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Polycystic ovary syndrome

Long-term health aspects

SOFIA PERSSON



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Abstract

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Polycystic ovary syndrome (PCOS) is a common endocrine disorder among women of reproductive age, affecting many aspects of women's health such as fertility, cardiovascular health and quality of life. However, less is known about later life stages, as well as the impact of hyperandrogenism and PCOS itself, apart from risks associated with excess body weight. The overall aim with this thesis was therefore to study how PCOS, and its hyperandrogenic phenotype, is associated with general health, with focus on diabetes, hypertension, dyslipidaemia and fertility at a longer span of time than during the fertile period.

Studies I-III were register-based cohort studies, linking six Swedish national registers. In total, >50 000 women with PCOS and \approx 250 000 controls were included and followed up for up to 20 years. Study IV was a clinical cross-sectional study including 124 women with PCOS and 74 controls.

The main results were that PCOS has a great impact on women's lives extending beyond the fertile period. While women with PCOS achieve a first childbirth as often as non-PCOS women, they have fewer children overall and give birth at a later age. In addition, PCOS is a moderate risk factor for type 2 diabetes, hypertension and dyslipidaemia, regardless of body weight. The risk appears to be more pronounced in those with the hyperandrogenic phenotype. Finally, hyperandrogenism persists largely in midlife for women with PCOS, both through ongoing symptoms such as hirsutism and by having a negative impact on the women's quality of life.

In conclusion, women with PCOS should be informed that they have a good chance of conceiving, but that some may need assisted reproduction. We suggest that PCOS in general and the hyperandrogenic phenotype in particular be included as independent risk factors when counselling women on their likelihood to suffer from cardiovascular disease and its risk factors in particular such as type 2 diabetes, hypertension and dyslipidaemia.

Keywords: PCOS, Hyperandrogenism, Hirsutism, Fertility, Type 2 diabetes, Hypertension, Dyslipidaemia

Sofia Persson, Department of Women's and Children's Health, Reproductive Health, Akademiska sjukhuset, Uppsala University, SE-751 85 UPPSALA, Sweden.

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To my family

List of Papers

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

- I. **Persson, S.**, Elenis, E., Turkmen, S., Kramer, M.S., Yong, E.-L., Sundström-Poromaa, I. (2019) Fecundity among women with polycystic ovary syndrome (PCOS) - a population based study. *Human Reproduction*, 34 (10):2052-2060
- II. **Persson, S.**, Elenis, E., Turkmen, S., Kramer, M.S., Yong, E.-L., Sundström-Poromaa, I. (2021) Higher risk of type 2 diabetes in women with hyperandrogenic polycystic ovary syndrome. *Fertility and Sterility*, 116 (3):862-871
- III. **Persson, S.**, Turkmen, S., Sundström-Poromaa, I., Linden-Hirschberg A., Elenis, E. (2023) Hypertension and dyslipidaemia in women with polycystic ovary syndrome: a population based register study in Sweden. *Manuscript*.
- IV. **Persson, S.**, Ubhayasekera, K.A., Bergquist, J., Turkmen, S., Sundström-Poromaa, I., Elenis, E. (2022) Hyperandrogenic symptoms are a persistent suffering in midlife women with PCOS; a prospective cohort study in Sweden. *Biomedicines*, 11 (96)

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Additional work

In addition to the work presented in this thesis, during the PhD studies, contribution was made to the following paper:

- Elenis E., Desroziers E., **Persson S.**, Sundström Poromaa I., Campbell R.E. (2021) Early initiation of anti-androgen treatment is associated with increased probability of spontaneous conception leading to childbirth in women with polycystic ovary syndrome: a population-based multiregistry cohort study in Sweden. *Human Reproduction*, 36 (5): 1427-1435

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Abbreviations

AE-PCOS	Androgen Excess and Polycystic Ovary Syndrome Society
aFR	Adjusted Fecundity Ratio
aHR	Adjusted Hazard Ratio
AMH	Anti-Müllerian Hormone
ART	Assisted Reproduction Technology
ATC	Anatomic Therapeutic Chemical
BMI	Body Mass Index, kg/m ²
COC	Combined Oral Contraceptive
COVID	Coronavirus Disease
CVD	Cardiovascular Disease
DHEAS	Dehydroepiandrosterone Sulphate
DHT	Dihydrotestosterone
EE	Ethinyl Estradiol
ESHRE	European Society of Human Reproduction and Embryology
FAI	Free Androgen Index
FR	Fecundity Ratio
FSH	Follicle Stimulating Hormone
GLP	Glucagon Like Peptide
GnRH	Gonadotropin Releasing Hormone
HA	Hyperandrogenic
HR	Hazard Ratio
HRQoL	Health-Related Quality of Life
ICD-10	International Statistical Classification of Diseases, version 10
IVF	In Vitro Fertilisation
KI	Konfidensintervall
LH	Luteinizing Hormone
MBR	Medical Birth Register
mFG	Modified Ferriman-Gallwey score
MHT	Menopausal Hormonal Treatment
NA	Normoandrogenic
NIH	National Institutes of Health
NPR	Swedish National Patient Register

OHSS	Ovarian Hyperstimulation Syndrome
PCOM	Polycystic Ovarian Morphology
PCOS	Polycystic Ovary Syndrome
PCOSQ	PCOS Health-Related Quality of Life Questionnaire
SF-36	Short Form - 36
SHBG	Sex Hormone Binding Globulin
SPDR	Swedish Prescribed Drug Register
SPSS	Statistical Package for the Social Sciences
T2D	Type 2 Diabetes
TPR	Total Population Register

Introduction

Definition of polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is characterised by hyperandrogenism (clinical and/or biochemical), ovarian dysfunction and ultrasonographic findings of polycystic ovaries (1). It is the most common endocrine disorder among fertile-aged women, with a prevalence of 8 to 13% (2).

In 1990, The National Institutes of Health (NIH) consensus conference concluded that the major criteria for PCOS should include: I) hyperandrogenism (HA), II) menstrual dysfunction and III) exclusion of other known disorders (3). In 2003, at the expert conference in Rotterdam, the recommendation was revised into fulfilment of at least two out of three diagnostic criteria: I) oligo- and/or anovulation, II) clinical and/or biochemical hyperandrogenism and III) polycystic ovarian morphology (PCOM), given that other androgen excess or related disorders are excluded (1). Androgen Excess and Polycystic Ovary Syndrome Society (AE-PCOS) in 2006 suggested further revisions of the definition of PCOS to include I) hyperandrogenism and II) ovarian dysfunction; oligo-anovulation and/or polycystic ovaries, and III) exclusion of other androgen excess or related disorders (4).

Since polycystic ovarian morphology is commonly seen in women without the syndrome, especially in young women, PCOM should not be used as a diagnostic criterion in adolescents (5-7). In Sweden the 2003 Rotterdam criteria are currently in use, but both NIH and Rotterdam criteria were used during the study periods of the studies in this thesis (8, 9).

PCOS phenotypes

Extended knowledge on PCOS has led to adjustments of the diagnostic criteria, which has hampered the comparability between different studies and PCOS populations. In order to overcome this limitation, the NIH consensus panel recommended in 2012 to maintain the 2003 Rotterdam definition of PCOS, while identifying phenotypes of PCOS for scientific purposes (10). It was suggested to use four phenotype groups, A-D (11). An overview of the phenotypes is shown in Table 1.

Table 1. *PCOS phenotypes.*

	PCOS phenotype groups			
	A	B	C	D
Hyperandrogenism	+	+	+	-
Ovulatory dysfunction	+	+	-	+
Polycystic ovaries	+	-	+	+

+ = present, - = absent

The phenotypes A-C are hyperandrogenic and are considered the most severe (12). The normoandrogenic phenotype D has been widely debated and AE-PCOS even suggested that hyperandrogenism should be mandatory for PCOS diagnosis (13).

