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COMMON RISK FACTORS ASSOCIATED WITH ACUTE MYOCARDIAL INFARCTION

Population-based studies with a focus on gender differences

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Abstract

The overall aim of this thesis was to explore how the risk for AMI, and the associations between several of its risk factors, differs by gender. Information was collected from four Swedish cohorts: one patient cohort from Skara (1992-1993, n=1149) and three population-based cohorts, i.e. from Skara (1993-1994, n=1109), Vara (2001-2004, n=1811), and Skövde (2003-2005, n=1005). Participants were examined and filled out questionnaires regarding lifestyle and health. An interaction between gender and type 2 diabetes was found with regard to the risk of fatal AMI, showing that the impact of type 2 diabetes on AMI is greater in women than in men. Gender differences were also found in several AMI risk factors and in the associations between these risk factors:

• Basal salivary cortisol was generally higher in women than in men (and in older subjects in general as compared to younger).

• Low morning salivary cortisol and low diurnal variation of cortisol was associated with high waist-hip ratio solely in women.

• Leisure time physical activity was negatively associated with insulin resistance and obesity in both men and women. However, the association with regard to insulin resistance and leisure time physical activity was stronger in women than in men.

• Occupational physical activity was positively associated with insulin resistance and general and abdominal obesity in women only.

• Factor analysis of AMI risk factors yielded three identical clusters in both genders: a "metabolic factor", a "vitality factor", and an "addiction factor". However, these factors partly differed in their association with left ventricular hypertrophy, with significant associations with regard to the metabolic factor in both genders, and to the addiction factor in men.

These findings show that gender differences with regard to AMI are present both in the risk of developing the disease and in how different risk factors for the disease interrelate. Thus, this thesis highlights the need for considering and addressing potential gender differences at all stages of preventive and interventional work aiming to reduce the burden of AMI.

Key words: AMI, gender differences, population-based, type 2 diabetes, hypertension, abdominal obesity, insulin resistance, cortisol, physical activity, left ventricular hypertrophy

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- Low morning salivary cortisol and low diurnal variation of cortisol was associated with high waist-hip ratio solely in women.
- Leisure time physical activity was negatively associated with insulin resistance and obesity in both men and women. However, the association with regard to insulin resistance and leisure time physical activity was stronger in women than in men.
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Abbreviations

AMI	Acute myocardial infarction
CHD	Coronary heart disease
HOMA-ir	Homeostasis model assessment of insulin resistance
HPA-axis	Hypothalamic-pituitary-adrenal axis
HDL-cholesterol	High-density lipoprotein cholesterol
IDL-cholesterol	Intermediate-density lipoprotein cholesterol
LDL-cholesterol	Low-density lipoprotein cholesterol
VLDL-cholesterol	Very low-density lipoprotein cholesterol

List of publications

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

I. Larsson CA, Gullberg B, Merlo J, Råstam L, Lindblad U. Female advantage in AMI mortality is reversed in patients with type 2 diabetes in the Skaraborg Project. Diabetes Care 2005;28(9):2246-8.

II Larsson CA, Gullberg B, Råstam L, Lindblad U. Salivary cortisol differs with age and sex and shows inverse associations with WHR in Swedish women: a cross-sectional study. BMC Endocrine Disorders 2009;9:16.

III Larsson CA, Krøll L, Bennet L, Gullberg B, Råstam L, Lindblad U. Leisure time and occupational physical activity in relation to obesity and insulin resistance: A population-based study from the Skaraborg Project in Sweden. Metabolism – Clinical and Experimental. *Accepted for publication*

IV Larsson CA, Daka B, Gullberg B, Råstam L, Lindblad U. Clusters of AMI risk factors and their association with left ventricular hypertrophy: A population-based study within the Skaraborg Project, Sweden. *Manuscript*

Introduction

Acute myocardial infarction (AMI) originates from an interaction between lifestyle, genetics, and psychosocial factors. In spite of a significant decrease in the incidence of AMI events in the past 20 years it is still the most common cause of death world-wide, including Sweden. Whereas men have a higher over-all risk for AMI than women, primarily due to the earlier manifestation in men, AMI is also the most common cause of death in women. Furthermore, men and women differ in their risk factor profiles related to AMI, and some of the risk factors have a higher impact on AMI risk in women than in men. Thus, more knowledge is needed with regard to gender-specific mechanisms to help improve primary as well as secondary prevention, and ultimately the prognosis for both men and women. The aim of this thesis was to explore gender differences with regard to the risk of AMI and with regard to the association between some of its most acknowledged risk factors.

AMI

The most common cause of death in the world is coronary heart disease (CHD) [1]. In Europe alone, CHD accounts for 1.92 million deaths each year [2]. CHD refers to different conditions of failing circulation of the heart and includes acute myocardial infarction (AMI), which is the one CHD that causes most deaths. AMI is defined by the ischeamia and succeeding necrosis of the heart muscles that follows from a dramatic reduction of the blood flow in the heart. This blood flow reduction is caused by a thrombosis formation that can be initiated from an erosion or from a disruption of an atherosclerotic plaque in the coronary artery [3]. Atherosclerosis relates to the accumulation of lipids and lipoproteins in the endothelium and is characterized by a chronic inflammation [4] that is also involved in the plague rupture and thrombosis [5]. In 2008, 37 156 persons suffered an AMI in Sweden and 10 509 died as a result of AMI [6, 7].

The risk of AMI is strongly age- and sex-dependent. For men and women together, the incidence in Sweden in 2008 was three times as high in subjects aged 70-74, as compared to those aged 50-54 [6]. Furthermore, the age-standardized incidence rate was 689 per 100 000 men and 365 per 100 000 women in those aged 20 years and above [6]. The corresponding mortality rates was 197 per 100 000 men and

103 per 100 000 women [6]. The higher risk of AMI in men is mainly due to the earlier manifestation of the disease in men, which usually takes place about a decade sooner than in women.

Even though AMI is still the most common cause of death in Sweden, both the incidence and mortality of AMI have decreased considerably in Sweden and in the last two decades [6, 8]. This decrease is attributable to both improved medical treatment [9] and to reductions in major risk factors [10].

The gender aspect

Men in general suffer their first AMI event about a decade earlier than women and as a consequence they have a higher total risk of AMI. Since the risk in women starts to rise after they reach menopause, a protective effect from sexual hormones has been postulated as the reason for women's later manifestation of AMI. This theory was strengthened in the 1990's when observational studies showed preventive effects of hormone replacement therapy on CHD risk. However, this was not confirmed in later randomised clinical trials, which on the contrary even showed increased risk of CHD in some cases [11, 12]. Still, there are new data indicating that the risk of CHD may vary according to initiation time of therapy in relation to menopause, with increased risk in women starting hormone replacement therapy distant from menopause and no apparent risk in women starting soon after menopause [13]. Findings with regard to endothelial dysfunction has also generated a theory explaining the increased risk in women with age as a detrimental age-dependant change in the effect of estrogen on nitric oxide synthase [14]. Thus, the most plausible reason for the later manifestation of AMI in women can probably still be attributable to some type of reproductive hormonal effect. Interestingly, increased levels of male reproductive hormones in women, and decreased levels in men, are both associated with increased risk of CHD in both genders respectively [15-17]. Furthermore, a recent review [18] highlights the potential importance of also considering the explanatory value of other risk factors that pertain solely to women, such as history of preeclampsia, and possibly polycystic ovarian syndrome.

Even though men have a higher total risk of AMI, women seem to be disadvantaged in other ways. For example, the decrease of AMI that has taken place in the last two decades seems to be somewhat lower in women than in men [6, 8]. For example, between 2001 and 2008, the age-standardized incidence of AMI in Sweden decreased with 20% in men compared to 15% in women. However, the difference between genders in the decrease of first event of AMI in 2008 was smaller, with an 18% decrease in men and 16% decrease in women.

Other differences between men and women regarding outcome, treatment, clinical presentation, and baseline characteristics have been extensively discussed; however, it is not always easy to distinguish whether women or men are at the greater disadvantage. Nevertheless, further elaboration regarding gender differences in outcome and baseline characteristics can be found below in the descriptions of common risk factors for AMI.

Risk factors

AMI is a multifactorial disease and there are thus several risk factors apart from age and gender. Most of these risk factors are lifestyle related and many vary according to gender.

Type 2 diabetes and Insulin resistance

Diabetes mellitus is a disorder leading to chronic hyperglycaemia. The disorder involves disturbed metabolism of carbohydrates, fat, and protein, and is caused by defects in insulin secretion or insulin action, or both [19]. In type 1 diabetes there is a destruction of the insulin producing beta cells, and frequently this is attributable to an autoimmune process [19]. In type 2 diabetes, the failing insulin secretion is instead usually preceded by a process of increasing loss of insulin sensitivity in several tissues, such as skeletal muscle, adipose tissue, liver, and endothelium [20]. This insulin resistance initially leads to increased insulin secretion to keep glucose homeostasis in balance; however, in many subjects the "cost" of this hyperinsulinemia is ultimately a failing insulin secretion and consequently hyperglycaemia and type 2 diabetes [21]. Insulin resistance with increasing hyperinsulinemia often goes on for years before overt type 2 diabetes develops. In a smaller proportion of subjects, the development of type 2 diabetes is a result of a primary defect in insulin secretion and thus not associated with insulin resistance [20].

The specific causes for insulin resistance are not firmly established but seem to involve lifestyle and environmental factors such as caloric and sodium intake, physical inactivity, and exposure to stress in interaction with genetic factors that control glucose metabolism, oxygen uptake, and the development of fat and muscle tissue [22]. Consequently, the risk of type 2 diabetes increases with genetic susceptibility [23], obesity (especially visceral), physical inactivity, age, smoking, and alcohol intake less than 10 grams per day [20]. Type 2 diabetes is also more frequently occurring in individuals with dyslipidemia and hypertension [24], and in women with a history of gestational diabetes [19]. Furthermore, suboptimal pre-

natal growth has been associated with various biological abnormalities in adult life, including insulin resistance [25] and type 2 diabetes [26].

According to the WHO guidelines from 1998 [19], diabetes mellitus is ascertained by the criteria shown in Table 1. For epidemiological and population screening purposes, a single measure of the fasting plasma value or the 2-h plasma glucose value after a standard 75 g glucose load in a glucose tolerance test may be used alone. For clinical purposes, however, the diagnosis of diabetes should always be confirmed by a repeated test on another day unless there is unequivocal hyperglycaemia with acute metabolic decompensation or obvious symptoms [19].

Table 1. Diagnostic criteria for diabetes mellitus and other categories of hyperglycaemia as recommended by WHO in 1998 [19].

	Glucose Concentration (mmol/l)		
	Whole blood		Plasma
	Venous	Capillary	Venous
Diabetes mellitus			
Fasting	≥6.1	≥6.1	≥7.0
and/or			
2 h post glucose load	≥10.0	≥11.1	≥11.1
Impaired glucose tolerance (IGT)			
Fasting	<6.1	<6.1	<7.0
and			
2 h post glucose load	≥6.7 and <10.0	≥7.8 and <11.1	≥7.8 and <11.1
Impaired fasting glycaemia (IFG)			
Fasting	≥5.6 and <6.1	≥5.6 and <6.1	≥6.1 and <7.0
2-h (if measured)	<6.7	<7.8	<7.8

Table 1 also shows the diagnostic criteria for impaired glucose tolerance and impaired fasting glycaemia. Impaired glucose tolerance is defined as a stage of impaired glucose regulation intermediate between normal glucose homeostasis and diabetes. Impaired fasting glycaemia is a related condition with fasting blood glucose levels above normal but below the levels that define diabetes [19]. Both these conditions are recognised as risk factors for definite type 2 diabetes in similarity to insulin resistance, which precedes impaired glucose tolerance and type 2 diabetes [19].

Both insulin resistance in itself (i.e. without fully developed diabetes) and type 2 diabetes are associated with an increased risk of CHD. The risk of CHD in subjects with diabetes is about doubled when looking at men and women together [27]. This seems to be an effect of several interacting metabolic changes in the pre-diabetic state, such as development of atherogenic dyslipidemia, impaired endothelial function, increased levels of free fatty acids, subclinical inflammation, changes in adipokines, and changes in thrombosis and fibrinolysis [28].

In women with diabetes the relative risk of CHD is larger than in corresponding men and the advantage generally seen in women seems to be more or less absent [27, 29]. The underlying reason for this phenomenon has been under debate, and frequently this has been suggested to be a result of lack of adjustments for differences in other classic CHD risk factors [29, 30], even though this argument does not seem to explain all of the difference [27]. In addition to this explanation, three other possible reasons for the high CHD risk in women with type 2 diabetes have been postulated [31]:

- "A major impact of some cardiovascular risk factors and/or diabetes per se on cardiovascular disease in women with diabetes."
- "Differences in the structure and function of heart and vessels."
- "Disparities in medical treatment as well as gender differences in treatment response."

Interestingly, a Danish study has shown that hormone replacement therapy is associated with increased risk of CHD in women with diabetes, while neither an increased risk nor a decreased risk was seen in non-diabetic women [32]. Still, hormone replacement therapy does not seem to explain all of the risk increase in CHD in women with diabetes [33].

In Sweden, around 300 000 people have type 2 diabetes and it is somewhat more common in men than in women [34]. Whereas the prevalence has been increasing in the last decades as an effect of increasing survival rates, the incidence seems to be fairly stable despite increasing levels of obesity in the population. However, since type 2 diabetes takes many years to develop, it is highly possible that an increase in its incidence is yet to come [34].

Hypertension

According to WHO´s international guidelines [35], hypertension is defined as a systolic blood pressure of 140 mmHg or greater and/or a diastolic blood pressure of 90 mmHg or greater in subjects who are not taking antihypertensive medication.

In Table 2 below, blood pressure categories in adults over the age of 18, as suggested by WHO, are described [35].

Table 2. Definitions and classifications of blood pressure levels (mmHg)

Category	Systolic	Diastolic
Optimal	< 120	< 80
Normal	< 130	< 85
High-Normal	130-139	85-89
Grade 1 hypertension (mild)	140-159	90-99
Subgroup: borderline	140-149	90-94
Grade 2 hypertension (moderate)	160-179	90-109
Grade 3 hypertension (severe)	≥ 180	≥ 110
Isolated systolic hypertension	≥ 140	< 90
Subgroup: borderline	140-149	< 90

When a patient's systolic and diastolic blood pressure fall into different categories, the higher category should apply.

In some subjects with hypertension, the increased blood pressure can be traced to an underlying disease that affects the regulatory mechanisms of the blood pressure (often renal or renovascular) [36]. This type of hypertension is referred to as secondary hypertension, and is generally rare. Instead, the vast majority of hypertensive subjects suffer from essential or primary hypertension [36]. Whereas the specific mechanisms are yet to be revealed, the origin of elevated blood pressure and hypertension are associated with vasomotor tone and sodium and fluid balance, as they are the main regulators of blood pressure [37]. These are in turn affected by genetic factors and various lifestyle factors, such as obesity, physical inactivity, and poor diet, including high intake of alcohol and salt [36]. Blood pressure is also known to vary by age, sex, and ethnicity [36]. Many of the lifestyle factors have a negative influences in themselves on the level of blood pressure, but many of them also act as risk factors because of their potentially weight-increasing effect. Excess body weight contributes to blood pressure levels from infancy and is the most important factor causing a predisposition to hypertension. In addition, overweight and obesity increase the risk of insulin resistance [38], another risk factor for hypertension [39]. Furthermore, the importance of psychological factors and chronic stress in the development of

hypertension is becoming increasingly acknowledged [40]. This is not only because of an association with the adoption of less healthier lifestyle habits associated with hypertension, but also because of other more direct effects on long-term blood pressure levels, involving both the sympathetic nervous system and the hypothalamic-pituitary-adrenal-axis (HPA-axis) [40].

According to large observational studies, high blood pressure is related to CHD in both men and women, and the risk rises progressively with increasing blood pressure [41-44]. This risk-increase with increasing blood pressure does not only refer to subjects with hypertension but also to normotensive subjects [41-43, 45]. In the INTERHEART study [44], history of hypertension was associated with an almost doubled risk of AMI. Interestingly the association was stronger in women. Whereas tendencies towards similar gender differences have been seen before with regard to the risk of CHD [46] and cardiovascular disease [45], the confidence intervals overlapped in these studies. Furthermore, in Selmer et al's study [46], as well as in the INTERHEART study [44], confounding might apply. However, in a recent Swedish study women were found to have a more gradual risk increase of cardiovascular mortality with regard to increasing blood pressure, in contrast to men where only very high levels showed a significant risk increase [47].

The effect of blood pressure on the risk of CHD is mostly an effect of different mechanisms leading to vascular remodelling [37]. Furthermore, the same regular dysfunction that causes high blood pressure might also in itself have other negative effects on the cardiovascular system, i.e. irrespective of high blood pressure [36]. Nor-adrenaline and adrenalin, for example, do not only increase arterial resistance, but may also in a direct way have detrimental effects on the heart muscle cells [36]. The risk of myocardial injury from a relative decrease in oxygen supply might also be a consequence of blood pressure damage to the heart that reduces the ability to respond to increased demands [36].

In a SBU-rapport from 2004, the prevalence of hypertension in Sweden was estimated at approximately 27% for both men and women aged ≥20 years [48]. In a population-based study from the Skaraborg project from 2001-2005, where repeated measures of blood pressure were used, the age-standardized prevalence of hypertension was 20% in both genders between 30 and 75 years of age [49]. The mean blood pressure in the same cohort was 124/72 in men and 119/69 in women.

General and abdominal obesity

The association between obesity and CHD is well established [50] and considering the epidemic proportions of obesity in the western society today the negative implications for public health are evident. Overweight and obesity are often

estimated by body mass index, which is calculated as the body weight in kilograms divided by the square of the height in meters (kg/m^2). An adult person is considered overweight if the body mass index is between 25.0 and 29.9 kg/m^2, and obese if the body mass index is ≥30.0 kg/m^2 [51]. Obesity not only increases the risk for CHD but also the risk for other CHD risk factors such as type 2 diabetes, hypertension, dyslipidemia, endothelial dysfunction, systemic inflammation, prothrombotic state, abnormal left ventricular geometry, and insulin resistance and the metabolic syndrome [52].

The importance of abdominal obesity as a risk factor for CHD has also been increasingly acknowledged and is now more or less considered the driving force behind the detrimental effects of obesity. Different measures have been used to estimate the amount adiposity, for example waist circumference and waist-hip ratio, and there is increasing evidence that these measures to a greater extent than body mass index predict CHD/AMI. A report from the INTERHEART study [53] indeed concluded that waist-hip ratio is a better predictor of AMI than body mass index and that waist circumference is intermediate between those two measures. Similar findings to those from INTERHEART have also been seen in prospective studies [54, 55]. While no apparent gender differences were seen in INTERHEART [53] or in the meta-analysis by de Koning et al [55], other studies suggest that the strength of the association between abdominal obesity and CHD might be stronger in women than in men [54].

The greater CHD risk for abdominal obesity as compared to general obesity is proposed to be a result of intra-abdominal fat accumulation. This type of fat has been shown to be more metabolically active with more lipolytic activity [56] and higher sensitivity to glucocorticoids [57]. Adipose tissue also acts as an endocrine organ as it produces different adipokines, which contribute to the association seen with insulin resistance, proinflammation, hypertension, and thrombotic state [Depres 2008]. Furthermore, in contrast to subcutaneous fat that is drained by the systemic circulation, the intra-abdominal fat is cleared by the portal circulation and thereby exposes the liver to high concentrations of free fatty acids. This in turn has been associated with various metabolic disturbances such as hyperinsulinemia, glucose intolerance, and hypertriglyceridemia [58].

According to self-report information gathered by The Swedish National Institute of Public Health [59], the prevalence of obesity in Sweden in 2010 was generally equal between men and women (13%), while overweight was more common in men (41%) than in women (29%). In a population-based study from Vara within the Skaraborg Project [60], the prevalences of obesity and overweight as measured by the study nurses in 2001-2005 were 20% and 52% in men, and 25% and 34% in women. When looking at the trend over the last three decades, both overweight and obesity has increased [34). However, over the past years the prevalence of

overweight has been fairly similar, while the prevalence of obesity has kept increasing in both men and women [34, 59].

Dyslipidemia

Dyslipidemia refers to several different abnormalities of the lipid levels in the blood. Lipids are considered to play a key role in the development of atherosclerosis and ultimately CHD because of its ability to build up on artery walls and form atherosclerotic plaque. As lipids are not soluble in blood, they are carried by certain proteins (apolipoproteins) for transport in the blood stream, and such packages of proteins and lipids are called lipoproteins [61]. The lipids that long have been considered the most relevant in the area of coronary heart disease are low-density lipoprotein cholesterol (LDL-cholesterol), high-density lipoprotein cholesterol (HDL-cholesterol), and triglycerides. There are, however, other lipids that are considered atherogenic too, like very low-density lipoprotein cholesterol (VLDL-cholesterol) and intermediate lipoprotein cholesterol (IDL-cholesterol). Whereas high levels of LDL-cholesterol, VLDL-cholesterol, IDL-cholesterol, and triglycerides are considered harmful, high levels of HDL in the blood are beneficial, mainly due to the ability of HDL to transport cholesterol out of the artery walls and back to the liver (reversed cholesterol transport) [61, 62]. However, other potential beneficial mechanisms also exist with regard to HDL, such as inhibiting effects on of thrombosis, oxidation and inflammation [61]. Different ratios between the aforementioned lipoproteins are often used to estimate the balance between the atherogenic and the antiatherogenic lipoproteins. However, in the last decades increasing interest has been directed towards measuring apolipoproteins instead of lipoproteins. Two examples are apolipoprotein A1 and apolipoprotein B. Apolipoprotein A1 is the principle apolipoprotein in HDL, whereas apolipoprotein B is present in several different lipoproteins, such as LDL, VLDL, and IDL. Measuring apolipoprotein B will thus give information about all the atherogenic lipoproteins in contrast to a measure of just LDL-cholesterol for example. Consequently, the ratio between apolipoprotein B and apolipoprotein A1 (apolipoprotein B/apolipoprotein A1 ratio) is becoming increasingly used to estimate lipid balance instead of the traditional ratios [63, 44].

