI or civil status”).

### ***Public health aspects***

As obesity is a rapidly increasing global health problem, there is a great need of reducing the CV risk in these individuals. A general intervention could maybe reduce the overall CV incidence. However, other CV risk factors up- and down-regulate the CV risk, making obese individuals a heterogeneous group where some individuals are more vulnerable than others. These individuals would gain even more from intervention and are therefore important to identify. Global risk assessment is a way to quantify CV risk in obese people more accurately, to identify individuals who will



benefit the most of intervention. It is also a possible way of risk reduction by not only weight loss, but with other prevention strategies, e.g. smoking cessation and physical activity. This is important as it is very difficult for many obese to obtain a sustained weight loss (135).

According to the results in this thesis, global risk assessment should include smoking, occupation, marital status, measurement of inflammatory markers, physical activity and assessment of abdominal adiposity. Smoking cessation is often associated with weight gain, however improvement in insulin sensitivity has been shown despite of this disadvantage, and with simultaneous dietary intervention and use of nicotine replacement weight gain was reduced (136). Occupation and marital status are factors that are hard to intervene on from an individual perspective; however the results from this thesis indicate that social support and economy are important factors that can be promoted by society. There are certainly more subgroups that are particularly vulnerable; others have identified subjects with increased alcohol consumption and physical inactivity (137-139).

The “epidemiological triad” consists of host, vector and environment, and intervention may be performed at all these parts (140). The host may be modified by biological, behavioural and physiological strategies like education and medical intervention, the vectors include factors that limit overconsumption of energy and physical inactivity, and the environment constitute physical, economic and socio-cultural aspects which can be modified by legislation and social changes (140).

Weight loss can be achieved by decreased dietary intake, increased exercise, liposuction or bariatric surgery. Pharmacological treatment is also used; however the effect has been controversial (7, 55). Beneficiary effects of weight loss on CV risk profile have been found with all these techniques, however Klein et al recently reported that subcutaneous abdominal liposuction did not improve metabolic abnormalities (3, 17, 51, 65, 141). These data suggest that a negative energy balance is critical for achieving metabolic benefits. Moreover, long-term weight maintenance after weight loss is unusual, which is thought to be a result of regulatory factors that aim at re-establish the balance before weight loss (135). The mechanisms behind this

regulation are incompletely understood. Bariatric surgery seems to be the only existing method to obtain a permanent weight loss (142).

Recent data from Gothenburg, Sweden, show that 11% of women and 15% of men between 25 and 64 years old are obese ( $\text{BMI} \geq 30.0 \text{ kg/m}^2$ ) and that the prevalence is increasing (4). In the same age category, 38% of the women and 58% of the men are overweight ( $\text{BMI} \geq 25.0 \text{ kg/m}^2$ ). With these data, it seems rational to focus on primary prevention to reduce the weight of these individuals before they develop complications, and to establish preventive programs that take into account the complicated interactions between modifiable risk factors.

## CONCLUSIONS

This thesis elucidates that obese people constitute a heterogeneous group in which the susceptibility for CVD differs substantially according to subsets of other biologic and socio-demographic circumstances. The results indicate a possibility to identify obese individuals with an increased risk of CV morbidity and mortality with global risk assessment. The separate papers have the following conclusions:

- Obesity is associated with an increased incidence of CE and death in men. The risk associated with obesity is substantially increased by exposure to other atherosclerotic risk factors, i.e. smoking, hypertension, diabetes and hyperlipidemia, of which smoking seems to be the most important.
- Obesity is associated with single status and manual job in men. Adjusted for lifestyle and biological risk factors, the increased risk of CE and death for obese men with manual jobs was applicable only to those who were single. Being single significantly increases the CV risk associated with obesity.
- The CV risk varies widely between obese or overweight men with high and low ISP. Relationships with ISP contribute to, but cannot fully explain, the increased CV risk in obese men.
- Body fatness is a risk factor for CV complications, i.e. CE, stroke and death from CVD; however there is a sex-specific effect where BF% seems to be more strongly related to complications in women than in men. The raised CV risk associated with high BF% is reduced by physical activity.
- The effect of body fat distribution on CV risk is modified by the level of overall body weight and by gender. WHR adds to the CV risk in women at all levels of BMI and in men with normal weight.

## POPULÄRVETENSKAPLIG SAMMANFATTNING (SUMMARY IN SWEDISH)

Fetma räknas som ett av världens tio viktigaste hälsoproblem enligt WHO och ökar i snabb takt. Man beräknar att över 1 miljard av världens vuxna befolkning är överviktig, d.v.s. har ett kroppsmasseindex (BMI)  $\geq 25.0$  kg/m<sup>2</sup>, varav 300 miljoner är feta, d.v.s. har ett BMI  $\geq 30.0$  kg/m<sup>2</sup>. I Sverige är 38% av kvinnor och 58% av män mellan 25 och 64 år överviktiga, och 11% respektive 15% är feta. Fetma ökar risken för allvarliga sjukdomar som diabetes typ 2, cancer i bl.a. tjocktarm, livmoder, bröst och matstrupe, och hjärtkärlsjukdom. I Sverige beräknas ca 45% dö i hjärtkärlsjukdom, vilket gör det till den vanligaste dödsorsaken. Det finns många bakomliggande riskfaktorer som dessutom kan interagera med varandra. Trots att fetma är en känd riskfaktor för både hjärtinfarkt, slaganfall (stroke) och för tidig död, är det långt ifrån alla feta individer som drabbas. Vilka faktorer som samvarierar med fetma och därmed kan öka eller minska risken är oklart. Syftet med denna avhandling var att undersöka heterogeniteten hos feta individer och ta reda på om skillnader i livsstil och biologi förändrar sambandet mellan fetma och hjärtkärlsjukdom.

