Polycystic ovary syndrome in a lifetime perspective

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“I have never tried that before,
so I think I should definitely be able to do that”

-Astrid Lindgren (Pippi Longstocking)

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is common, affecting 9-18% of women. PCOS is associated with symptoms due to hyperandrogenism and ovarian dysfunction, but is also associated with the metabolic syndrome including obesity, insulin resistance and elevated blood lipids. The post-menopausal consequences are uncertain, due to the lack of long-term studies. **Aim:** To increase the knowledge about ageing women with PCOS. **Material and method:** Two cohorts of women with PCOS and their age-matched controls have been followed prospectively: cohort 1 (PCOS n = 33 and controls n = 94) on two occasions, and cohort 2 (PCOS n = 37 and controls n = 120) on three occasions. The women with PCOS from the two cohorts together cover an age range from 20 to 91 years. **Results:** Women with PCOS reached the menopause four years later than controls. Parity and nulliparity did not differ. 19% of the women with PCOS had developed type 2 diabetes mellitus (T2DM) at perimenopause, vs. 1% of controls, but all women who developed T2DM were obese and had a high waist hip ratio already at mean age 30 years. Health-related quality of life did not differ at mean age 52 years. Women with PCOS had persistently lower FSH up to a mean age of 81 years, where hirsutism was more frequent (33 vs 4%), but biochemical hyperandrogenism did not differ. Cardiovascular disease (CVD) events, CVD-related or all-cause mortality did not differ at this age. **Conclusion:** Women with PCOS did not suffer from increased mortality or increased CVD events, despite increased risk factors. This might be caused by possible protective factors such as a delayed menopause, and hormonal factors that differed from those of the controls at senescence.

Keywords: cardiovascular disease, FSH, hyperandrogenism, insulin resistance, menopause, metabolic syndrome, PCOS, postmenopausal, type 2 diabetes mellitus, quality of life

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SAMMANFATTNING PÅ SVENSKA

Målet med denna avhandling är att öka kunskapen kring vad som händer när kvinnor med polycystiskt ovarialsyndrom (PCOS) åldras. Mellan nio och 18% av alla kvinnor uppfyller kriterierna för PCOS, således utgör det ett mycket vanligt tillstånd! Trots detta har få studier gjorts på kvinnor med PCOS i högre åldrar, delvis beroende på att diagnosen endast kan ställas under de fertila åren. PCOS kan i yngre åldrar innebära glesa menstruationer, infertilitet, ökad mängd manliga könshormoner och hårväxt av manlig typ. PCOS är också associerat med övervikt, högt blodtryck, höga blodfetter, diabetes, cancer i livmodern, psykisk ohälsa och försämrad livskvalitet. Det är oklart om dessa skillnader jämfört med kvinnor utan PCOS kvarstår eller förändras med ökande ålder, då studier saknas.

Vi har undersökt två grupper av kvinnor med PCOS och åldersmatchade kontroller. Kontrollerna utgörs av ett slumpmässigt urval av kvinnor ur Göteborgs befolkning som tidigare har deltagit i studien World Health Organisation (WHO) MONItoring of trends and determinants for CArdiovascular disease (MONICA) studien. Grupperna har undersökts vid flera tillfällen under en 24-årsperiod respektive en 32-årsperiod, och kvinnorna med PCOS täcker åldersspannet 20–91 år.

Vi har funnit att kvinnor med PCOS kommer i klimakteriet fyra år senare än kontroller. Kvinnor med PCOS föder barn i samma utsträckning som kontrollerna och andelen utan barn skiljer sig inte mellan grupperna. En stor andel kvinnor med PCOS utvecklar diabetes typ 2, men uppföljningen visar att samtliga kvinnor med PCOS som i åldern runt klimakteriet hade utvecklat diabetes (19%) hade fetma redan i 30-årsåldern. I åldern runt klimakteriet hade kvinnor med PCOS samma upplevda livskvalitet som kontroller. Upp till en medelålder på 81 år har kvinnor med PCOS fortsatt ökad hårväxt av manlig typ, men vid mätning av manliga könshormoner i blodet finns det inte längre någon skillnad jämfört med kontroller. Trots flera riskfaktorer för hjärtkärlsjukdom i yngre åldrar fanns ingen ökad sjuklighet i hjärtinfarkt eller stroke. Kvinnorna med PCOS, följda till över 80 års ålder, hade ingen ökad dödlighet jämfört med kontroller, varken i form av hjärtkärlrelaterad dödlighet eller total dödlighet.

LIST OF PAPERS

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

I. Forslund M, Landin-Wilhelmsen K, Schmidt J, Brannstrom M, Trimpou P, Dahlgren E. **Higher menopausal age but no differences in parity in women with polycystic ovary syndrome compared with controls.**
Acta Obstet Gynecol Scand. 2019;98(3):320-6.

II. Forslund M, Landin-Wilhelmsen K, Trimpou P, Schmidt J, Brannstrom M, Dahlgren E. **Type 2 diabetes mellitus in women with polycystic ovary syndrome during a 24-year period: importance of obesity and abdominal fat distribution.**
Hum Reprod Open. 2020;2020(1):hoz042

III. Forslund M, Landin-Wilhelmsen K, Krantz E, Trimpou P, Schmidt J, Brannstrom M, Trimpou P, Dahlgren E. **Health-Related Quality of Life in Perimenopausal Women with PCOS.**
Manuscript.

IV. Forslund M, Schmidt J, Brannstrom M, Landin-Wilhelmsen K, Dahlgren E. **Reproductive hormones and anthropometry: A follow-up of PCOS and their age-matched controls from perimenopause to a mean age above 80 years.**
The Journal of Clinical Endocrinology & Metabolism 2020.10.1210/clinem/dgaa840.

V. Forslund M, Schmidt J, Brannstrom M, Landin-Wilhelmsen K, Dahlgren E. **No evidence of increased morbidity and mortality in PCOS: A prospective follow-up up to mean age above 80 years.**
Manuscript.

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ABBREVIATIONS

AMH	Anti-müllerian hormone
BMI	Body mass index
CVD	Cardiovascular disease
FAI	Free androgen index (calculated as total testosterone/SHBG x 100)
FFQ	Food frequency questionnaire
fP-Glc	Fasting Plasma Glucose
FSH	Follicle-stimulating hormone
HOMA	Homeostatic model assessment of insulin resistance (calculated as fasting insulin x fasting glucose / 22.5)
HR	Hazard ratio
HRQoL	Health-related quality of life
IGT	Impaired glucose tolerance
IQR	Inter-quartile range
LH	Luteinizing hormone
mFG score	modified Ferriman Gallwey score (measures hirsutism)
NIH	National Institute of Health
OGTT	Oral glucose tolerance test
OR	Odds ratio
PCOM	Polycystic ovary morphology

PCOS	Polycystic ovary syndrome
P-Glc	Plasma Glucose
SF-36	Short Form 36 (used to measure HR-QoL)
SHBG	Sexual hormone-binding globulin
T2DM	Type 2 diabetes mellitus
TIA	Transient ischemic attack
WHO	World Health Organization
WHO MONICA	World Health Organization MONItoring of trends and determinants for CArdiovascular disease
WHR	Waist hip ratio

1 INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common condition in women of reproductive age, affecting more than every tenth woman. It is clinically characterized by signs of increased levels of androgens, such as hirsutism or severe acne, and irregular periods. PCOS is also strongly associated with the metabolic syndrome including obesity, insulin resistance and elevated blood lipids. The consequences in the fertile woman may include infertility due to anovulation, type 2 diabetes mellitus and hypertension, but also psychiatric morbidity, including depression and eating disorders.

The consequences in postmenopausal women are more uncertain due to the lack of studies in these ages. The few available studies are often cross-sectional and may have identified possible PCOS cases in peri- or postmenopausal women. There are no prospective follow-up studies on women with PCOS until senescence, an age appropriate to study cardiovascular disease and mortality.

In this thesis, women with PCOS have been followed prospectively, and the women with PCOS from the two cohorts together cover an age range from 20 to 91 years. Unique data on anthropometry, reproduction, reproductive hormones, health-related quality of life, co-morbidity and mortality are presented. Women with PCOS are often diagnosed during early adulthood and the ambition with this thesis is to increase the knowledge about PCOS in a lifetime perspective.

1.1 PCOS DIAGNOSIS

What we refer to today as PCOS has been diagnosed in different ways over the years.

The Stein-Leventhal Syndrome

Already in the early 1920s it was noticed that some obese women had irregular periods or amenorrhea and that hirsute women more often than others had infertility problems. Irving Freiler Stein (1887-1976) and Michael Leo Leventhal (1901-1971) performed explorative laparotomies on these women and found ovaries of increased size, often with multiple small cysts. In the classic article "Amenorrhea associated with bilateral polycystic ovaries" published in 1935 in the American Journal of Obstetrics and Gynecology, they were the first to describe the classic triad of polycystic ovaries, hirsutism and oligo-/amenorrhea. They also described the typical histopathological features of the ovary, which became a tool for diagnosis. The condition was referred to as Stein-Leventhal Syndrome. (Stein and Leventhal 1935)

Diagnosis through biochemical markers

With time, elevated levels of luteinizing hormone (LH) were found in women with polycystic ovaries, and later also elevated levels of testosterone, androstenedione and estrone. The diagnostic criteria drifted and the diagnosis was made using biochemical markers, rather than histopathology. (Dahlgren 1992)

The NIH Criteria

Over the years, there has been an ongoing discussion about the diagnostic criteria of PCOS. At the National Institute of Child Health and Human Development conference on PCOS in April 1989, a questionnaire was distributed to the attending experts to explore which diagnostic criteria the majority could accept. Fifty-eight of the attending experts responded.

The general agreement was that the major research criteria of the PCOS diagnosis should include:

1) hyperandrogenism and/or hyperandrogenemia; 2) oligo-ovulation; and 3) exclusion of other disorders such as Cushing's syndrome, hyperprolactinemia and congenital adrenal hyperplasia. These criteria are referred to as the National Institute of Health (NIH) criteria. (Zawadzki and Dunaif 1992)

The Rotterdam Criteria

In 2003, a new workshop was held on the diagnostic criteria, with the aim to achieve consensus. The definition of PCOS that resulted from this workshop is called the Rotterdam criteria, where at least two of the three criteria should be present for a diagnosis. The Rotterdam criteria include, 1) oligo-/anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, and 3) polycystic ovaries, defined as twelve or more follicles in an ovary measuring 2-9 mm and/or increased ovarian volume > 10 ml. As with the NIH criteria, it is included to rule out the major differential diagnoses before making the PCOS diagnosis. With the Rotterdam criteria it was no longer mandatory to have increased androgens to get the PCOS diagnosis. With these criteria it was also possible to describe different phenotypes of PCOS, depending on which criteria that applied. (Fauser et al. 2004)

Updated criteria from the international evidence-based guidelines

In 2018, international guidelines were published with the aim to be as evidence-based as possible (Teede et al. 2018). In these new guidelines, both the assessment and management of PCOS were reviewed, to provide the best possible evidence for diagnosis, assessment and treatment. In the new guidelines, the Rotterdam criteria were updated and defined in greater detail. Still, two out of three criteria are mandatory for a diagnosis. The criteria, including definitions, are the following:

- Irregular cycles and ovulatory dysfunction, describes as having irregular cycles. It was also defined what irregular cycles means. Within the first year after the menarche, irregular cycles are normal and, thus, this criterion is not applicable. Within 1-3 years post the menarche, a cycle length < 21 or > 45 days is considered abnormal; if more than three years have passed since the menarche, irregular periods are defined as < 21 or > 35 days or < 8 cycles/years. This criterion also applies if primary amenorrhea by age 15 or if ovulatory dysfunction is confirmed by other means; for instance, by serum progesterone levels.
- Hyperandrogenism was again described as either clinical and/or biochemical. Clinical hyperandrogenism was defined as hirsutism, alopecia, and/or acne (in adolescents, severe acne or hirsutism). For biochemical hyperandrogenism, it was recommended to use calculated free testosterone, the free androgen index, or calculated bioavailable testosterone for the PCOS diagnosis. Biochemical hyperandrogenism cannot be assessed if the woman is using systemic hormonal treatment, such as hormonal contraception.
- The polycystic ovary morphology (PCOM) criterion was updated due to advances in ultrasound techniques. It was also specified that this criterion was not applicable until more than eight years

after the menarche, since PCOM is considered normal in younger ages. The update specifies that the follicle number (measuring 2-9 mm) per ovary should be ≥ 20 and/or ovarian volume ≥ 10 ml (without the presence of corpora lutea, cysts or dominant follicles) for this criterion to apply.

Exclusion of differential diagnoses

Other disorders may mimic PCOS and need to be excluded, clinically or biochemically. These disorders include androgen-producing tumors, non-classical adrenal hyperplasia and Cushing's syndrome. Ovulatory dysfunction due to other causes, including thyroid dysfunction and hyperprolactinemia, also needs to be considered.

1.2 PREVALENCE

The prevalence of PCOS differs, depending on which criteria are used for the PCOS diagnosis. When using the more stringent NIH criteria, about 7% of female blood donors in a small study from Spain had PCOS (Asuncion et al. 2000). A prevalence of 7% was also found in the United States, where women undergoing a pre-employment physical examination were included and the NIH criteria were used (Azziz et al. 2004).

In a larger study from Australia, a three-year birth cohort was traced. Initially, the women went through structured interviews. Those who had menstrual irregularities and/or a self-reported modified Ferriman Gallwey (mFG) score of ≥ 8 (a scoring of hirsutism) were invited to examinations with blood samples and vaginal ultrasound, and 39% consented. TSH, prolactin and 17-hydroxyprogesterone levels were measured to exclude other causes of menstrual dysfunction. The women had a mean age of 30 years (29-31 years). Thirty-eight per cent were using oral contraceptives. Ninety-four per cent were Caucasian. In the whole cohort, 21% had hirsutism (≥ 8 mFG score). When different criteria were applied, different rates of prevalence were found. The NIH criteria were met by 8.7%, a figure similar to that of other studies. When using the Rotterdam criteria, 11.9% had PCOS, and if data were imputated on missing vaginal ultrasounds, 17.8% had PCOS. Many of the women fulfilling the diagnostic criteria were undiagnosed. Of the women that fulfilled Rotterdam criteria, 69% were undiagnosed. On the other hand, of the women who stated that they had been diagnosed with PCOS, 34% did not fulfill the Rotterdam criteria, which might mean that the symptoms had resolved. (March et al. 2010)

1.3 REPRODUCTIVE HORMONES

Gonadotropins and AMH

Gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus in pulses. The amplitude and frequency regulate the release of gonadotropins, pituary follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In women with PCOS, the hypothalamic GnRH pulse frequency differs from that in non-PCOS women (Coyle and Campbell 2019; Rebar et al. 1976). GnRH, in turn, is regulated both from direct inhibiting feedback by the gonadotropins and by kisspeptin and neurokinin B, and the kisspeptin-neurokinin B pathway might be involved in PCOS development (Tang, Ding, and Zhu 2019; Skorupskaite et al. 2020).

