Endogenous sex hormones and cardiometabolic risk factors –

population-based studies within the Skaraborg Project

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Abstract

Cardiovascular diseases are the major contributors to mortality in Sweden and globally. Men have a higher incidence of cardiovascular diseases compared to women, until women reach the menopause. Levels of sex hormones might explain these sex differences beyond known differences in risk factors. The overall aim of this thesis was to investigate the associations between sex hormones and known cardiometabolic risk factors.

Cohort studies in Vara and Skövde, based on a random sample of the population, were conducted. The first visit took place in 2002-2005 including 2,816 participants aged 30-74 years (50% men). The second visit in 2012-2014 included a representative sample of 1,327 participants. Papers I-II are based on this cohort. In 2018 we analyzed eight different sex hormones by a validated high sensitivity liquid chromatography-tandem mass spectrometry in a subset of 240 women who were ≥50 years of age at visit 1. Papers III-IV are studying this sub-cohort.

Study I showed a significant inverse association between testosterone and insulin resistance in men, both in the cross-sectional analysis and after approximately 10 years' follow-up. However, no significant association between insulin resistance at visit 1 and testosterone levels at visit 2 was found. Study II found a strong and significant inverse association between levels of sex hormone-binding globulin and insulin resistance in both men and women, also when the female group was stratified for age (</≥ 50 years old) or menopausal status. Study III addressed the association between known cardiometabolic risk factors and sex hormones in postmenopausal women. The waist-to-hip ratio was mainly associated with androgens and BMI was associated with estrogens. Study IV showed significant positive associations between estrone, progesterone and testosterone and the revised Framingham stroke risk profile after adjustments were made for confounders.

These studies found significant associations between levels of sex hormones and cardiometabolic risk factors. This new knowledge will contribute to the understanding of sex differences in the development of cardiovascular diseases and may contribute to add further precision to the risk stratification of individuals.

Keywords: Sex hormones, sex hormone binding globulin, cardiometabolic risk factors, insulin resistance, post-menopause, cardiovascular diseases
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Sammanfattning på svenska

Hjärt- och kärlsjukdomar är vanliga hos både män och kvinnor och är den ledande orsaken till sjuklighet och dödlighet i Sverige och globalt. Det finns könsskillnader i förekomsten av hjärt- och kärlsjukdomar. Kvinnor anses vara relativt skyddade mot hjärt-kärlsjukdomar fram till klimakteriet, jämfört med män i samma ålder.

Det övergripande syftet med denna avhandling var att undersöka sambandet mellan könshormoner och kända riskfaktorer för hjärt-kärlsjukdom, hos män och kvinnor, i en befolkningsstudie inom Skaraborgsprojektet. Studierna som ingår är observationsstudier, med både tvärsnitts- och longitudinella analyser.

Kohorten i alla delarbeten är Vara Skövde-kohorten (VSC), som består av ett slumpmässigt urval av befolkningen i två kommuner i sydvästra Sverige. Den första undersökningen utfördes under 2002-2005. Deltagandefrekvensen var 76 % och 2816 (49,7 % män) deltagare i åldrarna 30-74 år var inkluderade i studien vid besök 1. En uppföljningsundersökning gjordes 2012-2014 på ett representativt urval, med 1327 deltagare. Deltagarna undersöktes av särskilt utbildade sjuksköterskor och blodprover togs efter en natts fasta. Samtliga blodprover försvarades nedfrysta i biobank. Samma undersökningsprotokoll användes vid båda besöken. Under 2018 utfördes kompletterande analyser på frysta prover av åtta olika könshormoner med en validerad högkänslig vätskekromatografi-tandem mass-spektrometri i en grupp av 240 kvinnor som var ≥50 år vid besök 1. Denna grupp studerades i artikel III-IV. I artikel I var det specifika syftet att fastställa det möjliga dubbelriktade sambandet mellan testosteron och HOMA-Ir, ett mått på insulinresistens, hos män. Studien fann signifikanta samband mellan låg testosteronnivå och högre HOMA-Ir både i tvärsnittsanalysen och efter cirka 10 års uppföljning, även efter att justeringar gjorts för möjliga förväxlingsfaktorer. Det fanns dock inget signifikant samband mellan HOMA-Ir vid besök 1 och testosteronnivåer vid besök 2. Artikel II syftade till att fastställa sambandet mellan det hormonbindande proteinet SHBG, och HOMA-Ir hos män och kvinnor. Det framkom starka och signifikanta omvända samband mellan SHBG-nivåerna och HOMA-Ir hos både män och kvinnor, även när gruppen kvinnor var uppdelad i åldersgrupper (</≥ 50 år) eller utefter

om de genomgått klimakteriet. Resultaten återfanns även i de longitudinella analyserna. Artikel III syftade till att hitta sambandet mellan kända kardiometabola riskfaktorer och åtta olika könshormoner (17-α-hydroxyprogesteron, estron, dihydrotestosteron, estradiol, androstenedion, testosteron, dehydroepiandrosteron, och progesteron) hos kvinnor som passerat klimakteriet. Midje-höftkvoten var huvudsakligen associerad med androgener och BMI var associerad med östrogener. Samma grupp av kvinnliga deltagare studerades i artikel IV, som syftade till att undersöka sambandet mellan de åtta könshormonerna och den reviderade Framingham Stroke Risk Profile, ett mått på uppskattad risk för stroke efter 10 år. Signifikanta positiva samband mellan östron, progesteron och testosteron hittades i dessa analyser.

Huvudslutsatserna i denna avhandling är att låga testosteronkoncentrationer hos män, och låga SHBG-koncentrationer hos både män och kvinnor är associerade med insulinresistens, både i tvärsnittsanalyser och efter 10 års uppföljning. Av de kardiometabola riskfaktorerna är variabler som rör kroppskonstitution i störst utsträckning relaterade till nivåerna av könshormoner hos kvinnor efter klimakteriet, och vi fann även att testosteron, östron och progesteron är förknippade med högre uppskattad risk att utveckla stroke efter 10 år hos dessa kvinnor. Studierna bidrar till kunskap om könshormoner och deras samband med kardiometabola riskfaktorer. Kunskap om könsskillnader när det gäller förekomst av hjärt- och kärlsjukdomar, och sambandet mellan könshormoner och hjärt- och kärlsjukdomar i olika åldrar kan ge ytterligare precision i riskskattning av individer.

List of papers

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I: **Ottarsdottir K**, Nilsson A G, Hellgren M, Lindblad U, and Daka B. The Association between Serum Testosterone and Insulin Resistance: A Longitudinal Study. Endocrine Connections, 2018, Vol. 7, Iss. 12, Pp. 1491-.1500 7.12 (2018): 1491-500. Print

II: **Ottarsdottir K**, Hellgren M, Bock D, Nilsson A G, and Daka B. Longitudinal Associations between Sex Hormone-binding Globulin and Insulin Resistance. Endocrine Connections, 2020, Vol. 9, Iss. 5, Pp. 418-.425 9.5 (2020): 418-25. Print.

III: **Ottarsdottir K**, Tivesten Å, Li Y, Lindblad U, Hellgren M, Ohlsson C, and Daka B. Cardiometabolic Risk Factors and Endogenous Sex Hormones in Postmenopausal Women: A Cross-Sectional Study. Journal of the Endocrine Society 6.6 (2022): Bvac050. Web.

IV: **Ottarsdottir K**, Tivesten Å, Ohlsson C, Li Y, Hellgren M, Lindblad U and Daka B. Endogenous sex hormones levels are associated with the revised Framingham Stroke Risk Profile in postmenopausal women – a cross sectional study in a Swedish cohort. Manuscript.

Paper by the author not included in the thesis

V: Osmancevic, A., **Ottarsdottir K**., Hellgren M., Lindblad U, and Daka B. (2022). High C-reactive protein is associated with increased risk of biochemical hypogonadism: a population-based cohort study, Endocrine Connections, 11(7), e220141

Contents

Abbreviations

BMI	Body mass index
BP	Blood pressure
CVD	Cardiovascular disease
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DM	Diabetes mellitus
HDL	High-density lipoprotein
HOMA-Ir	Homeostasis model assessment of insulin resistance
HPA-axis	Hypothalamic pituitary adrenal axis
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LDL	Low-density lipoprotein
LTPA	Leisure time physical activity
MS	Mass spectrometry
PCOS	Polycystic ovarian syndrome
SHBG	Sex hormone binding globulin
SWAN	Study of Women’s health Across Nations
TG	Triglycerides

VSC	Vara Skövde Cohort
WHI	Women´s health initiative
WHR	Waist-to-hip ratio

Introduction

Cardiometabolic health in men and women

Cardiovascular diseases, including coronary heart disease and stroke, are the leading causes of death in Sweden[1] and globally[2]. Even though the incidence of cardiovascular diseases has decreased during the last decade in both men and women, the incidence differs between men and age-matched women[3]. Until menopause, women are relatively protected against both morbidity and mortality from coronary heart disease and stroke. However, after menopause, this difference decreases and women have an even higher incidence of stroke than men in the higher age groups[4], and women also have a higher death rate from stroke than men[5]. The reason for these sex differences in incidence of cardiovascular diseases between groups may be explained by sex differences in lifestyle habits related to known cardiovascular risk factors such as smoking, alcohol consumption and stress. However, adjusting for above-mentioned risk factors could not completely explain these differences when it comes to the incidence of myocardial infarction [6]. Therefore, other biological markers such as sex hormones might also partially explain these differences. The relatively sharp increase in incidence of CVD in women during the years after menopause suggests that the hormonal change may play an important role in their total risk profile.

Diabetes mellitus type 2 is the most prevalent diabetes type, accounting for more than 90 % of diabetes cases[7]. Globally, the prevalence of diabetes is only slightly lower in women than in men, and increases with age[7]. Impaired glucose tolerance and impaired fasting glucose, are forms of pre-diabetes that have a lower prevalence than diabetes[7].

Cardiometabolic risk factors

Among the risk factors for cardiovascular disease, some are not modifiable, such as age, sex, ethnicity and family history, whereas others are modifiable through lifestyle changes and medication. These include high blood pressure, high LDL-cholesterol, high BMI, high fasting plasma glucose, smoking, alcohol use and sedentary life style[8,9]. Among risk factors not discussed in detail in this thesis are air pollution, kidney dysfunction, ethnicity, family history, dietary risk factors and socioeconomic status[8].