PCOS phenotyping is currently not reflected in the International Classification of Diseases (ICD) used in the clinical everyday life. Hence, few data exist on PCOS phenotypes in subjects with PCOS identified in the general population (11). One important aspect of this thesis was therefore to come up with a reasonable alternative definition of the hyperandrogenic phenotype, which could in turn be used in future epidemiologic studies.

Hyperandrogenism

Hyperandrogenism, as a PCOS feature, can be identified either biochemically or clinically. Assessment of biochemical hyperandrogenism is challenging since there is a variety of androgens to measure and assays to use, as well as definition of normal ranges. Testosterone is circulating in plasma mostly bound to albumin and sex hormone-binding globulin (SHBG) and in a small percentage unbound (free). The free testosterone levels reflect the clinical condition/status more accurately than the total plasma levels (14). The current recommendation is, therefore, to use total testosterone and/or the calculated free testosterone (bioavailable testosterone) by estimating the free androgen index (FAI) expressed as: [FAI = (total testosterone/SHBG) as a per cent] or [FAI = 100 x (total testosterone/SHBG) as a ratio] to assess biochemical hyperandrogenism in the diagnosis of PCOS (15). Other androgens [such as dehydroepiandro sulfate (DHEAS) and androstendione] should only be used if total or free testosterone are not elevated and should therefore be avoided due to their poorer specificity (15).

Clinical signs of HA are hirsutism (abnormal hair growth of dark hair in specific regions of the body), female pattern hair loss and acne (16, 17). Hirsutism is the most common clinical sign of HA and is evaluated by using the

modified score described by Ferriman and Gallwey (mFG) (18) and currently considered the best marker for biochemical HA (15). The score evaluates hair growth in nine body parts, with score ranging between 0-4 (18). A cut-off at mFG score >4-6 has been suggested for hirsutism even though normative data on hirsutism in a Nordic setting is lacking (1, 9, 15). Female pattern hair loss is evaluated with the Ludwig scale, which classifies hair loss into three grades (19). Grading of acne could be helpful for clinicians, but currently no universal scoring system is recommended (20).

About half of women with PCOS present with self-reported hirsutism (21, 22) and up to 80% of women with PCOS have biochemical hyperandrogenism (23), with the proportion varying between studies (24). On the other side, up to 80% of women with hirsutism also have PCOS (25), with the two conditions being closely intertwined.

Anovulation and menstrual dysfunction

Normal menstrual cycle length ranges between 21 and 35 days, three years post menarche (26). Oligo-anovulation is defined as menstrual cycle duration longer than 35 days or less than eight menstruations per year (15, 27). Approximately 75-90% of women with PCOS present with menstrual dysfunction (27-29). In a fraction of these women, the ovulatory dysfunction presents as frequent bleeding while the majority of women with PCOS have cycles longer than 35 days (29).

Polycystic ovarian morphology

The morphologic criteria of PCOS refers to the finding of polycystic ovaries during the ultrasound assessment. Over time technical advancements in transvaginal ultrasound equipment have led to a change in the cut-offs for PCOM. The current definition of PCOM corresponds to an antral follicle count of ≥ 20 follicles per ovary and/or ovarian volume ≥ 10 mL in at least one ovary, instead of ≥ 12 follicles in previous guidelines (1, 9). However, PCOM is not specific for the polycystic ovary syndrome, and can be prevalent in up to 30% of healthy normally ovulating women below 35 years of age. The prevalence of PCOM decreases with age (5, 30, 31).

Exclusion of other androgen excess or related disorders

A number of other conditions should be excluded before the PCOS diagnosis can be considered (15), conditions that either lead to ovulatory dysfunction such as thyroid dysfunction, hyperprolactinemia, preterm ovarian insufficiency or to hyperandrogenism such as androgen-secreting ovarian and adrenal tumours, Cushing's syndrome and congenital adrenal hyperplasia (32).

PCOS pathophysiology

The pathophysiology of PCOS is multifactorial including genetic, epigenetic, endocrine, metabolic and environmental factors. PCOS run in families, and daughters of women with PCOS have an increased risk to be diagnosed with the syndrome (12). The aetiology of the disorder is incompletely understood, but seems to be the result of increased androgen secretion induced by hyperinsulinemia and elevated levels of luteinizing hormone (LH) (33).

Hyperandrogenism in PCOS originates from over-production of androgens, mainly from the ovaries but with a variable contribution also from the adrenal glands, which results in elevated circulating androgens (34). Hypothalamic gonadotropin releasing hormone (GnRH) secretion regulates the release of the gonadotropins LH and follicle stimulating hormone (FSH) from the pituitary gland. The pulse frequency of GnRH secretion is altered in women with PCOS, which results in increased pulse frequency and amplitude of LH and decreased pulse frequency of FSH (35). LH pulse frequency alterations are more common in lean than obese women with PCOS (36). LH and FSH both act on the ovaries; theca cells respond to LH by producing androgens and granulosa cells are stimulated by FSH in order to convert androgens into oestrogens through aromatisation (35). Nevertheless, in PCOS, the aromatization process in granulosa cells is insufficient, which further increases the androgen levels (37).

Normal ovulation on the other hand results from synchronised signalling from the hypothalamus, pituitary, ovarian theca and granulosa cells and the developing follicle (38). In comparison with regularly cycling women, the endocrine milieu in women with chronic anovulation is characterised by “steady state” where gonadotropin- and sex steroid concentrations vary relatively little during the cycle (38). In PCOS in particular, the follicular activation is increased, but the growth of follicles is arrested before maturation (39). The failure to select a dominant follicle, due to low circulating FSH, results in anovulation (40). The numerous small antral follicles and pre-antral follicles produce anti-Müllerian hormone (AMH), a hormone that inhibits the production of aromatase, preventing the conversion of androgens to oestrogens, further contributing to hyperandrogenism (12). The altered gonadotropin secretion, chronic hyperandrogenemia and hyperinsulinemia all contribute to the anovulatory state (38).

A high proportion of women with PCOS are insulin resistant, 75% of lean and 95% of overweight women (41). In insulin resistant women, hyperinsulinemia occurs and plays an important role in the establishment of hyperandrogenemia by multiple pathogenic pathways (34). Insulin resistance in PCOS is tissue selective, where some tissues (i.e., skeletal muscle) are highly resistant and other tissues (i.e., ovaries and adrenals) are still insulin sensitive (23). Insulin has several mechanisms of action contributing to the hyperandrogenic state; it acts as a co-gonadotropin by stimulating ovarian androgen production

but also upregulates adrenal androgen production and down-regulates SHBG production in the liver (34, 42, 43).

Androgen excess is a major mechanism in the oligo-anovulation and cutaneous manifestations of PCOS. It also facilitates insulin resistance and metabolic dysfunction by favouring abdominal and visceral adiposity (43-45). Adiponectin, an insulin sensitising cytokine released from subcutaneous adipose tissue, is lower in women with PCOS and contributes to insulin resistance (46, 47). Treatment with metformin increases the levels of adiponectin (46) while androgens reduce adiponectin secretion (48). The association between PCOS and type 2 diabetes (T2D) is largely driven by insulin resistance as well as by excess body weight, but not only due to it. Together, insulin resistance and obesity worsen the metabolic risk in women with PCOS (49).