Typically, women with PCOS have increased LH levels and normal or low FSH levels, resulting in an increased LH/FSH ratio. This, in turn, results in disturbed follicle development and ovulation. Follicles arrest in the stage of antral follicles, and an increased number of antral follicles is one of the diagnostic criteria in PCOS.

Anti-Müllerian Hormone (AMH) is synthesized by the granulosa cells in the primary follicle stage and is secreted at its highest concentration in the antral stage. AMH acts to inhibit the transition from primordial to primary follicle and inhibits the FSH-dependent cyclical recruitment. If AMH levels are high, the follicles grow slower and accumulate (Dewailly et al. 2016). AMH strongly reflects the antral follicle count; it declines with age and becomes undetectable in menopausal women, when the follicle pool is exhausted (Visser and Themmen 2005). Therefore, it is thought to serve as a marker of the ovarian reserve (Visser and Themmen 2005). Women with PCOS have higher AMH levels compared with controls in reproductive ages (Pigny et al. 2003). In PCOS, AMH excess may be one of the factors disturbing the cycle, as it seems to act as a gatekeeper (Dewailly et al. 2016).

Androgens and SHBG

Hyperandrogenism is one of the major characteristics of PCOS and is also one of the criteria for diagnosis. In women, androgens are produced in the ovaries, the adrenal cortex and in peripheral tissue.

In the ovary, steroids are produced by theca and granulosa cells combined (Hillier, Whitelaw, and Smyth 1994). In theca cells, LH stimulates the conversion of cholesterol to androstenedione, via pregnenolone, progesterone and 17-OH-progesterone. In the granulosa cells, androstenedione, which enters through diffusion, is converted to estrone or testosterone, which can then be converted to estradiol by aromatase, which is stimulated by FSH.

Three androgens are synthesized in the cortex of the adrenal gland: dehydroepiandrosterone sulfate (DHEAS), androstenedione and testosterone, and the synthesis is stimulated by pituary adrenocorticotrophic hormone (ACTH).

In women with PCOS, androgen secretion occurs mainly in the ovarian theca cells. In addition, more than 20% of women with classic anovulatory PCOS have adrenal androgen excess, as determined by DHEAS (Kumar et al. 2005). In healthy women, both the ovaries and the adrenals contribute to androgen production after the menopause (Markopoulos et al. 2011).

In the new guidelines for assessment of PCOS, the use of calculated free testosterone, free androgen index, or calculated bioavailable testosterone is recommended for the PCOS diagnosis (Teede et al. 2018). This is due to the fact that only 0.5-3% of testosterone is free; about 65-70% of circulating testosterone is bound to sexual hormone-binding globin (SHBG), and 30-35% is bound to albumin (Dunn, Nisula, and Rodbard 1981).

Free testosterone and bioavailable testosterone can be calculated using the formula of Vermeulen et al. (Vermeulen, Verdonck, and Kaufman 1999). Free testosterone refers to testosterone that is not bound to SHBG or albumin, and bioavailable testosterone refers to a combination of free testosterone and testosterone bound to albumin, since the binding to albumin is looser and easier to use. The free androgen index (FAI) is calculated as total testosterone (nmol/L)/SHBG (nmol/L) x 100.

The reason for hyperandrogenism in PCOS is not only high levels of androgens, but also that women with PCOS have lower levels of SHBG than women in general (Pinola et al. 2015). In a non-PCOS female population, SHBG decreased from the age of 20 to around 60 years, when the levels started to increase gradually (Maggio et al. 2008). Low levels of SHBG are associated with an increased risk of type 2 diabetes mellitus. The mechanism behind this is unclear, but polymorphism in SHBG has been associated with insulin resistance, which might indicate its involvement in the pathogenesis (Muka et al. 2017).

1.4 ETIOLOGY AND PATHOPHYSIOLOGY

The pathophysiology of PCOS is complex, as it is a heterogeneous condition with different clinical presentations and is not yet fully understood. It is characterized by hyperandrogenism and insulin resistance. When symptoms present, often during adolescence, a vicious circle involving neuroendocrine, metabolic and ovarian disturbances has already begun. The pathophysiology is summarized in Figure 1.

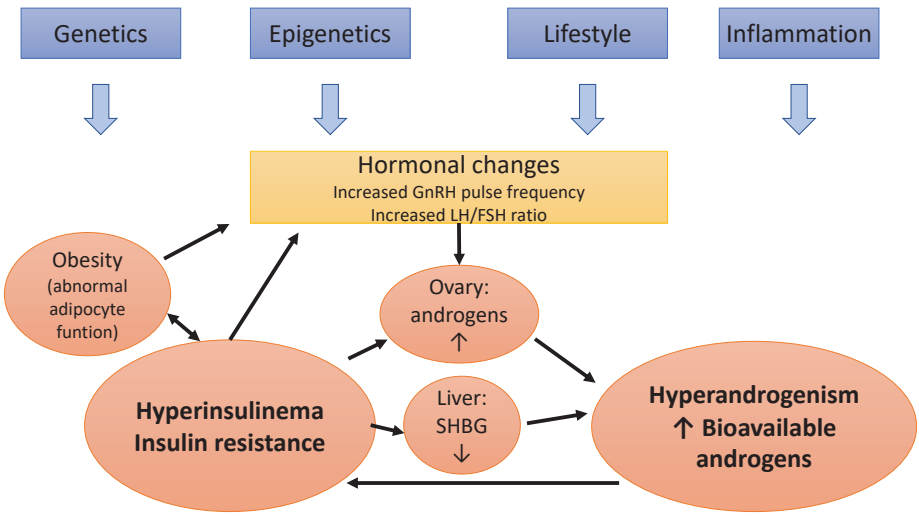

Figure 1. The vicious circle of the PCOS pathophysiology, with hyperinsulinemia and hyperandrogenism as important characteristics.

A genetic component is present in the PCOS pathophysiology, with clusters of PCOS in some families. The genetic basis is complex and over 200 genes have been identified as possibly related to PCOS (Laven 2019). However, most of these have not been confirmed in repeat

studies. Through genome association studies (GWAS), 18 genes have so far been identified as being significantly associated with PCOS, among these, variants in the LH gene, FSH-receptor genes and the FSH-β gene (Laven 2019).

It is also thought that epigenetic alterations influence the PCOS development. Since an hyperandrogenic intrauterine environment has been associated with changes resembling PCOS features in animal models, it has been proposed that hyperandrogenism may induce epigenetic changes prenatally (Li et al. 2016b). Epigenome-wide association studies (EWAS) have been performed on women with PCOS and controls, and although the results are interesting, with changes, i.a., the insulin-receptor gene, the results have so far been inconsistent, maybe because PCOS is such a heterogenic disorder (Li et al. 2016b).

Women with PCOS have increased levels of inflammatory markers, independently of BMI (Escobar-Morreale, Luque-Ramírez, and González 2011). Obesity, especially if mainly as visceral fat distribution, is in itself a proinflammatory condition and adipose tissue releases pro-inflammatory cytokines, which are involved in insulin resistance. Low-grade inflammation may play a role in the development of PCOS.

Alterations in the hypothalamic GnRH pulse frequency have been found in women with PCOS (Coyle and Campbell 2019; Rebar et al. 1976) and result in an increased LH over FSH ratio. LH stimulates ovarian androgen production, leading to increased androgen levels. Hypothalamic kisspeptin and neurokinin B are important regulators of GnRH, and recent studies show that the kisspeptin-neurokinin B pathway may play a role in PCOS development (Tang, Ding, and Zhu 2019; Skorupskaite et al. 2020).

Insulin can stimulate ovarian androgen production, both directly and indirectly through synergism with LH. In addition, hyperinsulinemia can influence the GnRH pulse frequency, so the LH over FSH ratio

increases, and decrease SHBG levels; thus, further increasing the bioavailable androgens (Diamanti-Kandarakis and Dunaif 2012). Androgens, on the other hand, can increase insulin resistance and also increase visceral adiposity, thereby further accentuating the vicious circle of hyperandrogenism and insulin resistance (Diamanti-Kandarakis and Dunaif 2012). In addition, obesity is associated with lower SHBG levels, which further increase the bioavailable androgens.

Ovarian dysfunction with follicle arrest and anovulation is part of the PCOS pathology. In an ovulatory cycle, FSH stimulates the follicle from the late antral follicle stage. With increasing estrogen levels, a dominant follicle expressing the highest level of FSH receptors will develop, which will eventually ovulate after an LH peak. Anti-Müllerian hormone (AMH) is produced by primary follicles until the small antral stage (Figure 2), after which they become more susceptible to FSH, and AMH inhibits the action of FSH (Dewailly et al. 2016). In PCOS, the balance between FSH, and AMH is disturbed, as women with PCOS have higher AMH levels compared with controls (Tehrani et al. 2010). This, together with a relative FSH deficiency, hyperinsulinemia and hyperandrogenism is thought to impair the final stages of follicle development, leaving an accumulation of small antral follicles, 2-9 mm in size (Franks, Stark, and Hardy 2008).

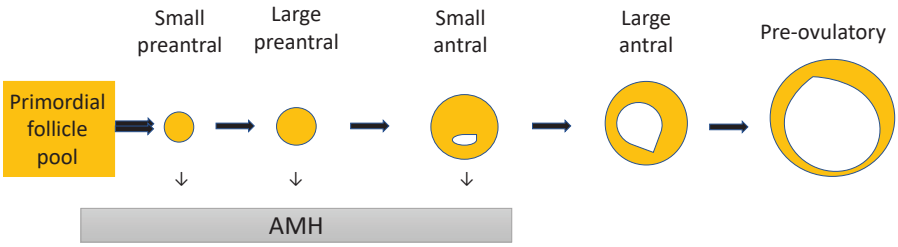

Figure 2. AMH is synthesized by small, growing follicles and acts as a gatekeeper by inhibiting the effect of FSH. In PCOS, AMH levels are high and FSH levels normal or low, which contributes to arrested follicular development with accumulation of small antral follicles.

1.5 PARITY AND MENOPAUSE IN PCOS

Parity

Infertility is a well-known consequence of PCOS, due to anovulation. Whether lifetime parity and nulliparity differ between women with PCOS and women in general is of interest, not only to the medical society providing infertility treatments, but also to young women with PCOS who worry about their future chances of getting pregnant. Although there are numerous publications on the effects on infertility treatment of women with PCOS, not much is published on overall parity rates at the end of the reproductive span.

In a follow-up of the Northern Finland Birth Cohort 1966, women with self-reported symptoms of PCOS were compared with women without such symptoms (West et al. 2014). The results showed that women with self-reported PCOS had the same prevalence of nulliparity as controls, but they had a lower mean parity number, 1.9 vs. 2.4 (West et al. 2014).

Menopause

Menopause is defined as the ending of menstrual periods, followed by one year without further bleeding, and where the reason for the cessation of bleeding is not due to other biological or physiological causes. Therefore, menopause is always defined retrospectively. The mean age of menopause in the general population is around 51 years (Santoro 2016; Li et al. 2016a).

Reproductive aging in women starts already in utero, when the number of oocytes is the greatest, around 500 000 –1 000 000 in total (Faddy et al. 1992). The depletion of the oocytes is due to a combination of atresia, recruitment towards a dominant follicle and ovulation (Faddy et al.

1992). When the number of ovarian follicles is reduced below a critical threshold (approximated to around 1000 follicles), ovulation ceases and menopause occurs (Faddy et al. 1992). The age of menopause differs between women. Whether the difference depends on the size of the original follicle cohort or is due to differences in the depletion rate of the follicles is not yet known.

The changes in the perimenopausal transition in non-PCOS women is summarized in a review by Santoro et al. (Santoro 2016). The loss of the ovarian reserve that accompanies the menopausal transition occurs before actual ovarian failure, which means that the granulosa cells are unable to respond to FSH. At the early phase of the menopausal transition (mean age 47 years), the ovarian follicle cohort has become critically low, and some menstrual periods will be missed. Cycles vary more and FSH starts to be elevated. The rise in FSH causes folliculogenesis to occur more rapidly, with shorter follicular phase length. At the late phase (mean age 49), menstrual cycles are often irregular and periods are scarce. Estrogen levels are more likely to be low, but can be high if ovulation occurs. In this review, the final menstrual period; *i.e.*, the menopause, occurred at a mean age of 51 years (Santoro 2016). In a prospective study by Burger et al., hormones were followed during the menopausal transition (Burger et al. 1999). Mean FSH levels increased greatly around the menopause, from 18 IU/L 1.5 years before the menopause, to 48 IU/L at the time of the menopause, and 101 IU/L 1.5 years after the menopause. Whereas levels of FSH increased progressively at the time of the menopausal transition, mean estradiol levels were characterized by a large variability during the premenopausal phase (Burger et al. 1999).

There are only a few reports on menopausal age in women with PCOS. A British study from 2000 found no difference in menopausal age between women with PCOS and women in general; however, a majority of the women with PCOS in that study had undergone ovarian surgery with a diminished ovarian reserve as a consequence (Wild et al. 2000a). In addition, only 38% of the original PCOS cohort participated in the

study (Wild et al. 2000a). In a recent Swedish study, women about to have a mammography screening filled out a questionnaire about common diseases and their menstrual and reproductive history. In this study, the median age of the natural menopause was 52 years in women without intercurrent diseases, but women reporting a PCOS diagnosis had a median menopausal age of 56 years (Li et al. 2016a). Attempts have also been made to predict the menopausal age in women with PCOS. Women with PCOS have higher AMH levels than women in general (Tehrani et al. 2010), and since AMH levels are highly correlated to the antral follicle count on ultrasound (Visser and Themmen 2005), it is likely that women with PCOS have a greater follicle reserve than women in general. Several studies have used AMH levels to calculate the menopausal age of women with and without PCOS (Tehrani et al. 2010; Depmann et al. 2016; Minooee et al. 2018). Based on the results of such calculations, the menopausal age of women with PCOS was postulated to be two years later than controls (Minooee et al. 2018).