In this thesis the terms sex and gender will be used. The term sex refers to the genetic expression of the individual, and the hormonal profiles and reproductive anatomy that are results of that genetic expression. Gender refers to the social or cultural roles, behavior and norms associated with being a woman or a man in a society[10,11].

High blood pressure

High blood pressure, hypertension, is the risk factor contributing the most to morbidity due to cardiovascular disease[8]. The current European guidelines define grade 1 hypertension as blood pressure ≥ 140 mmHg systolic and/or ≥ 90 mm Hg diastolic[12], whereas the current American guidelines according to the American Heart Association define 130-139 systolic or 80-89 mm Hg diastolic as stage 1 hypertension[13]. (Table 1).

Table 1: Current definitions regarding blood pressure levels.

American guidelines			European guidelines		
	SBP	DBP		SBP	DBP
Normal BP	<120	<80	Optimal BP	<120	<80
Elevated BP	120-129	<80	Normal BP	120-129	80-84
Stage 1 HT	130-139	80-89	High normal BP	130-139	85-89
Stage 2 HT	≥140	≥ 90	Grade 1 HT	140-159	90-99
			Grade 2 HT	160-179	100-109
			Grade 3 HT	≥180	≥110

SBP Systolic blood pressure, DBP diastolic blood pressure in mmHg. BP Blood pressure, HT Hypertension

Observational studies and meta-analyses have consistently shown strong associations between higher blood pressure levels and morbidity and mortality in CVD[14,15]. Another large meta-analysis has shown significantly reduced risks of CVD events such as myocardial infarction, heart failure and stroke with decrease in systolic blood pressure[16]. The prevalence of hypertension is high, and WHO estimates that about 1.4 billion people have hypertension globally[17]. It influences stroke risk in a similar manner in both men and women, who both benefit from decreasing high blood pressure levels[5]. Healthy women typically have lower blood pressure than men, but at the same time they seem to have a higher risk of CVD at lower blood pressure levels compared to men[18].

Insulin resistance and type 2 diabetes

Diabetes is a major risk factors for developing CVD, in both men and women[19]. Insulin resistance is a state where the insulin-sensitive tissues such as liver, muscle and adipose tissue become less sensitive to insulin effects, leading to less uptake of glucose and subsequent higher blood glucose levels[20]. Initially, impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) are observed[20]. Both states associate with development of type 2 diabetes. Later on beta cell dysfunction contributes further to the higher blood glucose and impaired glucose tolerance[20]. Both

insulin resistance and beta-cell dysfunctions are pre-diabetic states, since they occur early in type 2 diabetes development, however there is no consensus document on how to define pre-diabetes (Table 2). According to the World Health Organization (WHO)[21], IFG corresponds to fasting glucose value of 6.1-6.9 mmol/L and IGT is present when an oral glucose tolerance test (OGTT) results in blood glucose levels of ≥ 7.8 <11.1 mmol/L, with a fasting glucose of <7.0 mmol/L. According to the same guidelines, diabetes is diagnosed if fasting glucose ≥7.0 mmol/L or glucose level after a 2-hour OGTT is ≥ 11.1 mmol/L. The measurements have to be repeated or combined in order to confirm the diagnosis. American Diabetes Association (ADA) has the same limits, with the exception that impaired fasting glucose is defined as fasting glucose between 5.6-6.9 mmol/L [22].

Table 2: Blood glucose levels defining pre-diabetic states and diabetes according to WHO and ADA.

	WHO (mmol/L)	ADA (mmol/L)
Normal fasting glucose	<6.0	<5.6
IFG	6.1-6.9	5.6-6.9
IGT (OGTT)	≥7.8-11.0	7.8-11.0
Diabetes (fasting glucose)	≥7.0	≥7.0
Diabetes OGTT	≥ 11.1	≥ 11.1

Both diabetes[20] and insulin resistance[23] are associated with an increased risk for developing cardiovascular diseases. However, diabetes is a stronger risk factor for stroke and coronary heart disease in women than in men[11,24], and the protective role of female sex on myocardial infarction seems to disappear when women develop diabetes[25]. This is believed to be a result of endocrine factors such as the attenuated effect of protective estradiol on the blood vessels, but may also be because women with type 2 diabetes are diagnosed later than men, and at a higher BMI[11], and receive different treatment for secondary prevention compared to men[24].

Overweight and obesity

Overweight (BMI ≥ 25 kg/m^2) and obesity (BMI ≥ 30 kg/m^2) have complex genesis, with genetic, environmental and metabolic dysregulation factors acting together[26]. The prevalence of obesity has increased over time, and doubled since 1980 in more than 70 countries worldwide[27]. In Sweden, the prevalence of obesity is about 16% in the adult population, with 51% having a BMI of 25 kg/m^2 or above[28]. The prevalence of overweight increases with age, and is higher in men than in women. However, there are no sex differences in the prevalence of obesity in the Swedish adult population[28]. These are nevertheless self-reported data and may be subject to under-reporting[29].

Obesity is a strong risk factor for developing insulin resistance and type 2 diabetes[20]. Excess adipose tissue, especially the metabolically active visceral adiposity, is associated with elevated free fatty acids and release of adipocytokines, inflammatory signaling proteins which increase insulin secretion and contribute to insulin resistance [26,30]. The excess free fatty acids are associated with dyslipidemia which is a risk factor for coronary heart disease, and the link between obesity and cardiovascular diseases is further mediated by obesity-induced hypertension[26]. According to a large meta-analysis of 97 prospective cohorts, both overweight and obesity were significantly associated with higher risk of stroke and coronary heart disease. However, most of the excess risk was mediated through high blood pressure, high cholesterol and high blood glucose levels[31].

Dyslipidemia

There is strong evidence from epidemiological studies[32], genetic studies[33] and randomized controlled trials[34] that high levels of cholesterol, in particular low density lipoproteins (LDL) are strong risk factors for CVD. The main pathobiological mechanism is via the atherosclerosis-promoting effect of the accumulation of LDL in the arterial wall, leading to plaque formation[35]. There is evidence stating that higher total cholesterol is a stronger risk factor for men than women when it comes to coronary heart disease, whereas the risk increase is not as evident regarding stroke in either men or women[36].

Lifestyle factors

Maybe the most well-known risk factor for CVD is smoking, which is well studied with regard to both malignant diseases, lung diseases and cardiovascular diseases[37]. On average, individuals who are cigarette smokers die about 10 years before non-smokers[37]. Globally, men report a higher prevalence of current and former smoking[38], but the effect of smoking on the risk of myocardial infarction is higher in women than in men also after adjustments for other known risk factors for CVD[39,40].

Low physical activity and particularly sedentary behavior such as high level of total daily sitting time has been associated with increased risk of all-cause mortality, CVD mortality and incident diabetes mellitus type 2 in a meta-analysis of over 1,000,000 participants[41]. The mechanism behind this may be a reduction in muscle mass, decreased insulin sensitivity and an increase in adipose tissue[42]. Globally, women tend to have lower rate of recommended daily physical activity than men[43,44].

Alcohol consumption has long been known to be associated with cardiovascular diseases, but the association seems to be J-shaped, with a protective association with low-moderate (occasional daily consumption, ≤ 2 drinks per day for men, ≤ 1 drink per day for women) intake and a more deleterious effect with heavy alcohol intake (≥ 5 binge drinking episodes/month)[45]. It is worth noting that among the participants who register no intake of alcohol, there may be different reasons behind this, for instance previous alcohol problems, other illnesses, or medications[45], which may affect the associations. Furthermore, there is a U-shaped association between alcohol consumption and risk of type 2 diabetes[46]. Globally, men report higher alcohol intake than women[38], but women experience more adverse effects of alcohol at the same amount of intake, due to different alcohol metabolism and smaller body water volume, and generally smaller bodies [45].

Age and sex

Among the non-modifiable risk factors for cardiovascular disease are age and sex. As mentioned above, female sex is associated with lower cardiovascular risk profile and with less cardiovascular events, however, this changes after menopause. Age is a risk factor, contributing to a considerably increased risk of developing cardiovascular disease[47]. There is also

evidence that there is an interaction between age and many of the traditional risk factors, suggesting that many of the traditional risk factor lose their significance with aging, with regard to association with incident cardiovascular disease[48].

Risk assessment

One way of determining the risk of developing CVD is to use different scoring tools, where many of the risk factors are registered and put into an algorithm. The SCORE2 (Systematic coronary risk estimation) is recommended for use in clinical practice and estimates the 10-year risk of CVD for people aged 40-69 years old, whereas patients aged ≥70 years old are assessed with SCORE2-OP[49].
The original Framingham Stroke Risk Profile[50] and the revised Framingham Stroke Risk Profile[51], estimate the 10-year risk of stroke, taking age, current smoking, prevalent cardiovascular disease, prevalent atrial fibrillation, diabetes, hypertension and systolic blood pressure into account.

Endogenous sex hormones – physiology and synthesis

Figure 1 illustrates the biosynthesis of steroid hormones in both men and women. The main endogenous estrogens are estradiol, which is the most potent and prevalent in pre- and perimenopausal women, estriol (produced during pregnancy), and estrone, the dominant hormone in women after menopause. Among the endogenous androgens discussed here are dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), androstenedione and testosterone. Progesterone and 17-α-hydroxy-progesterone are called progestogens.

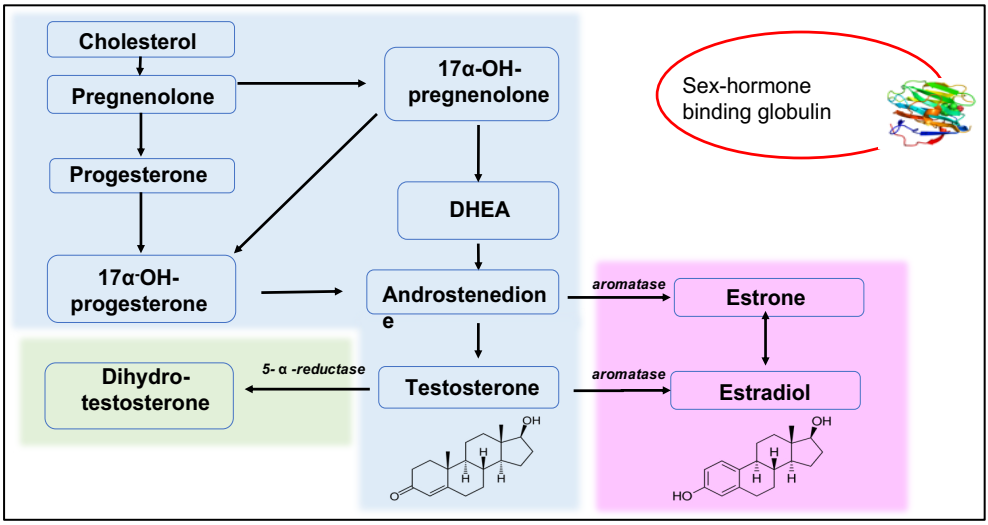

Figure 1 Sex steroid synthesis in men and women. The major source of sex steroid hormones is cholesterol. The first step of the steroidogenesis is placed in the adrenal cortex, the ovaries, and testes, respectively (blue box). The conversion from androgens to estrogens occurs mainly in the ovaries but also in peripheral tissues such as the adipose tissue (pink box). Testosterone is converted to DHT mainly in peripheral organs such as prostate, skin and epididymis (green box).