Obesity in PCOS

Obesity is highly prevalent in the PCOS-population with 50-80% of women being overweight or obese (50). In addition, numerous studies have shown that women with PCOS have increased abdominal fat distribution, even among those who are not obese (48). The mechanisms behind the high prevalence of obesity in PCOS are not fully understood, but low-quality evidence points towards some differences in life-style with self-reported higher calorie intake and lower physical activity, as well as some differences in appetite related hormones (15). Obesity affects female fertility negatively, irrespectively of whether PCOS is present or not (45). In PCOS women, the severity of obesity and insulin resistance is worst in phenotype A, with declining prevalence to the normoandrogenic phenotype D (13).

Visceral fat tissue has endocrine activity, with sex steroids being aromatised (peripheral conversion of androgens into oestrogens) and inflammatory cytokines being produced on site, contributing to chronic low-grade inflammation (13). The endocrine activity of visceral adipose tissue facilitates androgen excess by having direct effects on the ovaries and adrenals and indirectly by inducing insulin resistance and hyperinsulinemia (43).

Furthermore, obese women have increased activity of the enzyme 5- α -reductase, which converts testosterone to the more potent androgen dihydrotestosterone (DHT), compared with normal weight women (34). The increased enzyme activity leads to enhanced conversion of cortisol into break down products, activation of the hypothalamo-pituitary-adrenal axis and further enhanced adrenal androgen synthesis (34).

Obesity is one of the components within the metabolic syndrome, a clustering of metabolic risk factors in an individual that affect their long-term health; elevated blood pressure, increased fasting blood glucose and dyslipidaemia are the rest (49). The metabolic syndrome, among other risk factors for cardiovascular disease (CVD), is more prevalent in women with PCOS

and it is more common in the anovulatory than in the ovulatory PCOS phenotypes (49, 51-53). Women with PCOS develop metabolic syndrome at a younger age than non-PCOS women do (54), and the prevalence in the general population is 33% as opposed to 46% in women with PCOS (55).

Quality of life

The symptoms of PCOS cause psychological morbidity (anxiety and depression) and have a negative impact on women's health-related quality of life (HRQoL) (56, 57). Both depression and anxiety are common disorders in women with PCOS (15), with a prevalence of depression of 26% in 46-year-old women with PCOS vs 14% in controls in a Finnish study (58). Presence of hirsutism and high body mass index (BMI) are associated with a higher risk of depression in women with PCOS (59); however, the risk of depression remains elevated even after adjusting for BMI indicating that other mechanisms besides obesity lie behind this association (57). There is limited data on whether PCOS phenotype has an impact on the risk of depression (57). In addition, hormonal contraception, which is first-line medical treatment for PCOS, has been associated with a higher risk of depression, but this question has not been addressed in a PCOS-population in particular (60).

When data from eight studies using Short Form-36 (SF-36) to measure quality of life (QoL) were pooled and analysed, women with PCOS scored lower QoL than controls, however, the certainty of evidence was low (15). Most studies on quality-of-life focus on women during the reproductive years and recruit participants from infertility settings, a fact that can confound the findings since infertility per se is a cause of significant psychological suffering (56, 61).

Thus far, there is one tool developed to measure HRQoL specifically in PCOS, the PCOS Health-Related Quality of Life Questionnaire (PCOSQ), which has been translated into several languages (62).

In addition to lower quality of life and enhanced risk of depression, women with PCOS also report lower sexual function with reduced sexual satisfaction and sexual attractiveness with negative impact of excess hair growth than controls, which was not related to androgen levels (15, 63, 64). Further, women with PCOS have lower self-scored self-esteem than controls (61).

Treatment strategies in PCOS

There is currently no cure for PCOS and its management is mainly symptomatic aiming at relieving the different symptoms. Education, self-empowerment, multidisciplinary care and lifestyle interventions are prioritised as treatment in all women with PCOS, with a generally healthy life-style and prevention of weight gain being the basic treatment (15, 17).

The primary approach in the management of PCOS among overweight and obese individuals concerns weight loss, where a loss of 5% of body weight can decrease insulin resistance, improve fertility outcomes by leading to more regular ovulations, improving body composition and adipose tissue dysfunction (15, 33). When life-style interventions and weight loss are insufficient, medical treatment is needed instead. Current evidence is recommending combined oral contraceptives (COCs) as first-line treatment for hyperandrogenism, menstrual irregularities and for contraception in PCOS (65).

Anti-androgenic treatment

The anti-androgenic mechanisms of action of COCs are inhibition of GnRH secretion and LH-dependent ovarian androgen production, enhanced SHBG production and potentially also blockade of the androgen receptor (66-69). Swedish national guidelines on anti-androgenic treatment in women with PCOS, in place during the study period, suggested that COCs containing ethinylestradiol (EE) and anti-androgenic progestogens should be used as first line treatment for hirsutism (8). Recently, it has been reported that treatment with fourth generation progestogens results in lower BMI and a more favourable lipid profile compared with third generation progestogens (70). When COCs are contraindicated, unsuitable or failed to improve HA symptoms after 6 months of use, other anti-androgenic agents could be tried (15, 65).

Oral anti-androgenic treatment includes spironolactone (71), 5-alpha-reductase inhibitors (finasteride, dutasterid) (72) and androgen receptor blockers (flutamide, bicalutamide) (73). Spironolactone is an aldosterone antagonist, with anti-androgenic properties by being a competitive inhibitor of the androgen receptor and inhibitor of 5-alpha-reductase (74). 5-alpha-reductase inhibitors (finasteride, dutasterid) inhibit the conversion of testosterone to dihydrotestosterone (72). Flutamide binds to the androgen receptor and blocks it (75). Topical medication with eflornithine cream, an irreversible inhibitor of ornithine decarboxylase, affects hair follicle growth and can improve mild facial hirsutism in women (76). In addition to medical treatment, cosmetic therapy such as mechanical laser and light therapies should be considered in order to treat facial hirsutism (15).

Cyproterone acetate-containing COCs in combination with EE may relieve hirsutism more efficiently than other COCs, but are not recommended as first-line treatment because of the higher risk of venous thrombotic events (70). There is evidence that spironolactone and flutamide are effective in relieving

hirsutism, with spironolactone being equal to COCs in lowering levels of free testosterone (77, 78). However, concerning female pattern hair loss, there is no difference in treatment results between spironolactone and cyproterone acetate (79).

Regarding acne, first-line treatment is topical agents, while oral antibiotics are used in moderate acne (20) and isotretinoin, which is highly effective but also teratogenic, is being used in severe cases. (80). In women with acne, COCs have a similar effect as oral antibiotics and can be tried in women requiring contraception (80). Cyproterone acetate and chlormadinone acetate are superior to COCs containing levonorgestrel in relieving acne (78) Thus far, there are no randomised studies comparing single anti-androgenic agents with COCs. Moreover, most of existing studies on hirsutism treatment in PCOS included fertile-aged women only, resulting in a lack of data in midlife- and post-menopausal women (65).

Insulin sensitisers

Since insulin plays a crucial role in the pathogenesis of PCOS, insulin sensitisers are part of the treatment rationale: metformin has been widely used since the 1990s to treat insulin resistance in PCOS, and with declining weight and insulin levels, hyperandrogenism can be alleviated as well (81). Metformin is recommended primarily for the management of metabolic features among women with BMI ≥ 25 kg/m², in addition to COCs or alone (17). However, insulin sensitisers alone are not an effective treatment for hirsutism, and are instead recommended together with COCs (77). Inositols, which belong to the Vitamin B complex group, seem to reduce testosterone and increase SHBG levels, however, meta-analyses exploring their effect have not shown a clear advantage (82). They do not seem to ameliorate hirsutism, and the use of inositols is not recommended against hyperandrogenism, but could be offered as an alternative for metabolic reasons (15). It should be noted though that inositols are not medical substances, and are at a high financial cost for patients (15).