High levels of LDL-cholesterol have been associated with increased risk of CHD in both men and women in several studies [62, 64]. Increased risk of CHD in both men and women has also been seen with regard to low levels of HDL-cholesterol; however, the risk increase appears to be higher in women than in men [65, 62]. Whereas the ratio between LDL and HDL cholesterol is associated with increased CHD risk, the apolipoprotein B/apolipoprotein A1 ratio has been found to be an even better predictor than LDL/HDL ratio and other of the conventional ratios

between different lipoproteins [63]. Regarding triglycerides, numerous studies have linked also this type of lipid to an increased risk of CHD [66-68]. Although doubts have been raised concerning whether this association is independent of other risk factors [61, 69], triglycerides seem to be an independent risk factor for CHD under certain circumstances [61, 70], such as in populations without pre-existing CHD [70] and perhaps especially in women [61, 66, 68, 71]. Furthermore, there is evidence of an inverse association between triglycerides and HDL-cholesterol [70], as often seen in subjects with the metabolic syndrome (see below).

The Metabolic Syndrome

The metabolic syndrome refers to a diagnostic entity of a set of cardiovascular risk factors that are known to cluster in the same individuals more often than can be explained by chance [72]. It was first identified around the 1980´s and it has also been referred to as the "insulin resistance syndrome" [73] and "the Syndrome X" [74]. Different criteria for identifying subjects with the syndrome have been developed throughout the years [19, 73, 75], and even though the specific criteria are still not universally agreed upon, the risk factors usually considered to be key components are obesity/abdominal obesity, hypertension, dyslipidemia, and insulin resistance (with or without glucose intolerance or diabetes. In addition, the NCEP [75] also considers proinflamatory (clinically measured by C-reactive protein) and prothrombotic state (clinically measured by plasma plasminogen activator-1 and fibrinogen) to be part of the syndrome. When clusters of these risk factors have been evaluated for principal components, abdominal [Lempiäinen P, 1999]/general obesity [76, 77] and insulin resistance [76, 77] have emerged as major factors and are often believed to be the underlying driving force of the syndrome.

Despite the fact that the specific causal chain behind the development of the metabolic syndrome has yet to be defined, there are some general features that are usually considered vital. This involves the release of excess free fatty acids, angiotensin II, and adipokines from abdominal adipose tissue. Excess free fatty acids have an inhibiting effect on glucose uptake by muscles, and thus contribute to insulin resistance [78]. Since the adipose tissue is drained by the portal vein, the free fatty acids also act more directly on the hepatic metabolism, thus increasing the secretion of glucose, prothrombotic proteins, and triglycerides [72]. The higher levels of triglycerides in turn make lipoptotein carry less HDL-cholesterol in favour of triglycerides [78]. Furthermore, increased inulin levels in response to insulin resistance, together with the high levels of free fatty acids and angiotensin II, have damaging effect on the beta-cells in the pancreas, whereas angiotensin II

also has blood pressure increasing effects. Different cytokines induce inflammation, which may diminish the efficacy of insulin and also increase blood pressure.

The metabolic syndrome has been associated with an increased risk of CHD and cardiovascular disease in several studies [79, 80]. The risk seems to be higher in women than in men [80, 81], which is in accordance with the greater impact on CHD and cardiovascular disease in women that has been seen or indicated with regard to several of the individual components, such as type 2 diabetes [27, 29], abdominal obesity [54], hypertension [44-46], and dyslipidemia [61, 62, 65, 66, 68, 71]. However, it has lately been questioned whether the syndrome really imposes a greater risk increase than the individual components do, and therefore the utility of the term has been challenged [82, 83].

The prevalence of the metabolic syndrome has been found to be approximately 20% in subjects aged 48-68 years from Malmö [84], in 60-year-old subjects from Stockholm [85], and in a study of 70-year-olds in Gothenburg [86]. Men seem to be affected to a somewhat higher extent than women [85, 86], which is in agreement with findings from other parts of Europe [87].

Smoking

The risk-increasing effect of tobacco smoking on CHD is well established [88-91], and there is a clear dose-response relationship with CHD [91]. The INTERHEART study [44] identified smoking as the second most important risk factor for AMI world-wide (OR: 2.87, adjusted for all other included risk factors) and this was consistent with regards to sex, ethnicity, and geographical region. The detrimental effects of smoking on the cardiovascular system seem to act through promotion of vasomotor dysfunction, atherogenesis (e.g. inflammation and dyslipidemia), and thrombosis in multiple vascular beds [92]. Whereas the specific mechanisms behind these effects are yet to be fully understood, free radical-mediated oxidative stress seems to be a central feature [92]. The negative effects of smoking on the cardiovascular system are known to be more severe in women than in men. A recent meta-analysis and review [93] estimated than women had a 25% higher risk of CHD than men after adjustment for other classic cardiovascular risk factors. The reasons for this gender difference are not clear, but one hypothesis is that a greater amount of toxic agents are extracted in women than in men from the same quantity of cigarettes smoked [93].

In 2010, the over-all prevalence of every-day-smoking in Sweden was 14.7% in women and 12.5% in men according to the ULF-study [94], and 13% and 12%, respectively, according to data from the Swedish National Institute of Health [59].

These differences were not statistically significant; however, in women (24.2%) and men (13.8%) aged 45-54 years in the ULF-study [94] the gender-difference was significant. Furthermore, there is a clear socio-economic gradient in both genders, with more daily smokers in workers, in those with lower educational level, and in those with low income [59].

Alcohol Consumption

A moderate intake of alcohol has been increasingly recognized as a protective factor for CHD; however, the exact nature of the association has been under some debate. A recent review and meta-analysis [95] concluded that the association for both CHD incidence and CHD mortality is L-shaped, in that a moderate intake (≤1 drink/day or 2.5-14.9 g alcohol) was equally protective (25-35% risk reduction) as a high intake when compared to non-drinkers. Interestingly, this effect was at least as strong in women as in men. It is important to note that while moderate drinking is also protective of stroke and all-cause mortality, heavier drinking (>60 g alcohol/day) increases the risk of these conditions [95].

The potential causal link between the protective effect of alcohol use and CHD seems to be mediated by several mechanisms. Another recent review and meta-analysis on interventional studies of the effects of alcohol intake on biological markers for CHD in healthy adults [96] revealed higher levels of HDL-cholesterol (and apolipoprotein A1) and adiponectin, and lower levels of fibrinogen in subjects with a moderate alcohol intake.

According to the data from the Swedish National Institute of Health in 2010 [59], the prevalence of "risky drinking" (based on how often a person drinks alcohol, how much a person drinks on a typical drinking occasion, and how often a person drinks a greater quantity) among Swedish adults were significantly higher in men (16%) than in women in 2010 (10%). Furthermore it was more common among women (17%) to be abstainers compared to men (12%). However, according to data from the Swedish Monitor project, the prevalence of abstainers in 2008 was as high as 26.9% in women and 17.5% in men [97]. Nevertheless, these data are based on random telephone interviews with a high refusal rate. According to the data from the Swedish National Institute of Health, the consumption of alcohol also differed by level of age, education, and region [59].

Physical inactivity

Physical inactivity is a significant risk factor for CHD and AMI and it has been widely studied since the 1950's. Physical inactivity can be defined as "a level of

activity less than needed to maintain good health" [98]. There are different ways of assessing physical inactivity and different terms are thus used in association with this concept, e.g. physical activity, exercise, and physical fitness. Physical activity can be defined as; "bodily movement produced by skeletal muscles that requires energy expenditure"; exercise as "a type of physical activity defined as planned, structured, and repetitive bodily movement done to improve or maintain one or more components of physical fitness"; and physical fitness as "a set of attributes that people have or achieve that relates to the ability to perform physical activity" [98]. Further distinctions of physical inactivity that are often made are that between leisure time physical activity and occupational (work) physical activity.

Physical activity has several beneficial effects on the cardiovascular system and the most important ones are associated with increased myocardial oxygen supply and decreased myocardial work and oxygen demand [36]. Consequently, physical inactivity can be assumed to increase the risk of opposite (i.e. negative) effects on the heart. Furthermore, physical inactivity also has indirect effects on CHD risk by affecting other risk factors for the disease, like obesity [99, 100], hypertension [101], fibrinolytic activity [102], markers of inflammation [103], dyslipidemia [64, 104], and insulin resistance/type 2 diabetes [99, 100, 105]. In addition, physically active subjects seem to be less likely to smoke [106, 107] and eat unhealthily [107, 108]; however, whether these associations are causal or not are under debate.

Several studies have found physical activity to decrease the risk of AMI/CHD [109-112]. INTERHEART, the large case-control study from 2004 [44], reported a significantly reduced risk of AMI in association with regular physical activity and 12.2% of the events of AMI in the populations studied were attributable to physical inactivity. The risk decrease from physical activity could be seen for both men and women; however, the decrease seemed stronger in women than in men. A tendency towards a more protective effect of physical activity on the risk of CHD and cardiovascular disease in women than in men was also seen in the review by Shiroma et al [109]. Whether these differences represent a real difference, or are an effect of methodological shortcomings, or lower physical activity in the female reference group than in the male reference group are, however, not clear.

Some studies have also seen protective effects on AMI/CHD when specifically investigating occupational physical activity [110, 111, 113, 114]. However, others have found no such association [115] or even tendencies towards the opposite (i.e. positive associations between occupational physical activity and AMI/CHD) [116-118]. Similar discrepancies in results have also been seen in studies on the association between occupational physical activity and different coronary risk

factors (e.g. obesity and insulin resistance) [99, 100, 119-122] and some of these results also differ according to gender [100, 123, 124].

According to the annual report from The Swedish National Institute of Public Health [59], the proportion of adult individuals with sedentary leisure time was similar for men (15%) and women (13%) in 2010. Similarly, there was no gender-difference seen when looking at the proportions of men (66%) and women (65%) that were engaged in at least 30 min of physical activity each day. Generally, no major changes have been observed in these patterns since 2004.

Psychosocial factors

In the past decades there has been an increasing interest in the importance of psychosocial factors in association with CHD and AMI. There are several factors of interest, such as socioeconomic status and different aspects of stress. Self-rated health is another factor often used as an over-all measure of people's perception of their general health, including psychological well-being.

Socioeconomic status

Income, occupational grade, and level of education are factors often used to define socioeconomic status [125]. These factors have been inversely associated with CHD mortality in both men and women in several studies [125]. For example, a large longitudinal study from 10 western European populations have found a significantly increased mortality risk from ischaemic heart disease in both men and women with low educational levels compared to those with higher levels [126]. Low socioeconomic status is associated with several modifiable and behavioural risk factors for CHD, and it is still under debate whether this explains all of the association between socioeconomic status and CHD or if there is some additional CHD-increasing risk from low socioeconomic status [127].

According to Statistics Sweden [128], the prevalence in 2010 of having solely primary school education was 22% in both men and women ≥25 years of age. Furthermore, the prevalence of having some form of higher education was 35% in women and 31% in men in the same age group. The gender-difference was most apparent in those between 25 and 34 years of age, where the prevalence of a higher education was 52% in women and 38% in men.

Self-rated health

Self-rated health (SRH), or self-assessed health, is often measured by the single question "In general, how would you rate your health?" with the five response options of "excellent, very good, good, fair, or poor". This, and similar questions,

were significantly associated with all-cause mortality in a meta-analysis of 22 cohort studies [129], and the association was similar for men and women. Furthermore, a large study from the U.S. [130] found self-rated health to be significantly associated with all-cause mortality as well as with death from circulatory disease (cardiovascular disease or diabetes) even after adjustments for age, gender, race, marital status, household size, region of residence, major activity limitations, bed days, restricted activity (in the past 14 days), and BMI. Interestingly, this study also found the association to be stronger in subjects with higher socioeconomic status compared to those with lower status. This has been seen previously [131] but is not in accordance with a Swedish study where the effect of self-rated health on survival was not dependent on education and income [132]. As discussed in the meta-analysis by DeSalvo et al [129], the specific mechanisms behind the capacity of self-rated health to predict mortality are unknown; however, several potential links has been proposed. For example, self-rated health might serve as a proxy for known covariates that predict health and resource needs [129]. Self-rated health might also reflect an individual's own perception about current health status as well as past current or future health risks [133] that might ultimately affect an individual's health clinically. Either way, given the fact that associations between self-rated health and mortality are persistent even after extensive multivariate adjustments, self-rated health clearly captures some aspect of health that is difficult to objectively measure [129].

Self-rated health has also been associated with different aspects of stress in both men and women, such as work stress [134], perceived level of stress [135], and long-lasting difficulties [136]. According to the yearly information gathered by the Swedish National Institute of Public Health [59], poor self-rated health is significantly more common in women (7%) than in men (5%). Women (70%) also report good self-rated health to a significantly lower degree than men (74%). According to these data, poor self-rated health differs significantly in both men and women according to age, education, income, employment rate, and country of birth. Poor self-rated health is thus being reported to a higher degree in subjects >44 years of age compared to 16-29 year-olds, in those with lower educational levels compared to higher levels, in those with low income compared to high, in those on sickness allowance compared to those working, and in those born outside of Europe compared to Swedish-born.

Stress

Strictly speaking stress refers to any physical, mental, or emotional adjustments after the homeostatic balance of the body has been altered. A stress response can thus be described as the bodily responses to maintain homeostasis [137], and a "stressor" can consequently be described as any factor that can lead to such bodily

stress responses. However, it should be noted that the word "stress" is often a bit carelessly used in reference to both stress response and stressors.

There have been numerous studies on the association between CHD/AMI and different aspects of stress. In INTERHEART [138], permanent stress defined as "feeling irritable, filled with anxiety, or as having sleeping difficulties as a result of conditions at work or at home" was significantly associated with AMI, as were financial stress, stressful life events, and depression, albeit, more modestly than with permanent stress. Other studies have also found CHD/AMI to be associated with anxiety, depression, low social support, different aspects of stress at work and in family life, as well as with different personality traits such as hostility and anger [139, 125]. However, whether all of these factors are independently associated with CHD/AMI remains to be ascertained.

The stress system

The stress responses in the body are subordinated under "the stress system", which can be defined as "an elaborate neuroendocrine, cellular, and molecular infrastructure" [140]. This system is located in the central nervous system as well as in the periphery [140] and its crucial functions are mediated by the HPA-axis and the autonomic nervous system [140]. It is thus through activation of these two "sub-systems" that the different stressors affect CHD risk.

The HPA-axis

Activation of the HPA-axis starts a release of corticoid releasing hormone and arginine vasopressin at the hypothalamic-pituitary unit. These hormones act as regulators of the adrenocorticotrophic hormone, which is released from the anterior pituitary gland. The secretion of adrenocorticotrophic hormone in turn results in release of cortisol from the adrenal gland [141]. The duration of the activation of the HPA-axis depends both on aspects of the stressor and on the ability of an individual to cope with the specific stressor [142]. The termination of the activated HPA-axis is mediated by negative feedback from cortisol at the hypothalamic level, at the pituitary level, and at the level of higher brain centres [143].

The "end-product" of the HPA-axis is thus cortisol. Cortisol is normally secreted in a specific diurnal pattern with a normal curve presenting a sharp peak in the early morning before awakening, to then gradually decreasing over the day and ending up very low in the evening and at night. Except for the increased secretion in stressful situations, there are also smaller peaks during the day when the body is exposed to exercise, food, and tobacco [144].

In the acute phase, cortisol has potential effects on the whole body metabolism since the body aims to utilize all energy resources to be able to meet the increased demands that a stressor enforces [140]. For example, proteolysis and insulin resistance in the muscles are promoted and lipolysis in fat depots [145]. Cortisol also has blood pressure increasing effects and suppresses inflammation [146]. This "catabolic shift" of the metabolism is usually reversed when the stressor is removed, however, chronic stress with prolonged activation of the HPA-axis and sustained cortisol secretion is expected to decrease muscle and bone mass and increase visceral obesity and insulin resistance [140].

The autonomic nervous system

The other main sub-system that controls the body's stress responses, the autonomic nervous system, works via the sympathetic or the parasympathetic system. Often the sympathetic and the parasympathetic system are involved simultaneously, with an increased parasympathetic activity working antagonizing on the sympathetic functions. The parasympathetic system can also by withdrawing, enhance the functions of the sympathetic system [147]. The autonomic nervous system, with the sympathetic and the parasympathetic system, affects many functions in the body, such as cardiovascular, respiratory, gastrointestinal, renal, and endocrine functions [140]. Activation of the autonomic nervous system in response to stress thus plays an important part in understanding the link between different aspects of stress and CHD/AMI. For example, acute increases of sympathetic activity in combination with chronic stress and metabolic risk may increase vasoconstriction, hypertension, and hypercoagulation and thereby increase the risk for cardiovascular events [140].

Potential biological mechanisms

While the acute stress responses are vital to maintain homeostasis in the body, a situation of prolonged stress activation is associated with numerous detrimental effects in the body. Considering the diverse biological and psychological effects of a stress reaction in the body, whether initiated by the HPA-axis or the autonomic nervous system, there are several potential causal links between different stressors and CHD, such as hypertension, insulin resistance, abdominal and general obesity, lipids, decreased heart rate variability, endothelial and vascular functions, and inflammatory and coagulatory processes [139, 125]. Thus, these factors are all potential effects of different stressors and they have also been associated with CHD and AMI. In addition, perceived stress has been linked to unhealthy behaviours (such as physical inactivity, smoking and unhealthy diet) [125, 139] and decreased adherence to behavioural change and cardiac medications [125], which indirectly increases the detrimental effects of stress. Recent epidemiological

studies have presented significantly increased risk of all-cause mortality, especially from cardiovascular causes, in association with elevated 24-h urinary cortisol [148] and flatter diurnal cortisol slope [149]. Furthermore, heightened cortisol reactivity in repsonse to mental stress has been associated with coronary artery calcification in healthy older men and women [150]. Thus, an inadequate activation of the HPA-axis seems to have negative effects on especially the cardiovascular system and these findings further add to the evidence of a causal link between different stressors and CHD.

Women are known to suffer from more stress-related conditions, such as anxiety disorders and mood disorders, than men [151]. However, women in the follicular phase or on oral contraceptives generally seem to have a lower HPA-axis and autonomic response to psychological and psychosocial stress than do men [152, 153]. In data collected by the Swedish National Institute of Public Health [59], the prevalence of subjects reporting to be very stressed was 5% among women and 3% among men in 2010. Women in the same study also reported reduced mental well-being (women: 21%; men: 14%), severe anxiety (women: 6%; men: 4%), and severe fatigue (women: 9%; men: 5%) to a higher degree than men.

Left ventricular hypertrophy

Left ventricular hypertrophy refers to the increase of left ventricular mass that takes place as an adaptive response to a long-term exposure of increased workload on the heart [154]. At the cellular levels, this increased haemodynamic stress on the ventricular wall leads to a compensatory expansion of cardiomyocytes with little or no increase in the number of cells [154]. In pathological types of hypertrophy this expansion also includes the development of fibrosis [155] and a compromised cardiac function [156], while this is not seen in eccentric types of hypertrophy, such as hypertrophy in response to growth and intensive physical activity (athlete's heart) [154].

Myocardial strain is increased with increasing blood pressure, and hypertension is consequently often considered the single most important risk factor for increase in left ventricular mass and for left ventricular hypertrophy. However, other factors are also of importance, such as age and obesity [157] (especially abdominal obesity in women) [158]. Obesity actually seems to explain part of the association between left ventricular mass/left ventricular hypertrophy and other risk factors, such as blood glucose [157, 158, 159] and cholesterol [157, 158], and even blood pressure to some extent [157]. In studies where gender-stratified analyses were performed, blood pressure and general or abdominal obesity have been found independently associated with left ventricular mass and left ventricular

hypertrophy in both men and women [160, 161], while blood glucose was solely an independent risk factor in men [160, 161].

Lifestyle factors such as smoking [162-166] and alcohol intake [167] have also been associated with left ventricular mass or left ventricular hypertrophy. However, null-findings also exist [168, 169] and there are indications that the risk with regard to alcohol intake is only present in men [170]. While intensive physical activity is known to lead to a benign increase of left ventricular mass [171-175], moderate physical activity seems to be associated with a decreased mass [176], especially in hypertensive subjects [177-182].