I avhandlingen utnyttjas två studiepopulationer. I "Malmö Förebyggande Medicin" (delarbete I-III) ingår 22 444 män som undersöktes 1974-1984. Undersökningen innefattade bl.a. vikt, längd, blodtryck, blodprover och ett frågeformulär om tidigare sjukdomar, livsstil m.m. I "Malmö Kost Cancer" (delarbete IV-V) ingår 28 098 män och kvinnor som undersöktes 1991-1996. Med hjälp av samkörning med etablerade register kan man se vilka studiedeltagare som sedan insjuknat i hjärtkärlsjukdom eller dött en för tidig död.

Delarbete I visade att fetma, mätt med BMI, är en riskfaktor för hjärtinfarkt och död, även efter att man tagit hänsyn till effekten av vissa andra riskfaktorer, såsom ålder, rökning, alkohol, diabetes, blodtryck, blodfetter, fysisk aktivitet, socioekonomiska faktorer och tidigare sjukdom. Risken att drabbas av en hjärtinfarkt var 18% högre hos överviktiga och 39% högre hos feta, jämfört med normalviktiga (BMI 20.0-24.9 kg/m<sup>2</sup>). Det fanns dock en tydlig skillnad i risk när man delade upp feta individer i subgrupper med avseende på diabetes, högt blodtryck, höga blodfetter och rökning. Så

många som 16% av de feta hade ingen av dessa riskfaktorer och hos dem var risken att drabbas av hjärtinfarkt inte förhöjd. Endast 2% var exponerade för alla riskfaktorerna, vilket innebar betydligt ökad risk. En särskilt utsatt grupp var feta män som dessutom var rökare, hos dessa var risken kraftigt förhöjd.

I delarbete II studerades effekten av yrke och civilstånd, faktorer som båda är relaterade till hjärtkärlsjukdom. Arbetare hade högre BMI än tjänstemän, och ensamboende hade högre BMI än sammanboende män. I en subgruppsanalys visade det sig att den ökade risken för hjärtinfarkt bara fanns kvar hos feta ensamboende arbetare, efter hänsynstagande till andra riskfaktorer. Att vara ensamboende ökade betydligt risken för feta män.

Bakgrunden till delarbete III var att inflammation har visats vara relaterat till hjärtkärlsjukdom och att halten inflammatoriska proteiner, d.v.s. proteiner som tyder på inflammation i kroppen, är högre hos feta än hos normalviktiga. Blodprov för inflammatoriska proteiner togs på 6193 män i "Malmö Förebyggande Medicin". Studien visade att risken för hjärtinfarkt och stroke varierade kraftigt mellan feta män med höga respektive låga nivåer av inflammatoriska proteiner, även efter att man tagit hänsyn till andra faktorer. Huruvida detta beror på att fetma ökar nivån av inflammation eller tvärtom är oklart.

BMI är ett omstritt mått på fetma, eftersom det inte tar hänsyn till andelen kroppsfett och hur fettet är fördelat i kroppen. Bukfetma har visats vara en farligare fetma än fetma på andra delar av kroppen ur hjärtkärlsynpunkt. Detta kan bero på att bukfett har högre fettomsättning än övrigt fett, och dessutom utsöndrar en rad produkter som påverkar sjukdomsprocessen för hjärtkärlsjukdom. I delarbete IV användes s.k. bioimpedansteknik, som ger ett mått på procenthalt kroppsfett, BF%, hos en individ. Studien visade att BF% är en riskfaktor för hjärtinfarkt, stroke och död i hjärtkärlsjukdom, även efter att man tagit hänsyn till vissa andra faktorer. Hög BF% var en starkare riskfaktor hos kvinnor än hos män. Vidare visades att den ökade risken minskades av fysisk aktivitet hos individer med hög BF%, d.v.s. feta individer kan minska sin risk genom fysisk aktivitet.

I delarbete V studerades s.k. midje-höftkvot (WHR) som mått på bukfetma, i relation till BMI. WHR ökade risken för hjärtkärlsjukdom hos både normalviktiga, överviktiga

och feta kvinnor. Hos män fanns den ökade risken relaterad till WHR bara hos normalviktiga, inte hos överviktiga och feta.

Då fetma är ett snabbt växande globalt folkhälsoproblem är det viktigt att minska risken för hjärtkärlsjukdom hos feta individer. Eftersom de utgör en heterogen grupp med avseende på risken att insjukna i hjärtkärlsjukdom, vore det ur folkhälsosynpunkt bra att kunna identifiera särskilda högriskindivider bland de feta, för att i första hand behandla dessa. Viktminskning kan uppnås via olika metoder, t.ex. minskat energiintag, ökad fysisk aktivitet, läkemedel och operationer som förminskar magsäcken, som alla visats ha positiva effekter på hjärtkärlrelaterade riskfaktorer. Många har dock svårt att upprätthålla en lägre vikt när de uppnått den. Om man kan identifiera särskilda högriskindivider genom att göra en riskskattning som tar hänsyn inte bara till vikt, utan även till andra hjärtkärlrelaterade riskfaktorer, såsom blodtryck, blodfetter, diabetes, rökning, socioekonomi, inflammation, fysisk aktivitet och fettfördelning, kan man försöka hjälpa dessa individer att minska sin risk, inte bara genom viktminskning utan även med andra metoder såsom fysisk aktivitet och rökstopp.

Slutsatsen i denna avhandling är att risken att drabbas av hjärtkärlsjukdom hos feta individer varierar beroende på andra biologiska och livsstilsrelaterade omständigheter. Fynden skulle kunna användas för att identifiera högriskindivider bland feta för att hjälpa dem att minska sin risk för hjärtkärlsjukdom.

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