In men

The main **androgens** in men are testosterone (largest amount), DHT, and androstenedione, secreted by the Leydig cells of the testes. A small amount (< 5%) of active androgens is produced by the adrenal gland. Similar to all sex hormones, androgens are steroid compounds derived mainly from cholesterol. Testosterone is to 97% bound to sex hormone-binding globulin (SHBG) or albumin in the blood stream, reaching its target tissues, such as the prostate, where it is converted to DHT [52]. It is believed that the testosterone concentrations in men slowly decline with age, whereas the binding protein SHBG increases, resulting in a larger decline in free testosterone[53]. Low levels of testosterone in men have been associated with obesity[54], hypertension[55] and type 2 diabetes[56]. Subsequently, loss in visceral fat tissue has been associated with increase in testosterone levels in men[57].

There is also a smaller production of **estrogens** in males, about one-fifth of the amount in non-pregnant women. The largest amount of estradiol is produced by the liver by conversion from testosterone[52] but there is also conversion in peripheral tissues such as the adipose tissue[58] and prostate[59]. Low concentrations of estradiol, especially in combination with low testosterone levels, has been shown to predict mortality in elderly men[60], whereas high estradiol levels have been shown to be carcinogenic in the prostate gland epithelium and in animal models[59]. The role of estradiol in male cardiovascular health needs further investigation, as does the role of progesterone. **Progesterone** is produced in the adrenal cortex in men, and low progesterone as well as low estradiol associate with the prevalence of aortic aneurysm in men, but the role of progesterone in men with regard to cardiovascular disease is not fully understood[61].

Sex hormone-binding globulin is the main binding glycoprotein for sex steroids, and is produced in the liver. It rises with age in males[53]. Genetic[62,63] and epidemiological[64,65] studies have found an association between low SHBG and type 2 diabetes, the metabolic syndrome[66] and hypertension[67]. In a longitudinal study however, SHBG was significantly positively associated with increased risk of all-cause mortality, but not significantly associated with CVD mortality[68]. It is unclear however if the associations are mediated by other cardiometabolic variables or if the SHBG molecule itself has its own hormone-like activity[69-71].

Women

Premenopausal women have a cyclic secretion of sex hormones during the menstrual cycle. In the beginning of the cycle, estradiol from the ovaries is the dominant hormone, contributing to the maturation of follicles in the ovary. Mid-cycle, the mature follicle ovulates, and the cells of the follicle, called corpus luteum, start to produce high levels of estradiol and progesterone. If the egg is not fertilized, both estradiol and progesterone levels decrease sharply due to the degeneration of the corpus luteum. This results in a menstruation, and the cycle starts over. The cycle is controlled by the pituitary gland and will continue until menopause[52].

Estrogens are produced mainly in the ovaries and to a lesser extent in the adrenal gland. They are derived from cholesterol which is synthesized to progesterone or testosterone by the aromatase enzyme. Besides being

fundamental in puberty and pregnancy, estradiol has effects on body fat distribution, and also contributes to sodium and water retention in the kidneys[52]. Estradiol and estrone are converted in the liver to less potent estrogens such as estriol, and thereafter excreted via the bile or the urine. Estradiol in premenopausal women is believed to have a protective effect on the vessels, in promoting vasodilatation and being anti-atherogenic, besides improving the lipid profile[72,73].

Progesterone and 17-α-hydroxy-progesterone are called progestogens and have similar physiological effects. Progesterone is by far the most important since it is secreted in larger amounts. Progesterone is secreted from the ovaries during the luteal phase, and from the placenta during pregnancy. The main effect of progesterone before menopause is to prepare the endometrium of the uterus and the breast tissues for pregnancy. Progesterone is degraded in the liver[52].

Both estrogen and progesterone are transported in the blood stream by *sex hormone-binding globulin*, a glycoprotein that is inversely associated with cardiovascular risk factors such as blood glucose and adverse lipid profile[74], type 2 diabetes[62] and impaired glucose tolerance [75]. Low levels of SHBG are associated with polycystic ovarian syndrome (PCOS)[76], an hyperandrogenic state affecting about 5-18% of women[77]. PCOS is also associated with higher prevalence of type 2 diabetes, obesity, insulin resistance and dyslipidemia[77]. SHBG concentrations rise in pregnancy, with the use of oral contraceptives, and in states of anorexia[78].

During the natural **menopausal transition**, estradiol levels decline due to an age-related process where the function of the ovaries successively decreases[79]. This hormonal change is then followed by the cessation of menstruation. The perimenopausal period is from the start of irregularities of menstruation until 12 months after the final menstrual period[80]. Median age for menopause in Sweden is 52 years old [81], (globally 50 years old[82]), but the age of menopause is affected by complex interactions between genetic and socioeconomic health factors and can be affected by for instance smoking and body weight[83]. The menopausal transition is accompanied by weight gain[84], increases in carotid atherosclerosis, arterial stiffness[82], and an increase in cardiovascular disease incidence[85], although the increase in CVD incidence is probably also affected by aging[86]. The SWAN study found an increase in total cholesterol and LDL one year after menopause[87]. These changes were independent

from aging, whereas in the same study other cardiovascular risk factors such as blood pressure and blood glucose were correlated with age and not menopause status.

In **postmenopausal women**, estradiol and progesterone concentrations remain low. The evidence concerning the association between estrogens and cardiovascular diseases is conflicting. A positive association between estradiol and coronary heart disease[88] has been shown, whereas no association between estradiol and stroke was found[89]. The assumed protective role of exogenous estrogens on cardiovascular disease in the early postmenopausal period, and later detrimental effect in the late postmenopausal period has been called the timing effect [90]. This hypothesis is supported by both observational studies such as the Nurse's health study [91] and RCTs such as ELITE[92]. These studies investigated the exogenous estradiol in menopausal hormonal therapy, indicating that the effect of estradiol on vasodilatation may differ depending on the time elapsed since menopause[93].

The adrenal glands continue to produce androgens such as DHEA which is a precursor in the steroid synthesis. Conversion from DHEA into active sex steroids takes place in the peripheral tissues through a mechanism called intracrinology[94]. This conversion is made in many peripheral tissues such as vascular endothelium, bone, and brain tissue[95], except for the endometrium [96]. Higher levels of androgens in postmenopausal women have been associated with elevated breast cancer risk and visceral fat deposition[95], whereas a higher level of testosterone has been associated with a lower risk of hip fracture in postmenopausal women[97]. Regarding the risk of cardiovascular disease, studies have shown conflicting results. Among the more recent studies using reliable techniques on measurements of hormonal levels such as LC-MS, DHT has been positively associated with all-cause mortality, but not with CVD mortality[68], whereas another study found negative associations between testosterone and major adverse vascular events and also negative associations between DHEA and major adverse vascular events[98]. Yet another study found no association between testosterone and stroke in postmenopausal women[99].

The role of SHBG in postmenopausal women is to bind the sex hormones in the blood stream. There is evidence that low levels of SHBG are associated with type 2 diabetes[62], insulin resistance[100] and higher BMI and

WHR[101]. SHBG production declines with age in women, especially after menopause[76].

Finally, there is a large body of evidence showing that sex hormones are strongly associated with different cardiometabolic factors and may play a role in the cardiometabolic health of both men and women. However, the results are in many areas still inconclusive, and to a great degree this early research was conducted using less precise methods. Hence further investigations are needed.

Aims

General aim

The general aim of this thesis was to determine the associations between endogenous sex hormones and various cardiometabolic risk factors in women and in men, in cross-sectional and longitudinal study designs.

Specific aims

Study I

In this study we aimed to determine the association between testosterone and HOMA-Ir, as a measure of insulin resistance, cross-sectionally and after approximately 10 years of follow-up, in men. The purpose was also to explore if the association is bidirectional.

Study II

This study aimed to examine the association between SHBG and HOMA-Ir, in men and women, cross-sectionally and longitudinally after 10-years' follow-up.

Study III

In this study, the purpose was to explore which cardiometabolic risk factors were associated to the largest extent with 8 endogenous sex hormones in postmenopausal women.

Study IV

In Study IV the aim was to investigate the association between endogenous sex hormones and the revised Framingham Stroke Risk Profile, an estimated risk score for the 10-year risk of stroke, in postmenopausal women.

Subjects and methods

The Vara-Skövde Cohort

Participants

The Skaraborg Project started in 1977 with a medical care program in primary care aiming to improve blood pressure control and reduce the risk of developing cardiovascular diseases in patients with hypertension in the community[102]. Their prognosis was systematically surveilled[103]. When this trial was closed, the research program developed into a community-based epidemiological study of major risk factors of the metabolic syndrome, including life-style, behaviors and genetics, starting with a study of patients in Skara primary care[25,104] followed by a population survey in the same municipality[25]. In order to establish a younger and larger cohort, and using more advanced techniques, the Vara-Skövde Cohort (VSC) was added to the Skaraborg Project in 2002-2005.

The VSC consisted of a random computer-generated sample of a population aged 30-74 years living in two municipalities in south-western Sweden (Vara and Skövde)[105,106]. Participants were invited to take part in the survey between 2002-2005, and a total of 2,816 individuals were consecutively recruited into the study. From Vara, 1,811 individuals were included (participation rate 81%), and in the Skövde cohort 1,105 individuals were included (participation rate 70%). There was a threefold oversampling of individuals aged 50 years old and younger. A representative sample of 1954 participants were summoned to follow-up. In 2012 to 2014, 1,327 individuals were studied at the follow-up visit. The participation rate at follow-up was approximately 68 % and the study protocol was the same as at baseline (Figure 2).