1.6 METABOLIC DISTURBANCES IN PCOS

Obesity and abdominal fat distribution

Excessive body fat is linked to increased morbidity and mortality in the general population. For practical reasons, BMI is often used to asses obesity. According to the World Health Organization (WHO), BMI is classified as (WHO 1995; Seidell and Flegal 1997):

- ≥ 25 but < 30 kg/m^2 - overweight
- ≥ 30 but < 40 kg/m^2 - obesity
- ≥ 40 kg/m^2 - morbid obesity

This classification is based on the association between BMI and mortality (WHO 1995). Abdominal (visceral) fat distribution is associated with higher morbidity and mortality compared with peripheral fat distribution. Common ways to asses abdominal fat distribution is to measure the waist circumference or to calculate the waist-hip-ratio, with sex-specific cutoffs as markers of an increased morbidity risk (Seidell and Flegal 1997).

Women with PCOS have an increased prevalence of being overweight and obese compared with the general population. A review and meta-analysis of women with PCOS found a pooled prevalence of 61% for overweight and obesity combined, and a pooled prevalence of 49% for obesity (Lim et al. 2012). Women with PCOS also have an increased risk of abdominal fat distribution, with a RR of 1.7 compared with controls (Lim et al. 2012). As in most of the PCOS studies, mainly premenopausal women were included in this meta-analysis.

Dyslipidemia

Dyslipidemia has been shown to be the most important modifiable risk factor for myocardial infarction (Yusuf et al. 2004). As described by the National Cholesterol Education Program Adult Treatment Panel III guidelines, the primary target in order to prevent atherosclerosis and CVD is LDL (Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III) 2001). Both HDL and triglycerides, on the other hand, are well-described components of the metabolic syndrome, described later. Women with PCOS have lower HDL levels and higher levels of LDL and triglycerides than controls, which are all unfavorable changes with regard to cardiovascular risk (Diamanti-Kandarakis et al. 2007). In the evidence-based guidelines for the assessment and management of PCOS, overweight and obese women with PCOS are recommended to have a fasting lipid profile, including total cholesterol, LDL, HDL and triglyceride levels, at the time of diagnosis, regardless of age (Teede et al. 2018). Thereafter, the frequency of further lipid profiles should be based on the total CVD risk and the results from the first lipid profile (Teede et al. 2018).

Insulin resistance and type 2 diabetes mellitus

Women with PCOS have higher levels of insulin than women in general, and insulin is part of the vicious circle of androgens and insulin, as shown in Figure 1. Insulin resistance is present in 75% of lean, and 95% of overweight women with PCOS (Stepto et al. 2013). The insulin resistance in PCOS involves both extrinsic (BMI-related) and intrinsic mechanisms, where abnormal signaling in the insulin pathway has been found in women with PCOS (Diamanti-Kandarakis and Papavassiliou 2006; Stepto et al. 2013).

Diabetes mellitus is a condition characterized by elevated glucose levels, which cause microvascular damage, and it is associated with increased morbidity due to microvascular as well as macrovascular complications. According to the World Health Organization (WHO), the diagnostic criteria for abnormal glucose metabolism are as follows (WHO 2006):

- Diabetes: fasting plasma glucose (fP-Glc) level ≥ 7.0 mmol/L *or* a 2h plasma glucose level (p-Glc) ≥ 11.1 mmol/L after an oral glucose tolerance test (OGTT).
- Impaired glucose tolerance (IGT): fP-Glc < 7.0 mmol/L *and* a 2h p-Glc ≥ 7.8 and < 11.1 mmol/L after an OGTT.
- Impaired fasting glucose: fP-Glc 6.1 - 6.9 mmol/l (and, if measured, a 2h P-Glc after OGTT < 7.8 mmol/L).

Type 2 diabetes mellitus (T2DM) is characterized by hyperinsulinemia, insulin resistance and β cell failure, constituting more than 90% of all cases of diabetes mellitus (Chatterjee, Khunti, and Davies 2017).

Since insulin resistance is a key PCOS feature, PCOS is also considered a risk factor for T2DM. A recent meta-analysis showed that women with PCOS have threefold increased odds of IGT compared with controls, and also an increased risk of T2DM (Kakoly et al. 2018). The risk of IGT was increased independently of BMI, but the increased risk of T2DM disappeared when matching for BMI (Kakoly et al. 2018). Most of the included studies were cross-sectional and mainly included women around the age of 30 and younger; only two studies included women with a mean age above 50 years (Kakoly et al. 2018). There are few available studies involving peri- and postmenopausal women with PCOS. Comorbidities in women with PCOS above 40 years of age were recently reviewed, and the results, although based on few studies, suggest that the risk of T2DM remains elevated in peri- and postmenopausal women with PCOS, but the risk increase was uncertain if the results were adjusted or matched for BMI (Cooney and Dokras 2018).

The evidence-based guidelines for the management of women with PCOS recommend assessment of glycemic status at the time of diagnosis (Teede et al. 2018). After an initial assessment, women with PCOS should be assessed every one to three years, depending on the considered total risk, for early detection of IGT and/or T2DM (Teede et al. 2018).

1.7 CARDIOVASCULAR RISK FACTORS AND CARDIOVASCULAR DISEASE IN PCOS

Hypertension

The link between high blood pressure, both diastolic and systolic, and an increased cardiovascular disease (CVD) risk is well known. The risk of CVD increases in a log-linear way with increasing blood pressure, and a 20 mm Hg increase in systolic blood pressure and a 10 mm Hg increase in diastolic blood pressure are each associated with a doubled risk of death due to stroke or heart disease (Whelton et al. 2018). According to guidelines from the American College of Cardiology and the American Heart Association, hypertension is categorized into two stages, where a systolic blood pressure of 130 – 139 mm Hg and/or a diastolic blood pressure of 80 – 89 mm Hg are classified as stage 1 hypertension, and a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥ 90 mm Hg are categorized as stage 2 hypertension (Whelton et al. 2018). Treatment is recommended for stage 1 hypertension (130/80) as secondary prevention of recurrent CVD in patients with earlier CVD events, and as primary prevention with increased risk of CVD, whereas treatment is recommended for stage 2 hypertension (140/90) as primary prevention of CVD for adults without any other major risk factors (Whelton et al. 2018).

The prevalence of hypertension is increased in PCOS compared with controls in most studies, although results have been conflicting (Ollila et al. 2018; Chang et al. 2011; Pinola et al. 2017; Schmidt et al. 2011b). The evidence-based guidelines for the management of women with PCOS recommend that blood pressure be measured annually or more often, based on their CVD risk profile (Teede et al. 2018).

The metabolic syndrome

The metabolic syndrome is a cluster of risk factors for cardiovascular disease and T2DM. Different diagnostic criteria for the metabolic syndrome have been used by different organizations, but in 2009 a joint statement was published regarding the definition of the metabolic syndrome (Alberti et al. 2009). The risk factors included in the metabolic syndrome are elevated blood pressure, increased triglyceride levels, low HDL levels, increased fasting glucose and central obesity, where the presence of at least three of the five criteria qualify a person

Table 1. Criteria for the metabolic syndrome. Presence of at least three of the five criteria qualify a person for the diagnosis.

Criteria	**Definition**
Waist circumference	Population-specific. For Europeans/Caucasians, the most commonly used limit is ≥ 88 cm.
Elevated triglycerides	≥ 1.7 mmol/L and/or drug treatment for elevated triglycerides.
Reduced HDL	< 1.3 mmol/L in females and/or drug treatment for reduced HDL.
High blood pressure	Systolic ≥ 130 and/or diastolic blood pressure ≥ 85 mm Hg, and/or anti-hypertensive drug treatment due to hypertension
Elevated fasting glucose	≥ 5.6 mmol/L and/or drug treatment due to elevated glucose levels.

for the diagnosis. The criteria, according to the harmonized definition of the metabolic syndrome, are summarized in Table 1 (Alberti et al. 2009).

Women with PCOS have an increased prevalence of the metabolic syndrome compared with the general population. In a recent review and meta-analysis, women with PCOS had an OR of 3.4 for the metabolic syndrome compared with controls, and the prevalence remained significantly higher in BMI-matched studies (Lim et al. 2018). The studies in the review included almost entirely women of young ages; only two out of the 59 reviewed studies included women with a mean age above 40 years (Lim et al. 2018).

Cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of death in the United States (Virani et al. 2020). The CVD incidence increases with age, with the highest incidence above the age of 80 years (Virani et al. 2020). CVD is uncommon in women below 50 years of age (Bhatnagar et al. 2016). As discussed above, a number of modifiable risk factors for CVD have been identified, and women with PCOS have excessive risk factors for CVD compared with controls, already at young ages.

In addition to the increased risk factors for CVD, there is also evidence of increased subclinical vascular disease. Endothelial dysfunction precedes the development of CVD, and can be measured with flow-mediated dilatation (FMD), a non-invasive method (Sprung et al. 2013). A meta-analysis, including 21 studies comparing women with PCOS and controls, showed a significant decrease in FMD in PCOS, indicating an increased CVD risk (Sprung et al. 2013). However, all the studies included women with a mean age below 35 years (Sprung et al. 2013). Another non-invasive way to measure subclinical

atherosclerosis is to measure the carotid intima media thickness. Nineteen studies were included in a meta-analysis comparing women with PCOS and controls, and showed that women with PCOS had a greater mean carotid intima thickness, indicating more atherosclerosis; however, in 18 of the included studies, the mean age was below 30 years (Meyer et al. 2012). Measures of coronary artery calcium is a non-invasive way to measure coronary atherosclerosis, and studies have also found evidence of increased atherosclerosis in young women with PCOS (Shroff et al. 2007; Talbott et al. 2000). Thus, not only increased risk factors, but also signs of increased atherosclerosis in younger women with PCOS, strengthen the assumption that women with PCOS have an increased risk of CVD events and increased CVD mortality. As a contrast, women previously diagnosed with PCOS were included in a follow-up study at a mean age of 51 years, where women with PCOS had a smaller carotid intima thickness and a similar calculated ten-year cardiovascular disease risk (Meun et al. 2020).

The results regarding manifest CVD and CVD-related mortality have, however, been conflicting. The initial publication on CVD risk by our group from 1992 proposed a sevenfold increased risk of myocardial infarction (Dahlgren et al. 1992a). In the first follow-up of that cohort, at a mean age of 71 years, no increased risk of CVD events was found (Schmidt et al. 2011b). A number of studies regarding CVD in PCOS have been published, with varying results. Many of the studies reporting increased CVD events have included only or mostly premenopausal women, thus with low absolute risks (Glintborg et al. 2018; Ollila et al. 2018; Mani et al. 2013; Hart and Doherty 2015). Other studies have not found a difference in CVD events, but these have been cross-sectional or retrospective and with a mean age around the menopause or somewhat later (Wild et al. 2000b; Elting et al. 2001), and some of these studies have defined women with presumed PCOS at peri- or postmenopausal ages (Meun et al. 2018; Krentz, von Muhlen, and Barrett-Connor 2007).

As reviewed by Gunning et al., whether women with PCOS have an increased risk of CVD and CVD-related mortality remains unknown (Gunning and Fauser 2017). There is no available study where women with PCOS have been followed prospectively until the senescence, at an age where CVD events are common.

1.8 DEPRESSION/ANXIETY AND HEALTH-RELATED QUALITY OF LIFE IN PCOS

Depression and anxiety

Previous studies have shown that women with PCOS have a greater prevalence of depression and anxiety compared with controls (Hung et al. 2014; Cesta et al. 2016; Mansson et al. 2008) and that women with PCOS were more often hospitalized due to mood disorders (Hart and Doherty 2015). They also score higher on depression and anxiety scales, including the Beck Depression/Anxiety Inventory (BDI) and the Hospital Anxiety and Depression Scale (HADS) (Cooney et al. 2017; Cinar et al. 2011). However, these studies only included younger women. Women with PCOS also have a higher risk of other psychiatric disorders, and a common genetic pathogenesis between PCOS and psychiatric disorders has been suggested, since some data show that siblings, including brothers, of women with PCOS also had an increased risk of psychiatric disorders (Cesta et al. 2016; Cesta et al. 2017). To our knowledge, the Northern Finland Birth Cohort is the study with the highest mean age (46 years) to report on the prevalence of depression and anxiety in PCOS (Karjula et al. 2017). In that study, a self-reported PCOS diagnosis was associated with a higher prevalence of depression (25% compared with 14% for controls) and higher depression and anxiety scores. In a recent meta-analysis, including 30 studies comparing women with PCOS and controls, the median prevalence of depression (including mild) was 36.6% among PCOS women and 14.2% among controls (Cooney et al. 2017). The mean ages in the included studies were 20 – 30 years.

Health-related Quality of Life (HRQoL)

Health-Related quality of life (HRQoL) is used to measure the way a disease or disorder (and the therapy used to treat that disease or disorder) affects a person's ability to function, from that person's point of view (Krantz 2019). Different methods to measure HRQoL are available, both generic, which can be used regardless of the illness or condition, and disease-specific, adapted to certain diseases. Generic instruments give the opportunity to compare with other groups but, on the other hand, they may lack sensitivity for problems associated with specific conditions (Krantz 2019). The most commonly used generic instrument in PCOS is the short form 36 (SF-36), and the most commonly used disease-specific questionnaire is the PCOS questionnaire (PCOSQ) (Jones et al. 2008).

There is convincing evidence that women of fertile age with PCOS have reduced HRQoL compared with controls, with regard both to mental and physical aspects (Li et al. 2011; Jones et al. 2008). The typical features of PCOS, such as hirsutism, obesity, menstrual disturbances and infertility, have been shown to be associated with a decrease in HRQoL in younger women with PCOS (Hahn et al. 2005; Jones et al. 2008; Bazarganipour et al. 2015). If and how HRQoL is affected in peri- and postmenopausal women has not been studied.