Weight loss

Medical treatment for weight loss in PCOS often starts with the insulin sensitiser metformin, which improves weight loss, insulin resistance and lipid profile in overweight women with PCOS (15). Recently approved treatment for diabetes type 2 includes glucagon like peptide (GLP)-1 analogues, such as exenatide, liraglutide and semaglutide (15, 83), have also been evaluated in PCOS women. GLP-1 is a physiological hormone that has multiple actions in glucose and appetite regulation. Treatment with GLP-1 analogues results in reduced glucose levels, body weight and body fat (84). Few studies compared GLP-1 analogues with placebo in PCOS women; it seems though that semaglutide and liraglutide both have beneficial effects on metabolic parameters, bleeding pattern and hyperandrogenism compared with placebo (83, 85, 86).

Treatment with GLP-1 analogues gives a higher proportion of individuals reaching the 5% weight loss target compared with placebo or orlistat (a weight loss agent), while simultaneously lowering blood pressure (87). Orlistat is a reversible pancreatic lipase inhibitor that inhibits the absorption of ingested fat (88). One study comparing the combination orlistat + EE/drosperinone-containing COC with COC only showed significantly lower BMI after combination treatment, but no difference in androgen parameters (89).

In addition to life-style interventions and medical treatment, the treatment arsenal for obesity includes bariatric surgery. The most commonly described surgical methods are Roux-en-Y gastric bypass and vertical sleeve gastrectomy (90). Bariatric surgery improves both hyperandrogenism and metabolic symptoms in obese women with PCOS (91-93). After bariatric surgery, important changes in adipose tissue function are induced, possibly related to the degree of weight loss (33).

Infertility

Infertility in women with PCOS is often the result of anovulation, and treatment therefore mainly starts by inducing ovulation. Use of metformin can restore ovulation to some degree, but often further treatment is needed (15). Ovulation induction can otherwise be done by using aromatase inhibitors (letrozole)(first line treatment), oestrogen receptor modulators (clomiphene citrate), or gonadotropins (15). Women with PCOS phenotype A are more often resistant to letrozole treatment (53). If ovulation induction is unsuccessful, in vitro fertilization (IVF) is recommended instead (15). The fertility potential in hyperandrogenic women with PCOS seems also to be positively affected by early initiation of anti-androgen treatment (i.e., before 20 years of age) compared with late usage (94).

Treatment of PCOS symptoms is important in order to alleviate the suffering caused by them, and to prevent future negative health impact.

Long term health aspects

There are two major knowledge gaps in research on long-term health effects in PCOS: what is the independent role of PCOS for the long-term outcomes and what is the role of the hyperandrogenic PCOS phenotype? To address these knowledge gaps, studies should optimally be large, prospective, categorising the study population in PCOS phenotypes, adjusting for BMI and following participants for a long time or at least to the post-menopausal stage of life.

Fertility

Polycystic ovary syndrome is the main cause of anovulatory infertility, in 80-90% of cases (95, 96). Even though there is a clear association with reduced fertility, the fertility rate is restored after PCOS diagnosis, suggesting that infertility in women with PCOS is treatable (97). Generally, women with PCOS are older than non-PCOS women when giving birth and are more likely to use assisted reproductive treatment (ART) to achieve pregnancy (98). Fecundity is reduced in obese women, even among those with normal menstrual cycles (55).

There are studies that indirectly suggest that the cumulative probability of childbirth in women with PCOS over the long term might be similar to that in women without PCOS. Women with PCOS might eventually achieve the family size they desire, or at least, have 1-2 children (99-102), but that might take longer time. Further, women with PCOS can have sustained fertility with advancing age compared to infertile eumenorrheic women (103, 104). However, population-based data on fecundity in women with PCOS has not yet been published.

Diabetes

PCOS is associated with obesity, insulin resistance, type 2 diabetes and the metabolic syndrome (49, 105-107). Data is however contradictory as to whether all women with PCOS are at risk for T2D or only those with concomitant overweight or obesity (108-111). It has been discussed that obesity might be the main driver behind the elevated risk of diabetes in women with PCOS since obesity itself is a known risk factor to develop T2D, and obese women with PCOS seem to be at higher risk than normal weight PCOS women (112). In a long-term follow-up of a Swedish cohort of women with PCOS and controls, abdominal obesity was associated with a higher risk of diabetes (113).

Androgen excess exacerbates insulin resistance with higher testosterone and lower SHBG, being risk factors for T2D development (114, 115). Although there are several studies in women with type 1 diabetes indicating a higher prevalence of hyperandrogenism and PCOS (116), the situation is unclear when exploring the coupling of hyperandrogenism, PCOS and T2D. More specifically, it is yet unknown if women with the HA PCOS phenotype are at higher risk of T2D than normoandrogenic women with PCOS, irrespectively of body weight (108, 113).

Cardiovascular risk and metabolic syndrome

Cardiovascular disease is one of the leading causes of death in women in developed countries today, increasing simultaneously with the increasing prevalence of overweight and obesity in the population (117).

Isolated hypertension is more common in women with PCOS (50) and blood pressure during reproductive years is higher than in non-PCOS women despite adjustment for BMI (52). Dyslipidaemia is highly prevalent in reproductive-aged women with PCOS, with them exhibiting higher levels of triglycerides and low-density lipoprotein (LDL) cholesterol and lower high-density lipoprotein (HDL) cholesterol than women without PCOS (24, 118). The incidence of dyslipidaemia is even higher in obese and hyperandrogenic women. Hypertension and dyslipidaemia both are important factors in the atherosclerosis process, eventually leading to cardiovascular events (50)

One problem when assessing cardiovascular risk is that cardiovascular events are more common in the older population and many studies have a short time of follow up or investigate people at a young age, where the events are relatively uncommon (119, 120). Many studies on long-term health risks of PCOS are small (112, 113, 121) and very few studies separate PCOS phenotypes in their analyses (112, 122), which may underestimate the role of hyperandrogenism.

Generally, oestrogens act preventive on CVD in women which explains why the prevalence of CVD is higher after menopause (120). Studies relying on data in young women precede the years with highest incidence of CVD and therefore hinder us from drawing conclusions for women in peri- or postmenopausal stages of life (112).

It has been reported that even young women with PCOS have an elevated risk of coronary events compared with normal population, when adjusted for BMI (123), but no difference in cardiovascular mortality has been established (119). One recent meta-analysis by Wekker *et.al.* analysed 23 studies on CVD risk in PCOS and concluded that women with PCOS had a substantially increased risk for hypertension and T2D but not for non-fatal coronary events (50). The included studies did not however stratify upon PCOS phenotypes, only a handful of them adjusted for BMI, and it was not possible to make point estimates that account for obesity rates among women with PCOS (50).

Persistence of symptoms

When women are ageing, the distribution within the PCOS phenotypes A-D is changed, with a decrease of the HA phenotypes (A-C) and an increase of the ovulatory phenotype (D), with some women no longer fulfilling the diagnostic criteria (124, 125). More women with PCOS gain regular menstrual cycles when aging (126) and limited available data suggests that they enter menopause at a later age than non-PCOS women (127). However, women with PCOS are more obese and have higher levels of androgens than non-PCOS women throughout the reproductive years. Even though hyperandrogenism often improves with age, post-menopausal women with PCOS continue to have elevated androgens and are more hirsute than controls (125, 128, 129).

A Swedish study following PCOS patients and controls until age above 80 years, showed similar body composition and biochemical hyperandrogenism, but higher prevalence of hirsutism in women with PCOS (129). It is still unknown whether persistence of symptoms affects the wellbeing of women and if the cardio-metabolic risk seen during reproductive years continues to increase in midlife and after menopause.

Menopause itself can be considered as a state of relative androgen excess, when oestrogen and SHBG levels are declining, increasing the free androgen levels, leading to mild hyperandrogenic symptoms even among non-PCOS women (130-132).