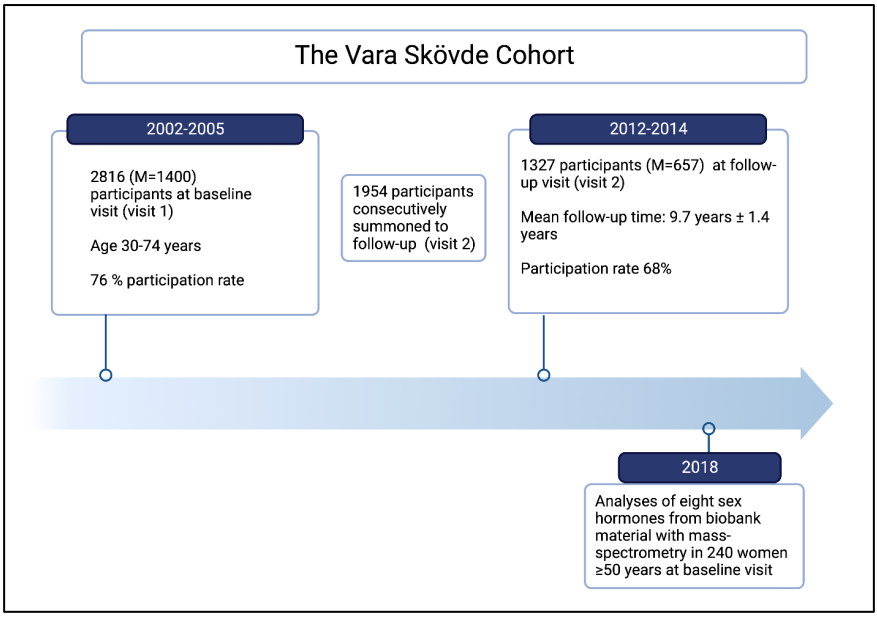

Figure 2: The Vara-Skövde Cohort within the Skaraborg Project. Created with BioRender.com.

The studies included are summarized in Table 3.

In Study I only men were included. After excluding subjects using insulin therapy, 1,282 men were included in the baseline study, and 546 men in the follow-up study. Only men with valid values of sex hormones, insulin resistance, and potential confounding covariate variables both at baseline and follow-up were included in the analyses.

In Study II all participants with valid information on insulin resistance and SHBG were included. Individuals with insulin therapy or hormone replacement therapy were excluded from the analyses. Furthermore, women who said that they had undergone pharmacological menopause were also excluded. Only individuals who could provide full information at baseline and follow-up were included in the analyses. A total of 1,193 (649 men; 323 women who were younger than 50 years old; 221 women 50 years old or older) individuals were included in the baseline analyses and 1,110 (649 men; 130 women who were younger than 50 years old; 331 women 50 years old or older) individuals at follow-up.

Study III: this study population included only women from the VSC who were 55 years old or older at baseline, and had measurements of sex hormones analyzed with LC-MS/MS. Women who reported usage of sys-

temic hormone replacement therapy were excluded as were participants who had estradiol concentrations ≥20 pg/mL. As no self-reported information regarding menopausal status was available, we selected participants that were 55 years of age or older. After exclusion, 146 women were included in the study.

In Study IV, participants from the group of women who had sex hormones analyzed by LC-MS/MS were included. The individuals were ≥55 years old. Participants who were using systemic hormone replacement therapy (n=32) and those with a history of previous stroke (n=4) were excluded. Only participants with estradiol concentrations less than 20 pg/mL were included, in order to limit the risk of including premenopausal women.

A total of 133 women were included in Study IV. When we conducted the regression analyses with the coronary heart risk score, the number of participants included was 125 due to exclusion of participants with previous cardiovascular disease.

Procedures

All participants were physically examined by specially educated and trained nurses. Blood pressure was measured in a supine and sitting position, and body height, waist circumference and body weight were measured with participants wearing light clothing. Fasting venous blood samples were drawn in the morning, and an oral glucose tolerance test was performed. The blood samples were immediately frozen at -82 degrees Celsius. The participants filled out questionnaires about smoking habits (current daily smoker yes/no), and alcohol intake (grams per week). Leisure time physical activity (LTPA) was defined in four different categories, sedentary, light LTPA, moderate LTPA and heavy LTPA[106]. Medical history and information about current medication were obtained. Hypertension was defined as a blood pressure above 140/90 mmHg in accordance with the JNC8[8], and diabetes was diagnosed according to the WHO definition[107].

Assessment of sex hormones and SHBG

Radioimmunoassay

In Studies I and II, radioimmunoassays (RIA) were used for analyzing testosterone and sex hormone-binding globulin (SHBG). Bioavailable testosterone was calculated using the formula by Vermeulen et al[108] (Study I). Due to a change in the reagent used in the analysis method (From Access Testosterone assay Beckman-Coulter to Elecsys Testosterone II Assay, Roche diagnostics) during the follow-up time, testosterone levels were higher in the follow-up survey compared to baseline levels. Since we did not have access to an algorithm to adjust for this change in methods, change in testosterone over time was not possible to use in the analyses.

Liquid chromatography-tandem mass spectrometry

In 2018, we completed measurements of eight different sex hormones with a high-sensitivity liquid chromatography-tandem mass spectrometry assay[61] in 240 women who were all 50 years of age and over at baseline, and who also participated in the second visit. The hormones measured were: 17-α-hydroxy-progesterone, androstenedione, DHEA, DHT, estrone, estradiol, progesterone, and testosterone. The variables from these analyses were used in Studies III-IV.

Description of outcomes

Papers I-II: Insulin measurements were made with immune assay Roche Cobas (baseline) and DxL Beckman (follow-up). Due to this change in methods the insulin values were about 35 % higher at follow-up compared to baseline. The insulin values were therefore re-calculated using the formula new method=1.3544 x old method-0.3237, giving a correlation between methods r^2=0.9974. Glucose was measured at fasting in the morning. HOMA-ir was calculated using the formula by Matthews et al[109].

Paper III: Outcome variables were the sex hormones measured by LC-MS as described above. The hormones were natural logarithm transformed and used as continuous variables.
Paper IV: The revised Framingham Stroke Risk Profile was calculated according to the formula by Dufouil et al.[51] and used as a continuous variable. It takes numerous variables in account: age, current smoking, prevalent cardiovascular disease, prevalent atrial fibrillation, diabetes, hypertension and systolic blood pressure. We also made regression models with estimated risk for coronary heart disease as outcome variable. This algorithm by Wilson et al[110] includes age, total cholesterol, high-density lipids, blood pressure, smoking status and diabetes.

Statistical analyses

Paper I

The study population was described using descriptive statistics, both at baseline and at follow-up. The outcome variable (HOMA-Ir) was log-transformed due to skewness in the variable. Linear regression models were built based on theoretical models and thereafter linear regression analyses were performed to investigate the cross-sectional associations at baseline and follow-up respectively. Either testosterone or bioavailable testosterone were used as explanatory variables, and adjustments were made for possible confounders (age, smoking, LTPA, alcohol intake, WHR, LDL, CRP, hypertension and diabetes). In the longitudinal analyses we also adjusted for HOMA-Ir at baseline, besides the above-mentioned baseline variables. In order to investigate the variance in HOMA-IR in different quartiles of testosterone levels, we performed a general linear model, where pairwise comparisons were made and the lowest quartile was the reference. We also completed the analyses with age-stratified analyses with participants over and under 50 years of age at baseline. All analyses were done using IBM SPSS Statistics, version 24.

Paper II

Descriptive statistics were used to describe the study population at baseline and follow-up respectively. The functional form of the relationship between SHBG and HOMA-Ir was graphically investigated using splines, and a loglinear association was assumed. HOMA-Ir was log-transformed prior to the analyses. The analyses were stratified for age and sex, and the participants were divided into groups; women aged ≥ 50, women aged < 50, and men. We used a linear regression model with logHOMA-Ir as the dependent variable, and SHBG, group, and interaction term SHBG*group as independent variables. Potential confounders were identified from theoretical models and adjusted for age, current smoking, alcohol intake, WHR, LDL, CRP, hypertension, and diabetes in linear regression models. All variables were standardized to zero mean prior to the analyses and unit standard deviation. The results were thereafter presented as standardized regression coefficients with 95% confidence intervals and P-values. The cross-sectional analyses were performed separately for baseline and follow-up. In order to investigate the possible predictive association between SHBG at baseline and HOMA-Ir at follow-upon at group level, we conducted a linear regression model with logHOMA-IR (follow-up) as a dependent variable and SHBG and group belonging as predictive variables. As a covariate baseline HOMA-Ir was used, as well as the covariates mentioned above. Based on previous studies, we defined insulin resistance as the highest quartile in each group. The correlation between SHBG level at baseline and insulin resistance at follow-up was investigated in a logistic regression model using the same covariates as above. The results from these analyses were reported as odds-ratios with 95% confidence interval and p values. Analyses were performed in IBM SPSS statistics version 24, and SAS software (SAS institute inc.).

Paper III

Descriptive statistics were used to present the study population. The distribution of sex hormone concentrations was presented separately for each hormone and a normality test was performed using the Shapiro Wilks method. Due to skewness in the concentrations the hormone variables were log-transformed. Backward variable selection with Akaike information criterion was performed. In this method we used a set of

cardiometabolic variables (BMI, smoking, WHR, diabetes, hypertension, alcohol consumption, LTPA, age, LDL, hsCRP, HOMA-Ir, creatinine, triglycerides, and HOMA-Bc) to see which of the variables could best explain the hormone levels. In order to reduce the uncertainty that variable selection can introduce, bootstrap stability investigations were performed. This method draws N resamples from the original data, and repeats the variable selection in each set of resamples. We drew 1,000 sets of resamples, and variables that were selected in the variable selection in more than 50% of the resamples, and were significantly associated with the outcome were selected in the multiple regression model. All analyses were performed using R version 4.0.2.