1.9 MORTALITY IN PCOS

There are few available studies on mortality and PCOS. A retrospective cohort study from UK with a mean age of 57 years found no difference in CVD-related death or in total mortality (Wild et al. 2000b). In a prospective follow-up from our research group, women with PCOS had no increased total mortality, or mortality due to CVD, at mean age 71 years (Schmidt et al. 2011b). A population-based cohort study using data linkage showed an increased all-cause mortality in women with PCOS compared with controls, but the mean age was only 36 years (Hart and Doherty 2015).

Based on these findings, evidence regarding mortality is sparse and inconclusive.

2 AIM

The overall aim of this thesis was to increase the knowledge about ageing women with PCOS. The hypothesis was that ageing women with PCOS would have better health than expected, considering the increased risk factors for morbidity and mortality, due to increased protective factors associated with PCOS.

Specific aims:

To study if women with PCOS reach the menopause later than women without PCOS.

To study parity at the end of the reproductive period, compared with women without PCOS.

To study the development of type 2 diabetes mellitus in women with PCOS and explore which factors earlier in life are associated with an increased risk.

To study anthropometric changes and changes in reproductive hormones (including hyperandrogenism both clinically and biochemically) longitudinally in women with PCOS, and explore if they differ from those in women without PCOS.

To study health-related quality of life in women with PCOS after the reproductive period, and compare it with women without PCOS.

To study co-morbidity and mortality, including all-cause mortality and mortality related to cardiovascular disease, in post-menopausal women with PCOS, compared with women without PCOS.

3 PATIENTS AND METHODS

This thesis includes two cohorts of women with PCOS and their controls. The age ranges of the women in the different cohorts are illustrated in Figure 3. The women with PCOS from the two cohorts together cover an age range from 20 to 91 years.

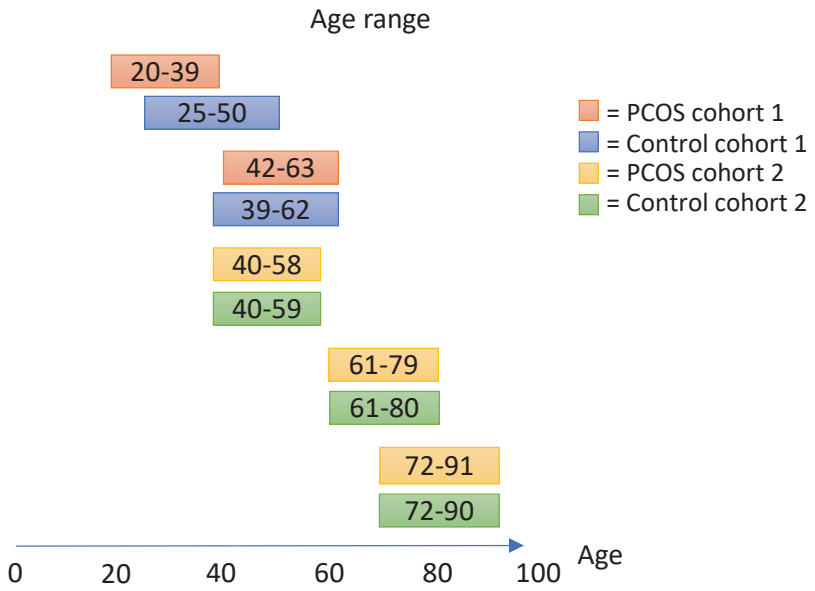

Figure 3. Age range of the women with PCOS and controls from two cohorts, at the different follow-up times. The age of the women with PCOS ranges between 20 and 91 years.

Paper I-III:

PCOS cohort 1 and Control cohort 1

Examined twice:

1) At mean age 30 years (1992, PCOS cohort 1) and mean age 40 years (1995, Control cohort 1)
2) At mean age 52 years (2016 PCOS cohort 1; 2008 Control cohort 1)

Paper IV-V:

PCOS cohort 2 (the Gothenburg PCOS cohort) and Control cohort 2

Examined three times:

1) At mean age 50 years (1987)
2) At mean age 71 years (2008)
3) At mean age 81 years (2019)

3.1 WOMEN WITH PCOS AND CONTROLS

PCOS cohort 1

A flow chart of PCOS cohort 1 is shown in Figure 4.

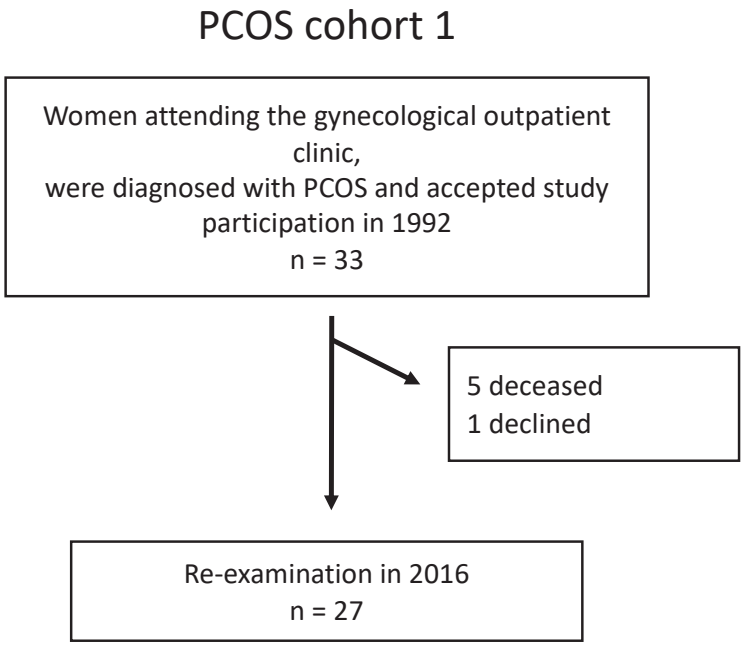

Figure 4. Flow chart of PCOS cohort 1. The women with PCOS were recruited when attending the gynecological outpatient clinic at Sahlgrenska University hospital due to PCOS-related complaints, and accepted to participate in a study regarding hormonal treatment.

In 1992, 33 women diagnosed with PCOS according to the NIH criteria, were included in a study regarding hormonal treatment for hirsutism, which included clinical examination, blood sampling and structured interviews before the intervention (Dahlgren et al. 1998). The women, at that time aged 20-39 years, were initially attending Sahlgrenska University Hospital for infertility or hirsutism.

A follow-up study was carried out in 2016. Five of the 33 women with PCOS were deceased and one declined participation. Thus, twenty-seven women with PCOS were included, with a mean age of 52 years, range 42-63 years. Of these, 25 participated in all parts of the follow-up study and two completed the interviews only. The protocols from 1992 were reviewed to establish the PCOS phenotype. All 27 women with PCOS had both clinical hyperandrogenism and oligo-/amenorrhea, meaning that both the NIH criteria and the Rotterdam criteria were fulfilled.

Control cohort 1

A flow chart of Control cohort 1 is shown in Figure 5.

A randomly selected sample from the general population was examined in 1994-1995 (25-64 years, n=872 women) as part of the World Health Organization (WHO) study, MONItoring of trends and determinants for CArdiovascular disease (MONICA) 1995, Gothenburg, Sweden (Wilhelmsen et al. 1997). They were re-examined in 2008, with a physical examination and structured interviews using standardized questionnaires, with a participation rate of 65% (Trimpou et al. 2012). In the age group 39-78 years, 317 women participated in the 2008 follow-up (Krantz 2019). To create a control group for the PCOS cohort examined in 2016, the women who participated in the 2008

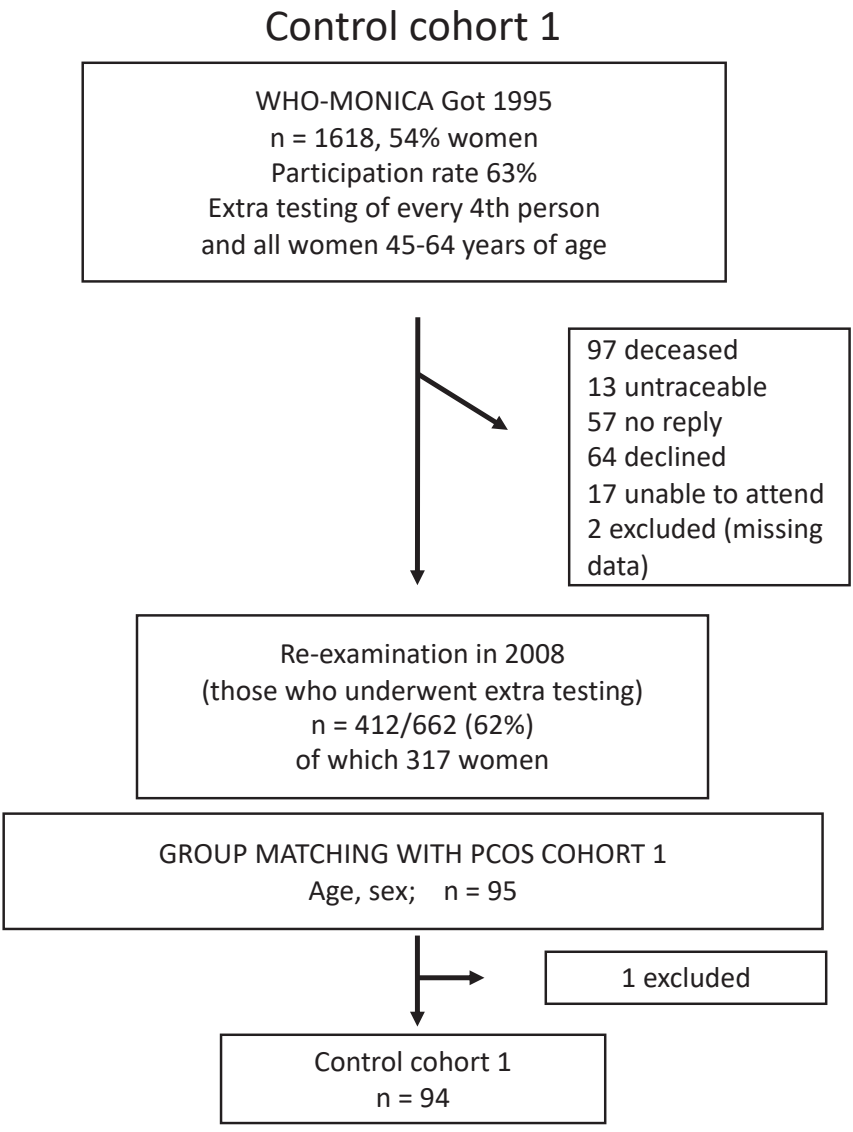

Figure 5. Flow chart of Control cohort 1. Controls were recruited from the WHO-MONICA Got 1995 study, which consisted of a randomly selected sample from the general population. A group-matching model, based on age and gender, was used to create a control group for PCOS cohort 1.

follow-up were used and an age-matched control group consisting of 95 women was created, using a group-matching model.

One of these women was excluded due to a testosterone level above the reference interval at that time, leaving a final control group of 94 women in the age range 38-68 years. The data used were retrieved from the examinations in 1995 and 2008. The women in the control group came from the same geographic area as the women with PCOS.

PCOS cohort 2 (the Gothenburg PCOS cohort)

A flow chart of the Gothenburg PCOS cohort is shown in Figure 6.

This cohort was first identified in 1987, when hospital records for all women undergoing wedge resection between 1956-1965 (n = 49) at Sahlgrenska University Hospital, Gothenburg, Sweden, were screened. All original histopathology specimens were re-evaluated and the histopathology was identified as typical of Stein-Leventhal in 38 cases. In 1987, three of the 38 identified women with Stein-Leventhal were deceased. The remaining cohort of 35 women were invited and 33 participated in a study regarding the long-term consequences of PCOS (Dahlgren et al. 1992b; Dahlgren et al. 1992a; Dahlgren et al. 1994).

In 2008, a new follow-up focusing on long-term consequences was carried out (Schmidt et al. 2011a; Schmidt et al. 2011b; Schmidt et al. 2012). Earlier study protocols were re-evaluated to confirm the PCOS diagnosis according to the Rotterdam criteria (Fauser et al. 2004). All 38 women had typical ovarian features on histopathology, and a typical ovarian histopathology is known to correlate with the PCOM ultrasound diagnosis (Takahashi et al. 1994). In 37 of the 38 women, either hyperandrogenism and/or oligo-/amenorrhea during their reproductive years was confirmed. One woman did not fulfill the Rotterdam criteria