Aims

The overall aims of this thesis were to explore how PCOS is associated with fertility and health during a longer time span than during the reproductive years.

The specific aims of the individual studies were:

- I. To determine the cumulative probability of childbirth and fecundity ratio (FR) in women with PCOS, with and without assisted reproduction, compared with age-matched women without PCOS.
- II. To study the long-term risk of developing type 2 diabetes in women with PCOS, with special reference to BMI and the hyperandrogenic phenotype of PCOS.
- III. To study the long-term risk of developing hypertension and dyslipidaemia in women with PCOS, after taking into account BMI and the hyperandrogenic phenotype of PCOS.
- IV. To study the degree of persistence of clinical and biochemical hyperandrogenism in women with PCOS older than 45 years compared to matched controls, and to evaluate the quality of life in women with PCOS.

Material and methods

Overview of the studies

Table 2. Overview of the studies.

Study	Study design	Participants	Outcome
I	Population-based cohort study	45 395 women with PCOS and 217 049 non-PCOS women	Cumulative probability of childbirth, fecundity ratio
II	Population-based cohort study	52 535 women with PCOS and 254 624 non-PCOS women	Type 2 diabetes
III	Population-based cohort study	50 969 women with PCOS and 246 246 non-PCOS women	Hypertension or dyslipidaemia
IV	Multi-centre cross-sectional study	124 women with PCOS and 74 matched controls	Hyperandrogenism, quality of life

Ethical considerations

The studies were approved by the Regional Ethical Review Authority in Uppsala, 9 August 2017, diary number 2017/309. The permit was extended on 29 March 2018 to include Falun and Stockholm as study sites (Study IV), diary number 2017/309/1.

Study design

The study design of papers I, II and III was population-based matched cohort using data from linked Swedish national registers. The study design of paper IV was a multi-centre cross-sectional study.

Data sources

Swedish National Registers

All individuals in Sweden are assigned a unique personal identification number upon birth or permanent residence permit, which enables linkage between registers (133). In the first three papers, data was retrieved after linking six Swedish national registers. The Swedish National Board of Health and Welfare provided data from the Swedish National Patient Register (NPR), the Swedish Prescribed Drug Register (SPDR), the Medical Birth Register (MBR), and the Register on Causes of Death. Statistics Sweden provided data from the Education Register and the Total Population Register (TPR).

The Swedish NPR includes nationwide information on visiting dates and diagnoses for in-patient hospital visits since 1964; the information is considered complete since 1987 (134). Since 1997, diagnoses are classified according to the International Statistical Classification of Diseases and Related Health Problems, version 10 (ICD-10) (134). From 2001 and onwards, outpatient hospital visits and visits to specialised healthcare (i.e., private gynaecologists) were included in the register.

The Swedish Prescribed Drug Register contains, since 2005, information on Anatomic Therapeutic Chemical (ATC) classification codes for prescribed drugs, dosage, date of prescription and date of purchase of the drug (135).

The Medical Birth Register contains data on 98% of all births in Sweden since 1973 and includes prospectively collected demographic and clinical data, including information on maternal BMI, smoking, complications during pregnancy, delivery and the neonatal period (136, 137).

The Register on Causes of Death provides information on cause of death and death date. The Education Register contains information on education of the population. The Total Population Register started in 1968 and provides information on country of birth and residential municipality (138).

Study population

The selection of study population was similar in papers I, II and III, with minor differences only. In all three studies, women with an existing ICD-10 diagnosis in the NPR of PCOS (E282), hyperandrogenism (E281) or anovulatory infertility (N970) were included in the study population as exposed subjects. Women with ICD-10 diagnosis of hyperprolactinemia (E22), congenital adrenal hyperplasia (E25), premature ovarian insufficiency (E283) or Turner syndrome (Q96) were excluded. Further, women diagnosed with PCOS before 12 years or after 50 years of age were excluded due to the risk of incorrect diagnosis.

For each woman with PCOS, up to five non-PCOS women born during the same month and living in the same municipality as the woman in the exposed group were randomly drawn from the TPR. Randomly selected women with a

diagnosis of PCOS, anovulatory infertility or a pregnancy achieved by ovarian stimulation were excluded since they may represent undiagnosed women with PCOS. Excluded women could not be replaced. A flowchart over the study population is shown in Figure 1. In paper I, the study period started when women turned 18 years of age and in papers II - III at the year when a woman was diagnosed with PCOS or included as a matched non-exposed woman.

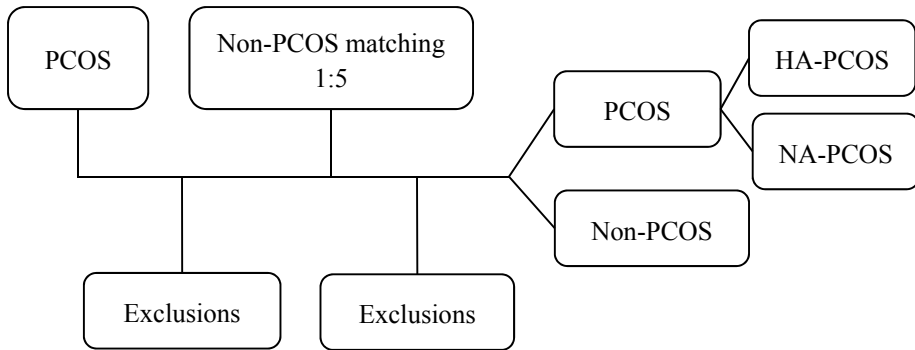


Figure 1. Flowchart over selection of study population of paper I-III. HA=hyperandrogenic, NA=normoandrogenic.

Paper I

All women born between 1971 and 1997 diagnosed with PCOS between 1st January 2001 and 31st December 2016 were included in the study population. In addition to the already mentioned exclusion criteria, women who were already parous when first registered in MBR were excluded. After exclusions, 45 395 women with PCOS and 217 049 non-PCOS women were included in the analyses. Follow-up started when women turned 18 years of age and ended when a participant reached the end of the observation period. Censoring occurred when childbirth was registered.

Paper II

All women born between 1950 and 1999 diagnosed with PCOS between 1st January 1997 and 31st December 2016 were included in the study population. Women diagnosed with diabetes mellitus type 1 or type 2 before the PCOS diagnosis were excluded. Similarly, non-PCOS women with diabetes before the index date were excluded. After exclusions 52 535 women with PCOS and 254 624 non-PCOS women were included in the analyses.

Follow-up started upon PCOS diagnosis or matching to an exposed individual. Censoring occurred when follow-up ended on 31st December 2016, at diagnosis of T2D or antidiabetic prescription or upon death.

Paper III

All women born between 1950 and 1999 diagnosed with PCOS between 1st January 1997 and 31st December 2016 were included in the study population. Women diagnosed with CVD, cardiac failure, stroke, hypertension or dyslipidaemia prior to receiving their PCOS diagnosis or matching to the exposed individual were excluded. All women who were prescribed spironolactone were excluded since the drug is used both against hypertension as well as anti-androgenic treatment. After the necessary exclusions, 50 969 women with PCOS and 246 246 matched non-PCOS women were included in the analyses.

Follow-up started upon PCOS diagnosis or matching to an exposed individual. Censoring occurred when follow-up ended on December 31, 2016, at the development of hypertension or dyslipidaemia (defined by either ICD-10 relevant diagnosis or antihypertensive or lipid-lowering medications respectively), or upon death.

Paper IV

The study was set at the departments of Women's Health at Uppsala University Hospital (Uppsala), Sundsvall County Hospital (Sundsvall), Karolinska University Hospital (Stockholm) and Falun County Hospital (Falun). The inclusion period lasted between January 2017 and December 2021, with an almost 18-month temporary stop during the COVID pandemic.