Left ventricular hypertrophy is a strong risk factor for cardiovascular disease in both men and women [183, 184] and has even been found to be a stronger risk factor than other classic cardiovascular risk factors, apart from age [184]. In a Framingham off-spring study in adult subjects [184], the prevalence of left ventricular hypertrophy was 15.5% in men and 21.0% in women when assessed by echocardiography.

Aims

General Aim

The aim of this thesis was to examine gender differences in the risk for AMI and to explore potential gender-related aspects of the associations between various risk factors related to the development of AMI.

Specific Aims

- To explore any potential interactions between gender, type 2 diabetes, and hypertension, with regard to the risk of fatal AMI in patients treated within primary care.
- To study the diurnal salivary cortisol pattern under basal conditions, stratified by age and gender, and to explore the relationship between cortisol levels and abdominal obesity in a large, randomly selected population of Swedish adults.
- To study occupational and leisure time physical activity in association with obesity and insulin resistance in in a large, randomly selected population of Swedish adults.
- To investigate how a number of widely acknowledged risk factors for AMI cluster in a large, randomly selected population of Swedish adults, and to study whether any of these clusters are related to LVH, used as a subclinical measure of CHD.

Methods

The Skaraborg Project

The Skaraborg Hypertension Project

The Skaraborg Hypertension Project was launched in 1977 in the county of Skaraborg to improve blood pressure control in the population, and to reduce the risk of AMI and acute stroke. The project entailed guidelines for detection, work-up, treatment, and follow-up of men and women with hypertension. In all primary health care settings in the program area, special out-patient clinics for hypertension were established.

Skara was one of the small towns included in the project. About half of the population lives in the countryside and the other half in an urban area and a high percentage are Swedish-born. At the end of 2003, Skara had 18 736 residents, of which 9 182 were men and 9 554 were women. Forty-eight percent of the population was 40 years or older.

At the Health Care Centre in Skara, a similar program as described above was organized for diabetic patients in the mid 1970´s. In 1986 the care organized for patients with hypertension and diabetes, respectively, were joined together. All the patients with diabetes or hypertension were seen for annual standardized controls, comprising medical history, risk factor control, a standard set of laboratory tests and re-evaluations of medical treatment. This Health Care Centre was the only primary health care service available in the area at the time.

Skaraborg Hypertension and Diabetes Project

Between June 1992 and September 1993 all 1149 patients with hypertension and/or diabetes who completed an annual check-up at the hypertension and diabetes outpatient clinic were consecutively surveyed. Specially educated and trained nurses conducted the study visit following the base-line procedure in the Skaraborg Hypertension Project. Medical history was registered in accordance with predefined criteria, and all current medication for hypertension and diabetes

was recorded. Physical examinations were also performed (see more details below.) Standard laboratory tests were analyzed at the local hospital (Kärnsjukhuset, Skövde) and four extra samples of blood were drawn and stored at -80º C for later analyses. A previously tested questionnaire inquiring about smoking habits, leisure time, physical activity, and alcohol consumption was also completed.

In 1993-1994 a population study was conducted as an age-stratified random sample drawn from the population census register. Subjects aged 40 or over were invited to the Health Care Centre for a health control. Among those aged 80 or more, 100 male and 100 female subjects were randomly selected and invited, as were 150 male and 150 female subjects from each ten-year age category between 40 and 79 years. Of these subjects, 1109 (80%) completed the study visit. The same protocol was used as for the extended annual check-up in the patient survey 1992-1993, and the same trained nurses at the hypertension and diabetes outpatient clinic performed the survey.

The Vara-Skövde Cohort

Between 2001 and 2005 two new and phenotypically better characterized population-based cohorts were established in the municipalities of Vara and Skövde. Vara is a small municipality with approximately 16 000 inhabitants. Ninety-five per cent of the residents are Swedish born and many are farmers. Skövde is the largest town in Skaraborg with approximately 50 000 residents, of which 90 per cent are Swedish born. Skövde is more urbanized than Vara and has a more developed infrastructure, with a hospital and a University.

From the population census register, computer-generated random samples, stratified by gender and five-year age groups, were generated from the total number of all individuals between 30 and 74 years residing in each municipality. There were no exclusion criteria and subjects between 30 and 50 years of age were intentionally over-sampled (three-fold), as compared to subjects over 50 years. There were 1811 subjects who fulfilled all requirements for participation from the Vara population (81% participation rate) and 1005 subjects from the Skövde population (70% participation rate). Those requirements included visiting the study nurse, completing the questionnaires, and having venous blood samples drawn. Compared to the Skara studies, several measurements were added to the protocol. In all participants without known history of diabetes, an oral glucose tolerance test was carried out measuring plasma levels of both insulin and glucose. Morning and evening saliva cortisol were also measured. Furthermore, in 1063 subjects from the Vara sub-cohort an echocardiography was performed (91% participation-rate) [185]. A more detailed questionnaire was added to the protocol

including questions about civil- and socioeconomic status, lifestyle behaviours, and psychosocial health. The field team who collected the data for the Vara-Skövde cohort included members of the team from the Skara studies, and they were all trained and calibrated in methodological procedures before the studies began.

Study Populations

Paper I

This study was based on the 1149 patients from the patient study in 1992-1993 and the 1109 subjects from the population survey in 1993-1994. After excluding 33 patients and 3 subjects with type 1 diabetes from the patient study and the populations survey, respectively, 1149 patients and 1106 population controls initially remained. All cases over 85 years of age at baseline were then excluded, leaving 1085 subjects in the patient sample and 804 in the population sample.

Paper II

Paper II was based on the initial 1811 subjects from the population survey in Vara 2002-2004. After excluding a total of 140 subjects because of pregnancies (n=5), use of cortisone medication (n=10), missing waist circumference measurements (n=1), or missing morning and/or evening salivary cortisol measurements (n=124), 1671 subjects remained.

Paper III

This study was based on the the Vara-Skövde cohort, which included the initial 1811 subjects from the population survey in Vara (2002-2004) and the initial 1005 subjects from the population survey in Skövde (2004-2005). Pregnant women (n=9) were excluded, as well as participants who had worked less than 20 h/week or less than 10 months during the past year (n=994) and those without full information on leisure time physical activity, occupational physical activity, body weight, and waist circumference (n=68). This left a total of 1745 subjects for further analyses.

Paper IV

The last study was also based on the Vara-Skövde cohort. From the initial total sample of 2816 subjects, pregnant women (n=9) were excluded, as were 323 subjects with known hypertension and/or diabetes. Another 156 subjects were excluded because they lacked information on the variables under study (leisure time physical activity (n=73), smoking (n=8), apolipoprotein B/apolipoprotein A1 (n=6), alcohol consumption (n=35), HOMA-ir (n=13), and general self-rated health (n=21)), leaving 2328 subjects for the initial factor analysis. For the analysis regarding left ventricular hypertrophy in association with the factors obtained from the factor analysis, those 852 subjects (all from the Vara sub-cohort) who also had information on left ventricular mass from the echocardiography were included.

General Methods

General procedures

Skara (Paper I)

After an overnight fast (10h), specially educated and trained nurses saw participants the following morning. Participants gave verbal informed consent and were then weighed on a calibrated scale and had their body height measured in light clothes and without shoes. The blood pressure was taken in a supine position and venous blood samples were drawn. The study nurses collected information regarding medical history and ongoing medication with a special focus on treatment for hypertension and diabetes. All participants also answered a questionnaire regarding smoking habits, alcohol consumption, and physical activity [186].

Vara-Skövde (Paper II-IV)

As in the Skara studies, participants were seen in the morning after an overnight fast (10h). Participants signed an informed consent form and venous blood samples were drawn. After collection of the fasting plasma samples, an oral glucose tolerance test was performed with an intake of a 75 g standard glucose load [19]. During the two-hour wait for the final blood drawing, participants filled out the same lifestyle questionnaire that was used in the Skara studies. Participants also filled out questionnaires regarding psychosocial health, stress, quality of life, and civil- and socioeconomic status. Participants were also provided with a

Salivette sampling device (cotton) along with both verbal and written instructions for usage. The instructions stated that participants were to: Collect saliva themselves at 0800 h and 2200 h (with a maximum of 30 minutes time shift) on one normal weekday within two weeks from the first study visit, abstain from food, drinks, snuff, smoking, tooth brushing, and exertion in the hour before saliva collection, rinse their mouths with water 15 minutes before the sampling, and rest for at least 15 minutes before sampling. Levels of saliva cortisol were analysed using a radioimmunoassay from Orion Diagnostica (Spectria™ Cortisol RIA) [187]. Approximately two weeks after the first visit the participants came for a second visit to the nurses. Particicipants were weighed on a calibrated scale and had their body height measured in light clothes and without shoes. They also had their blood pressure taken and provided detailed information on medical history and ongoing medication.

Medical History

Skara and Vara-Skövde (Paper I-IV)

The information collected regarding medical history was established according to predefined criteria. The variables included were acute myocardial infarction, angina pectoris, heart failure, acute stroke, intermittent claudication, diabetes mellitus, and smoking habits.

Physical Examination

Skara (Paper I)

Systolic and diastolic (phase V) right brachial arterial pressure was measured in a supine position after 5 minutes rest, as well as in a standing position after 1 minutes rest, and recorded to the nearest 2 mmHg. Tricuff™ for automatic adjustment of cuff size to arm circumference [188] was used and heart rate was registered simultaneously with blood pressure. The arm was supported by a pillow to be in level with the heart. Participants were weighed on a calibrated scale to the nearest 0.1 kg, and their body height was measured in light clothes and without shoes to the nearest cm for height as well as for waist and hip circumference.

Vara-Skövde (Paper II-IV)

The physical examination was performed according to the same procedure as in the Skara surveys, with waist circumference being measured between the lowest rib margin and iliac crest and hip circumference at the largest circumference between waist and thighs. From the Vara sub-cohort, 1063 consecutively invited subjects (91% participation rate) had an echocardiography performed for measurement of left ventricular mass (LVM = 0.8 × (1.04 [(diastolic dimension + posterior wall thickness + septal thickness)3 – (diastolic dimension)3] + 0.6g) [189]. The echocardiography was performed at a separate visit to a cardiologist using a General Electrics VingMed S5 System, operating with a 3.5 MHz-probe. The same cardiologist performed all echocardiographic measurements and data was stored in the EchoPac System.

Laboratory Examinations

Skara and Vara-Skövde (Paper I-IV)

Blood specimens at baseline were drawn in the morning after an overnight fast (10 hours minimum). Standard laboratory tests were used for serum cholesterol, fasting triglycerides, fasting blood glucose, and also HbA1c for subjects with diabetes. Micral® test was used to test for microalbuminuria in urine [190]. Tests for blood glucose and serum triglycerides were performed in a non-fasting state in insulin-treated patients with diabetes, as fasting tests were considered unsuitable for these patients. The analyses of fasting blood glucose were performed using a modified glucose dehydrogenase method from Hemocue (Hemocue AB, Ängelholm). Both glucose and HbA1c were analysed by the laboratory at the local hospital (Kärnsjukhuset, Skövde). Serum samples for further tests were frozen immediately at -82°C and analysed later for lipids at the Department of Clinical Chemistry, Skåne University Hospital, Lund. Serum insulin was also analysed later at the Wallenberg Laboratory (Malmö University Hospital), using an enzyme linked immunosorbent assay (ELISA) with <0.3% cross-reactivity for proinsulin using a kit from DAKO Diagnostics Ltd [191].

Questionnaires

Skara (Paper I)

Leisure time physical activity was measured based on four answer alternatives to the question “How physically active are you during your leisure time?”. The

question referred to the past year and the answer alternatives were: 1) Sedentary leisure time: reading, watching television, stamp collecting or other sedentary activity; 2) Light leisure time physical activity: walking, cycling, or other physical activity under at least four hours per week; 3) Moderate leisure time physical activity: running, Swimming, tennis, aerobic, heavier gardening, or similar physical activity during at least 2 hours a week; 4) Heavy training or competitive sport: heavy training or competitions in running, skiing, swimming, football, etc, which is performed regularly and several times a week.

Alcohol consumption was assessed by questions concerning the number of days during the past 30 days during which the subjects had consumed beer, wine, and strong liquor, respectively. Each of these questions was followed by questions concerning how many cans, glasses, and/or bottles that were normally consumed on such days. The total number of grams of alcohol consumed per week was then calculated by multiplying the number of days of alcohol consumption by the number of grams of alcohol contained in the consumed alcoholic beverage.

Cigarette smoking was measured by the question "Do you smoke?". The answer alternatives were: 1) No, I have never smoked; 2) No, I have smoked but have given it up; 3) Yes, I smoke.

Vara-Skövde (Paper II-IV)

Leisure time physical activity, alcohol consumption, and smoking were measured by the same questionnaire as that utilized in the Skara studies.

Occupational physical activity was measured by the question "Is your daily work physically light or heavy?". There were five alternative answers: 1) Very light – sitting work (e.g. driving a vehicle, reading, office work, teaching); 2) Light – Standing with little muscle activity (e.g. feeding, handing out medicine in medical care, washing up, precision mechanical work); 3) Moderate intensity – muscular activity with moderate intensity (e.g. walking around, lifting/carrying less than 5 kg, washing, making beds, tidying, carpeting, child care); 4) Heavy – Muscular work with quite high intensity and increased breathing (e.g. maintenance, heavier service work, handling patients within medical care, sweeping streets, heavier gardening, freighting/unloading goods); 5) Very heavy - muscular activity with high intensity and highly increased breathing (e.g. casting concrete, timbering, shoveling soil/sand, lifting/carrying more than 25 kg).

Educational level was assessed by a question with 10 alternatives reaching from primary school to PhD-exams. General self-rated health was defined based on five answer alternatives (from "very good" to "very poor") to the question "How would you rate your current health status in general?".

Diagnostic procedures

Skara (Paper I)

Subjects without an already existing diagnosis of hypertension were followed up with two further blood pressure measurements (1-2 weeks between each measurement) if the initial diastolic blood pressure was ≥90 mmHg [192]. In accordance with national and international guidelines at the time, all three DBP had to be at least 90 mmHg, for a diagnosis of hypertension to be confirmed [192, 193].

In subjects without a diagnosis of diabetes, further examinations of blood glucose were performed (1-2 weeks between measurement) if the initial fasting glucose value was >5.5 mmol L^{-1} [194]. Diagnosis of diabetes was confirmed after two fasting blood glucose values of ≥6.7 mmol L^{-1}. If the second fasting blood glucose test showed a value between 5.6 and 6.6 mmol L^{-1}, an oral glucose tolerance test was performed [194]. If the 2-h glucose value was ≥11.1 mmol L^{-1}, a diagnosis of diabetes was confirmed [194]. Differentiation between type 1 and type 2 diabetes was based on clinical criteria: i.e., age at onset, body weight, symptoms at initial stage, tendency of ketosis, requiring early insulin treatment, and in some cases C-peptide.

End-points included fatal or non-fatal first events of AMI (morbidity), all fatal AMI events (mortality), and all-cause mortality. According to a valid method [195, 196], information on end-points, from baseline through 2002, was ascertained by record linkage with the Swedish national mortality and in-patient registers.

Vara-Skövde (Paper II-IV)

In accordance with international standards [197, 35] subjects without a diagnosis of hypertension were followed up with two further blood pressure measurements (1-2 weeks between each measurement) if the initial diastolic blood pressure was ≥90 mmHg or the initial systolic blood pressure was ≥140. For a diagnosis of hypertension to be confirmed, all three consecutive measurements the diastolic blood pressure had to be ≥90 mmHg and/or the systolic blood pressures had to be ≥140 mmHg.

Diagnosis of diabetes was confirmed after two fasting plasma glucose values of ≥7.0 mmol L^{-1} (1-2 weeks between measurements), or after one 2-h plasma glucose value of ≥11.1 mmol L^{-1} in an oral glucose tolerance test [19]. Differentiation between type 1 and type 2 diabetes was based on clinical criteria: i.e. age at onset, weight, symptoms at initial stage, tendency of ketosis, treatment, and in some cases C-peptide.

Paper I

End-points included fatal events of AMI and all-cause mortality. Information on end-points, from baseline through 2002, was ascertained by record linkage with the Swedish national mortality and in-patient registers [195, 196].

Paper II

Measures of morning and evening values of salivary cortisol were used together with diurnal cortisol level (Δ-cortisol). Δ-cortisol was defined as the difference between logarithmic morning and evening cortisol, which corresponds to the difference in per cent between morning and evening values.

Waist-hip ratio was defined as the ratio of waist to hip circumference. Both waist-hip ratio and waist circumference were used to estimate abdominal obesity.

Paper III

Body mass index was calculated by the body weight in kilograms divided by the square of the height in meters (kg/m^2), and obesity was defined as body mass index ≥ 30 kg/m^2 [198]. Waist circumference >88 cm in women and >102 cm in men was defined as abdominal obesity [75].

Insulin resistance was estimated based on the Homeostasis Model Assessment of insulin resistance (HOMA-ir): fasting insulin × fasting blood glucose / 22.5 [191, 199].

All four categories of leisure time physical activity were used, while category four and five with regard to occupational physical activity were merged together because of low numbers in the last category.

Paper IV

WHR was defined as the ratio of waist to hip circumference. The apolipoprotein B/apolipoprotein A1 ratio was defined as the ratio between apolipoprotein B and apolipoprotein A1. Insulin resistance was estimated based on the Homeostasis Model Assesment of insulin resistance (HOMA-ir): fasting insulin × fasting blood glucose / 22.5 [191, 199]. Measures of systolic blood pressure were used.

Smoking was characterized as ”never smokers”, ”current smokers”, and ”previous smokers”. Alcohol consumption was measured as grams of alcohol per week. All

four categories of leisure time physical activity were used for data analyses, as were all five categories of general self-rated health.

LVM was indexed for height$^{2.7}$ [200] and left ventricular hypertrophy (LVH) was defined as LVM index >47.3 g/m$^{2.7}$ [201].

Statistical analyses

Paper I-IV

With regard to baseline characteristics, differences between men and women were examined by GLM (general linear model) in continuous variables and by logistic regression analyses in categorical variables. All statistical tests in this thesis were two-sided and statistical significance was assumed at $p < 0.05$.

Paper I

SPSS Base System for Macintosh 11.0 was used for data analyses. Baseline characteristics expressed as proportions were age-standardized in ten-year intervals using the total Skara population ≥40 years as standard. After controlling for proportionality, hazard ratios were examined by Cox proportional hazard model. Subjects who reached 85 years of age during the follow-up period were censored for further follow-up on their 85th birthday. Confounding by age, smoking, total cholesterol, BMI, and leisure time physical activity was controlled for in multiple Cox regression models and by stratification.

Paper II

SPSS Base System for Macintosh 11.0 was used for data analyses and all analyses were gender-specific. Baseline characteristics expressed as proportions were age-standardized in five-year intervals using the total Vara population 30-75 years as standard. Differences between groups in continuous variables were examined by general linear model and associations between continuous variables were analysed by linear regression. Associations between categorical variables were analysed by logistic regression and expressed as odds ratios with 95 per cent confidence intervals. For the logistic regression analyses of the associations between waist-hip ratio and morning cortisol/Δ-cortisol, waist-hip ratio above and below the mean was used as the dependent variable, and quartiles of morning and Δ -cortisol,

respectively, as independent variables. Pearson's correlation coefficient was used for testing the correlation between morning and evening cortisol. Confounding by differences in age, alcohol consumption, daily smoking (yes/no), leisure time physical activity, and use of oral contraceptives or estrogen replacements was controlled for in multivariate analyses and by stratification. As an oral glucose tolerance test was not performed in subjects with known diabetes (n=65); the analysis of 2 h blood glucose contains fewer subjects than the analyses of the other variables. Log transformation (10th logarithm) was used to induce normality in morning and evening cortisol measurements.

Paper III

SPSS Base System for Macintosh 17.0 was used for data analyses and all analyses were gender-specific. Differences in continuous variables across groups of leisure time physical activity and occupational physical activity were examined by general linear model, and tests for trends between categories were performed. Associations were also analysed by logistic regression analyses with body mass index and waist circumference, respectively, as dependent variables and occupational physical activity and leisure time physical activity, respectively, as independent variables. For these analyses, body mass index was dichotomized as <30/≥30, and waist circumference as ≤88/>88 cm in women and ≤102/>102 cm in men. Due to low numbers, the fifth and highest category of occupational physical activity was merged with the fourth category, leaving four groups (sedentary, low, moderate, high). The first of the four categories of occupational physical activity and leisure time physical activity (sedentary) was used as the reference category in all analyses, apart from the logistic regression analyses of leisure time physical activity. For these analyses the fourth category was merged with the third category, due to low numbers, and this last category was then used as the reference. Logistic regression analyses for the association between occupational physical activity and body mass index and waist circumference, respectively, were also performed stratified by high (category 3-4) and low (category 1-2) leisure time physical activity. Gender differences regarding occupational physical activity were examined by two-way interaction terms with body mass index, waist circumference, and HOMA-ir, respectively, as dependent variables. For these analyses, body mass index and waist circumference were dichotomized as described above for the logistic regression analyses, while HOMA-ir was dichotomized at the 90th percentile. In the analyses between HOMA-ir and occupational physical activity/leisure time physical activity, all subjects with diabetes were excluded (n=50). In all analyses with fasting insulin and HOMA-ir, log transformation (natural logarithm) was used to induce normality and coefficient of variation (cv) was used to describe dispersion. Confounding by age

(Model 1); age, alcohol consumption, smoking, education, study area (Vara or Skövde), and leisure time physical activity/occupational physical activity (Model 2); and age, alcohol consumption, smoking, education, area, leisure time physical activity/occupational physical activity, and body mass index (Model 3), was controlled for by multivariate analyses and stratification.