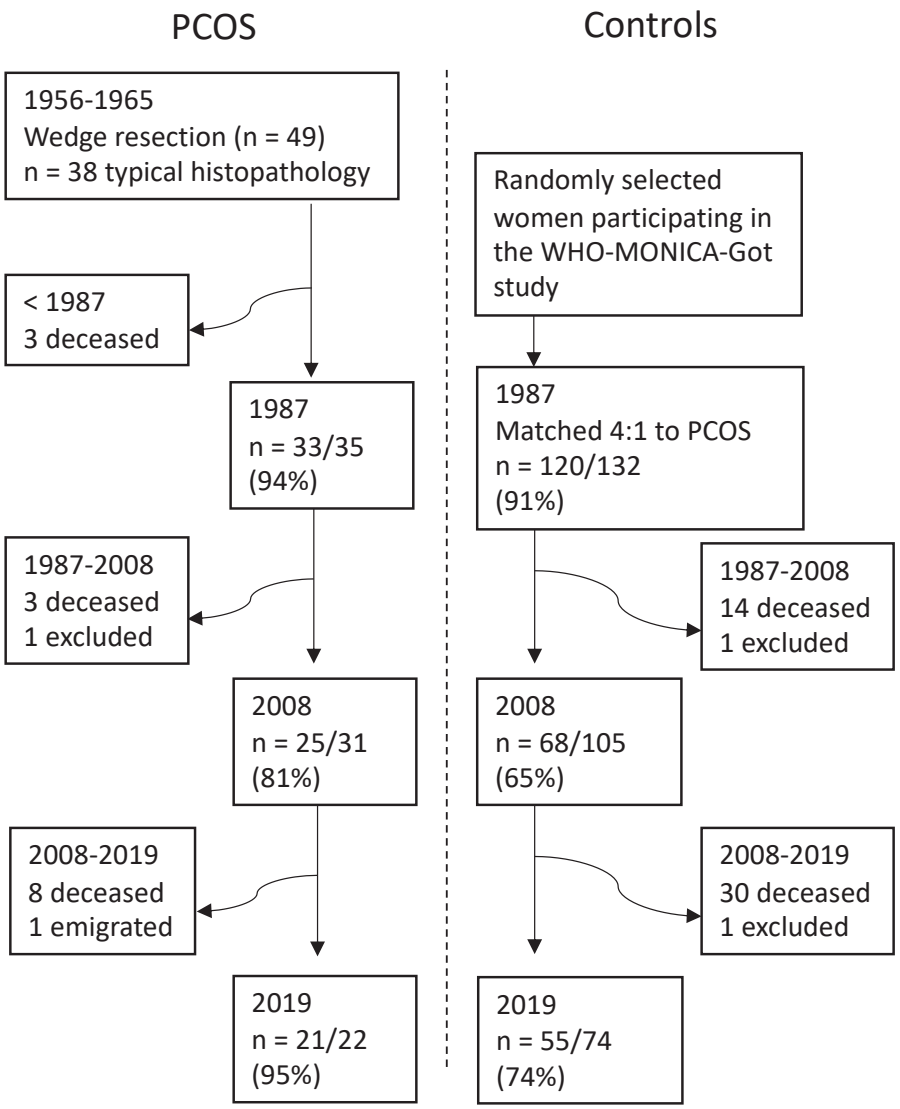

Figure 6. *Flow chart of PCOS cohort 2 (the Gothenburg PCOS cohort) and Control cohort 2. Controls were recruited from the WHO-MONICA Got 1985 study, which consisted of a randomly selected sample from the general population. Controls were matched in a 4:1 ratio with the women with PCOS who participated in the first follow-up in 1987.*

and was excluded. Of the 37 remaining women, three were deceased before 1987 and three more died between 1987 and 2008. Twenty-five women with PCOS participated in the 2008 study and morbidity data were available for 32 of them.

In 2019, 14 of the women with PCOS were deceased, eight of whom had died between 2008 and 2019. One woman had moved abroad and was lost to follow-up. The 22 eligible women with PCOS were invited to participate in the 2019 study. One of these women could not be reached, 21 (95%) participated in structured interviews, and 14 of these also underwent clinical examinations including blood sampling.

Control cohort 2

A flow chart of the control population is shown in Figure 6.

Randomly selected women from the general population of the same city as the women with PCOS (Gothenburg, Sweden) had participated in the WHO MONICA study 1985 (Wilhelmsen et al. 1997). Age-matched controls for the 33 women with PCOS who participated in the 1987 study were obtained from the participants of the WHO MONICA study at a 4:1 ratio (Dahlgren et al. 1992b; Dahlgren et al. 1992a; Dahlgren et al. 1994). Of the 132 invited controls in 1987, 120 participated. The controls were also comparable with the women with PCOS regarding BMI (Dahlgren et al. 1992b; Dahlgren et al. 1992a; Dahlgren et al. 1994).

Of the 120 controls, 106 were alive in the 2008 follow-up (Schmidt et al. 2011a; Schmidt et al. 2011b; Schmidt et al. 2012). To exclude PCOS cases among the controls, a re-evaluation was made according to the Rotterdam criteria introduced in 2003 (Fauser et al. 2004). If a woman in the control cohort only had one of the two criteria, hyperandrogenism (clinical or biochemical) or irregular bleeding, they remained as

controls. One control had a history of both irregular periods and hirsutism and was excluded. In 2008, 68 of the controls participated in all examinations and morbidity data were available for 95 of the women.

In 2019, the previous study protocols were recaptured again, and in addition to the woman who was excluded in 2008, one more woman, who declined participation in 2008 but accepted in 2019 was excluded, since she had a history of a combination of hirsutism and irregular bleeding. Of the remaining 118 controls, 44 were deceased (during the time period 1987-2019), 30 of them during the period 2008-2019. The remaining 74 women were invited to the 2019 follow-up study. Fifty-five (74%) women participated in structured interviews, twelve could not be reached and seven declined participation. Of the 55 women who participated, 28 underwent the clinical examinations, including blood sampling.

3.2 CLINICAL EXAMINATION

Body weight was measured in light clothing to the nearest 0.5 kg, and height was measured to the nearest 0.5 cm. Self-reported weight and height were used for those who only participated in the interviews but not in the clinical examination. Body mass index (BMI) was calculated as body weight divided by squared height (kg/m^2).

Waist was measured to the nearest cm in the standing position over the umbilicus and hip circumferences was measured at the maximum circumference over the buttocks. Waist-hip-ratio (WHR) was calculated.

Blood pressure was measured twice in the right arm, in the supine position, after 15 minutes of rest with an electronic blood pressure device. The means from the two measurements were used for systolic and diastolic blood pressure, respectively.

3.3 STRUCTURED INTERVIEWS AND REGISTER DATA

Structured interviews were made regarding social factors, smoking, reproductive history, previous and current diseases, current medications, physical activity levels, quality of life, and dietary habits.

For women who were deceased, data were retrieved from the National Board of Health and Welfare, using the Cause of Death Register, the Swedish Cancer Register and the National Patient Register (inpatient and outpatient registers).

Physical activity levels during leisure time and at work were measured using the Saltin-Grimby Physical Activity Scale. This grading is well-known and was originally used to assess the relationship between physical activity and risk of myocardial infarction (Saltin and Grimby 1968; Wilhelmsen et al. 1976). Physical activity at work was graded as: 1) predominantly sedentary; 2) some walking and standing but no stairs or heavy lifting; 3) walking, including stairs or walking uphill and/or lifting heavy objects, or 4) corresponding to heavy physical labor. Physical activity during leisure time was graded as 1) predominantly sedentary; *i.e*., reading or watching television; 2) moderate activity like walking, riding a bicycle, and/or light gardening at least four hours per week; 3) regular exercise such as running, swimming, tennis, heavy gardening at least two to three hours per week, and 4) regular athletic training and/or participation in competitive sports several times per week. The four possible levels of activity were then grouped for analysis purposes into “sedentary” (grade 1 and 2) or “active” (grade 3 and 4) lifestyle, during leisure and work, respectively.

Dietary habits were measured using a food frequency questionnaire (FFQ). A very similar FFQ has been used and validated in the Northern Sweden Health and Disease Cohort (Johansson et al. 2002). The

questionnaire contained questions on how often the women consumed 83 different groups of food items, with nine possible answers ranging from a score of 1 (never) to 9 (4 times per day or more). For the later statistical analysis, the scores were recoded into frequencies per week: if a food item was never used, used a few times per year or 1-3 times per month it was recorded as 0 times/week. The other possible answers were recorded as: 1 time/week = 1 time/week; 2-3 times/week = 2.5 times/week; 4-6 times/week = 5 times/week; 1 time/day = 7 times/week; 2-3 times/day = 17.5 times/week; 4 or more times/day = 30 times/week. To compare dietary habits, three categories were created reflecting food with 1) a high fat content (food items with a high content of saturated fat such as butter, margarine, cheese, cream, French fries, pizza, sausage, hamburgers, meatballs, liverwurst, bacon, crisps, chocolate, ice cream, cakes and pastry were included in this category); 2) high sugar content (including food items such as buns, cookies, pastry, cakes, sweets, sweet beverages, sugar and jam), and 3) a Mediterranean diet (where vegetables, fruit, berries, vegetable oils and fish were included). Total scores based on the frequencies for the different diets were then calculated. Possible ranges were a) high fat content – 0-630, b) high sugar content – 0-240, and c) Mediterranean diet – 0-540; with a higher score corresponding to greater use of that kind of food.

The Short Form 36 questionnaire (SF-36) – The SF-36 questionnaire is a generic instrument for measurement of HRQoL. The SF-36 contains 36 questions, yielding scores where a high score represents a better HRQoL (Sullivan, Karlsson, and Ware 1995). The scores from the 36 questions are calculated with specific software, to yield scores in eight areas: Physical Functioning, Role Physical (limitations due to physical problems), Bodily Pain, General Health, Vitality, Social Functioning, Role Emotional (limitations due to emotional problems), and Mental Health. All areas receive scores from 0 to 100, where a low score represents poorer HRQoL. Two summary scores are also generated, the Mental Component Summary Score (based on the subscales Vitality,

Social Functioning, Role Emotional, and Mental Health), and the Physical Component Summary Score (based on the subscales Physical Functioning, Role Physical, Bodily Pain, and General Health). The summary scores also have a range of 0-100, and are designed to have a population mean score of 50 with a standard deviation of 10, where a lower score represents lower HRQoL. The SF-36 and other commonly used generic tools, such as the Nottingham Health Profile (NHP) and the Psychological General Well-Being Index (PGWB), have been shown to correlate well with the each other (Krantz et al. 2019). The SF-36 was chosen, since it is the most commonly used tool in women with PCOS (Jones et al. 2008; Li et al. 2011).

Definitions of lifestyle factors and disease

Type 2 diabetes mellitus (T2DM) – In Paper II-III: diagnosis was self-reported, but was later verified in medical records. All women who reported having a T2DM diagnosis also used anti-glycemic treatment. In Paper V: diagnosis was defined as a fasting plasma glucose level ≥ 7.0 and/or current use of medication for T2DM, verified in medical records, or, for deceased women, a T2DM diagnosis in the medical registers described earlier.

Hypertension was defined as treated hypertension, and all of the women who reported having hypertension used antihypertensive drugs (Paper III). In Paper V, a systolic blood pressure above 139 mm Hg and/or a diastolic blood pressure above 89 mm Hg was used as an alternative definition.

Hypothyroidism was defined as using levothyroxine and/or having a thyroid-stimulating hormone (TSH) > 4.2 mIU/L at the examination.

The metabolic syndrome was defined as fulfilling at least three out of the five criteria: 1) waist > 88 cm; 2) S-TG ≥ 1.7 mmol/L; 3) S-HDL

≤ 1.3 mmol/l; 4) Systolic blood pressure ≥ 130 mm Hg and/or diastolic blood pressure ≥ 85 mm Hg and/or use of anti-hypertensive drugs; 5) fasting plasma glucose ≥ 5.6 mmol/L or use of anti-glycemic drugs (Alberti et al. 2009). This definition is according to the harmonized definition of the metabolic syndrome, and is used, among others, by the American Heart Association (Alberti et al. 2009).

Overweight was defined as BMI ≥ 25 kg/m^2 but < 30 kg/m^2.

Obesity was defined as BMI > 30 kg/m^2.

Myocardial infarction was defined as reporting having had a myocardial infarction. All cases were later verified in medical records. In Paper V, an alternative for deceased women was a myocardial infarction diagnosis in the medical registers described earlier.

Stroke was defined as reporting having had a cerebrovascular hemorrhage or thrombosis. All cases were verified in medical records. In Paper V, an alternative for deceased women was a stroke diagnosis in the medical registers described earlier.

Transischemic attack (TIA) was defined as stating having had a TIA. All cases were verified in medical records. An alternative for deceased women was a TIA diagnosis in the medical registers described earlier.

CVD events were used as a combined term for any of the events: myocardial infarction, angina pectoris, stroke, and/or TIA.

Gynecological cancer included ovarian, endometrial and cervical cancer. All cases were self-stated and verified in medical records; for deceased women the diagnoses were retrieved from the medical registers described earlier.

Cancer as a cause of death was defined as any cancer diagnosis registered as a cause of death in the Cause of Death Register.

Depression and/or anxiety - Current diagnoses of depression and anxiety were consolidated into one group to increase power and were defined according to medical records.

Psychopharmacological medication - Current medication was coded according to the Anatomical Therapeutic Chemical (ATC) Classification System. The proportion of patients using medications from ATC groups N05 and N06, denoting antidepressants, anxiolytic and sleeping pills, was analyzed.

Hirsutism – In 2019, the modified Ferriman Gallwey Score (mFG score) (Ferriman and Gallwey 1961; Yildiz et al. 2010) was used. A score of ≥ 5 was considered manifest hirsutism, in line with the recommendations in the international evidence-based guidelines (Teede et al. 2018). In 1987, hirsutism was assessed with the question: "Have you noticed considerably increased excess hair on your face, chest, stomach, legs or arms?". In 2008, hirsutism was defined as having a subjective feeling of excessive, coarse hair and a need to remove hair at least twice a week.

3.4 BIOCHEMISTRY

Fasting venous blood samples were taken in the supine position after at least 15 minutes of rest and the analyses were performed at the accredited (SWEDAC ISO 15189) Laboratory for Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg, Sweden.

Since immunoassays were the principal method used in the earlier publications in 1987, 1992 and 2008, immunoassays were also chosen for the steroid analyses, including testosterone, in 2016 and 2019. Immunoassays tend to give higher values than mass spectrometry, because of cross-reactivity with steroid precursors and metabolites (Handelsman and Wartofsky 2013). In a study reporting age-specific testosterone levels in healthy elderly women (aged 70-95 years) using mass spectrometry, testosterone levels were around 0.5 nmol/L (Davis et al. 2019), similar to the present controls.

Serum androstenedione was determined using a competitive chemiluminescent method (Immulite® 2000XPi, Siemens Medical Solutions Diagnostics AB). The coefficient of variation (CV) was 12% at 4 nmol/l and 9% at 13 nmol/l.
Serum dehydroepiandrosterone sulfate (DHEAS) was analyzed with the Access DHEA-S-assay (Beckman Coulter), a competitive binding immunoenzymatic assay with a CV of 10% at 3.5 and 14 μmol/l.
Serum total testosterone was determined using an electrochemiluminescent immunoassay with competitive analysis (ECLIA) (Cobas 8000 Roche Diagnostics Scandinavia AB, Ängelholm, Sweden) in the 2019 follow-up and for PCOS cohort 1. The CV was 6% at 2.0 nmol/l. The lower detection limit was 0.4 nmol/L. Serum total testosterone for Control cohort 1 was determined by nonextraction competitive RIA (ICN Biochemicals, Inc. Diagnostics Division, Costa Mesa, CA, USA). The CV for total testosterone levels was 16.3% at 2.0 nmol/l.