Paper IV

Descriptive statistics were performed to present the baseline characteristics of the study population. The revised Framingham Stroke Risk Profile was thereafter calculated for each participant and log-transformed. Regression analyses were conducted using the log-rFSRP as the dependent variable. Regression models were built, in order to also adjust for additional possible confounding factors. Model 1 adjusted for BMI and model 2 adjusted for BMI, CRP and total cholesterol, since these variables are not included in the algorithm of rFSRP. All of the eight sex hormones were used as explanatory variables. We also calculated an estimation of the 10-year risk for coronary heart disease according to Wilson et al, and similar regression models were performed using that score system as dependent variable. All analyses were performed using SPSS version 20 or R version 4.0.2.

Paper	Design	Subjects	Statistical methods	Exposure	Outcome(s)
I	Prospective l study	Vara-Skövde cohort (men) N=1282	Linear regression, general linear models	Testosterone	HOMA-Ir
II	Prospective study	Vara-Skövde cohort N=649 men, 544 women	Linear regression, logistic regression	SHBG	HOMA-Ir
III	Cross-sectional study	Sub-cohort from the Vara-Skövde cohort, postmenopausal women, N=146	Variable selection, bootstrap stability investigation, linear regression	BMI, smoking, WHR, diabetes, hypertension, alcohol consumption, leisure time physical activity, age, LDL, hsCRP, HOMA-Ir, creatinine, triglycerides, and HOMA-Bc	estradiol, estrone, dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), androstenedione, progesterone, testosterone, and α17-α-hydroxyprogesterone
IV	Cross-sectional study	Sub-cohort from the Vara-Skövde cohort, postmenopausal women, N=133	Linear regression	estradiol, estrone, dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), androstenedione, progesterone, testosterone, and 17-α-hydroxyprogesterone	Revised Framingham Stroke Risk Profile

Table 3. Summarizing table of the included studies.

Ethical considerations

All participants in the Skaraborg Project have given their written informed consent to participate in the study. The participants were informed about the purpose and benefits of the project and potential harm that was associated with the study before inclusion in the study. The potential harm of participating could be discovery of not previously known diseases or laboratory results indicating disease. In those cases, the participant was offered a referral to his or her health care center for follow-up. This may lead to distress and anxiety, but there may also be benefits from revealing potentially harmful diseases early, and so be able to initiate preventive care. The Regional Ethical Review Board in Gothenburg, Sweden has approved the study (Studies I-IV: D-nr, Ö199-01; Studies I-II: D-nr 036-12).

Results and discussion

Paper I

A significant inverse association between testosterone levels and HOMA-Ir was found in this study. Analyses were made cross-sectionally at baseline (n=1282), at follow-up (n=546) and thereafter a longitudinal analysis (n=578) was conducted. The mean follow-up time was 9.7 (SD 1.4) years and the mean ages at baseline and follow-up were 47.3 (SD 11.4) years old and 57.7 (SD 11.6) years old respectively.

At baseline, we found an inverse association between testosterone and HOMA-Ir in the crude model, when adjusting for age, and when adjusting for known possible confounders such as smoking, diabetes, hypertension, WHR, leisure time physical activity, alcohol intake, LDL and CRP (β=-0.140, $p<0.001$). The results were similar in the cross-sectional analyses at follow-up: (β=-0.181, $p<0.001$, fully adjusted model). Similar associations were observed when we investigated the association between bioavailable testosterone and HOMA-ir, however the significance of the association was lost in the final model when WHR was included. This may be a result of overadjustment, given the close correlation between WHR and HOMA-Ir . The results remained the same even after stratification into age groups above and under 50 years of age. Meanwhile, in the cross-sectional analysis at follow-up, bioavailable testosterone was significantly inversely associated with HOMA-Ir also in the fully adjusted model (β=-0.114, p=0.004).

In the longitudinal analyses, we used the same models for adjustments as in the cross-sectional analyses, with additional adjustment for HOMA-Ir-level at baseline (Table 4). Here, we found a significant inverse association in all models for both testosterone (β=-0.096, p=0.006) and for bioavailable testosterone (β=-0.079, $p<0.035$). To further investigate the variance of HOMA-IR in different quartiles of testosterone we performed general linear models. Men having testosterone concentrations within the lowest quartile of testosterone at baseline had the highest HOMA-Ir at follow-up, and this was significantly different from quartile 2 (p=0.008)

and quartile 3 (p=0.001), although not significantly higher than quartile 4 (p=0.052). To further explore if there was a bidirectional association between HOMA-Ir and testosterone, regression analyses were performed with HOMA-Ir as the explanatory variable and testosterone/bioavailable testosterone as dependent variables. In these analyses, the crude models were significant (testosterone: β=-0.164, p=0.001) (bioavailable testosterone β=-0.089, p=0.032). However, when adjustments were made for testosterone levels at baseline, no significant associations were present in any model of the multivariable regression analyses.

This study found that there was a strong longitudinal association between testosterone at baseline and HOMA-Ir at follow-up, even when adjustments were made for confounding factors including WHR. However, there were no significant associations found in the other direction. The role of testosterone in men with regard to cardiovascular and metabolic health has been studied for many decades[111,112] and the results of Study I in this thesis are in line with many of these studies. In the Massachusetts male aging study, low testosterone levels predicted development of the metabolic syndrome in men with BMI<25 km/m^2 [113] and low bioavailable testosterone concentrations in men >20 years old in the NHANES study have been shown to associate with diabetes regardless of BMI[114]. However, in a cohort of elderly men there were only associations between DHT and diabetes, but not with free ztestosterone[115]. Likewise, in a study of both young and elderly men, there was a significant association between DHT and fat mass and insulin resistance[116]. There are furthermore studies investigating the effects of exogenous testosterone administration, stating beneficial effects on insulin resistance and lipid profile in hypogonadal men with metabolic syndrome or type 2 diabetes[117], suggesting a role of androgens in glucose metabolism.

In this study no bidirectional association between testosterone and insulin resistance was found. The evidence for this direction of causality is scarce. Insulin resistance is closely related to obesity, and the theoretical model behind the bidirectional theory might reflect the bidirectional relationship between obesity and sex hormones, as shown in Figure 3. However, one study found an association between the metabolic syndrome and lower testosterone levels 11 years later in middle-aged men, although the insulin level at baseline was not significantly associated with testosterone levels at follow-up, suggesting other mechanisms than insulin resistance behind this association[118].

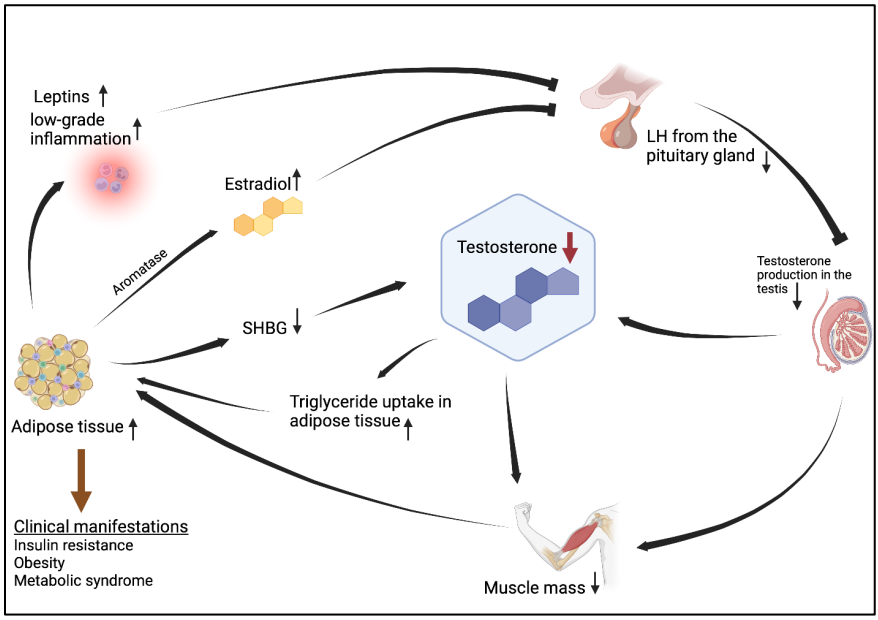

Figure 3. Illustration of the bidirectional relationship between low testosterone concentration and obesity/metabolic disease.
Low testosterone concentrations increase the levels of free fatty acid uptake in adipocytes, leading to an increase in the visceral adipose tissue. Aromatase activity increases proportionally in the adipose tissue, increasing the conversion from testosterone to estradiol. The metabolically active visceral fat deposit releases inflammatory mediators, and together with estradiol the inflammatory mediators suppress the hypothalamic pituitary regulation and decrease in LH release leading to a decrease in total testosterone. Visceral adiposity is associated with lower levels of SHBG. Created with BioRender.com.

Table 4: Results from longitudinal analyses showing the association between insulin resistance measured as lgHOMA-Ir at follow-up and sex hormones (total testosterone and bioavailable testosterone) at baseline.

Total serum testosterone (*N* = 578)		**Bioavailable testosterone** (*N* = 578)	
β	*P*	*β*	*P*
Model 1 Adjusted for age and baseline lgHOMA-Ir			
−0.147	<0.001	−0.114	0.003
Model 2 Adjusted for age, baseline lgHOMA-Ir, smoking, alcohol intake and PA			
−0.140	<0.001	−0.109	0.005
Model 3 Adjusted as in model 2 + whr			
−0.102	0.004	−0.083	0.027
Model 4 Adjusted as in model 3 + LDL, CRP, DM, HT			
−0.096	0.006	−0.079	0.035

Dependent variable: log-transformed HOMA-Ir.
CRP, c-reactive protein; DM, diabetes mellitus; HT, hypertension; LDL, low density lipoprotein; PA, physical activity; Whr, waist–hip-ratio.

Paper II

In this study, we studied the cross-sectional and longitudinal associations between sex hormone-binding globulin and HOMA-Ir, in men and women within the Skaraborg Project, with a mean follow-up time of 9.7 (SD 1.4) years. The mean age was 49.2 (SD 11.6) years old for men. Women were stratified based on age <50 years old and ≥50 years old and the mean ages of those groups were 40.9 (SD 5.4) years old and 60.8 (SD 6.9) years old respectively. The number of participants included, after exclusion of individuals with insulin therapy or hormonal therapy, was 1,193 (649 men, 323 women < 50 years of age, 221 women ≥50 years of age) at baseline. At follow-up the corresponding numbers of participants were 1,110 (649 men, 130 premenopausal women, 331 postmenopausal women).