Paper IV

SPSS Base System for Macintosh 18.0 was used for data analyses and all analyses were gender-specific. For the analyses with regard to baseline characteristics smoking was dichotomized as daily smoking or not, education was dichotomized as primary school only or above, leisure time physical activity as sedentary/low or moderate/high, and self-rated health as "good" ("good" + "very good") or "reasonable/poor" ("reasonable", "poor", and "very poor"). Factor analysis was used to explore the clustering of the following factors: systolic blood pressure, smoking (all three categories used), apolipoprotein B/apolipoprotein A1, HOMA-ir, waist-hip ratio, leisure time physical activity (all four categories used), alcohol consumption, and general self-rated health (all five categories used). Principal components with eigenvalues >1 were retained and variables were rotated using varimax rotation. Correlations were considered significant for variable loadings ≥0.40. The association between left ventricular hypertrophy and the factor scores obtained from the factor analysis was analyzed by logistic regression analyses with left ventricular hypertrophy as dependent variable and the respective factor scores as independent variables. Logistic regression analyses were also performed between left ventricular hypertrophy and the independent risk factors. In these analyses the categorical risk factors were dichotomized as described above, while one standard deviation was used as the unit for calculating odds ratios and 95% confidence intervals with regard to the continuous variables. Confounding by age and educational level was controlled for by multivariate analyses and by stratification in ten-year age groups and in three levels of education (primary school only, secondary school only, higher education), respectively.

Results and discussion

Paper I:

Female advantage in AMI mortality is reversed in patients with type 2 diabetes in the Skaraborg Project

Results

At baseline all patient samples generally had a more atherogenic risk factor profile than subjects in the reference population (Table 3). During a mean follow-up time of 7.8 years there were 52 events of fatal AMI in men and 29 events in women. Mortality rates were considerably lower in women (2/10 000 person years) than in men (39/10 000 person years) in non-diabetic subjects, while higher in women (116/10 000 person years) than in men (95/10 000) in patients with T2DM. Hazard ratios for AMI mortality for patients with type 2 diabetes compared to the population were 5.0 (2.4-10.8) in women and 1.9 (1.1-3.2) in men, adjusted for age and hypertension. The only significant interaction that was revealed was between gender and type 2 diabetes, showing a female disadvantage represented as a tripled risk of fatal AMI (Table 4), with a synergy index of 4.0. All results remained statistically significant after adjustment for smoking, total cholesterol, body mass index, and leisure time physical activity.

Table 3. Characteristics in patient categories and reference population from Skara, the Skaraborg Project 1992-1994.

Characteristics	**HT only**		**HT+T2DM**		**T2DM only**		**Population**	
Men	*n=277*		*n=119*		*n=94*		*n=401*	
	means (sd)		**means (sd)**		**means (sd)**		**means (sd)**	
Age, years	63.5	12.0[†]	70.1	9.5[*]	68.8	9.2[*]	60.8	13.1
Waist, cm	97.4	9.5[*]	99.4	9.7[*]	96.2	9.5	94.4	9.6
BMI, kg m^{-2}	27.6	3.59[*]	28.7	3.65[*]	27.2	3.61[†]	26.0	3.63
SBP, mm Hg	152	16.7[3]	162	17.0[3]	144	16.9[3]	134	16.9
DBP, mm Hg	87	8.6[*]	89	8.8[*]	82	8.7[*]	76	8.8
FB-glu, mmol L^{-1}	5.5	1.17[*]	7.9	1.19[*]	8.8	1.17[*]	4.8	1.18
Hba1c, % units	-	-	6.2	1.42[†]	6.8	1.41	-	-
LDL, mmol L^{-1}	4.1	0.96	3.9	0.98[†]	4.0	0.97[‡]	4.3	0.98
HDL, mmol L^{-1}	1.00	0.23[†]	0.97	0.23[†]	1.01	0.23	1.05	0.22
Triglycerides mmol L^{-1}[a]	1.48	0.94[*]	1.65	0.95[*]	1.42	0.94[‡]	1.21	0.94
Tot chol, mmol L^{-1}	5.9	1.04	5.7	1.06[‡]	5.7	1.05	5.9	1.06
	n	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
BP <160/90 mm Hg	111	42[*]	22	18[*]	62	68[†]	343	86
FB-glu <6.7 mmol L^{-1}	252	96[†]	28	24[*]	10	22[*]	399	100
Hba1c <6.5%	-	-	66	70[†]	38	52	-	-
Previous CVD	53	16.4[‡]	31	19.0[‡]	13	10.6	46	10.7
Daily smokers	43	16.7[‡]	14	8.9	23	33.2	97	24.7
Low LTPA	16	7.5[‡]	25	23.2[‡]	10	13.3	39	10.2
Microalbuminuria	64	21.9[†]	42	33.4[*]	25	25.4[†]	59	14.4

Table 3 continued

Women	*n=418*		*n=121*		*n=87*		*n=423*	
	means (sd)		**means (sd)**		**means (sd)**		**means (sd)**	
Age, years	66.0	12.2*	70.5	9.6*	71.1	11.0*	59.7	12.5
Waist, cm	87.2	11.0*	92.6	11.2*	89.2	11.1*	83.1	11.4
BMI, kg m^{-2}	28.0	4.81*	29.6	4.85*	27.7	4.83†	26.0	4.92
SBP, mm Hg	154	17.2*	160	17.3*	149	17.3*	136	17.6
DBP, mm Hg	82	9.5*	86	9.7*	79	9.6*	74	9.8
FB-glu, mmol L^{-1}	5.3	1.20*	8.1	1.22*	9.0	1.21*	4.8	1.23
Hba1c, % units	-	-	6.5	1.59	6.8	1.60	-	-
LDL, mmol L^{-1}	4.4	1.07	4.2	1.08	4.1	1.07‡	4.3	1.09
HDL, mmol L^{-1}	1.17	0.24*	1.05	0.25*	1.07	0.25*	1.24	0.25
Triglycerides mmol L^{-1}[a]	1.32	0.67*	1.68	0.67*	1.44	0.68*	1.14	0.68
Tot chol, mmol L^{-1}	6.3	1.15	6.1	1.17	5.9	1.16	6.1	1.19
	n	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
BP <160/90 mm Hg	190	48*	25	25*	46	59*	364	83
FB-glu<6.7 mmol L^{-1}	389	97‡	34	42*	13	25*	421	99
Hba1c <6.5%	-	-	51	58	43	52	-	-
Previous CVD	78	15.1*	28	15.6*	13	12.0	19	6.1
Daily smokers	43	13.5‡	15	25.5	10	19.4	89	19.6
Low LTPA	62	13.0‡	21	13.4	18	15.9	26	8.4
Microalbuminuria	64	21.9†	42	33.4*	25	25.4†	59	14.4

Data are based on all 1940 subjects from the Skara studies. Means are adjusted for age and proportions are age-standardised in ten-year intervals using the whole Skara population ≥40 years as standard. Subjects treated with insulin were excluded in analyses of fasting blood glucose and serum triglycerides. Differences between patient categories and the population (reference) were examined by analyses of covariance (Ancova) in continuous variables and by logistic regression in categorical variables. Age was used as covariate and tests were expressed as ‡=p<0.050; †=p<0.010, *=p<0.001

HT, hypertension; T2DM, type 2 diabetes; SBP, systolic blood pressure; DBP, diastolic blood pressure; F-glu, fasting blood glucose; Tot chol, total cholesterol; Low LTPA, low leisure time physical activity (exercise, walking and gardening included, less than 4 hours per week).

[a]Geometric means.

Table 4. Outcomes showing the Cross product interaction between gender, hypertension, and type 2 diabetes.

	Gender*			HT			T2DM			HT × gender*			T2DM × gender*			HT × T2DM		
Outcomes	HR	(CI)	p-value	HR	(CI)	p-value	HR	(CI)	p-value	HR	(CI)	p-value	HR	(CI)	p-value	HR	(CI)	p-value
Adjusted for age																		
AMI mortality	0.1	(0.04-0.4)	0.001	1.5	(0.5-2.3)	0.297	2.3	(1.1-5.1)	0.035	2.3	(0.8-6.8)	0.137	3.1	(1.2-8.2)	0.021	0.7	(0.2-1.7)	0.401
All-cause mortality	0.5	(0.3-0.7)	0.001	1.2	(0.9-1.7)	0.248	1.6	(1.1-2.4)	0.018	1.01	(0.7-1.7)	0.760	1.3	(0.8-2.0)	0.258	1.1	(0.7-1.7)	0.816
Adjusted for age, smoking, and total cholesterol																		
AMI mortality	0.1	(0.03-0.4)	<0.001	1.8	(0.8-3.7)	0.137	2.6	(1.1-6.1)	0.029	2.5	(0.8-8.1)	0.124	3.3	(1.2-9.1)	0.018	0.6	(0.2-1.6)	0.295
All-cause mortality	0.4	(0.3-0.7)	<0.001	1.2	(0.9-1.8)	0.252	1.5	(0.9-2.2)	0.094	1.0	(0.6-1.7)	0.892	1.5	(1.0-2.5)	0.071	1.1	(0.7-1.8)	0.676

Hazard ratios (HR) with 95% confidence intervals (CI) were analysed with Cox regression, using the population sample as reference. The hypertensive group (HT) includes all patients with hypertension, with or without type 2 diabetes (T2DM). The T2DM group includes all patient with T2DM, with or without hypertension.
* Women compared to men.

Discussion

Whereas the overall incidence of AMI is lower in women, the female advantage is known to disappear in subjects with type 2 diabetes, and the risk is sometimes even considered to be higher in women than in men [202]. This has, however, been argued to depend mainly on lack of adjustments for other risk factors such as age, smoking, and total cholesterol [29, 30]. Since those were adjusted for in the present study, they could not explain the higher female risk found here. Still, the possibility of residual confounding should be considered. Another study largely explains the higher relative risk of CHD mortality in women with type 2 diabetes with the more favourable survival rate in women than men without type 2 diabetes [33]. Although this phenomenon can be seen in the current study too, the female disadvantage was seen with both absolute and relative measures, and a clearly significant interaction between sex and type 2 diabetes was found. Since the interaction is also supported by a Finnish study with regard to CHD risk [203], other mechanisms are probably also at play. For example, hormone replacement therapy has been found to increase the risk of AMI in women with diabetes, whereas no such effect was seen in non-diabetic women [32]. This might be due to an effect of endothelial dysfunction in response to estrogen, which has been hypothesized to take place as women age and in women with diabetes [14]. Unfortunately, none of this was accounted for in the present study. However, while it seems unlikely that hormone replacement therapy would explain all of the detrimental effect of type 2 diabetes seen in women with regard to AMI prognosis, the effect of hormone replacement therapy and post-menopausal hormonal changes in women with type 2 diabetes warrant further investigation. Moreover, although women with type 2 diabetes seem to consume more health care than men, there are indications that preventative and interventional measures are not applied to women to the same extent as men, which may have contributed to our results [204-206]. Still, this is not supported by a recent study from Sweden where the prognosis after an AMI event in women and men with diabetes was investigated [207]. Even though an under-use in certain treatment after an AMI was seen in women <65 years as compared to men in the corresponding age-group, this did not explain the poorer outcome that was also seen in women <65 years.

Hypertension is often present in subjects with type 2 diabetes [186, 208] and is considered a principal factor in the development of cardiovascular disease in subjects with type 2 diabetes [208]. Tight blood pressure control in subjects with type 2 diabetes in fact seems to improve the prognosis of CHD to a greater extent than tight control of blood glucose [209]. There are also some studies that indicate that hypertension in itself might have a greater impact in women than in men [44-46]. A potential interactional effect between hypertension and gender, and

hypertension and type 2 diabetes was thus also explored in the present study, but none was found. Furthermore, the patients with only type 2 diabetes were analysed separately from those with both type 2 diabetes and hypertension. Although power was insufficient to conclusively distinguish any effect of hypertension on the interaction between sex and type 2 diabetes, the association was stronger in patients with both conditions.

The interaction between gender and type 2 diabetes shows that the effect of type 2 diabetes on fatal AMI was significantly stronger in women than in men independent of other major cardiovascular risk factors. To improve the prognosis of female patients with type 2 diabetes, more research on sex-specific mechanisms is warranted, and special reference should be made to the effect of estrogen and hypertension.

Paper II:

Salivary cortisol differs with age and sex and shows inverse associations with waist-hip ratio in Swedish women: a cross-sectional study

Results

In both men and women evening cortisol was significantly higher in older subjects compared to younger, while the same pattern for morning cortisol was seen in men only (Table 5). In women Δ-cortisol (morning - evening cortisol) was significantly lower in older subjects than in younger (Table 5). Test for trends over increasing age groups revealed highly significant trends ($p \leq 0.001$) in all cortisol variables but morning cortisol in women ($p=0.215$) and Δ-cortisol in men ($p=0.088$).

Women in general were found to have significantly higher levels of morning cortisol than men (Table 5), and women <50 years were found to have higher levels of both morning and Δ-cortisol than men <50 years. The correlations (r) between morning and evening cortisol values were 0.297 ($p<0.001$) for women and 0.240 ($p<0.001$) for men.

In women, waist-hip ratio was significantly associated with morning cortisol (Table 6). These associations remained when also adjusting for multiple confounders (Table 6), and were also seen in both a linear ($p=0.006$) and a logistic regression analysis (OR 1.5, 1.0-2.2, $p=0.058$). Waist-hip ratio was also significantly and independently associated with Δ-cortisol and this association was seen in women both above and below 50 years of age. Moreover, a significant association between Δ-cortisol and waist-hip ratio was seen in a linear regression analysis ($p=0.003$) and in a logistic regression analysis (OR 1.9, 1.3-2.8), and both results remained statistically significant when adjusting for multiple variables. Body mass index did not affect any of the results above. In men, morning cortisol (Table 6) and Δ-cortisol were not associated with either waist-hip ratio or waist circumference. No associations were seen in either men or women with regard to evening cortisol.

Table 5. Male and female salivary cortisol levels in different age-groups in the Vara cohort, the Skaraborg Project 2001-2004, Sweden.

	Morning cortisol				**Evening cortisol**				**Δ-cortisol**			
Age-groups	n	m	(q1-q3)	p	n	m	(q1-q3)	p	n	m	(q1-q3)	p
Women												
30-39	242	12.4	(9.0-17.0)	ref.	242	1.7	(1.0-3.0)	ref.	242	5.8	(3.7-10.0)	ref.
40-49	291	12.4	(8.0-19.0)	0.892	291	2.2	(2.0-3.0)	0.834	291	5.7	(3.7-9.0)	0.768
50-59	146	11.6	(8.7-15.2)	0.260	146	2.5	(2.0-3.0)	0.030	146	4.7	(3.0-8.0)	0.005
60-69	107	12.7	(10.0-18.0)	0.732	107	2.7	(2.0-4.0)	0.004	107	4.7	(3.3-7.5)	0.015
≥70	52	15.1	(9.2-19.8)	0.029	52	3.6	(2.0-6.0)	<0.001	52	4.2	(2.3-9.0)	0.004
<50	533	12.4	(9.0-18.0)	ref.	533	2.2	(1.4-3.0)	ref.	533	5.8	(3.7-9.5)	ref.
≥50	305	12.5	(9.0-18.0)	0.773	305	2.7	(2.0-3.0)	<0.001	305	4.6	(3.0-8.0)	<0.001
Men												
30-39	254	10.4	(7.0-15.0)	ref.	254	2.0	(1.0-3.0)	ref.	254	5.3	(3.5-9.0)	ref.
40-49	276	10.6	(8.0-15.0)	0.634	276	2.2	(2.0-3.0)	0.101	276	4.9	(3.0-8.0)	0.288
50-59	141	11.8	(8.0-18.0)	0.030	141	2.4	(2.0-3.0)	0.007	141	5.0	(3.0-8.0)	0.460
60-69	106	12.5	(8.0-17.3)	0.006	106	2.5	(2.0-3.0)	0.001	106	4.9	(3.3-7.6)	0.459
≥70	56	12.3	(9.0-16.8)	0.049	56	2.9	(2.0-4.0)	<0.001	56	4.2	(3.0-6.5)	0.040
<50	530	10.5	(7.0-15.0)	ref.	530	2.1	(1.0-3.0)	ref.	530	5.1	(3.3-8.5)	ref.
≥50	303	12.1	(8.0-18.0)	0.001	303	2.5	(2.0-3.0)	<0.001	303	4.8	(3.0-7.7)	0.305

Δ-cortisol = logarithmic morning salivary cortisol - logarithmic evening salivary cortisol.
Differences between age-groups were examined by GLM (general linear model), using group 30-39 and group <50, respectively, as reference. Means and quartiles 1-3 (q1-q3) are geometric (anti-log).

Table 6. Comparisons of body composition between quartiles of morning salivary cortisol in women and men in the Vara cohort, the Skaraborg Project 2001-2004, Sweden.

	WHR				Waist circumference			
Women	**n**	**mean**	**sd**	**p-value**	**n**	**mean**	**sd**	**p-value**
Adjusted for age								
Qrtl 1 morning cortisol	188	0.843	0.069	ref.	188	86.63	13.52	ref.
Qrtl 2 morning cortisol	230	0.838	0.076	0.492	230	85.90	13.53	0.585
Qrtl 3 morning cortisol	203	0.826	0.071	0.025	203	83.99	13.52	0.055
Qrtl 4 morning cortisol	217	0.827	0.074	0.036	217	84.74	13.54	0.163
Test for linearity:								
between quartiles				0.013				0.079
continuously				0.006				0.024
Adjusted for age, leisure time physical activity, smoking, education, alkohol consumption, oral contraceptives, and estrogen replacements								
Qrtl 1 morning cortisol	176	0.842	0.080	ref.	188	86.08	13.82	ref.
Qrtl 2 morning cortisol	224	0.836	0.075	0.398	230	85.67	13.53	0.763
Qrtl 3 morning cortisol	198	0.826	0.070	0.041	203	84.05	13.55	0.146
Qrtl 4 morning cortisol	210	0.827	0.072	0.041	217	84.50	13.60	0.250
Test for linearity:								
between quartiles				0.021				0.128
continuously				0.013				0.072
Men								
Adjusted for age								
Qrtl 1 morning cortisol	193	0.945	0.056	ref.	193	94.61	10.03	ref.
Qrtl 2 morning cortisol	240	0.939	0.062	0.275	240	93.96	9.99	0.498
Qrtl 3 morning cortisol	210	0.945	0.058	0.979	210	94.55	10.00	0.950
Qrtl 4 morning cortisol	190	0.945	0.055	0.913	190	94.92	9.99	0.763
Test for linearity:								
between quartiles				0.784				0.613
continuously				0.980				0.556

Differences between quartiles (qrtl) of morning cortisol were examined by GLM (general linear model), using quartile 1 as reference.

Discussion

The age-related increase in cortisol levels found in the present study is supported by findings from several other studies, especially regarding nocturnal levels [210-215]. While the basal secretion of cortisol are known to remain fairly stable with age, the negative feedback regulation of the HPA-axis seems to become impaired in older subjects [216, 217]. This would lead to prolonged periods of increased cortisol secretion in response to for example stress and consequently increased general cortisol levels.

The general pattern of higher cortisol levels in women than men has been seen in some previous studies [218-220], but the opposite has also been reported [210, 211, 213]. These incongruities might be due to differences in measurements of cortisol, differences in timing of measurements, and differences in type and size of study sample.

The significant inverse association seen in women between morning cortisol and waist-hip ratio is in concordance with other studies [221-224], including a recent study from Whitehall II [225). These findings might be explained by an enhanced local clearance rate of cortisol, which has been hypothesized to impair the negative feedback control of the HPA-axis and lead to an increased cortisol secretion [226, 227]. This theory is supported by findings of increased total 24 h urinary cortisol out-put concurrent with decreased morning serum cortisol levels in abdominally obese subjects [221], and would explain the findings here and elsewhere of abdominal fat accumulation in spite of low morning levels. Furthermore, higher cortisol response to both physical and mental stress has been found in women with high waist-hip ratio compared to those with normal waist-hip ratio [221, 228]. Taken together, the findings indicate an abnormal activation of the HPA-axis in abdominally obese women, even though there are contradictory findings as well. An abnormal habituation to stress and ultimately hypocortisolism through dysregulation at some level of the HPA-axis [229, 230] could be an alternative hypothesis that needs testing in longitudinal studies where stress is also accounted for. Increasing interest is also being directed towards the tissue-specific actions of 11ß-hydroxysteroid dehydrogenase type 1 [231-232]. In adipose tissues, for example, this enzyme usually converts inactive cortisone to active cortisol [231-232], a process that has been found to be increased in obese individuals [233, 234]. However, these mechanisms are difficult to measure in a reliable and valid manner in humans and their relevance on a population level is yet to be determined.

With regard to the null-findings in men, differences in the metabolic clearance rate of cortisol could possibly hold some of the explanation, which is supported by some studies [235, 236] but not other [216, 237]. Furthermore, there are studies

that also in men have found inverse associations between morning cortisol and waist-hip ratio [238-241]. While the reasons for the null-findings in men in previous studies are not known, it might in the present study be explained by our men's lower prevalence and variation of abdominal obesity compared to our women, and consequently a decreased ability to identify an association with morning and Δ-cortisol.