Women with PCOS were identified through the outpatient registers at the collaborating clinics. Inclusion criteria for the exposed group were prior diagnosis of PCOS (E282) and present age above 45 years. Both the NIH and Rotterdam criteria were accepted for PCOS diagnosis. All women with a past PCOS diagnosis were included, irrespective of whether they still fulfilled the diagnostic criteria or not at the time of inclusion.

For each woman with PCOS, one control person was randomly selected, and invited for participation in the study. The controls were identified from the population register of Uppsala and Västernorrland Counties, respectively, and matched for age and area of residence. Due to the difficulties recruiting controls during the pandemic, adjustments in the study protocol were made by extending the cohort with a well-defined cohort of controls recruited during a previous study performed in 2006–2007 using the same inclusion criteria as above (99). None of the controls had a prior PCOS diagnosis in their medical records. In addition, at inclusion, we confirmed that controls had no prior history of oligomenorrhea/amenorrhea, hirsutism or anovulatory infertility. Exclusion criteria for all participants were inability to understand Swedish or attend the health care exam in-person.

In total, 124 women with PCOS and 74 controls were included.

Exposure

Paper I-III

The main exposure was a PCOS-related ICD-10 diagnosis. Anovulatory infertility was included as exposure since PCOS is the main cause of infertility due to anovulation (80-90% of cases) (95, 96). Women with PCOS were further characterised into having normo- and hyperandrogenic phenotypes with different definitions utilised in Paper II and III respectively. In paper I, phenotyping was not used.

Paper II-III

Women with PCOS were classified according to their PCOS phenotype (hyperandrogenic or normoandrogenic). Since the registers did not provide information on PCOS phenotype, except for women who were diagnosed with androgen excess (E281), we created a definition of hyperandrogenism by categorising women with PCOS as normoandrogenic (NA) or hyperandrogenic (HA) using information from SPDR. Three levels of specificity for the classification of hyperandrogenism were used: broad, intermediate, and specific subtype/phenotype. The broad HA phenotype comprised women with at least two filled prescriptions for anti-androgenic combined oral contraceptives (COC) and/or anti-androgenic drugs. The following substances were used: COC with ethinyl estradiol (EE) and drospirinone, EE and desogestrel, EE and dienogest or EE and cyproterone acetate; the anti-androgenic drugs finasteride and eflornithine, finasteride/dutasteride, flutamide/bicalutamide, or spironolactone.

The intermediate HA phenotype comprised women who filled at least two prescriptions for a cyproterone acetate-containing COC and/or the above-mentioned anti-androgenic drugs. We included COCs with cyproterone acetate in the intermediate phenotype exposure because acne is the only approved indication for the preparation in Sweden. Further, in contrast to all other hormonal contraceptives, which can be prescribed by midwives, cyproterone acetate-containing pills can only be prescribed by physicians.

Finally, the specific HA phenotype comprised women filling at least two prescriptions for spironolactone.

All women with a diagnosis of hyperandrogenism (E281) were considered having the HA phenotype, regardless of broad/intermediate/specific drug exposure. Remaining women with PCOS not included in any of the HA phenotypes were classified as normoandrogenic.

In paper II, the broad definition was used for the main analyses, whereas in paper III the intermediate definition was used since COCs are generally associated with hypertension and therefore not suitable as a marker for hyperandrogenism.

Sensitivity analysis was performed in paper I-III by separating PCOS diagnosis per se (E282) and anovulatory infertility (N970). It could be argued that

women with the diagnosis anovulatory infertility actively sought care for infertility, influencing thus the fecundity rates (paper I). The sensitivity analysis aimed in addition to explore whether results were similar when the diagnoses were separated since PCOS is the main reason for anovulatory infertility and diagnosed women could be assumed to have PCOS, even if they were not registered with a PCOS diagnosis (95, 96, 139).

In paper III, sensitivity analysis was done by separating dyslipidaemia diagnosis and prescription of lipid-lowering drugs, due to the fact that lipid-lowering drugs are sometimes prescribed as preventive measure against CVD events. We have further divided PCOS diagnosis into before or after 2005, since SPDR was introduced in 2005, and information on anti-androgenic treatment and COCs used for classifying the women in NA or HA phenotypes were not available before that.

Paper IV

PCOS was identified at the woman's medical record defined by the NIH or Rotterdam criteria during her reproductive years. Women with PCOS were classified as having a normoandrogenic or hyperandrogenic phenotype based on hyperandrogenic symptoms and androgen levels when first diagnosed with PCOS, identified either in the woman's medical record or according to patient recall. Among the cases where medical records were not available, or lacked relevant information on PCOS features/androgen levels, we relied on patient recall; if a participant stated that unwanted body hair or acne were the main causes for seeking health care at the time of PCOS diagnosis, they were classified as having the hyperandrogenic phenotype.

Outcomes

Paper I

The primary outcome was childbirth event, either after a spontaneous conception or after assisted reproduction.

Secondary outcomes were female age at childbirth, time to first and second childbirth, probability of a second childbirth, probability of childbirth after spontaneous conception, time to childbirth after spontaneous conception and fecundity ratio (FR). Fecundity was defined as the physiological maximum reproductive potential of an individual over its lifetime (140).

Data was collected from MBR regarding the year of childbirth/childbirths, female age at first childbirth and parity at the end of follow-up period. After 1995, data on the use of assisted reproduction is available in the MBR and pregnancies were classified as spontaneous if no assisted reproduction was recorded in the register.

Paper II

The primary outcome was T2D, defined as at least one of the following:

- 1) T2D diagnosis in the NPR according to ICD-10 non-insulin dependent diabetes mellitus (E11) or unspecified diabetes mellitus (E14).
- 2) Prescription of antidiabetics (ATC code A10B) according to the SPDR [excluding metformin (ATC code A10BA02)].

Secondary outcomes were age at T2D diagnosis and rate of T2D diagnosis.

Paper III

The primary outcomes were development of hypertension or dyslipidaemia defined as one of the following:

- 1) Hypertension (ICD-10 code I10) or dyslipidaemia (ICD-10 code E78) diagnosis in NPR.
- 2) Prescription of antihypertensive or lipid lowering drugs, according to the SPDR: (ATC code C02-C04, C07-C08 and C10, respectively).

Secondary outcome was age at diagnosis.

Paper IV

Four outcomes reflected presence of clinical hyperandrogenism: hirsutism, androgenic hair loss, self-reported bothersome acne and frequency of facial hair removal. To evaluate hirsutism, we used the modified Ferriman-Gallwey (mFG) score with score ≥ 8 indicating hirsutism. An accepted cut-off value of the mFG score for Scandinavian women is lacking and the cut off ≥ 8 was chosen because it has been widely used by other researchers (17, 141). Androgenic hair loss was assessed using the Ludwig scale, ranging from 0-III where stage III is the most severe grade (17). Current bothersome acne and frequency of facial hair removal were self-reported by study participants.

Biochemical outcomes were total testosterone levels, SHBG levels and FAI, which was calculated as total testosterone/SHBG ratio. Hyperandrogenism was defined as a ratio above 0.05.

Quality of life was evaluated using the PCOS Health-Related Quality of Life Questionnaire (PCOSQ), in women with PCOS only (62). The questionnaire has been translated to and validated in Swedish (142). The PCOSQ contains five domains where participants self-rate the impact on everyday life during the past two weeks: (i) Emotional concerns such as depressive symptoms, worries and low self-esteem as a result of having PCOS (8 items), (ii) Hirsutism (5 items), (iii) Weight (5 items), (iv) Infertility (4 items) and (v) Menstrual Disorders (4 items). Answer options ranged from serious problem/all the time to no problem/none of the time. We coded the answers from 1 (poorest function) to 7 (optimal function) (56, 62). Median values within each domain were calculated.