Serum sex hormone-binding globulin (SHBG) was analyzed with the sandwich principle (Cobas 8000 Roche Diagnostics Scandinavia AB, Ängelholm, Sweden) in the 2019 follow-up and for PCOS cohort 1. The CVs for SHBG were 7% at 40 nmol/l and 9% at 100 nmol/l. For Control cohort 1, SHBG was determined by IRMA (Orion Diagnostica Oy, Espoo, Finland), and the CV for SHBG levels was 4.2% at 19.7 nmol/l and 6.3% at 76.3 nmol/l.
Free testosterone and bioavailable testosterone were calculated using the formula of Vermeulen et al. (Vermeulen, Verdonck, and Kaufman 1999). Bioavailable testosterone refers to a combination of free testosterone and testosterone bound to albumin.
The free androgen index (FAI) was calculated as total testosterone (nmol/L) / SHBG (nmol/L) x 100.
Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were analyzed with chemiluminescent microparticle immunoassays (Architect i2000SR), with detection limits of 0.05 IU/L and 0.1 IU/L for FSH and LH, respectively. The CVs were 6-9% and 7-11% for the FSH and LH analyses, respectively.
Total, LDL and HDL cholesterol and triglycerides (TG) in serum were measured with an enzymatic method (Boehringer, Mannheim, Germany). The CV for total cholesterol was 3% at serum concentrations between 4 and 6 mmol/l, 4% for LDL at concentrations between 2 and 5 mmol/l, 5% for HDL cholesterol at concentrations between 1 and 2 mmol/l and 4% for TG at concentrations between 1 and 2 mmol/l.

Serum thyroid stimulation hormone (TSH) was analyzed with an electroluminescent immunoassay sandwich method, Cobas 8000, Roche Diagnostics Scandinavia, Ängelholm, Sweden. The CV was 7% at 0.4 mIU/l, 6% at 4 mIU/L and 5% at 20 mIU/L. A S-TSH level > 4.2 mIU/L was considered abnormal.

Plasma glucose concentrations were measured using an enzymatic hexokinase method (for controls in 2008), GLU, Roche/Hitachi; Roche Diagnostics, GmbH, Mannheim, Germany with a 4% coefficient of variation (CV) at concentrations between 5 and 15 mmol/l, and for

women with PCOS in 2016 and for the 2019 follow-up, GLUC3, Roche/Cobas; Roche Diagnostics Scandinavia, with a 3% CV at concentrations between 5 and 15 mmol/l.

Serum insulin concentrations for controls in 2008 were measured with an immunometric two-step sandwich method and chemiluminescence technology (Insulin Elecsys, Roche Diagnostics GmbH, Mannheim, Germany) with a 10% CV at 6, 20 and 70 mU/l. For the women with PCOS in 2016, the analysis was similar; an ElectroChemiLuminiscenseImumunoAssay (ECLIA) sandwich method (Insulin Elecsys, Roche Diagnostics Scandinavia, Ängelholm, Sweden) with a 10% CV at 6, 20 and 180 mU/l.

The homeostasis model assessment of insulin resistance (HOMA-IR) was used to estimate insulin sensitivity, and was calculated as fasting insulin (µU/L) x fasting glucose (nmol/L)/22.5 (Matthews et al. 1985).

The hyperinsulinemic, euglycemic glucose clamp (DeFronzo, Tobin, and Andres 1979) was performed at baseline in 1992 in women with PCOS, to further estimate insulin sensitivity further (Krotkiewski et al. 2003). After an overnight fast, an indwelling catheter was put in an antecubital vein for administration of glucose, insulin and potassium. A second catheter was put in a hand vein of the contralateral arm for blood sampling. After a ten-minute infusion of a priming dose of insulin, a constant dose of insulin was given, with an infusion rate of 0.08 IU/kg body weight/min, which corresponded to insulin levels of approximately 200 mU/L. Blood glucose levels were kept constant at 4.9 – 5.0 mmol/L by continuous infusion of glucose. Potassium was infused at a rate of 50 ml/h. The clamp test was performed for two hours and the glucose disposal rate was calculated from the steady state glucose infusion rate over the last 30 minutes. During this steady state, the infused glucose equals the metabolized glucose. The glucose disposal rate was expressed as mg/kg lean body mass/min., since the most important pools of glucose elimination in response to insulin are the skeletal muscles.

3.5 STATISTICS

Conventional methods were used for means, standard deviations (SD), medians and interquartile ranges (IQR) and ranges. Intergroup comparisons of normally distributed continuous variables were calculated with Student's t test, and non-normally distributed data were tested with the Mann-Whitney U test. Categorical comparisons were calculated using Fischer's exact t test or the chi square test. For changes within groups between the different follow-up examinations, the paired t test was used for normally distributed continuous variables and the Wilcoxon signed rank test for not normally distributed variables. Odds ratios (OR) were computed with a multiple variate logistic regression analysis

SPSS Statistics, version 25, was used for the above analyses.

Kaplan-Meier curves were used for time to event analyses, with log-rank test and Cox proportional hazard models. SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used for these analyses.

SF-36 scores (Paper III) were calculated using scoring software obtained from Optum™ license number QM03712, and Mental and Physical component scores were calculated using 1998 US norms.

P values < 0.05 were considered significant.

3.6 ETHICS

All women participating in the studies gave their written informed consent.

The studies were approved by the Regional Ethical Review Board in Gothenburg.

Paper I – III: (Cohort 1) 2016 reg.no. 221-16, 2006 reg.no. 088-06, and 2011 reg.no. T282-11.

Paper IV – V: (Cohort 2) 2007 reg.no. 321-01 and 2018 reg.no. 551-18.

4 RESULTS

Results Paper I:

At a mean age similar to the average menopausal age, 37% of women with PCOS and 57% of controls were postmenopausal. Serum FSH levels were higher in controls, 52 vs. 31 IU/L. The mean menopausal age were 49 years in controls and 53 years in women with PCOS.

There was no difference in parity between women with PCOS and controls, 1.9 vs. 1.7. The proportions of women with different parity numbers are shown in Figure 7. Importantly, the proportion of women who were nulliparous did not differ at the end of the reproductive period. However, more than 50% of the women with PCOS had undergone infertility treatment at least once.

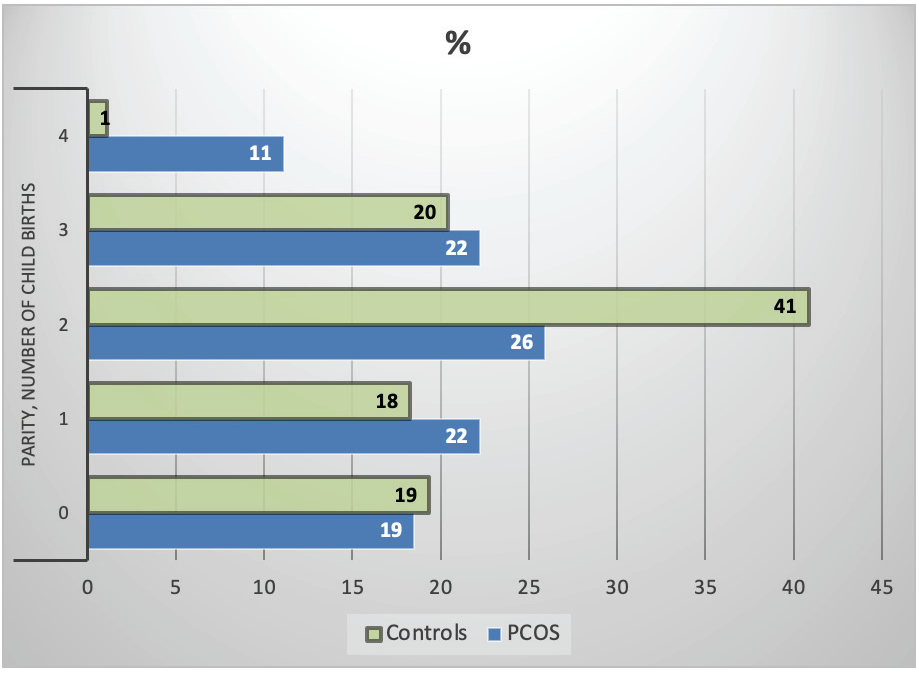

Figure 7. Proportion of women with PCOS and controls, respectively, with different parity numbers.

Results Paper II:

At perimenopausal age, 19% (5/27) of the women with PCOS had T2DM, compared with 1% (1/94) of controls. The women with PCOS who had developed T2DM had higher BMI, waist circumference and WHR already 24 years earlier, compared with the women with PCOS who did not later develop T2DM. In fact, all women with PCOS who had developed T2DM were obese (BMI ≥30 kg/m^2) and had a WHR ≥ 0.85 at mean age 30 years. In addition, fasting serum insulin levels were higher and HOMA were lower at baseline, and results from a glucose clamp test at baseline showed a lower mean glucose infusion rate per lean body mass, indicating a higher insulin resistance already at that age. SHBG, total testosterone and FAI did not differ between those who later developed T2DM and those who did not.

At mean age 52 years, women with PCOS had higher body weight (86 vs. 70 kg), BMI (31 vs. 26 kg/m^2), waist circumference (102 vs. 87 cm) and WHR (0.90 vs. 0.83), compared with controls. Since the duration of the follow-up period differed between women with PCOS and controls, the increase/year in BMI and WHR was calculated and there was no difference between the groups. HOMA levels were still higher in the women with PCOS, as was fP-Glc, but no differences were found in testosterone or FAI at this age.

Dietary habits and activity levels were compared at perimenopausal age. There was no difference in sedentary lifestyle, either at work or during leisure time. No differences were found between women with PCOS and controls regarding usage of a high-fat diet or a Mediterranean diet, but fewer women with PCOS than controls used a diet containing high sugar levels.

Results Paper III:

At perimenopausal age, there was no difference in HR-QoL in any of the eight dimensions. There was no difference in the physical summary score, median (IQR) 54 (43-58) vs. 56 (49-59), or in the mental summary scores, 53 (36-58) vs. 53 (47-58), for women with PCOS and controls, respectively. The prevalence of depression/anxiety did not differ at this age (22 vs. 15%), neither did usage of psychopharmacological medication (22 vs. 22%).

Similar HR-QoL was observed in the two groups, despite increased co-morbidity in women with PCOS. Thirty-seven per cent of the women with PCOS had hypertension, compared with 14% of controls, and 48% of women with PCOS had metabolic syndrome, compared with 12% of controls, indicating an elevated risk of later CVD.

Results Paper IV:

At mean age 81 years, there were no differences in BMI, body weight or WHR between women with PCOS and controls.

Women with PCOS still had lower FSH levels (50 vs. 70 IU/L). No differences were found in the levels of LH, SHBG, testosterone (including total, calculated free and calculated bioavailable testosterone), androstenedione, DHEAS or FAI.

Hirsutism was still more prevalent in women with PCOS than in controls, 33% vs. 4%.

Both testosterone levels and FAI decreased continuously during the 32 years of follow-up, whereas waist, WHR and SHBG increased continuously in the women with PCOS.

Results Paper V:

At mean age 81 years, there were no differences in T2DM (24 vs. 18%), hypertension (67 vs. 66%), myocardial infarction (10 vs. 13%), angina pectoris (14 vs. 16%), stroke (10 vs. 19%), or TIA (5 vs. 6%) between the PCOS and the control group. Neither did total CVD events differ; 38% vs. 41%.

Levels of glucose, insulin, HOMA-IR, thyroid hormones and blood lipids were similar between groups.

There was no difference in HR during the 32 years of follow-up for T2DM, CVD events, breast cancer, gynecological cancer, hypertension, stroke or myocardial infarction.

Total mortality was similar between women with PCOS and controls (HR 1.1, ns), as was CVD-related mortality (HR 1.7, ns). When comparing baseline data between the women with PCOS who were deceased and those still alive, there were no differences in total testosterone, FAI, SHBG, BMI or HOMA, but the deceased PCOS women had higher WHR at baseline, 0.87 vs. 0.80.

Combined results from the two cohorts

The women with PCOS in cohort 1 were more obese and had increased abdominal obesity, as measured by WHR and waist circumference, compared with controls; whereas in cohort 2, women with PCOS and controls had similar BMI and WHR. Anthropometry from the two cohorts at different ages are shown in Table 2.

Table 2. Age and anthropometry from the two cohorts at the different examinations. Women with PCOS vs. controls. Significant differences are marked in bold.

Cohort	1	1	2	2	2
Mean age, years	**30 vs. 40**	52 vs. 52	49 vs. 50	70 vs. 71	81 vs. 81
Mean BMI, kg/m^2	**27 vs. 24**	**31 vs. 26**	26 vs 25	27 vs. 26	26 vs. 26
Obesity	**26% vs. 4%**	**41% vs. 11%**	19% vs. 14%	24% vs. 19%	10% vs. 22%
WHR	**0.83 vs. 0.79**	**0.90 vs. 0.83**	0.82 vs. 0.80	0.85 vs. 0.87	0.89 vs.0.86
Waist ≥ 88 cm	**48% vs. 12%**	**80% vs. 39%**	**41% vs. 22%**	58% vs. 51%	64% vs. 50%

Table 3 shows comparisons of reproductive hormone levels between women with PCOS and controls from the two cohorts, at different ages. FSH levels are lower in women with PCOS than in controls in both

Table 3. Comparisons between women with PCOS and controls from the two cohorts regarding reproductive hormone levels at different ages, PCOS vs. controls, where the arrows indicate significant differences. N/A = not applicable.

Cohort	1	1	2	2	2
Mean age years	30 vs. 40	52 vs. 52	49 vs. 50	70 vs. 71	81 vs. 81
FSH	N/A	↓	↓	↓	↓
LH	N/A	↓	↓	=	=
Total testosterone	↑	=	↑	=	=
SHBG	=	=	↓	↓	=
FAI	↑	=	↑	↑	=

cohorts, from perimenopausal age until senescence. FAI levels were increased initially in both cohorts, but the difference disappeared with increasing age, already during the perimenopause in cohort 1 and at mean age 81 years in cohort 2. LH levels were lower in women with PCOS in both cohorts at perimenopausal age, but FSH levels were proportionally even lower, so the LH to FSH ratio was increased in women with PCOS in both cohorts at this age.