Large population-based studies in both men and women within this research area are scarce or even lacking. Thus, our results offer important knowledge about the descriptive characteristics of cortisol in relation to age and gender. Moreover, it shows that the associations previously seen between cortisol and abdominal obesity in smaller, selected samples, can also be seen on a population level. Future studies should account for reproductive hormones and stress exposure, as well as 24-h urinary cortisol and metabolites. The need for prospective studies is also evident.

Paper III:

Leisure time and occupational physical activity in relation to obesity and insulin resistance: A population-based study from the Skaraborg Project in Sweden

Results

As shown in Figure 1, an age-adjusted (Model 1) inverse association in both men and women was seen between leisure time physical activity and body mass index. Similar significant associations were also seen between leisure time physical activity and waist circumference and HOMA-ir, respectively. All associations remained statistically significant when adjusting for multiple confounders (Model 2), including body mass index (Model 3) in the analyses of waist circumference and HOMA-ir. Furthermore, cross-product interaction analyses between leisure time physical activity and gender in association with HOMA-ir revealed protective effects of being physically active in women in all models (Model 3: p=0.030). Leisure time physical activity was also significantly associated with low resting heart rate in both men and women and this association remained statistically significant in both Model 2 and Model 3.

Occupational physical activity in women was positively associated with general obesity when adjusting for Model 1 (Figure 2) and Model 2. The pattern was generally robust when stratifying for high and low leisure time physical activity. The corresponding analyses for waist circumference and HOMA-ir showed similar associations as for body mas index, albeit somewhat weaker in general and non-significant in Model 3.

In contrast to women, no associations between occupational physical activity and obesity (whether general or abdominal) were observed in men, while the association with insulin resistance was in an opposite and dose-dependent direction and statistically significant in Model 2 but not in Model 3. Gender differences were confirmed by statistically significant age-adjusted interaction terms between gender and occupational physical activity in association with all aforementioned effect variables (body mass index: p=0.018; waist circumference: p=0.041; HOMA-ir: p=0.006). Resting heart rate was also significantly increased with increasing levels of occupational physical activity, however, only in men. All

aforementioned associations remained in both men and women when also adjusting for self-reported sleep disturbances and daily life stress.

In a sub-analysis, leisure time physical activity and occupational physical activity were combined to form four categories of total physical activity. In general linear models, a consistent pattern of inverse associations was found in both men and women between the highest and the lowest category of total physical activity with regard to body mass index, waist circumference, and HOMA-ir, respectively. There was no statistically significant association between total physical activity and resting heart rate in either men or women.

Discussion

The inverse results in both men and women with regard to leisure time physical activity in association with general obesity, abdominal obesity, and insulin resistance, confirm findings from previous studies [99, 100, 242-248]. Also in agreement with previous studies [100, 244-248], the associations for insulin resistance and waist circumferences were independent of body mass index. However, in contradiction with two previous studies [246, 249] where associations between physical activity and fasting insulin were found only in men, a significant interaction between female gender and high leisure time physical activity was found here in association with HOMA-ir, which to our knowledge is a novel finding. Women are known to have more insulin sensitive muscle tissues than equally fit men [250-251], which was further indicated here when comparing means of HOMA-ir for men and women adjusting for age, BMI, and leisure time physical activity. Also, in a previous study [247], women similarly showed higher insulin sensitivity irrespective of energy expenditure from physical activity and percentage of body fat. However, those findings would still not explain the higher protective effect of physical activity on insulin resistance found here in women compared to men, and neither would the level of leisure time physical activity, since women in the present study reported less leisure time physical activity than men. In both men and women high levels of leisure time physical activity were found to be associated with low resting heart rate, which is a well-known indicator for physical fitness. These findings indicate a high internal validity of the leisure time physical activity questionnaire and also point to a good external validity of the results.

Occupational physical activity was positively associated with both general and abdominal obesity in women in this study, which is in general agreement with some previous studies [100, 122, 123, 124] but not others [99, 119-121, 252-256]. A positive association between occupational physical activity and insulin resistance was also found in women. None of the positive associations in women

with regard to occupational physical activity were replicated in men. The differences between men and women were confirmed in cross-product interaction analyses, which to our knowledge have not been seen before. Confounding by lifestyle factors seems like the most plausible explanation for the associations, and consequently, differences in these confounding factors between men and women might explain why the positive association was seen solely in women. For example, women in occupations with high occupational physical activity might differ from men in corresponding occupations with regard to some aspect not fully accounted for here, which could counteract the presumed biological benefits of their physically active work. Moreover, the question used here to assess occupational physical activity did not differentiate between types of physical activity, such as aerobic versus anaerobic types, which might have differing effects on metabolism and cardiovascular function [257]. Still, it seems unlikely that low frequency of "beneficial" types of occupational physical activity among women would do more than attenuate an expected inverse association with obesity/insulin resistance.

The analyses of total physical activity showed significant inverse associations with general and abdominal obesity in both genders, and also with insulin resistance in men, thus, completely masking the opposite associations of leisure time physical activity and occupational physical activity from the separate analyses. Taken together, these findings highlight the risk of introducing bias when using a measure of total physical activity.

The present study found conflicting results with regard to the beneficial aspects of leisure time physical activity and occupational physical activity in men and women, with gender differences apparent especially in the findings concerning occupational physical activity. While further exploration of the metabolic effects of occupational physical activity appears warranted, more thorough measurement of potential confounders is also vital in order to understand contextual effects. Our results also indicate that the work setting may be an important context to consider in cardiovascular prevention, especially in women. The stronger association between leisure time physical activity and insulin resistance in women than in men also calls for further investigation.

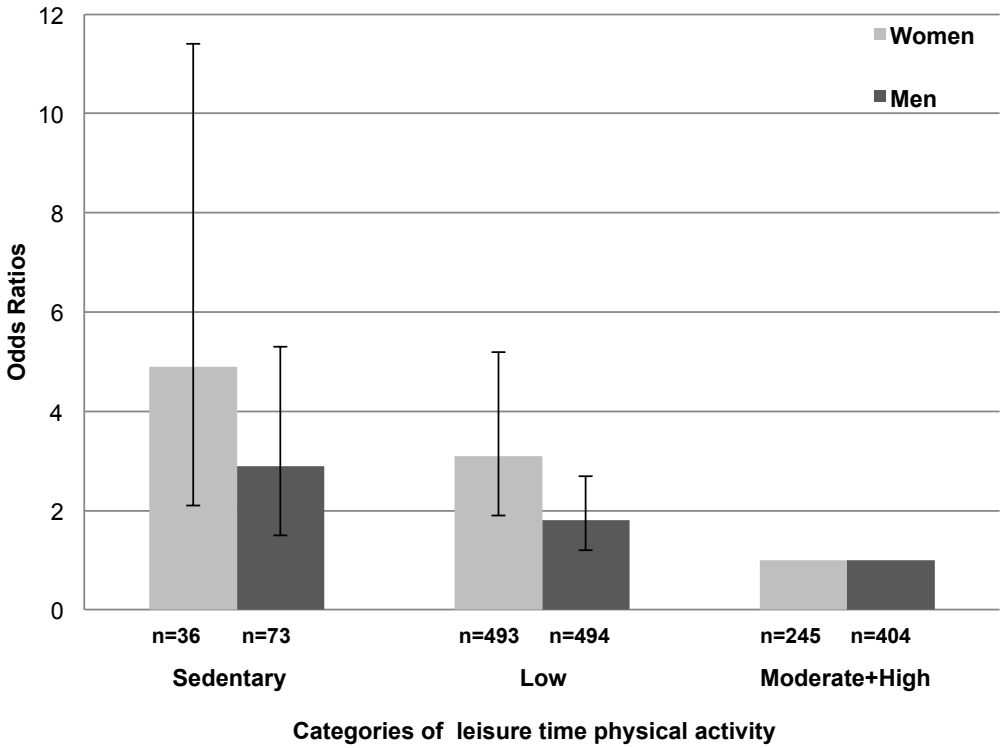

Figure 1. Age-adjusted odds ratios and confidence intervals of obesity (BMI ≥30 kg m^{-2} versus BMI <30 kg m^{-2}) in association with leisure time physical activity in the Vara-Skövde cohort, The Skaraborg Project 2001-2005, Sweden.

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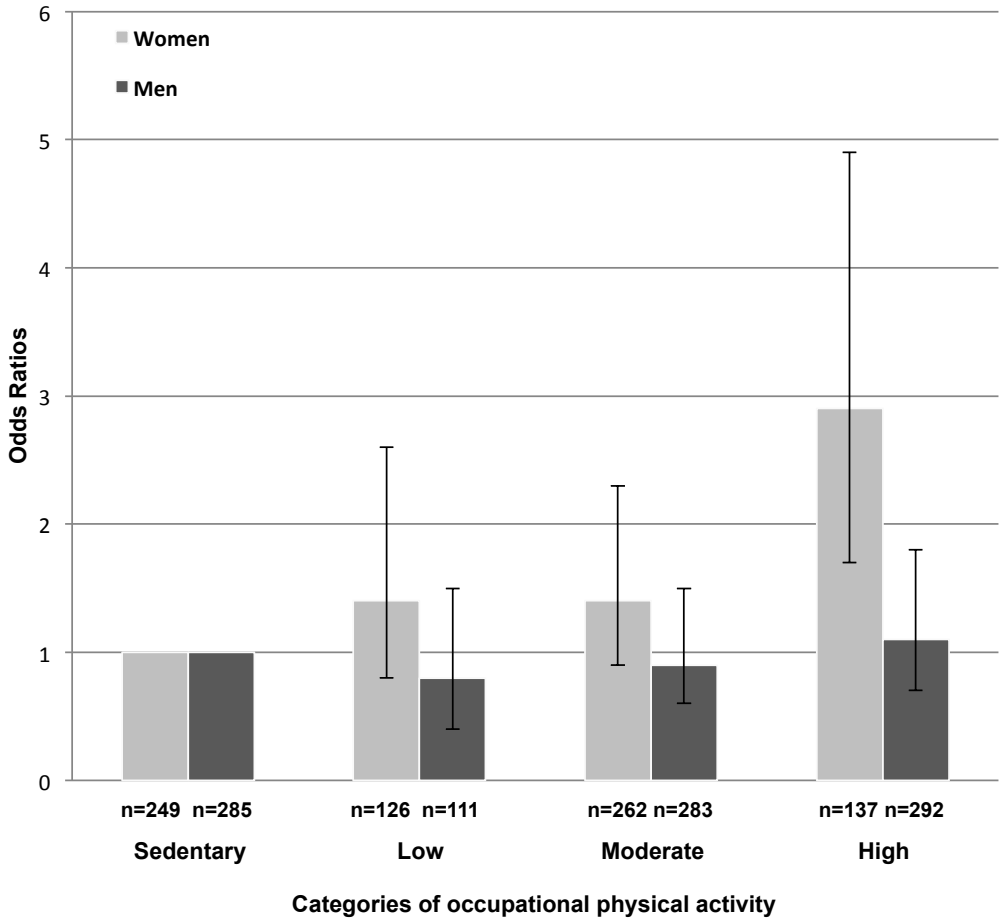

Figure 2. Age-adjusted odds ratios and confidence intervals of obesity (BMI ≥30 kg m^{-2} versus BMI <30 kg m^{-2}) in association with occupational physical activity in the Vara-Skövde cohort, The Skaraborg Project 2001-2005, Sweden.

Paper IV:

Clusters of AMI risk factors and the association with left ventricular hypertrophy: A population-based study within the Skaraborg Project in Sweden

Results

Men in general had a significantly more atherogenic risk factor profile than women, and they consumed more alcohol and had lower educational levels. However, women were significantly more often daily smokers and sedentary in their leisure time as compared to men. Women also had significantly higher 2-hour plasma glucose levels than men.

The factor analysis identified three factors that contained identical variables in men and women (Table 7). Waist-hip ratio, HOMA-ir, systolic blood pressure, and apolipoprotein B/apolipoprotein A1 loaded significantly on Factor 1 (the metabolic factor), with the strongest loading factor seen in waist-hip ratio in both men and women. Leisure time physical activity and self-rated health loaded significantly on Factor 2 (the vitality factor), and smoking and alcohol intake loaded significantly on Factor 3 (the addiction factor) (Table 7). When the factor analysis was performed stratified by the two sub-cohorts (i.e. Vara and Skövde, respectively), the results were generally the same.

The age-adjusted logistic regression analyses found the metabolic factor to be significantly associated with left ventricular hypertrophy in both men and women and the addiction factor to be significantly associated with left ventricular hypertrophy, however, in men only (Table 8). These associations were unchanged or even stronger in multivariate models where all factors and educational level were included (Table 8). Cross-product interaction terms also revealed a statistically significant interaction between the addiction factor and gender (age-adjusted p=0.023). When using the factors applying only to the Vara sub-cohort (retained from the stratified factor analysis), similar significant associations with left ventricular hypertrophy were seen as when using the factors from the whole cohort.

When the risk factors were studied separately in association with left ventricular hypertrophy, age, waist-hip ratio, HOMA-ir, systolic blood pressure, and leisure time physical activity were significantly associated with left ventricular hypertrophy in the age-adjusted analyses in both genders. However, when all risk factors where entered in the same model, age, waist-hip ratio, alcohol consumption, and systolic blood pressure in men were independently associated with left ventricular hypertrophy, while in women, only systolic blood pressure remained statistically significant.

Table 7. Factor analysis in women and men in the Vara-Skövde Cohort, The Skaraborg Project 2001-2005.

	Women			Men		
	Factor 1	**Factor 2**	**Factor 3**	**Factor 1**	**Factor 2**	**Factor 3**
	n=838			*n=833*		
WHR	**0.748**	0.066	0.075	**0.776**	0.121	0.166
HOMA-ir (logarithmic)	**0.675**	0.246	-0.217	**0.699**	0.212	-0.047
Systolic blood pressure	**0.653**	-0.067	0.081	**0.583**	-0.351	0.264
ApoB/ApoA	**0.609**	0.153	0.022	**0.550**	0.150	-0.169
Physical inactivity	0.049	**0.793**	0.000	0.233	**0.720**	0.015
General self-rated health	0.162	**0.744**	0.006	0.041	**0.748**	0.121
Smoking	0.067	0.241	**0.764**	0.149	0.165	**0.627**
Alcohol consumption	-0.022	-0.204	**0.717**	-0.129	-0.040	**0.773**
Variance explained, %	26.4	14.5	13.8	25.4	14.2	13.7
Cumulative variance, %	26.4	40.9	54.7	25.4	39.7	53.4
Initial eigenvalues	2.113	1.161	1.105	2.034	1.139	1.097

Principal components with eigenvalues >1 were retained and variables were rotated using varimax rotation. Correlations were considered significant for variable loadings ≥0.40.

Table 8. Associations between factor scores and left ventricular hypertrophy in subjects from the Vara sub-cohort 2001-2005.

	Women			Men		
	OR[a]	**95% CI**[a]	**p-value**	**OR**[a]	**95% CI**[a]	**p-value**
	n=437			*n=415*		
Adjusted for age						
Metabolic factor	2.4	**1.7-3.4**	**<0.001**	**1.9**	**1.4-2.7**	**<0.001**
Vitality factor	0.9	0.6-1.3	0.566	0.8	0.6-1.1	0.197
Addiction factor	0.8	0.5-1.1	0.167	**1.3**	**1.0-1.6**	**0.039**
Adjusted for age and all factors[b]						
Metabolic factor	**2.4**	**1.6-3.4**	**<0.001**	**2.1**	**1.5-3.0**	**<0.001**
Vitality factor	0.9	0.6-1.3	0.580	0.8	0.6-1.2	0.307
Addiction factor	0.8	0.6-1.2	0.250	**1.4**	**1.1-1.7**	**0.001**
Adjusted for age, education and all factors[c]						
Metabolic factor	**2.5**	**1.7-3.5**	**<0.001**	**2.2**	**1.5-3.1**	**<0.001**
Vitality factor	0.9	0.6-1.3	0.565	0.8	0.6-1.2	0.350
Addiction factor	0.8	0.6-1.2	0.257	**1.4**	**1.1-1.8**	**0.002**

[a] Odds ratios with 95% confidence intervals calculated by logistic regression analysis.

[b] The metabolic factor was adjusted for age, the vitality factor, and the addiction factor; the vitality factor was adjusted for age, the metabolic factor, and the addiction factor; and the addiction factor was adjusted for age, the metabolic factor, and the vitality factor.

[c] The metabolic factor was adjusted for age, education, the vitality factor, and the addiction factor; the vitality factor was adjusted for age, education, the metabolic factor, and the addiction factor; and the addiction factor was adjusted for age, education, the metabolic factor, and the vitality factor.

Discussion

The identified factors in the present study were identical for men and women. The factor with the highest eigenvalue comprised waist-hip ratio, HOMA-ir, apolipoprotein B/apolipoprotein A, and systolic blood pressure, thus, corresponding well with the typical cluster of risk factors associated with the metabolic syndrome [258]. The vitality factor and the addiction factor were more directly lifestyle-related, with no biological variable loading on them. The findings here were broadly in line with five comparable studies [259-262], where components of the metabolic syndrome generally loaded on one major metabolic factor, and the more directly lifestyle related variables loaded on other factors in various combinations.

Individuals who engage in one health-risk behaviour are more likely to also engage in another one, which is in agreement with alcohol consumption and smoking loading together on the addiction factor. Still, smoking has previously also been associated with physical inactivity, which in the present study loaded together with self-rated health. It is thus interesting to note that leisure time physical activity in the present sample was more strongly associated with self-rated health than with smoking. While the link between smoking and alcohol consumption probably has behavioural components, the link between self-rated health and physical activity might be biological, since physical activity is known to improve mood [263] and mood most likely will affect an individual's rating of his/her general health. Still, negative mood might also reduce the motivation to be physically active [264]. Since physical activity smoking, alcohol consumption, and self-rated health (used here as a proxy for e.g. stress) have previously been associated with several metabolic risk factors, it is interesting to note that none of these lifestyle variables loaded significantly with any of the metabolic risk factors in the present study.

To our knowledge, no other study has investigated how factor scores of cardio-vascular risk factors, also including lifestyle variables, predict left ventricular hypertrophy; however, a few studies have studied the prediction of CHD, and some of these have also included lifestyle variables [76, 259, 260, 265, 266]. Similarly to the present study, the "metabolic" factor identified in these studies predicted CHD to various degrees [76, 259, 260, 265]. However, comparable results in both men and women from such studies seem to be scarce, even though other types of studies support an association between metabolic variables and left ventricular mass/left ventricular hypertrophy [160, 161, 168, 267-269]. Interestingly, when traditional multivariate analysis was performed in the present study, only blood pressure was independently associated with left ventricular

hypertrophy in women, while also waist-hip ratio, age, and alcohol intake was associated with left ventricular hypertrophy in men.

The addiction factor was significantly associated with left ventricular hypertrophy in men only. While Lempiäinen et al [76] also found alcohol and smoking to load on their fourth factor together with total cholesterol, it was only seen in women and the factor did not predict CHD after 7 years of follow-up. The significant association found solely in men in the separate analysis between alcohol consumption and left ventricular hypertrophy, as previously seen [167, 170], and the lack of association with regard to smoking, indicates that the association between left ventricular hypertrophy and the addiction factor in men to a large extent could be an effect of their alcohol consumption. This gender difference is supported by findings from some studies [270, 271], but not from others [95]. Furthermore, effects from other variables not measured here that might cluster together with smoking and alcohol intake in men could also be at play.

Factor analysis is a useful tool to identify cluster of risk factors representing common underlying pathways and it offers a deeper understanding of the dynamics of complex conditions like cardiovascular disease, as compared to traditional multivariate analyses of independent risk factors. In women, multivariate analyses identified systolic blood pressure as a single independent risk factor for left ventricular hypertrophy, while the factor scores in association with left ventricular hypertrophy captured the full impact of the metabolic syndrome also in women. This illustrates the additional value of looking for clusters of factors that operate in the same pathway. However, factor analysis is sensitive to the set of variables included in the model, and differences in design and included study measures thus make it difficult to compare results between studies.

The identical principal factors identified in men and women suggest common underlying mechanisms in both genders for the most acknowledged AMI risk factors. In contrast, different pathways in the development of left ventricular hypertrophy between men and women are indicated by the findings of both biological mechanisms and lifestyle affecting the association with left ventricular hypertrophy in men, while only biological mechanisms seemed to matter in women. It remains to be studied whether these observations might explain some of the gender differences seen in the development of AMI. Finally, since left ventricular hypertrophy often precedes AMI, a deeper understanding of risk factors for left ventricular hypertrophy, including consideration of the supposed gender differences, can also be useful to explore for prevention strategies with regard to AMI.