In the cross-sectional analyses, there were significant inverse associations between SHBG and HOMA-Ir at baseline in both men (β= -0.20, p<0.001), women < 50 years old (β=-0.26, p=<0.001) and women 50 years old and older (β=-0.13, p=0.046) in all of the models including the fully adjusted model (adjustments were made for age, smoking, alcohol

intake, physical activity, WHR, LDL, CRP, diabetes status and hypertension). The results were similar in the cross-sectional follow-up analyses; men: β=-0.29, $p<0.001$, premenopausal women: β=-0.22, $p=0.001$, postmenopausal women β=-0.17, $p<0.001$ (fully adjusted models).

In longitudinal analyses (Table 5), we found significant associations between low levels of SHBG and high levels of insulin resistance in men: β=-0.16, $p<0.001$, and women <50 years of age: β=-0.16, $p<0.001$ in all models, however, in women ≥50 years of age significance was lost in the fully adjusted model: β=-0.07, $p=0.197$.
We further found that an increase in 10 nmol/L of SHBG was associated with a 18-22% decrease in odds of having insulin resistance at follow-up (men OR=0.81, CI: 0.65-0.99, P=0.042, women <50: OR=0.82, CI=0.71-0.94, p=0.006, women ≥50: OR=0.78, CI=0.64-0.94, p=0.009) in the fully adjusted model.

Table 5. Association between SHBG at visit 1 and logHOMA-Ir at visit 2.

Men (n = 649)		**Women <50 years of age at visit 1** (n = 130)		**Women ≥50 years of age at visit 1** (n = 331)	
β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Model 1, adjusted for age					
−0.17 (−0.24; −0.11)	<0.001	−0.19 (−0.28; −0.11)	<0.001	−0.20 (−0.31; −0.10)	<0.001
Model 2, adjusted for age, smoking, alcohol intake, PA, WHR					
−0.16 (−0.23; −0.09)	<0.001	−0.15 (−0.24; −0.06)	0.001	−0.17 (−0.28; −0.05)	0.005
Model 3, adjusted as in model 2 + LDL, CRP, DM, HT					
−0.16 (−0.23; −0.09)	<0.001	−0.16 (−0.25; −0.08)	<0.001	−0.07 (−0.18; 0.04)	0.197

Dependent variable: LogHOMA-Ir at visit 2. Due to the skewness of the variable, log-transformed HOMAIr was used in these analyses.
Adj, adjusted; CRP, C-reactive protein;DM, diabetes mellitus; HT, hypertension; LDL, low-density lipoprotein; PA, physical activity; SHBG, sex hormone-binding globulin; WHR, waist-hip-ratio.

This study revealed significant inverse associations between SHBG and HOMA-Ir in men, premenopausal and postmenopausal women in the cross-sectional analyses at both visit 1 and 2, and in the longitudinal model except for the group of women ≥50 years of age, in the fully adjusted model. The previous research on SHBG in premenopausal women has in large parts been conducted on women with PCOS, an hyperandrogenic state associated with low SHBG levels and insulin resistance[119]. In a cross-sectional cohort study of both premenopausal (n=125) and postmenopausal (n=104) women, a significant association was found between SHBG and HOMA-IR only in postmenopausal women[100]. In that study the mean BMI was 36kg/m^2 for the postmenopausal group, which is higher than in our cohort, however, the association was significant even after adjustments for BMI were made. Another cohort study including more than 700 women found inverse significant associations between SHBG and fasting insulin in both premenopausal and postmenopausal women, but the association between SHBG and fasting glucose was only significant in the postmenopausal group[120]. The association in men has been studied in prospective studies using HOMA-Ir as outcome, with similar results to our study[115,121]. Our results suggest that there is a link between SHBG and glycemic impairment that precedes the development of type 2 diabetes and shows that SHBG is associated with glycemic alterations even before diabetes has developed. If SHBG has its own hormonal effect on glucose metabolism, however, is unclear and needs further studies.

Paper III

This study aimed to explore which cardiometabolic risk factors were to the greatest extent associated with the variation in endogenous sex hormonal levels in postmenopausal women. In this study 146 postmenopausal women were included, all of whom were ≥55 years of age, and had no hormonal therapy, and did not have higher levels of estradiol than 20 pg/mL. While BMI was significantly associated with estradiol (B=0.054, p<0.001) and 17-α-hydroxy-progesterone (B=-0.023, p=0.028), WHR was inversely associated with DHT (B=-2.195, p=0.002) and testosterone (B=-1.541, p=0.004). HOMA-Ir was associated with androstenedione (B=0.071, p=0.032), estradiol (B=0.091, p=0.009), estrone (B=0.075, p=0.009) and 17-α-hydroxy-progesterone (B=0.157, p=0.001). Age was positively associated only with testosterone (B=0.017, p=0.042) and no significant associations with other hormones were observed. HsCRP showed a negative association with progesterone (B=-0.028, p=0.037). Furthermore, LDL was associated with estradiol (B=-0.093, p=0.049) and triglycerides were associated with DHT (B=-0.208, p=0.016). (Table 6).

Table 6. Multivariable linear regression analyses investigating the association between risk factors for cardiovascular disease and concentration of sex hormones in postmenopausal women

	β	95% CI	*P*	Standardized estimate
17-α-Hydroxyprogesterone				
HOMA-IR	0.157W	0.062 to 0.252	.001	0.224
Type 2 diabetes	–0.321	–0.704 to 0.062	.099	–0.108
BMI	–0.023	–0.044 to –0.002	.028	–0.120
Androstenedione				
HOMA-IR	0.071	0.006 to 0.136	.032	0.101
BMI	–0.016	–0.034 to 0.002	.075	–0.084
DHEA				
Age, y	–0.020	–0.040 to 0.001	.059	–0.101
DHT				
WHR	–2.195	–3.586 to –0.804	.002	–0.175
Triglycerides	–0.208	–0.377 to –0.040	.016	–0.137
Estradiol				
BMI	0.054	0.035 to 0.072	< .001	0.279
HOMA-IR	0.091	0.023 to 0.160	.009	0.130
LDL	–0.093	–0.185 to 0.000	.049	–0.083
Estrone				
BMI	0.015	0.000 to 0.030	.057	0.077
HOMA-IR	0.075	0.019 to 0.131	.009	0.107
Progesterone				
hsCRP	–0.028	–0.055 to –0.002	.037	–0.093
Testosterone				
Age, y	0.017	0.001 to 0.033	.042	0.086
WHR	–1.541	–2.579 to –0.504	.004	–0.123

The table presents the explanatory variables selected in the variable selection and showing greater than 50% inclusion in the bootstrap stability investigation. Dependent variable: sex hormone.
Abbreviations: BMI, body mass index; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; WHR, waist-to-hip ratio.

In this explorative study, sex hormones were associated with different risk factors for cardiometabolic disease and body composition. Interestingly, WHR was associated with androgens and BMI was associated with estrogens.

Although many studies have been performed to determine the association between sex hormones and cardiometabolic risk factors in postmenopau-

sal women, there is no consensus on the presence of these associations. More-over, many of the studies have used RIA methods which are not reliable enough when it comes to measuring levels of estrogens in postmenopausal women. Among the studies that have used LC-MS, our results are consistent with previous literature regarding the positive association between BMI and estradiol/estrone, that has been reported previously, as in the cross-sectional study of 101 postmenopausal women by Marchand et al[122]. We found inverse significant associations between WHR and DHT, however, in the study by Marchand et al, no significant associations between testosterone or DHT and WHR were found, in contrast to our results. In a study performed by Cote et al, an inverse association between visceral fat adiposity and DHT was found,[123], however, mean age in that study was 47.5 years old, and among the 60 women included only 10 were postmenopausal.

The only sex hormone associated with hsCRP was progesterone (negative association). This relation has been investigated with varying results. In studies using RIA both no significant associations[124], and positive associations have been found[125]. Studies investigating this association using LC-MS are scarce, and studies using RIA method should be interpreted with caution, due to the fact that there may be possible interference in the RIA assay between sex hormones and CRP[126]. The study by Liu et al using LC-MS found a positive association between progesterone and CRP in a Chinese rural cohort[127]; results opposite of what we found in our study. However, that study is difficult to compare to ours due to differences in detection limits of the assays, large differences in the mean progesterone levels, and considerable variations in the cohort characteristics. Furthermore, we found a significant positive association between HOMA-Ir and 17-α-hydroxy-progesterone. These results confirm previously described associations in a study using LC-MS that found positive associations between 17-α-hydroxy-progesterone and impaired glycemic control in postmenopausal women[128], and in mouse models[129].

Paper IV

Study IV included only postmenopausal women as described above. A total of 133 women were included in the analyses after excluding women with estradiol levels ≥20 pg/mL, women using hormonal therapy, or

women with previous stroke. The mean age in this group of postmenopausal women was 64.5 years old. The estimated 10-year risk for stroke according to the rFSRP was 3.5 % (SD 2.7 %). Progesterone was positively associated with rFRSP in the linear regression model (β=0.16 95% CI= 0.03;0.30, p=0.02) when adjusting for BMI, and also when additional adjustments were made for cholesterol and CRP (β=0.16, 95% CI= 0.03;0.30, p=0.03). 17-α-hydroxy-progesterone was also significantly associated with the rFRSP (β=0.20 95% CI= 0.00;0.20, p=0.03) in the model adjusting for BMI, and in the fully adjusted model: (β=0.20 95% CI= 0.00;0.20, p=0.04). Estrone showed a positive significant association with rFRSP in all models (full model: β=0.17 95% CI= 0.04;0.30, p=0.01). Testosterone was also positively associated with rFRSP when adjusted for BMI (β=0.119 95% CI= 0.00;0.24, p=0.018) and when adjusting for CRP and LDL (β=0.119 95% CI= 0.00;0.24, p=0.001). Estradiol showed a significant positive association with rFRSP in the crude model (β=0.17 95% CI= 0.03;0.31, p=0.02), however, the significance disappeared after adjustments were made for BMI. Regarding the other hormones included, DHEA, DHT, and androstenedione, no significant associations were found. The results from the association between sex hormones and the estimated risk for coronary heart disease (N=125) were similar for progesterone (β =0.13, p=0.01) and 17-α-hydroxy-progesterone (β =0.11, p=0.02) (adjusted for BMI and CRP) but there were no other significant associations for the other hormones.