Table 4 summarizes levels of plasma glucose, insulin, HOMA and blood lipids from the two cohorts at different ages.

Table 4. Comparisons of plasma glucose, insulin, HOMA and blood lipids, between women with PCOS and controls from the two cohorts at different ages. PCOS vs. controls, where the arrows indicate significant differences. HOMA = Homeostasis Model Assessment of Insulin Resistance. N/A = not applicable.

Cohort	1	1	2	2	2
Mean age years	30 vs. 40	52 vs. 52	49 vs. 50	70 vs. 71	81 vs. 81
insulin	=	=	=	=	=
glucose	N/A	↑	=	=	=
HOMA	N/A	↑	=	=	=
triglycerides	=	↑	↑	↑	=
Total cholesterol	↓	=	=	=	=
HDL	N/A	=	↑	=	=
LDL	N/A	=	↓	=	=

The two cohorts were both examined at perimenopausal age. The results from these examinations were combined to increase power, and the cohorts were then stratified into normal weight (BMI < 25 kg/m^2) and overweight and obese (BMI ≥ 25 kg/m^2). The mean age for the combined cohorts was 51 years, range 39-63 years. Women with PCOS and controls were compared. In the normal weight group, BMI was similar; 22.7 vs. 22.4 kg/m^2. In the overweight and obese group,

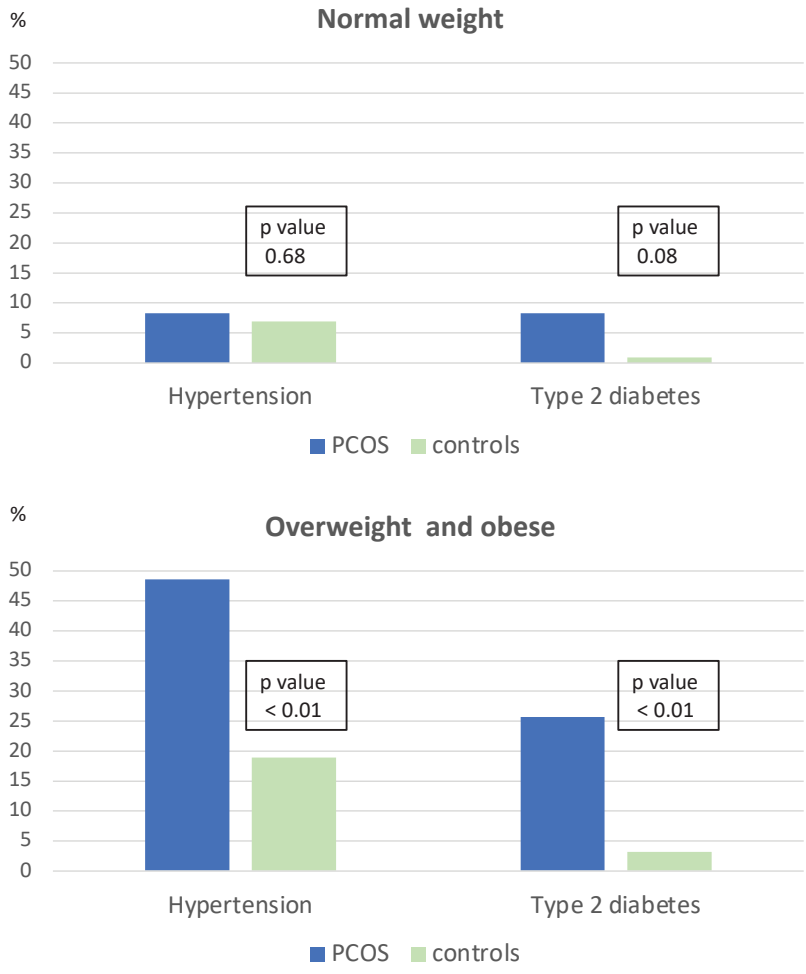

Figure 8. Prevalence of hypertension and type 2 diabetes at perimenopausal age in normal weight and overweight and obese women with PCOS and controls, respectively.

women with PCOS had a higher BMI; 32.2 vs. 28.9 kg/m^2, p value < 0.01.

As illustrated in Figure 8, in the normal weight group, there were no significant differences in the prevalence of hypertension or T2DM, but in the overweight and obese group, women with PCOS had more hypertension, 49 vs. 19%, p value < 0.01, and also more T2DM, 26 vs. 3%, p value < 0.01.

5 DISCUSSION

Reproductive hormones and hyperandrogenism

FSH levels are known to be decreased in women with PCOS of reproductive age, and this is thought to be one of the explanations for the anovulatory infertility in PCOS. Results from the two cohorts examined in this thesis show that this phenomenon exists also beyond the menopause. FSH levels have been lower in both cohorts at perimenopausal age, and have remained lower at mean ages 71 and 81 years, compared with controls.

The mechanism behind the lower FSH levels after the menopause is unknown. Alterations in FSH receptor genes as well as in the FSH-β genes have been linked to PCOS, but the exploration of the effect of these gene alterations has only begun, and, to date, only in fertile women with PCOS (Laven 2019). The GnRH pulse frequency in fertile women with PCOS differs from that in controls (Coyle and Campbell 2019; Rebar et al. 1976), and this is probably another mechanisms behind the lower FSH levels in fertile ages. It could be that the remaining lower FSH levels at older ages are due to a remaining difference in the GnRH pulse frequency, which, in turn, may be due to priming during the reproductive years, or to genetic differences. Both kisspeptin and neurokinin B are known to play a key role in the regulation of the hypothalamic-pituitary-gonadal axis, and emerging data suggest that changes in the kisspeptin-neurokinin pathway could play a role in the development of PCOS (Tang, Ding, and Zhu 2019; Skorupskaite et al. 2020). Beside the role of FSH in the ovary, FSH also plays a role in the regulation of bone mass, adipose tissue and cholesterol production, at least in animal models (Taneja et al. 2019). Whether the remaining lower FSH levels in women with PCOS after the menopause is one of the mechanisms protecting against additional BMI gain remains to be explored.

FSH is often used as a surrogate marker for the menopause, with different cutoff levels. In the well-known prospective study by Burger et al. on women in the general population, hormones were measured during the menopausal transition (Burger et al. 1999). FSH levels increased strongly around the menopause, from a mean FSH of 18 IU/L 1.5 years before the menopause; to a mean of 48 IU/L at the time of the menopause, and 1.5 years after the menopause, the mean FSH was 101 IU/L. Levels around and above 50 IU/L were thus indicative of the menopause in non-PCOS women in that study (Burger et al. 1999). Using that cutoff in women with PCOS from the cohorts of this thesis shows that many women with PCOS have lower levels well after the menopause. At mean age 71 years, 64% of the women with PCOS had levels below 50 IU/L, and 17% had FSH < 30 IU/L at this age. If FSH is to be used as a proxy for the menopause in PCOS, normative values for the menopausal transition in this specific group need to be developed.

Hyperandrogenism is one of the hallmarks of PCOS, and as a single measurement to detect biochemical hyperandrogenism, the free testosterone (measured as calculated free testosterone, FAI or calculated bioavailable testosterone) level provides the greatest accuracy (Teede et al. 2018). In the present thesis, FAI was elevated, compared with controls, up to mean age 71 years. The higher levels of testosterone might be an advantage for women with PCOS, as that could be one reason why women with PCOS do not suffer from more CVD events, in spite of the increased number of risk factors for CVD. The FAI ratio between women with PCOS and controls was attenuated with increasing age, and at mean age 81 years, there was no significant difference. In addition, in the younger cohort, the difference in FAI was present in reproductive ages, but disappeared already at perimenopausal age. These findings call for caution, since several studies have used high testosterone or FAI levels in peri- and postmenopausal ages as a way to define presumed PCOS cases. Our findings show that even in women with well-defined PCOS, who were in contact with the healthcare

system during their reproductive years for PCOS-related symptoms, androgen levels normalize in relation to controls with increasing age. Women with high androgens at peri- and postmenopausal ages may represent a subgroup, not representing PCOS generally, and the findings of studies defining PCOS with these methods are therefore not valid for PCOS in general. This also emphasizes the importance of prospective long-term follow-up studies with well-characterized PCOS populations.

Hirsutism, a clinical marker of hyperandrogenism, persisted up to a mean age of 81 years in women with PCOS, despite the similar biochemical androgen levels at this age. Hirsutism was measured with different tools in 1987, 2008 and 2019, and can therefore not be compared directly, but the hirsutism ratio between women with PCOS and controls were similar at all times. This is somewhat surprising, considering the decreasing biochemical androgen levels. One explanation could be genetic changes linked to PCOS and to hirsutism; for instance, alterations related to androgen receptor activity or 5α-reductase activity (which converts testosterone to dihydrotestosterone). Another hypothesis is that androgen receptors in the hair follicles are sensitized during the time period when androgen levels are higher.

Low SHBG levels have been linked to PCOS, and low SHBG is one of the mechanisms behind the higher levels of free testosterone that characterize PCOS. In the general female population, SHBG decreases from an age around 20 to 60 years, when the levels start to increase gradually with increasing age, thus forming a U-shaped curve (Maggio et al. 2008). This was also seen in cohort 2 in this thesis, where SHBG increased both in women with PCOS and in controls from perimenopausal age and onwards. At the first two follow-ups (mean age 50 and 71 years, respectively), SHBG levels were lower in the PCOS women compared with controls. At the last follow-up, at mean age 81 years, a similar difference was seen, although not significant. SHBG is known to be lower in overweight women, and this has been put forward as one explanation for the lower levels in PCOS; however, in cohort 2, BMI was similar compared with controls at all follow-ups. A cross-

sectional study on women in the general population found higher SHBG levels in women ≥ 70 years, and suggested that high SHBG could be a metabolic survivorship advantage (Davis et al. 2019). However, in the present longitudinal follow-up, it is evident that SHBG levels increase with increasing age also in older ages, and both in women with PCOS and controls, and there was no association between lower SHBG levels earlier in life and increased mortality.

Parity and menopause

PCOS is the major cause of anovulatory infertility, comprising around 20% of infertile couples (Laven 2019). In a population-based study, women with self-reported PCOS had lower fecundability and suffered more from infertility compared with controls (38% vs. 16%) (Koivunen et al. 2008). A follow-up of the same cohort of women showed that at age 44, nulliparity did not differ, but controls had larger family size (West et al. 2014). In the present thesis, parity in women with PCOS, initially recruited from a hospital setting when attending a gynecological clinic for PCOS-related problems, was compared with parity in controls. In this setting, at the follow-up around perimenopausal age, the prevalence of nulliparity was similar to that in controls, as was parity numbers, even though a large proportion of the women with PCOS (> 50%) had received infertility treatment at least once. It is known that BMI is a predictor of treatment resistance in pharmacological induction of ovulation (Legro 2012). With this in mind it is encouraging to see the good results on both spontaneous and assisted pregnancies in the PCOS group, despite the higher average BMI. That the chance of getting pregnant is similar to controls, although sometimes with help, is important to young women with PCOS, who are often concerned about their future reproductive capacity.

It has been suggested that women with PCOS have a longer reproductive life than controls. This is supported by studies showing that women with

PCOS develop more regular ovulating cycles later in their reproductive life and have a larger ovarian reserve than controls (Eiting 2000; Hudecova et al. 2009; Carmina, Campagna, and Lobo 2012). Women with PCOS also have higher AMH levels than controls, and as AMH levels are highly correlated to the antral follicle count on ultrasound, they can thus be used as a surrogate for follicle number (Visser and Themmen 2005). Since the menopause occurs when the number of ovarian follicles declines below a threshold level, it is biologically likely that women with PCOS would reach menopause later than controls (Hansen et al. 2008). AMH levels have also been used to calculate menopausal age, and the calculations suggest that the menopause occurs two years later in women with PCOS (Tehrani et al. 2010; Depmann et al. 2016; Minooee et al. 2018). We found that women with PCOS, followed prospectively, reached the menopause four years later than controls. A well-known confounder of menopausal age is smoking, which leads to an earlier menopause, but there was no difference in smoking between the groups (Hayatbakhsh et al. 2012). The women with PCOS were more obese than the controls, but whether obesity is a confounder of menopausal age is unclear, with diverging results from different studies, as reviewed by Al-Safi (Al-Safi and Polotsky 2015), but in the present cohort, no association was found between BMI and menopausal age. In the older cohort, no difference in menopausal age was found. This might be due to the fact that all women with PCOS in that study had undergone ovarian surgery (wedge resection), which leads to a decrease in follicle numbers and, thus, could lead to an earlier menopause.

The longer fertile period could be one factor in PCOS that protects against CVD events, since CVD events increase after the menopause in women in general (van der Schouw et al. 1996), and also after premenopausal prophylactic oophorectomy (Ingelsson et al. 2011).

Obesity and fat distribution

In cohort 1, women with PCOS had higher BMI and WHR compared with controls, both at mean age 52 years and at baseline (24 years earlier for women with PCOS and 13 years earlier for controls), whereas in cohort 2, the older cohort, BMI has been similar at all three follow-ups.

In the prospective study from the Northern Finland Birth Cohort 1966, women with a self-reported PCOS diagnosis at age 31 years were studied regarding weight changes from 14 - 46 years (Ollila et al. 2016). Data showed that women who later developed symptoms typical of PCOS had higher BMI compared with controls already at age 14, and also at age 31 and 46 years. In addition, the women with PCOS also had greater weight gain in early adulthood (14-31 years) compared with controls, but not later (31–46 years) (Ollila et al. 2016). In a prospective study from Australia, women with PCOS had greater weight gain compared with controls between 20 and 30 years of age. In cohort 1, BMI differed compared with controls at mean age 52 years, but using the baseline data, the increase in BMI/year from mid-fertile age (30 years for PCOS and 40 years for controls) did not differ, indicating that the difference in BMI occurred before the mid-fertile age. Data from cohort 2 also show that women with PCOS do not gain more weight or have a greater BMI increase than controls after the menopause. This could be due to lifestyle advice in connection with the PCOS diagnosis, but also to weight gain being driven by hormonal factors that differ greatly from controls during the younger years but become more similar to controls later. Such hormonal factors that could affect early weight gain could be hyperinsulinemia and hyperandrogenism, which we have shown do not differ in older ages in PCOS.