General discussion

In these population-based studies, gender differences for some of the most common risk factors for AMI were explored. In Paper I, a significant interaction was found between type 2 diabetes and gender with regard to the risk of AMI, whereby type 2 diabetes were shown to have a higher impact on AMI mortality in women than in men. In Paper II, basal salivary cortisol was found to be higher in women and in older subjects more generally, while a significant inverse association between waist-hip ratio and morning cortisol levels was found solely in women. In Paper III, leisure time physical activity was significantly associated with less obesity and insulin resistance in both genders. In contrast, occupational physical activity was associated with higher levels of obesity and insulin resistance, albeit solely in women. In Paper IV, clusters of AMI risk factors were explored and the three principal factors that emerged were identical for men and women. However, while left ventricular hypertrophy, used as a sub-clinical measure of CHD, was significantly associated with the main metabolic factor in both genders, left ventricular hypertrophy was associated with the addiction factor solely in men. These population-based results show that not only does the risk of AMI differ by gender, but also that the associations between different risk factors show gender differences. Gender differences were also observed with regard to both lifestyle [Paper III, Paper IV] and biological measures [Paper I, Paper II, Paper IV].

Despite the established knowledge that high and long-term increased cortisol levels lead to abdominal obesity (as seen in Cushing's disease), inverse associations between cortisol levels and abdominal obesity were found in women [Paper II). This is also in accordance with findings from some previous studies [221-225], and has been hypothesized to be a result of enhanced local clearance rate of cortisol that in turn might impair the negative feedback control of the HPA-axis and lead to an increased cortisol secretion [226, 227]. Thus, even though previous studies show that women with abdominal obesity appear to have lower levels of circulating morning cortisol [221-225], there are indications that they still have an increased cortisol secretion [221], which would also explain the findings in paper II of higher abdominal obesity in spite of low morning cortisol levels. Still, the reason for the gender differences found here are not clear. However, a study from Whitehall II [272] found a bidirectional effect of job strain on body

mass index in men, while there were indications of solely a positive association in women. Thus, in men in the highest body mass index quintile (>27) at baseline, job strain and low job control were associated with increased body mass index at follow-up, while the association in men in the lowest quintile of body mass index (<22kg/m^2) at baseline was reversed at follow-up. Even though it is speculative, given the fact that stress was not accounted for here, these findings may hold some of the explanation for the null-findings between cortisol and abdominal obesity in men [Paper II].

Furthermore, women with high levels of occupational physical activity were significantly more abdominally (as well as generally) obese and insulin resistant [Paper III]. Although educational level was adjusted for, residual confounding might still be present and women in professions involving high occupational physical activity might be more vulnerable to e.g. stress related to low socioeconomic status. It is therefore tempting to also ponder whether increased cortisol output in response to stress could be a potential part of the explanation for the higher occurrence of abdominal obesity and insulin resistance in these women. However, stress was not accounted for in Paper II, and when self-perceived stress was included in Paper III as a covariate, the relationship between occupational physical activity and obesity/insulin resistance remained. Thus, whether the findings from Paper II and Paper III are in fact interlinked remains uncertain, especially since the findings here and elsewhere with regard to cortisol are not fully understood. More thorough investigations with regard to stress, cortisol, and abdominal obesity are therefore needed to further elucidate if and how the current associations are interlinked and what the impact is on a population-level. Nevertheless, the potential importance of the dysregulation of the HPA-axis in relation to obesity, as well as to CHD, is further emphasised by recent findings from Whitehall II [149, 150, 225], as well as from another population-based study [148], where different measures of cortisol have been found to be significantly associated with obesity [225], coronary artery calcification [150], and cardiovascular mortality [148, 149].

When eight of the most acknowledged AMI risk factors were included in a factor analysis to identify clusters of risk factors [Paper IV], the three principal factors produced were identical for men and women. The first and main factor contained WHR, HOMA-ir, systolic blood pressure, and apolipoprotein B/apolipoprotein A1, thus perfectly matching the general perception of how cardiovascular risk factors cluster in the metabolic syndrome [73-75]. While leisure time physical activity has been found to be inversely associated with metabolic risk factors both here (Paper III) and elsewhere [273], the findings that it failed to load on the metabolic factor here and instead loaded together with self-rated health, suggests that it is involved in other important mechanisms too. Furthermore, the fact that all of the risk factors more directly related to lifestyle loaded on secondary factors

seems to indicate that the clustering of metabolic risk factors is stronger than any effect that lifestyle may have on the individual metabolic components.

Whereas the findings from the factor analysis [Paper IV] were identical in men and women, some interesting gender differences emerged when the factors' associations with left ventricular hypertrophy were examined. The metabolic factor was significantly associated with left ventricular hypertrophy in both genders; however, the addiction factor (containing alcohol and smoking) was significantly associated with left ventricular hypertrophy in men only. Still, since the separate analysis for smoking revealed no association with left ventricular hypertrophy in either gender, and since alcohol consumption alone was associated with left ventricular hypertrophy solely in men, it is tempting to draw the conclusion that alcohol consumption is the main driving force behind the finding with regard to the addiction factor. However, since women in this cohort consumed considerably less alcohol than men, it is difficult to elucidate whether the different results are thus an effect of differing causal mechanisms or whether it is merely a question of less exposure in women and thus less power to detect an association. Even so, it is also important to consider the possibility that the addiction factor comprise other factors that were not measured here and are thus yet to be revealed. Furthermore, it remains to be seen if prospective studies using left ventricular hypertrophy or AMI as an outcome can confirm these cross-sectional associations with regard to different clusters of risk factors.

Paper I is the only longitudinal study in this thesis and it also revealed some considerable gender differences regarding the impact of type 2 diabetes on the risk of AMI mortality. While the findings of women with type 2 diabetes losing their general advantage vs. men with regard to CHD risk is in accordance with previous studies, most previous studies have found gender differences in risk factor levels to be the explanation. In contrast, the interaction-term between type 2 diabetes and gender with regard to AMI mortality in Paper I remained statistically significant when adjusting for other well-known cardiovascular risk factors. This might also indicate that any potential gender differences in diabetes treatment, which have been seen before [205] and suggested as one of several possible explanations for the higher CHD risk in women with diabetes [31], would not explain the female disadvantage found in Paper I. Still, residual confounding might be at play, in so far as other risk factors than those accounted for could be of importance. For example, women with type 2 diabetes have been found to be in a higher pro-thrombotic state than men with type 2 diabetes [274], which might predispose them to plaque rupture and thrombosis [31], and thus increase their risk of AMI. Cardiovascular risk factors might not just be more common in women with type 2 diabetes, but could possibly have a higher impact in women than in men [31]. This has, for example, been seen with regard to dyslipidemia [203, 275-276] and hypertension [203, 277]. Gender differences in medical treatment after a CHD

event have also been suggested to explain the higher CHD mortality for women with diabetes than in men [31]. However, this is not supported by findings from a recent study concerning all patients admitted to Swedish coronary care units between 1995 and 2002 [207]. In that study gender differences in treatment after an AMI event did not explain the poorer outcome in women with diabetes that was found in those <65 years of age compared to men of corresponding age.

Lastly, structural or functional differences of the heart and blood vessels between men and women with diabetes have also been proposed as a possible explanation for the greater increase of CHD risk in women with type 2 diabetes [31]. One example is endothelial dysfunction, which seems to be more severe in women with type 2 diabetes than in men with type 2 diabetes [31]. In addition, genetic predisposition for endothelial dysfunction seems to predict AMI morbidity more strongly in women than in men with type 2 diabetes [278]. Furthermore, the influence of reproductive hormones and hormone replacement therapy in women with diabetes might also be of relevance. Early observational studies found hormone replacement therapy to have a beneficial effect on CHD risk, both in women in general [279], and in women with type 2 diabetes [280]. However, later randomised trials found no effect or even an increased risk in some cases [11, 12], and the protective effect seen in observational studies was then deemed to depend on a healthy user effect [281]. However, more recent studies indicate that time of hormone replacement therapy initiation might be of importance, in that there is a decreased risk in younger women receiving hormone replacement therapy within 10 years of menopause, whereas women starting on hormone replacement therapy later (and thus being older) have an increased risk [13]. White et al [14] recently proposed that estrogen might target nitric oxide synthases within the vascular wall. Normally in younger women, the primary product of estrogen action is nitric oxide, which contributes to vascular health in several ways. However, when women get older there are indications that nitric oxide synthase instead starts producing the detrimental reactive oxygen species, superoxide, in response to estrogen, which would explain the dual findings with regard to age in the risk of CHD induced by hormone replacement therapy. The authors [14] further proposed that diabetes, similarly to increasing age, might reverse the estrogen response with regard to nitric oxide synthase in favour of superoxide. This would thus explain why the general female advantage in CHD risk is lost in women with diabetes [14] and why HRT in some studies has been associated with CHD solely in women with diabetes [32]. HRT was not accounted for in Paper I. However, while any potential use of HRT in our women with type 2 diabetes might have strengthened the association seen between type 2 diabetes and CHD in women, the main risk increase would still most likely be attributable to diabetes itself, according to White et al's theory [14]. Thus, whereas all, or at least most, of the proposed explanations for the gender differences seen in paper I (and elsewhere) might be at

play to some extent, there is increasing evidence that type 2 diabetes in itself actually does have a more detrimental effect in women than in men.

In summary, AMI originates from both biological factors and lifestyle and there are considerable gender differences in the risk of developing the disease and also in the distribution of those risk factors generally seen to be associated with AMI. Gender differences are also apparent in how these different types of risk factors are interrelated, which have been further illuminated here. Thus, this thesis highlights the need for considering and addressing potential gender differences at all stages of preventive and interventional work aiming to reduce the burden of AMI.

Methodological considerations

Representativeness and generalizability

The cohorts used for the studies in the present thesis all showed high participation rates. In Paper I, the study data originated from the patient survey and the population survey from Skara. All patients with hypertension and/or type 2 diabetes who had their annual check-up at the out-patient clinic between 1992 and 1993 were included in the patient survey. As the out-patient clinic is the only clinic in the area [186], the included subjects in all likelihood covers the vast majority of subjects with known hypertension and type 2 diabetes in the municipality. However, a few subjects with hypertension and/or type 2 diabetes who were in need of frequent appointments with their family physician did not attend the out-patient clinic in Skara, and neither did some patients living in nursing homes. However, these non-participating subjects would probably be older than 85 years or have considerable co-morbidities and higher mortality rates. Thus, as the study was aimed mainly at primary and secondary prevention, these subjects would not be as relevant to include as younger patients or those with less co-morbidity. In the Skara population survey in 1993-1994, a computer-generated random sample was obtained from the population census register from Skara (no exclusion criteria). Of the selected subjects 80% participated. Apart from an intentional three-fold oversampling in those below 50 years of age, subjects from the Vara-Skövde cohort were selected in the same fashion as in Skara and the participation rates were 81% and 70% respectively. This high participation rate together with the random selection, are aspects that enhance the representativeness of the study sample. However, subjects who chose not to participate in surveys have in some studies been found to be less healthy and to have a higher mortality rate than those who do participate [282, 283]. Thus, even though not always

present [282], the risk of selection bias must always be considered when there is non-participation. Still, if any non-response bias applies to our cohort, such bias would most likely only have attenuated any potential associations found. Furthermore, even though the population in Vara and Skövde might differ in some respect compared to more urbanised areas in Sweden, our study samples might still be regarded as representative of the general Swedish population, and the results should thus also be generalizable to other countries that are similar to Sweden as a whole and possibly also to other countries.

Population-based studies differ to a great extent from experimental studies. Whereas the strength of experimental studies is the possibility to thoroughly measure numerous variables under controlled conditions, this is seldom possible in a population-based study, because of the large number of study subjects. Instead, the strength of epidemiological studies, such as those presented in this thesis, is the high number of study participants and that they usually to a higher degree than in experimental studies are representative of the source population, due to the random selection. Thus, any conclusion drawn from an adequate sized population-based study is more likely to be generalizable to the putative background population. Population-based studies can therefore, on a population level, provide a test of the relevance of biological mechanisms found in smaller, selected samples in experimental studies. Thus, in a translational perspective it is important to combine these two methods in order to put biomolecular findings into an epidemiological context.

Validity

Endpoints

Information on the end-points in Paper I, AMI mortality and all-cause mortality, was collected by record linkage with the Swedish national mortality and in-patient registers. Even though this has previously been deemed a valid method [195, 196], several precautions were taken to assure the validity of the data. For example, all death certificates were also obtained and examined, and the certificates showing multiple diagnoses were double-checked to ascertain that the underlying cause of death had been chosen according to general recommendations. Fatal events of AMI were accepted even when another diagnosis was listed as the underlying cause of death in the National Mortality Register, unless it was a diagnosis of a malignant disease. All certificates of patients dying outside of hospital were also especially examined, along with their diagnosis in the mortality register, to be able to establish a correct diagnosis in those cases where the amount of evidence was considered scarce. To further increase validity, two persons (Larsson CA, Jungå

K) created the end point register, working side by side to double-check all steps. The coordinator for the Skaraborg Project (Lindblad U) surveyed the process and took the final decision in spurious cases, blinded as to the exposure status of the study subjects in question. The examination of death certificates for persons dying outside of hospital revealed four cases with uncertain diagnoses. Furthermore, the examination of deaths with multiple diagnoses, which was conducted to correct inaccurate hierarchy of diagnoses, revealed six additional AMI events, whereas one event was excluded. While the validation used in Study I increases the correctness of the end-points, the overall impact on the validity of the data are most likely not substantial.

Risk factors

The field team who collected the data for the Vara-Skövde cohort included members of the team from the Skara studies. All team members were trained and calibrated in methodological procedures prior to the studies of the Skara cohorts and again prior to the studies of the Vara-Skövde cohort. Thus, inter-observer variation was kept at a minimum and standards maintained over time. To limit misclassification, the nurses on site briefly examined the questionnaires when collecting them, to make sure that they were filled out properly. The data was also entered twice, i.e. the same raw data was entered into two separate data base spread sheets, in order to detect errors at input. In addition, all variables have been checked by frequency listing to reveal implausible values.

In contrast to many other epidemiological studies, the diagnoses of hypertension and diabetes used in the present studies were based on repeated measurements when the first measurements at baseline showed values exceeding the diagnostic criteria. This strengthens the validity of the diagnoses, as using measurements from only one occasion might lead to over-diagnosing with regard to values only temporarily increased. However, the diagnostic criteria were, in line with international standards, higher in the beginning of the nineties than today. This is often a problem with longitudinal studies and in this case one that could not be corrected in retrospect because of the repeated measurements, which were only performed when the first measurement showed increased blood pressure or blood glucose. Due to the older, and higher, diagnostic thresholds employed in Paper I, the patient groups here were accordingly containing proportionally more patients at higher risk than they would have, if today's diagnostic criteria had been used. However, the population sample is also containing more subjects at increased risk and had the new criteria been used, the change in hazard ratios, if any, would most likely have been small and the conclusions drawn would probably still be the same. A possible limitation applying only to the surveys in Skara is the lack of routine screening for impaired glucose metabolism with oral glucose tolerance test in patients with hypertension and in the participants of the population survey.

Consequently, some patients might have been incorrectly categorised as having only hypertension when in fact they also had type 2 diabetes. Some subjects in the reference population might also have been deemed healthy when they should have been diagnosed with type 2 diabetes, and thus not included among the controls. Still, the number of subjects affected by such misclassification is most likely to have been small, and if anything, the effect of this would probably only have weakened our results. Since participants from the Vara-Skövde cohort all had an oral glucose tolerance test done, any potential limitation with regard to diagnosis of diabetes and hypertension does not apply to study II-IV.

In Study II, morning and evening cortisol were measured, which might have involved some potential sources of error. Cortisol was measured from saliva and the participants collected the saliva themselves at home. Since cortisol is a potent stress hormone there are obvious advantages associated with allowing the participants to collect the saliva themselves in a familiar environment. However, to ensure an accurate measure, participants were instructed to; 1) abstain from food, drinks, snuff, smoking, tooth brushing, and exertion in the hour before saliva collection; 2) to rinse their mouths with water 15 minutes before the sampling; 3) to rest for at least 15 minutes before sampling. Thus, considering that the procedure of the sampling did involve a fair amount of specific instructions that may have been perceived as inconvenient to follow, it is not unlikely that some participants might have failed to comply with all these instructions. Furthermore, the morning cortisol values are based on a single measurement in our study. Since cortisol levels rise very sharply in the morning there is a risk of misclassification due to lack of adjustment for time of awakening and actual time of sampling. However, there is no reason to believe that these potential sources of misclassification would be systematic, and if anything they would only have weakened our findings.

Other clinical risk factors investigated in Paper II-IV were all measured according to previously validated methods [188-191]. However, information about several of the lifestyle related risk factors were collected from questionnaires and thus based on self-report. Self-reported information from surveys has a disadvantage due to the risk of self-reporting inaccuracy. Unhealthy subjects might be more likely to underestimate risk behaviour and healthy people might be more prone to underestimate healthy behaviour. In large observational studies it is not always practically or economically possible to more objectively measure lifestyle variables such as physical activity, alcohol consumption, and smoking. However, it is becoming increasingly popular to do so, especially with regard to physical activity, for example by using pedometers. Still, these measures do not ensure valid data either, as e.g. a pedometer does not detect all kinds of movement, and also, participants who know that they are under observation might alter their behaviours. Nevertheless, the question about leisure time physical activity used in

the current studies has been extensively used previously and is considered valid [284, 285]. The statistically significant association between high leisure time physical activity and low heart rate (Paper III) also supports the internal validity of the question used. Furthermore, if misclassification were to apply with regard to leisure time physical activity, it would probably only have attenuated our associations. Still, the validity of the other questions related to lifestyle is less known and thus a potential methodological weakness.

Implications for public health strategies

The high prevalence of overweight and obesity seen in all the Skaraborg cohorts [Paper I-IV] and elsewhere [286, 287], and its association with several other AMI risk factors [Paper II-IV], call for interventions to combat these conditions. Since leisure time physical activity has been found to be associated with lower prevalence of both abdominal and general obesity here [Paper III] and elsewhere [10, 11, 242, 243], activating subjects physically should be the main focus. The focus on physical activity might be especially important as it not only targets obesity but also several other CHD risk factors, such as insulin resistance [Paper III], blood pressure, and lipid levels. In addition, physical activity is known to improve mood and relieve stress, which might have beneficial effects on cortisol levels as well. Even though increasing physical activity might be considered the most important intervention together with smoking cessation/prevention, other lifestyle modifications should also be considered, such as improved diet and drinking habits. The finding of an increased association between the addiction factor (comprising alcohol consumption and smoking) and left ventricular hypertrophy in men [Paper IV] suggests that this combination of risk factors should be especially considered.

The apparent gender differences, both with regard to the risk of AMI and in the association between different AMI risk factors, also call for more gender specific interventions. Whereas paper I did not confirm previous findings of higher risk factor levels in women with type 2 diabetes being the reason for their increased risk of AMI, women with type 2 diabetes should still be especially targeted with regard to reduction of all AMI risk factors levels, since residual confounding might be at play. The findings in paper III also stress the importance of considering the work setting for public health strategies, and perhaps especially with regard to women.

In general, the findings of this thesis emphasize the need for gender-specific interventions aiming at reducing AMI and its risk factors. Gender differences need to be addressed with regard to both biological and psychosocial aspects.

Future research

Even though type 2 diabetes has severe implications for men as well, further research is needed to understand the specific mechanisms behind the more pronounced risk increase of AMI in women than men once they develop type 2 diabetes. The influence of estrogen on nitric oxide synthases and its consequences in women with type 2 diabetes, might be of special interest.

Data on basal cortisol levels in both men and women and in different age-groups are needed from different population-based studies to confirm the findings in Paper II of generally higher basal cortisol levels in women than in men. More studies are also needed with regard abdominal obesity in association with cortisol levels. In Paper II morning salivary cortisol was inversely associated with abdominal obesity. Since this has been seen in previous studies concurrently with a positive association between total 24-h urinary cortisol secretion and abdominal obesity [221], future studies should include both of these cortisol measures as well as at least an evening measure too to explain these spurious dual associations. The relevance of peripheral cortisol metabolism also needs to be further investigated, especially on a population level. Since cortisol is a stress hormone, measures of stress should be taken into account and the bidirectional association previously found solely in men with regard to change in body mass index and work stress [272] highlights the need for longitudinal studies. Furthermore, previous studies have indicated that reproductive hormones have an effect on the regulation of the HPA-axis, and inclusion of reproductive hormones in future studies does thus seem prudent.

The different findings with regard to occupational physical activity and leisure time physical activity in association with obesity and insulin resistance warrant further investigation. To help identify targets for intervention, more thorough measurement of potential confounders is needed to assess whether confounding is in fact the reason for the positive finding in women, as suggested. Questions that differentiate between aerobic and anaerobic types of both occupational and leisure time physical activity would help to ascertain if different types of physical activity have different effects on the metabolism and if the occurrence or impact in that case differs between men and women. More studies are needed to further explore the interaction between female gender and high leisure time physical activity in association with insulin resistance.

The addiction factor was only associated with left ventricular hypertrophy in men [Paper IV]. Future studies are needed to investigate if some factor inherent in one or the other of the separate variables (alcohol consumption and smoking) that loaded on the addiction factor is particularly responsible for the findings, or if there is some other variable/s of importance that has not yet been identified but that also clusters together with smoking and alcohol consumption, and perhaps

especially so in men. The possibility of the male heart being particularly vulnerable to the cluster of variables, or to any of the single variables, also needs to be considered and further explored. As for all of the cross-sectional findings in this thesis, the associations with regard to left ventricular hypertrophy need to be confirmed in longitudinal studies to establish causality.