Covariates

In paper I, the covariates explored were maternal birth period, country of birth and level of education.

In papers II and III, we retrieved data on BMI in women who gave birth during the study period from MBR and presence of obesity diagnosis from NPR.

In paper IV, covariates recorded and used in analyses were age (years), BMI (categorised as normal weight $<25.0 \text{ kg/m}^2$, overweight $25.0\text{-}29.9 \text{ kg/m}^2$ and obesity $\geq 30.0 \text{ kg/m}^2$) and marital status (single/cohabitant/married).

Statistical methods

In papers I and II, we used the Statistical Package for the Social Sciences (SPSS) version 25 (SPSS Inc., Chicago, IL, USA) for the main statistical analysis and R version 3.6.2 packages Survival and Coxme for the clustered Cox regression. In paper III, the SPSS version 28.0.1.0 (142) and in paper IV, the SPSS version 26 were used for statistical analyses.

Paper I-III

In papers I – II, the cumulative probability of childbirth, mean time to childbirth, cumulative probability of T2D diagnosis and time to diagnosis were calculated by Kaplan-Meier survival analysis. Fecundity ratio (FR) (paper I) and hazard ratios (HR) (paper II-III) were estimated by clustered Cox regression with 95% confidence intervals. Fecundity ratio and hazard ratios were adjusted for birth period, country of birth and level of education. We used two models to adjust for the effect of body weight; Model one using BMI, which was retrieved from MBR, thereby only available for women who gave birth during the study period. Model two was adjusted for presence of ICD-10 diagnosis of obesity (E66.9) in NPR. In paper III, analyses were additionally stratified upon BMI-group ($<25 \text{ kg/m}^2$, $25.0\text{-}29.9 \text{ kg/m}^2$, $\geq 30.0 \text{ kg/m}^2$). Both models were adjusting for birth period, country of birth and education.

To evaluate our definition of the HA phenotypes, the two models were tested for each of the broad, intermediate and specific HA phenotypes in paper II.

Paper IV

Categorical variables were compared by Chi-square test and Fischer's exact test. Continuous variables were compared by Mann-Whitney U-test as some of the variables were not normally distributed. Multiple linear regression was used to assess associations between BMI and PCOS phenotypes on hirsutism, testosterone and FAI. A two-sided p-value of <0.05 was considered as statistically significant. Missing values were not imputed.

Analysis for quality of life in women with PCOS was analysed by Mann-Whitney U-test and median scores for each domain were calculated and used in the analysis.

Results

Demographic data, paper I-III

The study populations of papers I-III were similar but not identical. In all three studies, the most common period of birth was in the 1980s, the majority of study participants were born in the Nordic countries, with a higher proportion of women with PCOS born in the Middle East.

High bodyweight, either identified by BMI ≥ 25.0 kg/m² or obesity diagnosis, was more prevalent among women with PCOS than in women of the comparison group.

In papers II and III, where data was analysed based on PCOS phenotypes, a higher proportion of women with the HA phenotype remained childless throughout the study period than NA PCOS women and non-PCOS women. Demographic data are shown in Table 3, with PCOS phenotypes separated. Detailed demographic information for papers I and III is found in the respective papers.

Table 3. Demographic and clinical variables in the study population of paper II.

	Non-PCOS women n=254 624 n (%)	NA-PCOS n=31 383 n (%)	HA-PCOS n=21 152 n (%)
Birth period			
1950-1969	22 171 (8.7)	3 720 (11.9)	805 (3.8)
1970-1979	75 646 (29.7)	11 115 (35.4)	4 602 (21.8)
1980-1989	110 165 (43.3)	12 181 (38.8)	10 606 (50.1)
1990-1999	46 642 (18.3)	4 367 (13.9)	5 139 (24.3)
Parity			
No child	125 714 (49.4)	14 470 (46.1)	12 541 (59.3)
1 child or more	128 910 (50.6)	16 913 (53.9)	8 611 (40.7)
Country of birth			
Nordic countries	202 326 (79.5)	23 071 (73.5)	16 270 (76.9)
Europe	19 437 (7.6)	2 600 (8.3)	1 487 (7.0)
Middle East	11 194 (4.4)	2 635 (8.4)	1 998 (9.4)
India, Bangladesh or Pakistan	2 124 (0.8)	587 (1.9)	231 (1.1)

Africa	6 591 (2.6)	839 (2.7)	231 (1.1)
Remaining countries	12 952 (5.1)	1 651 (5.3)	935 (4.4)
Education			
≤12 years	117 450 (46.1)	1 5243 (48.5)	8 843 (41.8)
>12 years	132 830 (52.2)	17 545 (50.2)	12 157 (57.5)
Missing data	4 344 (1.7)	395 (1.3)	152 (0.7)
BMI			
<25.0 kg/m ²	83 120 (32.6)	7 846 (25.0)	4 516 (21.4)
25.0-29.99 kg/m ²	28 759 (11.3)	4 612 (14.7)	2 266 (10.7)
≥30.0 kg/m ²	12 460 (4.9)	3 740 (11.9)	1 480 (7.0)
Missing data	130 285 (51.2)	15 185 (48.4)	12 890 (60.9)
Obesity diagnosis			
No	243 147 (95.5)	26 185 (83.4)	18 465 (87.3)
Yes	11 477 (4.5)	5 198 (16.6)	2 687 (12.7)

Paper I

During the study period, the proportion of women not having children was similar between women with and without PCOS, but a lower proportion of PCOS-women than non-PCOS women gave birth to two children or more (26.5% vs. 32.8 %, $p < 0.001$). Women with PCOS were also older at first childbirth (mean age 28.4 ± 4.8 years, vs 27.0 ± 4.4 years in non-PCOS women, $p < 0.001$). Twice as many women with PCOS than non-PCOS women had their first childbirth after the age of 35 (10% vs 4.7%).

Cumulative probability of childbirth in women with PCOS was 80.2% (95% CI 79.5-80.9%), and in women without PCOS, the cumulative probability of childbirth was 78.2% (95% CI 77.9-78.5%), Figure 2. The mean time to first childbirth was longer in PCOS-women than in non-PCOS women, $p < 0.001$.

Cumulative probability of childbirth after spontaneous conception was 55.0% (95% CI 53.7-56.2%) vs. 73.8% (95% CI 73.4-74.3%) in PCOS and non-PCOS women respectively, Figure 3. Mean time to first childbirth after spontaneous conception was longer in women with PCOS (15.3 years, 95% CI 15.2-15.4) than in women without PCOS (13.0 years, 95% CI 13.0-13.0), $p < 0.001$.

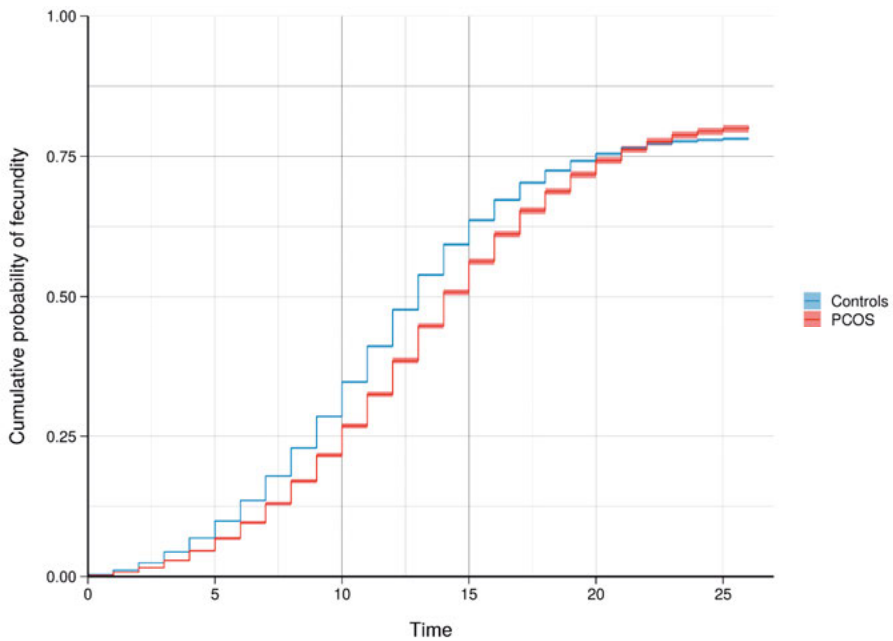


Figure 2. Time to first childbirth in years, irrespectively if spontaneous or by assisted reproduction. Cumulative probability of fecundity among women with PCOS and the comparison group.