In summary, the results from this study revealed a positive association between estrone, 17-α-hydroxy-progesterone, progesterone and testosterone and rFSRP respectively. These results were significant in the adjusted models when also adding BMI and CRP. Previous studies have found conflicting results about the association between sex hormones and stroke risk in women. One study from the MESA cohort found that higher testosterone was associated with higher risk of CVD[130], and Benn et al found that extremely high values of testosterone were associated with higher risk of CVD and death after 30 years of follow-up[131]. However, yet another study using RIA methods to define sex hormone levels from the Rancho Bernardo cohort[132] found that being in the lowest quartile of testosterone was associated with higher risk of coronary heart disease in postmenopausal women. These results were similar to another study including both pre-and postmenopausal women where low testosterone predicted all-cause mortality[133]. Due to the fact that these previous studies have used RIA-technique, it is difficult to easily compare these stud-

ies with ours. However, regarding testosterone measured with LC-MS, a study within the The Nurse's health study[99] found no associations between testosterone levels and ischemic stroke, whereas Islam et al[98] found an inverse association between testosterone and DHEA, a precursor of testosterone, and major adverse cardiovascular events (MACE) after 4 years of follow-up in women above 70 years of age. In the Rotterdam Study, using LC-MS for the testosterone analysis, no associations between testosterone or DHEA and incident stroke, coronary heart disease or CVD were found after approximately 11 years of follow-up[134].

The data on estrone in postmenopausal women with regard to stroke is limited. In the study by Islam et al[98], a significantly lower risk of MACE was found in participants in the second quartile of estrone compared with the first quartile, however, no consistent associations were found between estrone and MACE[98]. In our study no significant associations were found between estradiol and rFSRP after adjustments were made for BMI. The Three City Cohort study found positive significant associations between estradiol (by RIA) and risk of CHD and ischemic arterial disease in postmenopausal women even when adjusting for diabetes and BMI, but the association was non-significant for ischemic stroke [88].

The literature on progesterone and stroke risk is scarce. One study in line with ours found positive associations between progesterone/17-α-hydroxy-progesterone respectively and higher Hba1c, indicating an association with cardiometabolic risk factors[128]. In another study using LC-MS no significant differences were found in the 17-α-hydroxy-progesterone levels between the group with CVD (n=8) and the control group[135], but due to the low number of events, it is difficult to draw conclusions from this study.

General discussion

This thesis describes associations between endogenous sex hormones and important cardiometabolic risk factors in both cross-sectional and longitudinal designs in both men and women, with a special focus on postmenopausal women in Studies III and IV. The studies found significant associations between sex hormones and different risk factors, and provide new knowledge about the role of sex hormones in cardiometabolic health. Specifically, in Study I, a strong and significant association was found between testosterone and HOMA-Ir in men, both cross-sectionally and longitudinally, regardless of relevant confounding factors. However, there were no significant associations in the analyses of the bidirectionality of this association. In Study II, a significant cross-sectional association between sex hormone-binding globulin and HOMA-Ir in both men and women was found. Furthermore, levels of SHBG could predict the development of insulin resistance after 10 years. In postmenopausal women, we were able to explore the association between eight different sex hormone concentrations and cardiometabolic risk factors by using gold standard techniques on the determination of sex hormone levels. A pattern was found that to the highest degree could explain the variance in sex hormone levels in postmenopausal women. This pattern revealed that BMI was associated with estrogen and progesterone whereas WHR was associated with testosterone and DHT. Other important findings on the association between sex hormones, inflammation, HOMA-ir and lipids were also reported. Finally, in Study IV, a significant association between estrone, 17-α-hydroxy-progesterone and testosterone and the revised Framingham Stroke Risk Profile was found in postmenopausal women.

Sex differences in incidence of cardiovascular diseases are observed through the lifespans of men and women. A large part of these differences can be explained by differences in the modifiable risk factors between men and women e g obesity, smoking and alcohol consumption. Recent prospective data from the PURE study including a total of 21 countries from both low-income, middle-income and high-income areas, revealed that the modifiable risk factors were similarly associated with

CVD (stroke, myocardial infarction, CVD death, heart failure) in women and in men, although lipid markers and depression were stronger risk factors for men than women, whereas diet was a stronger risk factor in women[38]. Furthermore, the impact of type 2 diabetes on CVD is not proportionate in men and women, since diabetes contributes to a higher CVD risk in women, at least in high-income countries[6].

Another aspect of these differences is that we have less knowledge about CVD health in women compared to men. In fact, clinical research has historically included men to a larger extent than women, which could have led to misinterpretation of women's symptoms of stroke and ischemic heart disease[5]. Women have lower incidence of stroke but higher death rate from stroke and worse recovery outcomes post-stroke than men, which may be due to both gender and sex differences[5]. Gender-related factors that can influence women's worse outcome after stroke are for example different healthcare seeking behavior, delay in diagnosis, and different secondary preventive treatments[5]. It is also worth noting that age is a major confounder in these associations due to women's longer life expectancy, leading to stroke events later in life[136]. Ischemic heart disease is another area where there are both gender and sex differences, such as differences in plaques composition, healthcare seeking behavior, and differences in symptom presentation[136]. The known risk factors are also prevalent to different extent in men and women, for instance the prevalence of current or former smoking and alcohol consumption is higher in men than in women, according to recent data from the PURE study[38].

Although there is evidence that some of the differences between men and women seem to be explained by differences in risk factors there is evidence that differences between men and women remain, even when other risk factors are considered. In fact, an increase in CVD incidence is observed in women after the menopausal transition[5]. This may be due to hormonal changes, but aging per se also contributes to changes in risk factors[86]. In a study of women in the SWAN cohort of approximately 1,000 women going through the menopause, only LDL and total cholesterol changed substantially, during the year before and after menopause, whereas blood pressure, glucose, insulin and CRP changed linearly, suggesting an age-related increase[87]. Moreover, women who experience menopause before the age of 40 have a higher hazard ratio for developing many cardiovascular diseases than women who do not experience early

menopause, suggesting a protective role of hormones before menopause[137], although it is important to acknowledge that these associations may be confounded, for instance by smoking or socioeconomic status, which are also associated with an earlier menopause[83]. Nevertheless, information on early menopause has recently been included as a risk factor to consider in the guidelines of statin use, due to its contribution the higher CVD risk in these women[138].

Interestingly, in men there is a larger decline of bioavailable testosterone with aging, partially due to increasing levels of sex hormone-binding globulin, although total levels of testosterone only marginally decline during the lifespan [139]. This decrease in testosterone levels seems to be associated with increased risk for the metabolic syndrome, and type 2 diabetes independent of age and obesity.[140]. Although testosterone has been found to be associated with insulin resistance, the mechanisms behind this association and the possible bidirectional association are unclear. The results in Study I showed that low testosterone in men was strongly and significantly associated with insulin resistance. This evidence contributes to the understanding of pathways leading to increased risk for type 2 diabetes in men with low testosterone. Interestingly these associations seem to be independent of body composition (WHR or BMI) suggesting other mechanisms on the diabetogenesis of low levels of testosterone. The same study was not able to observe significant association in the other direction i.e., insulin resistance could not predict development of hypogonadism.

In Study II, SHBG and insulin resistance were significantly associated in cross-sectional analyses, longitudinal analyses and in both men and women, also after adjustments for relevant confounders. There was no difference in the associations between women before or after age 50, which was used as a proxy for menopause in this study. Even though previous studies have found an association between low SHBG and type 2 diabetes[62], few other studies have studied insulin resistance longitudinally, including both women and men in the same study. In a previous epidemiological study of women, there were inverse associations between SHBG and incidence of type 2 diabetes, although the association weakened when adjustments were made for waist circumference[141], which can also be seen in our study. However, our results were still significant in all groups except in the fully adjusted longitudinal model in postmenopausal women. We also detected a small difference in the asso-

ciation between the three groups (men and women before and after age 50), although since this association was small and only seen between men and postmenopausal women at visit 2, in the cross-sectional model, it was not considered clinically relevant. Even though SHBG levels differ between men and women, and also change differently during the lifespan[142,143], it seems that SHBG is associated with insulin resistance in the same way in both women and men, also before and after menopause. The results in Study II are similar to the associations seen in PCOS, although in this study we did not have information about the prevalence of PCOS among the female participants. Given the prevalence of about 5-18% in the female population[77], this may be a confounder in this study. However, our results in the group of women under 50 years of age showed similar results as in the other two groups, and the only significant difference in slopes was between postmenopausal women and men at visit 2, in the fully adjusted model. PCOS, a state with decreased SHBG and increased androgens and estradiol levels[144] is also associated with obesity and insulin resistance, even though the causal relationships between insulin and sex hormones in PCOS are not fully understood[77]. However, there is evidence that the hyperinsulinemia in PCOS decreases SHBG, and affects the androgen production from the ovaries positively via hypothalamic effects[77]. On the other hand, Ding et al[62] suggested a causal effect of higher SHBG levels on decreased risk of developing type 2 diabetes, in their prospective cohort study including also Mendelian randomization, a finding also in line with our findings in the prospective analyses in Study II.

The results of Study II suggest that SHBG is inversely associated with insulin resistance, in cross-sectional and longitudinal analyses in men and women. These results add knowledge to the known association between low SHBG and type 2 diabetes.

The lack of a reliable method to determine concentrations of sex hormone in the lowest range have led to lack of knowledge of their impact on CVD-risk. This applies especially to postmenopausal women; a group in which changes in hormonal levels are thought to partially explain the increase in CVD events. Therefore, we completed the hormonal assays for eight different hormones using LC-MS/MS in the Vara-Skövde Cohort. Due to the limitations in the RIA assays, with low precision in the lower hormone ranges, and possible interference with inflammatory parameters[126,145], many previous studies on postmenopausal women are dif-

ficult to interpret and compare with our results. It is not fully investigated how the eight hormones are associated with different cardiometabolic risk factors in postmenopausal women, which led to Study III: an explorative descriptive study investigating which cardiometabolic variables to the largest extent associate with the variance in hormonal levels. The main results of that study were that WHR was mainly negatively associated with the androgens whereas BMI was mainly positively associated with estradiol. DHT, a potent metabolite of testosterone was significantly inversely associated with WHR in our study, in line with a study showing similar associations between DHT and even more accurate measures of visceral adiposity than WHR, such as computer tomography[123] . This would suggest that postmenopausal women have similar associations between androgens and abdominal obesity as men[73]. The inverse association between DHT and triglycerides has not been reported elsewhere, however, a similar relation has been found between DHT and LDL[122]. Even though WHR is a less precise measure of visceral adiposity, the results from Study III are also in line with early studies that have studied the effect of exogenous DHT or other androgens in obese postmenopausal women,[146] in which the treatment led to a decrease in abdominal fat. However, further studies are warranted to shed light on the association between body composition and androgens in postmenopausal women.