AMI is a serious condition in both genders. However, as further highlighted in the present thesis, potential gender differences need to be taken into consideration in all kinds of future research within this area in order to improve the prognosis in both men and women. As genetic predisposition applies to most kinds of medical conditions, future studies should also explore the relevance of genetic mechanisms with regard to the finding of this thesis.

Conclusions

General conclusion

In these population-based studies, considerable gender differences emerged both in the risk of AMI and in the way in which different risk factors for AMI interrelate.

Specific conclusions

- Type 2 diabetes has a higher impact on AMI mortality in women than in men, independent of several major AMI risk factors.
- Salivary cortisol is generally higher in women than in men, and in older than younger individuals. Low morning cortisol and low diurnal variation in cortisol levels were associated with high levels of abdominal obesity; however, solely in women.
- Leisure time and occupational physical activity show different associations with regard to obesity and insulin resistance. While the associations with regard to leisure time physical activity were inverse in both men and women, the associations with regard to occupational physical activity were positive, albeit only in women.
- Common risk factors for AMI cluster in the same way in men and women. However, while the principal "metabolic" factor was significantly associated with LVH in both genders, the "addiction" factor (comprising alcohol and smoking) was associated with LVH solely in men, and the vitality factor (leisure time physical activity and SRH) neither in men nor in women.

Populärvetenskaplig sammanfattning

År 2008 insjuknade 37 156 personer i akut hjärtinfarkt i Sverige och av dessa dog 10 509 personer. Akut hjärtinfarkt är därmed den vanligaste dödsorsaken i Sverige, men även i resten av världen. Generellt sett insjuknar män ungefär 10 år tidigare än kvinnor i hjärtinfarkt och män har därför en högre total risk att insjukna, även om hjärtinfarkt är den vanligaste dödsorsaken även hos kvinnor.

Akut hjärtinfarkt definieras av den syrebrist och påföljande celldöd i hjärtmuskeln som uppkommer då blodflödet i hjärtat dramatisk minskar. Denna reduktion av blodflöde uppkommer av att en blodpropp bildats, antingen p g a en kärlskada, eller p g a att plack i kärlväggen lossnat. Bakgrunden till varför en blodpropp och därmed hjärtinfarkt uppkommer är mångfacetterad, men brukar uppfattas som en samverkan mellan livsstil, ärftlighet och socioekonomiska faktorer (är ”psykosociala faktorer” bättre?).

I denna avhandling har deltagare från Skaraborgsprojektet i Västra Götaland studerats. Mellan 1992 och 1993 genomfördes en studie i Skara av 1149 patienter med högt blodtryck och/eller diabetes. En likadan undersökning genomfördes 1993-1994 på ett slumpmässigt urval av 1109 personer från befolkningen i Skara. Mellan 2001 och 2005 genomfördes även två befolkningsstudier på ett slumpmässigt urval från invånarna i Vara (1811 personer) respektive Skövde (1005 personer).

Skillnader mellan män och kvinnor avseende dödligheten i akut hjärtinfarkt undersöktes i delarbete I hos personer med typ 2 diabetes och hypertoni (högt blodtryck) med en frisk population som jämförelsegrupp. Typ 2 diabetes hos kvinnor visade sig ha en mycket större negativ inverkan på risken att drabbas av en dödlig hjärtinfarkt än hos män. Detta fynd kunde inte förklaras av eventuella skillnader i ålder eller i förekomst av andra riskfaktorer, såsom blodtryck, blodfetter, fetma, och fysisk aktivitet.

I delarbete II studerades stresshormonet kortisol i relation till kön, ålder samt graden av bukfetma. Kvinnor hade generellt högre kortisolnivåer än män, medan äldre personer hade högre nivåer än yngre individer. Låga nivåer av kortisol på

morgonen och låg dygnsvariation av kortisol var associerat till bukfetma, men endast hos kvinnor.

Kopplingen mellan fysisk aktivitet på fritid respektive på arbetet undersöktes i delarbete III i relation till fetma och insulinresistens. Högre grad av fysisk aktivitet på fritiden var som förväntat relaterat till lägre grad av generell fetma, bukfetma och insulinresistens hos både män och kvinnor, vilket var förväntat. För fysisk aktivitet i samband med arbetet var dock sambandet det omvända hos kvinnor, d v s högre grad av fysisk aktivitet på arbetet var relaterat till högre grad av fetma och insulin resistens. Detta sistnämnda samband kunde inte förklaras av uppmätta skillnader i ålder, utbildning, fysisk aktivitet på fritiden, eller alkohol konsumtion. Skillnader i andra faktorer som ej mättes i studien, t ex kosthållning, är förmodligen den troligaste förklaringen till de omvända fynden beträffande fysisk aktivitet på arbetet samt till skillnaderna mellan könen. Eventuella skillnader i hur ämnesomsättningen reagerar på olika typer av fysisk aktivitet bör dock också beaktas, liksom inverkan av framför allt utdragen belastning i form av stress.

I delarbete IV undersöktes hur några av de mest erkända och betydande riskfaktorerna för hjärtinfarkt samvarierar. Detta gjordes genom en så kallad faktoranalys där man studerar hur de olika variablerna faller ut i grupper som förmodas representera gemensamma underliggande mekanismer. Intressant nog framkom ett mönster med tre dominerande faktorer som var identiska för båda könen. Riskfaktorer relaterade till det så kallade ”Metabola syndromet”, såsom bukfetma, insulinresistens, högt blodtryck och lipidrubbningar visade sig vara den starkaste faktorn hos både män och kvinnor (den metabola faktorn). Hos båda könen visade sig även fysisk inaktivitet på fritiden och låg självskattad hälsa höra samman (vitalitetsfaktorn), samt alkohol konsumtion och rökning (beroendefaktorn). Dessa tre faktorer undersöktes sedan i relation till vänsterkammarhypertrofi som anses vara en mycket stark riskfaktor för hjärtinfarkt och en icke-symtomgivande manifestation av kranskärlssjukdom. Medan den metabola faktorn var associerad med vänsterkammarhypertrofi hos båda könen, så var beroendefaktorn kopplad till vänsterkammarhypertrofi endast hos män, och vitalitetsfaktorn varken hos män eller kvinnor.

Denna avhandling visar att könsskillnader är påtagliga både beträffande risken att insjukna i hjärtinfarkt och hur olika riskfaktorer för hjärtinfarkt relaterar till varandra. Dessa fynd understryker vikten av att hänsyn till kön tas i alla stadier av förebyggande och behandlande arbete som syftar till att reducera bördan av hjärtinfarkt i befolkningen.

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Appendix

Paper I

Paper II

BMC Endocrine Disorders

BioMed Central

Research article

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Salivary cortisol differs with age and sex and shows inverse associations with WHR in Swedish women: a cross-sectional study

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Abstract

Background: Most studies on cortisol have focused on smaller, selected samples. We therefore aimed to sex-specifically study the diurnal cortisol pattern and explore its association with abdominal obesity in a large unselected population.

Methods: In 2001–2004, 1811 men and women (30–75 years) were randomly selected from the Vara population, south-western Sweden (81% participation rate). Of these, 1671 subjects with full information on basal morning and evening salivary cortisol and anthropometric measurements were included in this cross-sectional study. Differences between groups were examined by general linear model and by logistic and linear regression analyses.

Results: Morning and Δ-cortisol (morning – evening cortisol) were significantly higher in women than men. In both genders older age was significantly associated with higher levels of all cortisol measures, however, most consistently with evening cortisol. In women only, age-adjusted means of WHR were significantly lower in the highest compared to the lowest quartile of morning cortisol ($p = 0.036$) and Δ-cortisol ($p < 0.001$), respectively. Furthermore, when comparing WHR above and below the mean, the age-adjusted OR in women for the lowest quartile of cortisol compared to the highest was 1.5 (1.0–2.2, $p = 0.058$) for morning cortisol and 1.9 (1.3–2.8) for Δ-cortisol. All findings for Δ-cortisol remained after adjustments for multiple covariates and were also seen in a linear regression analysis ($p = 0.003$).

Conclusion: In summary, our findings of generally higher cortisol levels in women than men of all ages are novel and the stronger results seen for Δ-cortisol as opposed to morning cortisol in the association with WHR emphasise the need of studying cortisol variation intra-individually. To our knowledge, the associations in this study have never before been investigated in such a large population sample of both men and women. Our results therefore offer important knowledge on the descriptive characteristics of cortisol in relation to age and gender, and on the impact that associations previously seen between cortisol and abdominal obesity in smaller, selected samples have on a population level.

Background

Cortisol is a potent stress hormone and the secretion is regulated by the Hypothalamic-Pituitary-Adrenal-axis (HPA-axis). Cortisol is secreted in a specific diurnal pattern with a normal curve presenting a sharp peak in the early morning to then gradually decrease over the day and end up very low in the evening and at night. Except for the increased secretion in stressful situations, there are also smaller peaks during the day when the body is exposed to exercise, food, and tobacco [1]. Aging is hypothesised to alter the function of the HPA-axis in both men and women with increasing cortisol levels as a result, especially regarding nocturnal levels [2-7]. Previous studies have also indicated that cortisol levels differ between men and women [2-5,8,9]. However, while several of the studies have found men to have higher levels than women there are inconsistencies regarding in what age-groups these findings have been seen [2-5].

Patients with Cushing's syndrome are characterised by abdominal fat accumulation and decreased hip circumference, caused by their excess cortisol secretion. Consequently, numerous studies have investigated if increased cortisol levels are associated with abdominal obesity in otherwise healthy subjects too. While some studies have found total 24 h cortisol (urinary excretion or plasma/serum) to be positively correlated to abdominal obesity in women [10,11], others have found the opposite in women [12] or no association at all in either men [12,13] or women [14]. Furthermore, even though there are null findings too [10,15-18], morning cortisol seems to be negatively associated with abdominal obesity in both men and women [11,13,14,19-23].

The vast majority of previous studies of cortisol have focused on smaller selected groups. While these studies offer important contributions for understanding the underlying mechanisms of cortisol metabolism and its association with e.g. abdominal obesity, larger studies are needed to add information on what impact associations on smaller selected samples have on a population level. The present study was therefore designed to sex-specifically look at the diurnal salivary cortisol pattern under basal conditions and to explore the relationship between cortisol and abdominal obesity, in a large, randomly selected population.

Methods

Subjects

Vara is a small rural municipality in South-western Sweden with around 16 000 inhabitants. The population of Vara is a homogeneous population that generally resembles that of the total Swedish population. However, the Vara population has a lower level of education [24] and a lower level of foreign-born individuals [25]. Furthermore, both men and women in Vara are more obese [26], and women are less often smokers than the general Swedish population [24]. Between 2002 and 2004 a random sample from the Vara population was surveyed as part of a new population study within the Skaraborg Project. Participants were randomly selected, stratified by sex and five-year age groups, from all individuals between 30 and 74 years with a three times over-sampling in the ages 30 to 50 years as compared to those over 50 years. There were 1811 subjects who fulfilled all requirements for participation in the survey including visiting the study nurse, completion of the questionnaires, and having venous blood samples drawn (81% participation rate). After excluding a total of 140 subjects because of pregnancies (n = 5), use of cortisone medication (n = 10), missing waist circumference measurements (n = 1), and missing morning and/or evening salivary cortisol measurements (n = 124), 1671 subjects remained for further analyses in the present study. Informed consent was collected from all participants and the ethics committee at the University of Gothenburg, Sweden, approved the protocol.

Procedures

Specially educated and trained nurses saw participants in the morning after an overnight fast (10 h). Participants signed an informed consent form and were then weighed on a calibrated scale and measured in light cloths and no shoes. They had their blood pressure taken twice in a supine position and had blood samples drawn. An oral glucose tolerance test (OGTT) was performed with an intake of 75 g standard glucose load [27]. In the two hours wait for the final blood drawing participants filled in a questionnaire regarding civil- and socioeconomic status, including educational level. Participants were also provided with a Salivette sampling device (cotton) along with both verbal and written instructions for usage. The instructions stated that participants were to: Collect saliva themselves at 0800 h and 2200 h (with a maximum of 30 minutes time shift) on one normal weekday within two weeks from the first study visit; abstain from food, drinks, snuff, smoking, tooth brushing, and exertion in the hour before saliva collection; rinse their mouths with water 15 minutes before the sampling; and rest for at least 15 minutes before sampling. Levels of saliva cortisol were analysed using a radioimmunoassay from Orion Diagnostica (Spectria™ Cortisol RIA) [28]. Approximately two weeks after the first visit the participants came for a second visit to the nurses to provide detailed information on medical history and ongoing medication, and to fill in a validated questionnaire regarding smoking habits, leisure time physical activity, and alcohol intake.

Measures

Waist circumference was measured between the lowest rib margin and iliac crest and hip circumference at the largest

circumference between waist and thighs. Waist-hip ratio (WHR) was defined as the ratio of waist to hip circumference.

Diurnal cortisol level (Δ-cortisol) was measured as the difference between logarithmic morning and evening saliva cortisol, which corresponds to the difference in percent between morning and evening values.

Current smoking was defined as daily smoking (yes/no). Leisure time physical activity was characterized based on four answer alternatives to the question "How physically active are you during your leisure time?". Alcohol consumption was defined by questions on how many days over the last 30 days that the subjects had consumed beer, wine, and strong liquor, respectively. Each of these questions was followed by questions on how many tins, glasses, and/or bottles that were normally consumed on such days. The total gram of alcohol consumed per week was then calculated by multiplying the number of days of alcohol drinking with the gram of alcohol that the items of consumed alcoholic beverage contain.

Educational level was examined by a question with 10 alternatives reaching from primary school to PhD-exams.

Statistical analyses

SPSS Base System for Macintosh 11.0 was used for data analyses and all analyses were sex-specific. Baseline characteristics expressed as proportions were age-standardised in five-year intervals using the whole Vara population 30–75 years as standard. Differences between groups in continuous variables were examined by GLM (general linear model) and associations between continuous variables were analysed by linear regression. Associations between categorical variables were analysed by logistic regression and expressed as odds ratios (OR) with 95 per cent confidence intervals (CI). For the logistic regression analyses of the associations between WHR and morning cortisol/Δ-cortisol, WHR above and below the mean was used as dependent variable and quartiles of morning and Δ-cortisol, respectively, as independent variables. Pearson's correlation coefficient was used for testing the correlation between morning and evening cortisol. Confounding by differences in age, alcohol consumption, daily smoking (yes/no), leisure time physical activity, and use of oral contraceptives or estrogen replacements was controlled for in multivariate analyses and by stratification. Subjects treated with insulin were excluded from the analyses of 2 h blood glucose. Log transformation (10th logarithm) was used to induce normality in morning and evening cortisol. All tests were two-sided and statistical significance was assumed at $p < 0.05$.

Results

The mean age in both men and women was 48 years (Table 1). Fasting blood glucose and blood pressure were higher in men and they reported lower levels of leisure time physical activity. Women were more often smokers than men and had higher salivary cortisol and 2 h glucose in an OGTT (Table 1).

Basal salivary cortisol

Age-differences

In both men and women evening cortisol was significantly higher in older subjects compared to younger, while the same pattern for morning cortisol was seen in men only (Table 2). In women Δ-cortisol was significantly lower in older subjects than in younger (Table 2). Test for trends over increasing age-groups, revealed highly significant trends ($p \leq 0.001$) in all cortisol variables but morning cortisol in women ($p = 0.215$) and Δ-cortisol in men ($p = 0.088$).

Sex-differences

Women in general were found to have significantly higher levels of morning cortisol than men, and women under <50 years were found to have higher levels of both morning and Δ-cortisol than corresponding men (Table 1). The correlation (r) between morning and evening cortisol values were 0.297 ($p < 0.001$) for women and 0.240 ($p < 0.001$) for men.

Associations between salivary cortisol and abdominal obesity

In women, the age-adjusted mean of WHR was significantly lower in the two highest quartiles of morning cortisol compared to the lowest quartile (Figure 1 and Table 3). This association remained when also adjusting for leisure time physical activity, smoking, education, alcohol consumption, oral contraceptives, and estrogen replacements (Table 3), and was also seen in both a linear ($p = 0.006$) and a logistic regression analysis (OR 1.5, 1.0–2.2, $p = 0.058$). Similarly, the mean of WHR in the lowest quartile of Δ-cortisol in women was significantly higher than in the other three quartiles in the age-adjusted model (Figure 2 and Table 4), however, in the multivariate model the increase was only statistically significant in comparison with the second and the forth quartile (Table 4). The association between Δ-cortisol and WHR was also seen in women both above and below 50 years of age (data not shown). Moreover, a significant association between Δ-cortisol and WHR was seen in a linear regression analysis ($p = 0.003$) and in a logistic regression analysis (OR 1.9, 1.3–2.8), which both remained statistically significant when adjusting for multiple variables (Table 4). BMI did not affect any of the results above (data not shown).

Table 1: Characteristics in men and women

Characteristics	Women m.v.	m	sd (q1–q3)	Men m.v.	m	sd (q1–q3)	p-value
	n = 838			**n = 833**			
Age, years	0	48	12	0	48	12	0.875
Fasting p-glucose, mmol L^{-1}	0	5.3	1.1	0	5.5	1.1	0.001
2 h p-glucose, mmol L^{-1}	33	5.8	2.1	32	5.5	2.2	0.017
Systolic BP, mm Hg	0	121	14	0	125	14	<0.001
Diastolic BP, mm Hg	0	69	9	0	72	9	<0.001
Waist circumference, cm	0	85	12	0	95	12	<0.001
Hip circumference, cm	0	102	9	0	100	9	<0.001
WHR (waist-hip-ratio)	0	0.83	0.06	0	0.94	0.06	<0.001
BMI, kg m^{-2}	1	26.8	4.5	0	26.9	4.6	0.442
Morning cortsiol, mmol L^{-1}, all	0	12.5	(9.0–18.0)	0	11.1	(8.0–16.0)	<0.001
Morning cortsiol, <50 years	0	12.4	(9.0–18.0)	0	10.5	(7.0–15.0)	<0.001
Morning cortsiol, ≥ 50 years	0	12.5	(9.0–18.0)	0	12.1	(8.0–18.0)	0.491
Evening cortsiol, mmol L^{-1}, all	0	2.3	(2.0–3.0)	0	2.2	(2.0–3.0)	0.132
Evening cortsiol, <50 years	0	2.2	(2.0–3.0)	0	2.1	(1.0–3.0)	0.386
Evening cortsiol, ≥ 50 years	0	2.7	(2.0–3.0)	0	2.5	(2.0–3.0)	0.183
Δ-cortisol, mmol L^{-1}, all	0	5.3	(3.5–9.0)	0	5.0	(3.3–8.0)	0.086
Δ-cortisol, <50 years	0	5.8	(3.7–9.5)	0	5.1	(3.3–8.5)	0.004
Δ-cortisol, ≥ 50 years	0	4.6	(3.0–8.0)	0	4.8	(3.0–7.7)	0.469
Alcohol consumption, g/week[a]	6	22	(0–30)	2	61	(10–77)	<0.001[b]
	m.v.	n	%	m.v.	n	%	p-value
Oral contraceptives	-	64	6	-	-	-	-
HRT	-	26	5	-	-	-	-
Low physical activity, yes/no	8	50	7	11	67	7	0.094
Daily smoking, yes/no	3	182	21	4	128	15	0.001
Primary school only, yes/no	16	221	37	17	292	44	<0.001

All means (m) are adjusted for differences in age and proportions are standardised for age using the Vara population as standard. M.v. = missing values, P-glucose = plasma glucose, Δ-cortisol = logarithmic morning salivary cortisol – logarithmic evening saliva cortisol, HRT = hormone replacement therapy. For all cortisol variables means and quartiles 1–3 (q1–q3) are geometric (anti-log).

[a] 10 g alcohol is equivalent to approximately 1 glass of wine or 1 small beer.

[b] The p-value is accounted for the generally higher physiological acceptance for alcohol in men as compared to women.

When WHR was substituted by waist circumference in each of the analyses above, the patterns generally remained, however, with less degree of statistical significance (Table 3 and 4). In men, morning cortisol and Δ-cortisol were not associated with either WHR or waist circumference (Figure 1 and 2, Table 3 and 4).

For evening cortisol there was no significant association with WHR (Figure 3 and Table 5) or waist circumference in either women or men (Table 5). However, in women both morning ($p = 0.001$) and evening values ($p = 0.049$) were significantly associated with WHR when simultaneously entered in an age-adjusted linear regression model. This association was not seen for waist circumference.

Discussion

In this cross-sectional study we found significant age- and sex-differences in diurnal cortisol levels. Levels were generally higher in women than men and in older subjects compared to younger. We also found abdominal obesity to be significantly associated with low morning cortisol and low diurnal variation of cortisol, but only in women. To our knowledge, these associations have never before been investigated in such a large population sample of both men and women.

Basal salivary cortisol

Age-differences

In the present study age was associated with higher morning cortisol in men and with higher evening cortisol in both men and women. Furthermore, age and Δ-cortisol were significantly inversely associated in women. This age-related increase in cortisol levels is supported by findings from several other studies, especially regarding nocturnal levels [2-7]. It has been concluded that while the basal secretion of cortisol remains fairly stable with age the negative feedback regulation of the HPA-axis seems to become impaired in older subjects [29,30]. This would

Table 2: Male and female salivary cortisol levels in different age-groups.