Central obesity also increases in younger ages, with waist circumference increasing between 20-25 years and 40-45 years in women with PCOS (Carmina, Campagna, and Lobo 2012). However, that study did not include controls. In cohort 1, waist circumference and WHR were

higher, both at baseline and at follow-up during the perimenopausal age. However, the increase/year in WHR was not different compared with controls, indicating again that the difference in fat distribution has occurred earlier in life. In cohort 2, the WHR differed at perimenopausal age, but not at mean ages 71 or 81 years, indicating that controls catch up with PCOS women in terms of abdominal fat distribution, again perhaps due to the declining difference in hyperandrogenism after the menopause.

Dyslipidemia

Dyslipidemia is more common in women with PCOS of reproductive age than among women in general, and altered levels of triglycerides, HDL and LDL are more severe in hyperandrogenic women with PCOS (Fauser et al. 2012). In perimenopausal women, both in cohort 1 and cohort 2, women with PCOS had higher triglycerides than controls. This difference remained at mean age 71 years, whereas there was no difference at age 81 years.

At the first follow-up of cohort 2, at mean age 50 years, HDL levels were higher and LDL levels lower compared with controls, showing that the women with PCOS had a better risk profile at that age. This finding was not reproduced in cohort 1, nor was there a difference regarding HDL or LDL in cohort 2 at mean ages 71 and 81 years.

Insulin resistance and type 2 diabetes

Insulin resistance is one of the characteristic features of PCOS, and women of reproductive age have an increased risk of IGT and T2DM.

At mean age 30 years, the women with PCOS in cohort 1 were examined with a hyperinsulinemic-euglycemic glucose clamp. As expected,

glucose disposal was lower in obese than in lean women with PCOS (Krotkiewski et al. 2003). Dunaif and co-workers compared non-obese women with PCOS with non-obese controls, and obese women with PCOS with obese controls, and found that women with PCOS have a greater insulin resistance that is independent of obesity, but that, due to synergy, the insulin resistance increases significantly more in obese women with PCOS than in obese controls (Dunaif et al. 1989). This might be the reason why many of the women with PCOS had developed T2DM during the follow-up period; 19% had T2DM at perimenopausal age, compared with 1% of controls. In cohort 2, at similar age, 15% had developed T2DM compared with 2% of the controls, but the women in cohort 2 had similar BMI. Interestingly, at mean age 71 years, the prevalence had increased in both the PCOS group and the control group, but more in the control group. Twenty-two per cent of the women with PCOS had T2DM compared with 14% of the controls, and at mean age 81 years, 24% had T2DM compared with 18% of controls, with neither of these latter figures reaching significance. It seems that the controls catch up with the women with PCOS after the menopause, possibly also due to the decrease in androgens discussed earlier.

In cohort 1, baseline data were analyzed and women with PCOS who later developed T2DM were compared with those who did not. The results showed that all women with PCOS who developed T2DM during the follow-up were both obese (BMI $\geq$ 30) and had abdominal fat distribution (WHR $\geq$ 0.85). They also had a high HOMA index and low insulin sensitivity according to the hyperinsulinemic-euglycemic glucose clamp. Our results are in line with those of Ollila et al., where a population-based cohort of women were followed prospectively. The women with self-reported PCOS had 2.5 times higher odds of T2DM than controls, but no cases of T2DM were found in normal-weight women with PCOS at age 46 years (Ollila et al. 2017). As shown in Figure 8, T2DM was also found in normal weight women with PCOS at perimenopausal age in the present cohorts, but the effect of PCOS and overweight /obesity combined was much more pronounced than

overweight/obesity alone, also with women with PCOS recruited from a hospital setting. Our results further emphasize the importance of regular screening of women with PCOS. According to guidelines, screening for glycemic status every 1-3 years is recommended for women with PCOS, and for high-risk women, including women with a BMI > 25 kg/m^2, an oral glucose tolerance test (OGTT) is recommended (Teede et al. 2018). Nineteen per cent of women with PCOS developed T2DM from a mean age of 30 years, to a mean age of 52 years, but in the subgroup of women with PCOS and BMI ≥ 30, 71% developed T2DM during the same period, showing that obese women with PCOS are at extreme risk of developing T2DM.

At mean age 52 years, women with PCOS had a diet with less sugar compared with controls, but there was no difference regarding sedentary lifestyle or high-fat food use, or use of a Mediterranean diet. This might be due to dietary advice earlier, since lifestyle intervention is the first line of treatment for PCOS, but also to the fact that T2DM was more common among women with PCOS and they would then be advised to change their diet.

In the general population, weight loss, regardless of whether it is due to lifestyle changes, weight loss medication or bariatric surgery, has been shown to prevent or delay T2DM (Grams and Garvey 2015). It is likely that these kinds of interventions could also prevent or delay T2DM in women with PCOS, if timed properly.

Cardiovascular risk factors and cardiovascular disease

In 1992, Dahlgren et al. published a study on women with PCOS and risk of CVD (Dahlgren et al. 1992a). Based on the CVD risk factors of the model at the time and data from cohort 2 at perimenopausal age, the calculations predicted a sevenfold increased risk of CVD (Dahlgren et al. 1992a). That women with PCOS have increased risk factors for CVD

was later confirmed in other studies, but did not seem to be associated with an increase in CVD events (Wild et al. 2000b). In the follow-up of cohort 2, that is, the same cohort that the risk factor model was based on, we did not find an increase in CVD events or in CVD-related mortality.

Hypertension was one of the risk factors included in the model mentioned above, and high blood pressure is also included in the metabolic syndrome, which summarizes a group of interrelated risk factors for CVD (Alberti et al. 2009). In our cohorts, the prevalence of hypertension was elevated at perimenopausal age, in cohort 1, 37 vs. 14%; and in cohort 2, 39 vs. 11%, which shows that the figures are strikingly similar, although the women with PCOS in cohort 1 had a higher BMI. As shown in Figure 8, BMI has an impact on the prevalence of hypertension. In the normal weight group, there is no difference between women with PCOS and controls, but in the overweight and obese group, hypertension is more common both in PCOS and controls, but the effect of BMI on hypertension is much more pronounced in women with PCOS than controls.

At mean age 71 years, hypertension was still more common among women with PCOS, 69 vs. 41%, but at mean age 81 years, the prevalence was similar, 67 vs. 66%. Again, it seems as if the controls catch up with the women with PCOS with increasing age. Results from previous studies regarding CVD events have been diverging. There has been a long-felt need for longitudinal follow-ups of women with PCOS and controls reaching a high age, since CVD events occur more frequently with increasing age, and the highest incidence is seen above 80 years of age. (Virani et al. 2020). In the present follow-up, the women were followed from perimenopause until a mean age of 81 years. The incidence rates for CVD events were similar for women with PCOS and controls. This strengthens the hypothesis that women with PCOS do not only have increased risk factors for CVD, but also increased protective factors. These factors include a delayed menopause. Concerning hyperandrogenism, the biochemical difference is attenuated with

increasing age. Whether hyperandrogenism is a risk factor, or on the contrary, a protective factor, can be debated. Recent studies suggest that the elevated androgens may be an advantage for women with PCOS in terms of CVD development. Studies on postmenopausal women have found that elevated testosterone levels are not associated with increased mortality, and in postmenopausal women with diabetes, low testosterone levels were associated with elevated CVD mortality (Wehr et al. 2011). In fact, one study showed that in postmenopausal women not using hormone replacement therapy, high testosterone was associated with a decreased HR for CVD (Zhao et al. 2018). Another study, with a case-control design, showed that postmenopausal women with hormone replacement therapy who had suffered a stroke or myocardial infarction, had lower testosterone levels compared with women who were cardiovascularly healthy (Khatibi et al. 2007). It is possible that the increased levels of testosterone in women with PCOS are actually protective of CVD.

Depression and anxiety

Women with PCOS have an increased risk of psychiatric co-morbidity, and most well-documented is the increased risk of depression and anxiety (Cooney et al. 2017; Cesta et al. 2016). However, studies regarding psychiatric co-morbidity have involved premenopausal women. The study with the oldest mean age this far studied women with self-reported PCOS at a mean age of 46 years. In that study, 25% of the women with PCOS had depression, compared with 14% of the controls (Karjula et al. 2017). In our follow-up, at a mean age of 52 years, 22% of the women with PCOS had depression and/or anxiety, compared with 15% of the controls. The majority reported depression, not anxiety. Our figures were similar to those in the study referred to above, but with the difference that we included depression and anxiety; however, the difference in our study did not reach significance. The figures from both these studies are also similar to those reported from a meta-analysis for

moderate to severe depressive symptoms in younger women, 26 vs. 15% (Cooney et al. 2017). In addition, they are similar to a register-based study, with the aim to investigate familial factors in the association between PCOS and psychiatric co-morbidity (Cesta et al. 2016). In that study, more than 24 000 women with a PCOS diagnosis, their full siblings and controls were included. The results showed that 22% of the women with PCOS received at least one psychiatric diagnosis, compared with 16% of controls (Cesta et al. 2016). In addition, both sisters and brothers of women with PCOS had a significantly higher risk of psychiatric disease, compared with sisters and brothers of controls, which suggests that PCOS could be associated with genes increasing the risk of psychiatric disorders (Cesta et al. 2016).

The proportions of women who had current use of psychopharmacological medication at perimenopausal age in cohort 1 were compared, but this did not differ between the groups, 22% vs. 22%. These latter figures resemble those from cohort 2, at mean age 81 years, where 24% of the women with PCOS had depression, compared with 23% of controls.

Quality of life

Measures of HRQoL have been accorded increasing importance. In young women with PCOS, studies have repeatedly shown decreased HRQoL compared with controls in premenopausal ages. How PCOS affects women at older ages has not been studied previously. In younger women with PCOS, symptoms such as hirsutism, menstrual irregularities and obesity were associated with a decrease in HRQoL (Hahn et al. 2005; Bazarganipour et al. 2015). Infertility is also known to affect HRQoL (Chachamovich et al. 2010). In older ages, one could expect that women have learned to cope with hirsutism, their menstruations cease, and, as shown, there is no difference in nulliparity at the end of the reproductive period. With this in mind, the results, at a

mean age of 52 years, when no difference in HRQoL was found compared with controls, either with regards to mental or in physical aspects, are not surprising.

Mortality

In this long-term prospective follow-up of women with PCOS diagnosed in 1956-1965, there was no difference in all-cause mortality or in CVD-related mortality, compared with age-matched controls, when the women had reached a mean age of 81 years. This despite the increased number of risk factors associated with increased mortality in the general population. This raises the question of whether other, protective, factors balance these risk factors for increased mortality.

Baseline data were compared between deceased and living women with PCOS, and there was no difference in testosterone/FAI, SHBG or HOMA levels. Although these data are based on small numbers, no differences in hyperandrogenism or insulin resistance seem to predispose powerfully for increased mortality in the PCOS group. The women with PCOS who were deceased had a higher WHR at baseline compared with the women who were still alive, and increased abdominal adiposity, for which a high WHR is a surrogate marker, is a risk factor for all-cause mortality in the general population (Koster et al. 2015). This underlines further the importance of abdominal obesity as a modifiable and strong risk factor for future health among PCOS women.

CONCLUSIONS

Key findings in PCOS during a lifetime perspective:

- No difference in mortality compared with controls.
- No difference in CVD events compared with controls.
- Women with PCOS reached the menopause later than controls.

In many ways, women with PCOS and controls differed to a less extent with increasing age:

- Biochemical hyperandrogenism: the difference compared with controls diminished with increasing age and, eventually, there was no difference.
- Insulin resistance and insulinemia: no remaining difference at mean age 81 years.
- HRQoL: at mean age 52 years, there were no differences.
- Parity: no difference at the end of the reproductive period.

However, there were remaining differences attributable to PCOS up to the senescence:

- Hirsutism was more frequent in PCOS at mean age 81 years, and the difference compared with controls did not seem to be attenuated with age.
- FSH levels remained lower in PCOS from the perimenopause until the senescence.

One of the main findings was that women with PCOS did not have increased mortality compared with controls, neither all-cause mortality nor CVD-related mortality. This was despite the well-documented risk profile, including the increased prevalence of hypertension, T2DM, obesity and dyslipidemia. It seems that women with PCOS have other possible protective factors that counteract the increased risk of CVD. One such factor is the later menopausal age. The combination of PCOS and overweight and/or obesity greatly exacerbate co-morbidity, including hypertension and T2DM.

CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

The finding of similar mortality compared with controls needs to be repeated in other cohorts followed prospectively. This PCOS cohort was derived from a hospital setting and should represent a more severe form of PCOS, however, thereby also representing the women with PCOS that the healthcare system meets. The risk profile earlier in life in this cohort lead to the suspicion of an increased risk of both CVD events and mortality, but since this turned out to be wrong, the protective factors in women with PCOS need further exploration, preferably in larger cohorts. Pinpointing protective factors in women with PCOS could, in a broader perspective, also lead to the development of future gender-specific prophylactic treatments for other women with an increased risk of CVD.

In the two cohorts followed prospectively in this thesis, hyper-androgenism declined and the androgen levels increasingly resembled those in controls with advancing age. Several studies have defined PCOS cases in peri- and postmenopausal ages by using high androgen levels at these ages as a criterion. Whether women with significantly increased androgens also in older ages constitute a subgroup of PCOS or are expressing high androgen levels for other reasons needs to be further studied. However, conclusions from such studies should not be generalized to women with PCOS in general with our current knowledge. The importance of prospective studies to be able to make assumptions regarding future health issues for young women with PCOS in contact with the healthcare system must be recognized.

Women with PCOS seem to develop adiposity already at a young age, and this, together with the other features of PCOS, constitutes a major health risk. We found that more than 70% of women who had a BMI ≥ 30 kg/m^2 at mid-fertile age developed T2DM during a 24-year period. Whether powerful interventions, for instance bariatric surgery, in young women with PCOS will decrease the long-term risks of T2DM and other obesity-related states needs to be studied.

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