Even though there are sex differences between risk factors and their effect on CVD, hypertension seems to be the most important modifiable risk factor in both men and women when it comes to stroke incidence[38]. The event rate of cardiovascular events (stroke, myocardial infarction, death) in the sub-cohort of postmenopausal women in this thesis was too low to use as outcome. Therefore, a risk score for estimating stroke risk in ten years was used as outcome[51], to investigate the association between sex hormones and estimated stroke risk. Our results show significant associations between estrone, testosterone, progesterone and 17-α-hydroxy-progesterone and rFSRP, also when adjustments were made for confounders such as BMI (estradiol, estrone, progesterone, 17-α-hydroxy-progesterone) or WHR (testosterone, DHT, DHEA, androstenedione). Since age was also associated with testosterone in Study III, a methodological concern regarding age as a covariate was raised, since age is already included in the algorithm of rFRSP. Even though sensitivity analyses revealed similar estimates when the analyses were stratified based on median age (=64 years), there may be reason to believe that age is one of the main contributors to the association. When investigating the

association between levels of sex hormones and the risk score for CHD, the results were similar for progesterone and 17-α-hydroxy-progesterone, but not for estrone and testosterone. This may be due to the fact that the scores were based on different variables, but also that in that study we also excluded participants with previous CVD, which was not the case in the rFSRP (except for stroke). Given that previous research has been inconclusive when it comes to endogenous sex hormones and stroke, further large prospective studies with stroke incidence as outcome are warranted.

In summary, there are sex and gender differences in CVD incidence, among which the sex differences may, in part, be due to sex hormones. The hormonal levels in men and women are associated with various cardiometabolic risk factors. In men there are associations between low testosterone, and low SHBG and insulin resistance, both cross-sectionally and longitudinally, whereas in women there are similar associations between low SHBG and higher insulin resistance, regardless of age or menopausal status. In postmenopausal women, body composition is to the greatest extent associated with hormonal levels, especially WHR that was associated with concentrations of androgens and BMI that was associated with the concentration of estrogens. In the same cohort of postmenopausal women, positive associations were found between some of the hormones and the estimated stroke risk.

Methodological discussion

This thesis has studied the associations between endogenous sex hormones and various cardiometabolic risk factors in men and women. The strengths of the studies are the robust protocols at study visits, the long follow-up time and a high participation rate in both baseline and follow-up visits.

Cross-sectional observational studies lack the possibility to determine causality, which is a limitation in Studies III and IV. Further large cohort studies are warranted for further understanding of the biological mechanisms behind the associations found. Observational studies are also less reliable than randomized trials, however, since we were aiming to investigate the endogenous sex hormone levels with regard to cardiometabolic risk, interventional studies would not have answered the research question for this project. Using end-point such as stroke incidence from the Socialstyrelsen register would indeed have been a more reliable outcome compared to the estimated rFSRP – however in this relatively young cohort, the event rate was low and led to a power problem in the analyses.

The long follow-up time is indeed a strength in the thesis, although during the follow-up time the measurements of insulin and testosterone changed, leading to difficulties in interpreting the new versus the old values. The insulin measurements at follow-up were about 35 % higher than at baseline measurement, and therefore the insulin values had to be recalculated with a formula provided by the laboratory. The correlation between the methods was r^2=0.9974, and was used in Studies I and II, although due to this change the difference in insulin was not used. The analysis of testosterone also changed during the follow-up time, and the method led to higher values compared to the values found at baseline. Unfortunately, we did not have access to any algorithm that could adjust for this change, which is a limitation to Study I.

The use of LC-MS is stated by the Endocrine Society as the gold standard when measuring endogenous sex hormones [147,148] and allows for detection of very low levels of hormones, such as in children,

postmenopausal women and hypogonadal men. This method was used in Studies III and IV, and is a major strength of those studies. The hormonal levels in postmenopausal women are low and therefore the precision and low detection range of LC-MS is needed for studying associations in this particular group. In Studies I and II we did not have access to LC-MS/MS, although in this group of healthy men, testosterone levels are in a higher range, leading to less problems with the RIA method used[149]. Another disadvantage with RIA is that the RIA analysis of estradiol in men has shown to correlate with CRP, whereas LC-MS did not[126], suggesting an interference by inflammatory parameters, which may affect the results when assessing outcomes that may also be related to inflammation. In Study I, adjustments were made for CRP, without any change in significance of the estimate.

The outcomes of this thesis included HOMA-Ir (Studies I-II), the sex hormones measured by LC-MS (Study III), and the revised Framingham Stroke Risk Profile (Study IV). HOMA-Ir is calculated from fasting insulin and fasting glucose, and is considered a valid measurement of insulin resistance in cohort studies. The gold standard for measuring insulin resistance is euglycemic insulin clamp, however this is difficult to perform in larger cohort studies. The measurements of insulin and glucose were all performed in the morning after overnight fasting, as instructed in the HOMA-Ir algorithm. The sex hormones were the outcomes of Study III, and measured by LC-MS/MS, which is gold standard for measuring sex hormones. There was, however, only one measurement in each participant, whereas three measurements would have been optimal due to the fact that these hormones also have a diurnal rhythm to some extent.
The outcome of Study IV is based on several risk factors for stroke. The robust examination protocol and information about medication and medical history of the participants allowed us to use these variables in the rFSRP algorithm.
In Study II menopause status was not known at baseline. Instead, age 50 and above was used to determine postmenopausal state. This age of menopause is the median age of menopause globally[82] and has support in observational studies[150] but could lead to misclassifications of menopausal status in both directions. The question about menopause was later added to the questionnaire answered by the participants at the follow-up visit. In Studies III and IV, we wanted to investigate postmenopausal women only, and in order to further reduce risk of mis-classification, we used women aged 55 years and above in those studies.

The strength of Study II is its prospective design with a large cohort including both men, premenopausal women and postmenopausal women. The measurement of SHBG is done with a valid method. One limitation of the study is that we did not have the information about PCOS in women, a state that can confound the associations due to its association with low SHBG and altered glucose metabolism[119] .

The studies included in this thesis have been performed in a cohort of men and women of mostly Swedish origin and may not be fully generalizable to all men and women, even though many of our results are consistent with other studies including men and women of different geographical and ethnic origin.

In the follow-up, the participation rate was 68% (1,327/1,954 summoned). Sensitivity analyses for differences between responders and non-responders were performed in Studies 1 and 2 due to this, but no differences were found in the associations in the two groups in Study II. In Study I, there was a difference in smoking rate and age (both significantly higher in the non-response group).

Conclusions

General conclusions

This thesis found associations between levels of sex hormones, sex hormone binding globulin and cardiometabolic risk factors, in both men and women, and both in cross-sectional and longitudinal analyses. These findings add knowledge to previous research emphasizing the relationship between sex hormones and cardiometabolic diseases, and contribute to the understanding of sex-differences in the development of cardiovascular disease.

Specifically:

Study I found an association between testosterone and insulin resistance in men cross-sectionally and after 10 years of follow-up. These associations were significant regardless of confounding factors, but the association was not bidirectional.

In Study II, in both men and women, SHBG levels show an inverse association with HOMA-Ir cross-sectionally and longitudinally in both men and women, and adds knowledge about the previously known relationship between SHBG and type 2 diabetes.

Study III found that in postmenopausal women, BMI is positively associated with estrone and estradiol, while WHR is inversely associated with testosterone and DHT. In addition, hsCRP is inversely associated with progesterone. By using gold standard technique in measluring sex hormones, these studies add information about cardiometabolic risk factors and sex hormones, previously measured with other techniques.

Study IV showed that increases in endogenous levels of progesterone, 17-α-hydroxy-progesterone, estrone and testosterone in postmenopausal

women are associated with an increase in the estimated 10-year risk of stroke according to the revised Framingham Stroke Risk Profile.

Points of perspective

Sex differences in the incidence of cardiovascular disease, and the possible different impact of diabetes in men and women on cardiovascular risk are important for future studies to investigate. In daily clinical practice, it is important to acknowledge these differences, with the aim to reduce differences in incidence that possibly can be affected, and take gender and sex into account in risk scoring. The associations found between endogenous hormonal levels in postmenopausal women with regard to estimated stroke risk need further investigation, in larger cohorts and with actual stroke events as outcome. Study III added to the knowledge of how cardiometabolic risk factors associate with sex hormones and is important for deeper mechanistic knowledge about how sex hormones may affect cardiometabolic health. Similar studies in men should add further to this understanding, and give new insight into possible intermediate biological pathways behind the associations between sex hormones and CVD.

In Study I there was a strong and significant association between low testosterone levels in men and the development of insulin resistance. Further studies, with gold standard measurements, should investigate the complex interaction between testosterone and other sex hormones in men, such as progesterone and estradiol, and how these hormones may have different actions, with regard to cardiovascular risk factors.

In 2018 the measurements of eight different sex hormones by LC-MS further improved the validity of these cohort data. Study III added knowledge about which variables were to the greatest extent associated with the variance in the hormonal level in postmenopausal women. Descriptive studies are needed to further understand the mechanisms and pathways behind the somewhat conflicting data about sex hormones and cardiometabolic risk in women, especially during the menopausal transition. However, given the contradictory results in literature, larger cohort studies are warranted to confirm the associations found. Larger prospective studies using CVD events as outcome would also add higher levels of evidence to these associations.

Future studies using the Vara-Skövde Cohort but also assessing sex hormones by LC-MS in men would further add knowledge to this research field. It would be favorable to also include premenopausal women, even though their cyclic expression of hormones would demand planning of the time of measurement, to enable an accurate and valid measurement, perhaps by drop-in laboratories where all the hormones would be assessed on the same day of the menstrual cycle. The role of follicle-stimulating hormone (FSH) with regard to cardiovascular risk is unclear, and represents another field where epidemiological studies like the VSC could add new knowledge.

The robust and thorough study protocol used in the Vara-Skövde Cohort at two and – in the future – more time points, enables further prospective studies in this field, with focus on knowledge gaps, such as exploring biomarkers for CVD prevention or adding sex-specific risk factors to risk scores, with the aim to find ways to further reduce cardiovascular risk.

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