	Morning cortisol				**Evening cortisol**				**Δ-cortisol**			
Age-group	n	m	q1–q3	p	n	m	q1–q3	p	n	m	q1–q3	p
Women												
30–39	242	12.4	9.0–17.0	ref.	242	1.7	1.0–3.0	ref.	242	5.8	3.7–10.0	ref.
40–49	291	12.4	8.0–19.0	0.892	291	2.2	2.0–3.0	0.834	291	5.7	3.7–9.0	0.768
50–59	146	11.6	8.7–15.2	0.260	146	2.5	2.0–3.0	0.030	146	4.7	3.0–8.0	0.005
60–69	107	12.7	10.0–18	0.732	107	2.7	2.0–4.0	0.004	107	4.7	3.3–7.5	0.015
≥ 70	52	15.1	9.2–19.8	0.029	52	3.6	2.0–6.0	<0.001	52	4.2	2.3–9.0	0.004
<50	533	12.4	9.0–18.0	ref.	533	2.2	1.4–3.0	ref.	533	5.8	3.7–9.5	ref.
≥ 50	305	12.5	9.0–18.0	0.773	305	2.7	2.0–3.0	<0.001	305	4.6	3.0–8.0	<0.001
Men												
30–39	254	10.4	7.0–15.0	ref.	254	2.0	1.0–3.0	ref.	254	5.3	3.5–9.0	ref.
40–49	276	10.6	8.0–15.0	0.634	276	2.2	2.0–3.0	0.101	276	4.9	3.0–8.0	0.288
50–59	141	11.8	8.0–18.0	0.030	141	2.4	2.0–3.0	0.007	141	5.0	3.0–8.0	0.460
60–69	106	12.5	8.0–17.3	0.006	106	2.5	2.0–3.0	0.001	106	4.9	3.3–7.6	0.459
≥ 70	56	12.3	9.0–16.8	0.049	56	2.9	2.0–4.0	<0.001	56	4.2	3.0–6.5	0.040
<50	530	10.5	7.0–15.0	ref.	530	2.1	1.0–3.0	ref.	530	5.1	3.3–8.5	ref.
≥ 50	303	12.1	8.0–18.0	0.001	303	2.5	2.0–3.0	<0.001	303	4.8	3.0–7.7	0.305

Δ-cortisol=logarithmic morning salivary cortisol-logarithmic evening salivary cortisol
Differences between age-groups were examined by GLM (general linear model), using group 30–39 and group <50, respectively, as reference.
Means and quartiles 1–3 (q1–q3) are geometric (anti-log).

Table 3: Comparisons of body composition between quartiles of morning salivary cortisol in men and women.

	WHR				**Waist circumference**			
	n	m	sd	p	n	m	sd	p
Women								
Adjusted for age								
qrtl 1 morning cortisol	188	0.843	0.069	ref.	188	86.63	13.52	ref.
qrtl 2 morning cortisol	230	0.838	0.076	0.492	230	85.90	13.53	0.585
qrtl 3 morning cortisol	203	0.826	0.071	0.025	203	83.99	13.52	0.055
qrtl 4 morning cortisol	217	0.827	0.074	0.036	217	84.74	13.54	0.163
Test for linearity:								
between quartiles				0.013				0.079
continuously				0.006				0.024
Adjusted for model 2[a]								
qrtl 1 morning cortisol	176	0.842	0.080	ref.	188	86.11	13.82	ref.
qrtl 2 morning cortisol	224	0.836	0.075	0.379	230	85.66	13.53	0.740
qrtl 3 morning cortisol	198	0.826	0.070	0.038	203	84.04	13.55	0.137
qrtl 4 morning cortisol	210	0.827	0.072	0.038	217	84.49	13.60	0.237
Test for linearity:								
between quartiles				0.019				0.135
continuously				0.012				0.075
Men								
Adjusted for age								
qrtl 1 morning cortisol	193	0.945	0.056	ref.	193	94.61	10.03	ref.
qrtl 2 morning cortisol	240	0.939	0.062	0.275	240	93.96	9.99	0.498
qrtl 3 morning cortisol	210	0.945	0.058	0.979	210	94.55	10.00	0.950
qrtl 4 morning cortisol	190	0.945	0.055	0.913	190	94.92	9.99	0.763
Test for linearity:								
between quartiles				0.784				0.613
continuously				0.980				0.556

Differences between quartiles (qrtl) of morning cortisol were examined by GLM (general linear model), using quartile 1 as reference.
[a]Model 2 = age, leisure time physical activity, smoking, education, alcohol consumption, oral contraceptives, and estrogen replacements.

Table 4: Comparisons of body composition between quartiles of Δ-cortisol in men and women.

	WHR				Waist circumference			
	n	m	sd	p	n	m	sd	p
Women								
Adjusted for age								
qrtl 1 Δ-cortisol	205	0.849	0.072	ref.	205	87.56	13.50	ref.
qrtl 2 Δ-cortisol	223	0.829	0.075	0.006	223	84.26	13.47	0.012
qrtl 3 Δ-cortisol	192	0.833	0.069	0.032	192	86.04	13.45	0.262
qrtl 4 Δ-cortisol	218	0.823	0.074	<0.001	218	83.59	13.47	0.003
Test for linearity:								
between quartiles				0.001				0.016
continuously				0.003				0.041
Adjusted for model 2[a]								
qrtl 1 Δ-cortisol	194	0.846	0.070	ref.	194	86.70	13.34	ref.
qrtl 2 Δ-cortisol	217	0.829	0.074	0.018	217	84.19	13.29	0.056
qrtl 3 Δ-cortisol	181	0.833	0.081	0.104	181	86.13	13.33	0.680
qrtl 4 Δ-cortisol	216	0.824	0.073	0.003	216	83.56	13.30	0.018
Test for linearity:								
between quartiles				0.009				0.071
continuously				0.009				0.119
Men								
Adjusted for age								
qrtl 1 Δ-cortisol	206	0.942	0.057	ref.	206	94.11	9.99	ref.
qrtl 2 Δ-cortisol	212	0.942	0.058	0.963	212	94.22	9.99	0.912
qrtl 3 Δ-cortisol	216	0.946	0.059	0.512	216	94.50	9.98	0.684
qrtl 4 Δ-cortisol	199	0.942	0.056	0.947	199	95.12	10.02	0.307
Test for linearity:								
between quartiles				0.787				0.291
continuously				0.957				0.310

Differences between quartiles (qrtl) of Δ-cortisol (logarithmic morning salivary cortisol – logarithmic evening salivary cortisol) were examined by GLM (general linear model), using quartile 1 as reference.
[a]Model 2 = age, leisure time physical activity, smoking, education, alkohol consumption, oral contraceptives, and estrogen replacements.

lead to prolonged periods of increased cortisol secretion in response to for example stress and, thus, also increased general cortisol levels.

Sex-differences

We also found a consistent pattern of higher cortisol levels in women compared to men. While this have been seen in a couple of previous studies on older subjects [8,9], it is in contrast with others where men generally have been found to have higher cortisol levels [2,3,5]. However, in the latter studies there are some inconsistencies regarding in what age-groups the sex-differences have been seen. Different measurements of cortisol might be a plausible explanation for the incongruity between studies as some studies have measured the total cortisol levels in plasma or serum [2,5] while we and others [3] have measured the free cortisol levels found in saliva. This notion is supported by the findings of Kudelka et al [4] showing that while the total plasma cortisol seemed higher in older women than older men there was a significant reverse association regarding salivary cortisol. However, the analyses performed with saliva cortisol in previous studies [3,4] are still in contrast with our own results and the analyses with plasma cortisol [2,4,5] have not produced consistent results either, indicating other mechanisms behind the differing results. One might be that measurements of cortisol have been performed at different times of the day in different studies. Van Cauter et al [2] have found that the quiescent period tends to start earlier in women than in men. In two of the other previous studies [3,4] cortisol was in fact measured during the late afternoon when levels might have started sinking in women but not in men, thus, possibly explaining the higher levels found in men than women in these studies. Lastly, differences might partly be explained by most previous studies having used small, selected study groups, which make the results more unreliable and less representative of the general population.

Associations between salivary cortisol and abdominal obesity

Women

The significant inverse association seen in women between morning cortisol and WHR is in concordance with other studies [11,14,19,20], of which two have used salivary cortisol like us [14,19]. Thus, the fact that saliva cortisol, in contrast to plasma or serum cortisol, primarily reflects free and active cortisol does not seem to affect the

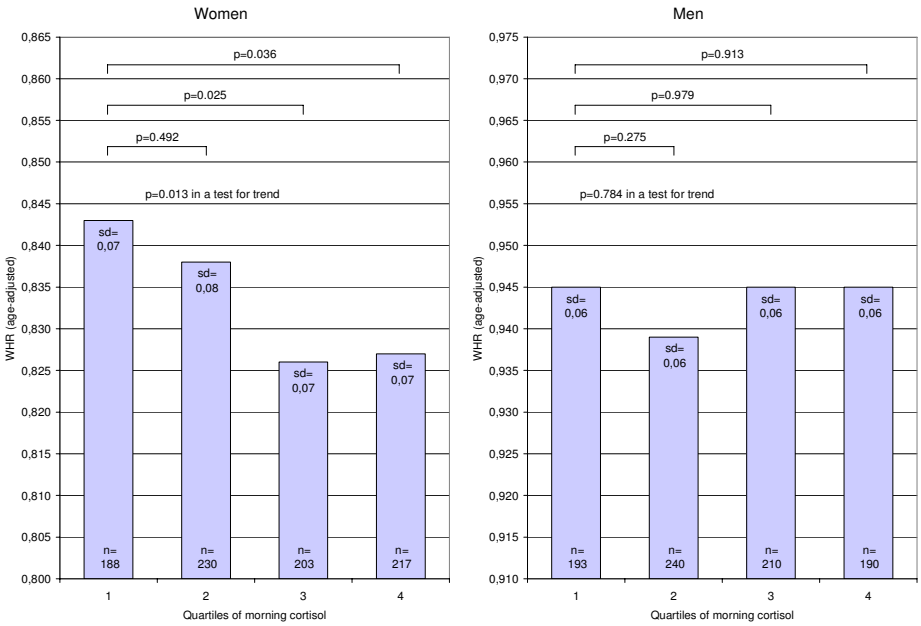

Figure 1
Age-adjusted means of WHR (waist-hip ratio) by quartiles of morning cortisol.

association seen between abdominal obesity and cortisol. This inverse association may instead be explained by an enhanced local clearance rate of cortisol in visceral fat depots since this type of fat has been found to harbour more glucocorticoid receptors than the subcutaneous type [31]. Higher metabolic transition rate of cortisol to its inactive metabolites [32] and impaired regeneration of cortisol from cortisone [33,34], have also been found in obese women compared to lean women. The enhanced clearance rate of cortisol is hypothesized to impair the negative feedback control of the HPA-axis and lead to an increased cortisol secretion [32,35]. This theory is supported by findings of increased total 24 h urinary cortisol out-put concurrent with decreased morning serum cortisol levels in abdominal obese subjects [11], and would explain our findings of abdominal fat accumulation in spite of low morning levels. Furthermore, higher cortisol response to both physical and mental stress has been found in women with high WHR compared to those with normal WHR [11,36]. Taken together, the findings indicate an abnormal activation of the HPA-axis in abdominally obese women. However, since there are also contradicting findings, the need for further studies, especially larger prospective ones, is vital. An alternative hypothesis may be that some of our abdominally obese women with low morning cortisol have been exposed previously to excessive cortisol output in response to stress, which caused their increased WHR. If the stress they experienced became chronic it might eventually have lead to the low cortisol levels seen, since chronic stress have been found to lead to an abnormal habituation to stress and ultimately hypocortisolism through dysregulation on some level of the HPA-axis [37,38].

In contrast to the morning values there was no significant association between WHR and evening cortisol in women. This is, however, not surprising since there is a lower variability in the evening values than in the morning values. Consequently, the inverse association between WHR and Δ-cortisol found in women is mainly an effect of the morning values. However, as the combined regression analysis of morning and evening cortisol with WHR

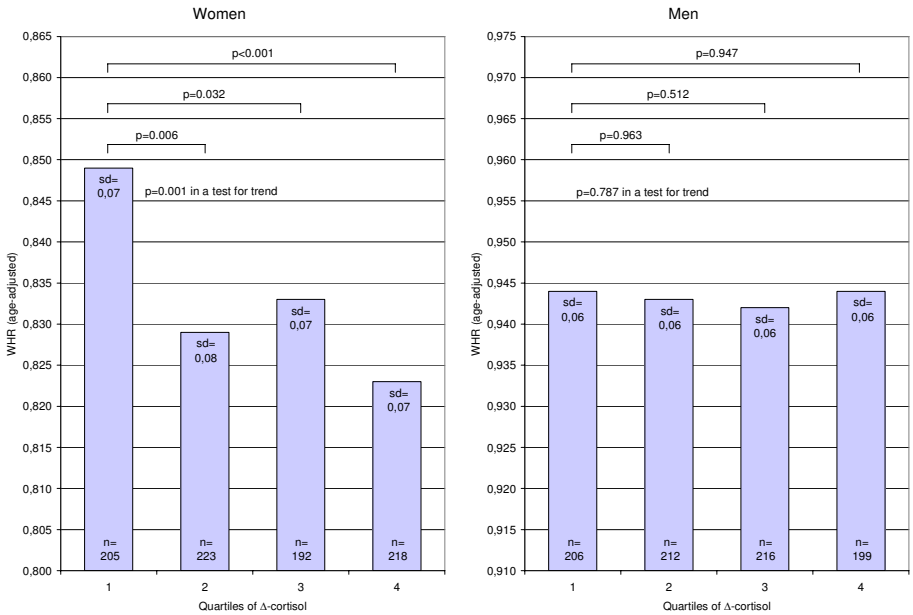

Figure 2
Age-adjusted means of WHR (waist-hip ratio) by quartiles of Δ-cortisol (logarithmic morning cortisol – logarithmic evening cortisol).

revealed, evening values are also of importance for the association with abdominal obesity in women. The stronger association found between WHR and Δ-cortisol also emphasizes that an intra-individual statistical analysis of cortisol levels for detecting associations with abdominal obesity is preferred to an inter-individual analysis where morning and/or evening cortisol values are used separately.

Similarly to the association between WHR and cortisol, a significant inverse association was seen between waist circumference and Δ-cortisol in women, as well as a consistent tendency of an association between waist circumference and morning cortisol. However, WHR was clearly a stronger variable than waist circumference in the association with cortisol. Interestingly, this is in agreement with the body constitution of patients with Cuching's syndrome, who not only accumulate fat around the stomach but often also have decreased hip circumference.

Men

The association between WHR and morning cortisol/Δ-cortisol found here in women was not duplicated in men and differences in the metabolism of cortisol could possibly hold some of the explanation. For example, while the metabolic clearance rate of cortisol has been found to be significantly higher in both obese men and women compared to lean subjects [29,33], some studies have only seen this in women [12,34]. Still, there are studies that also in men have found inverse associations between morning cortisol and WHR [13,14,21,22], as well as between morning cortisol and BMI [23]. Furthermore, while the reasons for the null-findings in men in previous studies are not known, it might be explained in the present study by our men's lower prevalence and variation of abdominal obesity compared to our women and consequently a decreased ability to identify an association with morning and Δ-cortisol.

Table 5: Comparisons of body composition between quartiles of evening cortisol in men and women.

	WHR				**Waist circumference**			
	n	m	sd	p	n	m	sd	p
Women								
Adjusted for age								
qrtl 1 evening cortisol	182	0.830	0.081	ref.	182	85.03	13.60	ref.
qrtl 2 evening cortisol	332	0.830	0.073	0.992	332	84.80	13.54	0.851
qrtl 3 evening cortisol	169	0.839	0.078	0.252	169	86.13	13.56	0.451
qrtl 4 evening cortisol	155	0.837	0.075	0.392	155	85.78	13.61	0.617
Test for linearity:								
between quartiles				0.209				0.402
continuously				0.288				0.695
Men								
Adjusted for age								
qrtl 1 evening cortisol	206	0.944	0.057	ref.	206	95.43	10.08	ref.
qrtl 2 evening cortisol	320	0.943	0.054	0.874	320	94.19	9.96	0.167
qrtl 3 evening cortisol	154	0.942	0.062	0.838	154	93.63	10.00	0.095
qrtl 4 evening cortisol	153	0.944	0.062	0.990	153	94.66	10.03	0.478
Test for linearity:								
between quartiles				0.986				0.382
continuously				0.933				0.500

Differences between quartiles (qrtl) of evening cortisol were examined by GLM (general linear model), using quartile 1 as reference.

Methodological considerations

The main advantages of the present study are the large unselected study sample and the high participation rate (81%), which supports the representativeness of the study sample. There are nevertheless some potential limitations to the study: Firstly, the participants collected the saliva samples themselves and it is not unlikely that some participants have failed to fully comply with the instructions. Secondly, the morning cortisol values are based on a single measurement in our study. Since cortisol levels rise very sharply in the morning there is a risk of misclassification due to lack of adjustment for time of awakening and actual time of sampling. However, there is no reason to believe that these potential sources of misclassification would be systematic, and if anything it would only have weakened our findings. Thirdly, we don't know to what extent waist circumference and WHR depend on visceral fat tissue as opposed to the subcutaneous type. However, while waist circumference has mostly been found to be a better estimate of visceral obesity than WHR [39], the correlation between visceral obesity and WHR has still been good [40] and we therefore feel fairly confident that this is the case in our population too. Furthermore, we did look at cortisol levels in relation to both waist circumference and WHR in our study and WHR came out strongest. Fourthly, we were not able to control for menstrual cycle in women or reproductive hormones in general. Again, this limitation is more likely to have lead to type 2 errors than type 1 errors. In addition, our findings are supported by the presence of an association between WHR and Δ-cortisol in women over as well as under 50 years of age. The findings also remained in stratified analyses of estrogen consumption (data not shown). Lastly, the cross-sectional design makes it impossible to establish causality. In this case we cannot be sure whether decreased cortisol levels lead to abdominal fat accumulation or vice versa, which could have been decided on in a prospective study. Still, these results lay a solid foundation for hypotheses to be tested in future studies.

Conclusion

While our findings of an age-related increase in basal cortisol levels are in concordance with previous studies, the results of generally higher cortisol levels in women than men of all ages are novel. We also confirm earlier findings of an inverse association between morning cortisol and abdominal obesity, however, only in women. Future studies should focus on intra-individual analysis and probably also account for reproductive hormones and stress exposure, as well as 24-h urinary cortisol and metabolites. Moreover, the possible association between cortisol and hip circumference needs to be further explored. To our knowledge, the associations in this study have never before been investigated in such a large population sample of both men and women. Our results therefore offer important knowledge on the descriptive characteristics of cortisol in relation to age and gender, and on the impact that associations previously seen between cortisol and abdominal obesity in smaller, selected samples have on a population level.

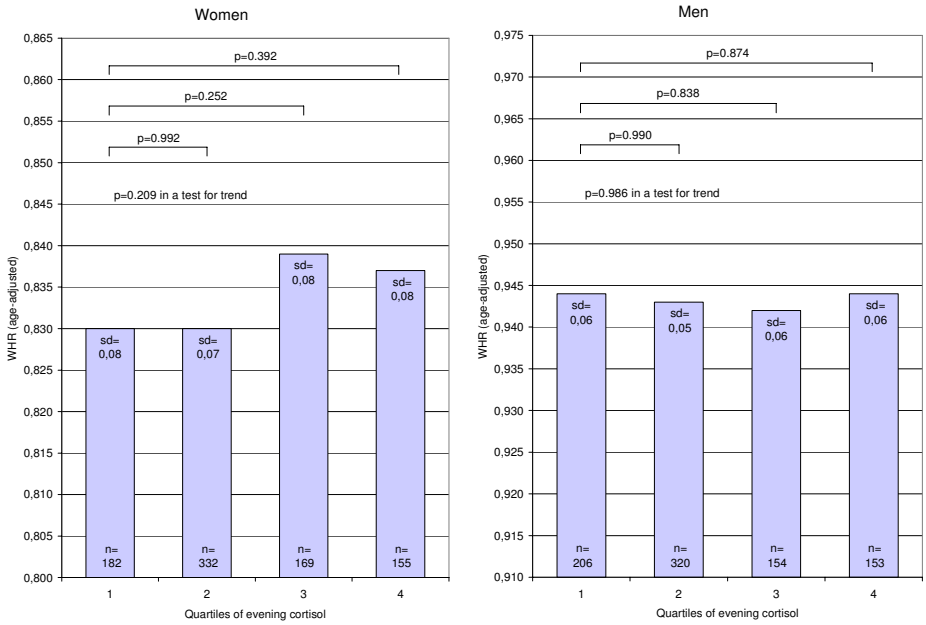

Figure 3
Age-adjusted means of WHR (waist-hip ratio) by quartiles of evening cortisol.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CAL prepared the data, performed the statistical analyses, drafted the manuscript, and took part in conceiving the study. BG offered statistical expertise and performed some of the statistical analyses. LR conceived the study and acquired the data. UL conceived and coordinated the study, and acquired the data. All authors took part in the design of the study, the interpretation of data, the revision of the manuscript, and read and approved the final manuscript.

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