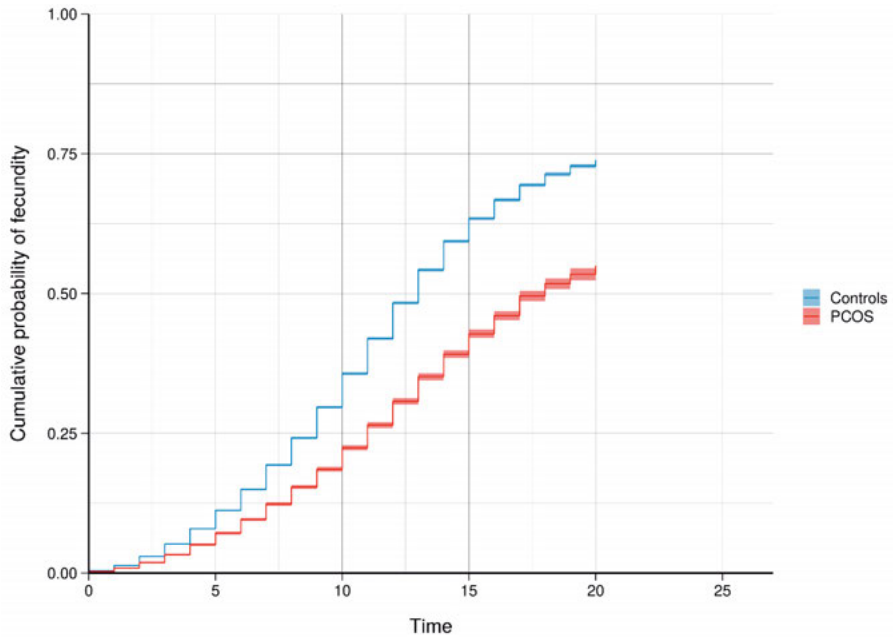


Figure 3. Time to first childbirth, in years, by spontaneous conception. Cumulative probability of fecundity among women with PCOS and comparison group. The analysis is restricted to women born 1977 or later.

Paper II

Women with PCOS, irrespectively of phenotype, had a higher incidence of T2D and were diagnosed at a younger age than non-PCOS women. In non-PCOS women, the cumulative incidence of T2D during the study period was $1.3 \pm 0.1\%$ and their median age at T2D diagnosis was 40.0 ± 0.3 years. Normoandrogenic women with PCOS had a $4.4 \pm 0.5\%$ cumulative incidence with a median age of 38.0 ± 0.5 years at diagnosis, whereas HA women with PCOS had a $14.2 \pm 4.2\%$ cumulative incidence and a median age at diagnosis of 37.0 ± 0.7 years.

Cox-regression analysis of Model 1 (adjusted for BMI, birth period, country of birth and education) resulted in a likelihood of T2D in NA PCOS-women two-fold higher than in non-PCOS women [aHR 2.52 (95% CI 2.15 - 2.96)], whereas women with the HA PCOS phenotype (broad definition) had a significantly higher likelihood [aHR 3.86 (95% CI 3.16-4.72)], Table 4. When the risk of T2D was estimated in women using the more specific HA phenotypes, the risk estimates were even higher.

Women born later during the study period, in the Nordic countries, and those with a higher level of education had lower rates of T2D and the highest rates were found in women born in South Asia. With increasing BMI, the rate of T2D showed a marked dose-response relation.

Table 4. Adjusted hazard ratios (aHR) among PCOS women for T2D, stratified by PCOS HA phenotype, adjusted for BMI, birth period, country of birth and education. (n=148 121, missing values n=678)

	Broad HA pheno- type aHR (95% CI)	Intermediate HA phenotype aHR (95% CI)	Specific HA phe- notype aHR (95% CI)
Group			
Non-PCOS	(ref)	(ref)	(ref)
NA-PCOS	2.52 (2.15, 2.96)	2.70 (2.32, 3.13)	2.76 (2.38, 3.19)
HA-PCOS	3.86 (3.16, 4.72)	4.30 (3.28, 5.65)	6.34 (4.25, 9.45)
Country of birth			
Nordic countries	(ref)	(ref)	(ref)
Europe	1.43 (1.09, 1.88)	1.43 (1.09, 1.88)	1.44 (1.10, 1.89)
Middle East	1.94 (1.53, 2.46)	1.94 (1.53, 2.46)	1.97 (1.56, 2.50)
India, Bangladesh or Pakistan	6.33 (4.29, 9.33)	6.38 (4.33, 9.41)	6.54 (4.43, 9.64)
Africa	1.96 (1.36, 2.84)	1.94 (1.34, 2.81)	1.94 (1.34, 2.81)
Remaining countries	2.32 (1.76, 3.06)	2.32 (1.76, 3.06)	2.35 (1.78, 3.10)
Education			
>12 years	(ref)	(ref)	(ref)
10-12 years	1.66 (1.42, 1.95)	1.65 (1.41, 1.94)	1.65 (1.41, 1.93)
≤9 years	2.24 (1.79, 2.81)	2.21 (1.77, 2.76)	2.20 (1.76, 2.75)
BMI			
<25.0 kg/m ²	(ref)	(ref)	(ref)
25.0-29.99 kg/m ²	3.10 (2.52, 3.82)	3.10 (2.52, 3.82)	3.09 (2.51, 3.81)
≥30.0 kg/m ²	9.96 (8.21, 12.08)	9.87 (8.13, 11.98)	9.83 (8.10, 11.93)

aHR= adjusted Hazard ratio; CI= confidence interval; HA = hyperandrogenism; BMI = body mass index

Paper III

The incidence of both hypertension and dyslipidaemia was lowest among non-PCOS women (hypertension 1.4% and dyslipidaemia 0.2%) and highest in women with the HA-PCOS phenotype (hypertension 7.5% and dyslipidaemia 2.0%).

Compared with non-PCOS women, the risk for hypertension was increased in women with PCOS, aHR of 2.08 (95% CI 1.92-2.25) in Model 1 adjusted for BMI and aHR 2.25 (95% CI 2.13-2.39) in Model 2, adjusted for obesity diagnosis. Women with the HA phenotype had an almost six times higher risk of hypertension than non-PCOS women independently of body weight, Table 5 and Table 6.

Women with PCOS had a three times higher risk for dyslipidaemia compared with non-PCOS women, [aHR 2.84 (95% CI 2.36-3.40) when adjusted for BMI and aHR 3.05 (95% CI 2.69-3.46) when adjusted for obesity]. Women with the NA-PCOS phenotype had a 2-fold increased risk for dyslipidaemia. The highest risk was seen in women with the HA phenotype, seven-fold increased risk, compared with non-PCOS women.

Table 5. Risk of hypertension and dyslipidaemia, Model 1, adjusted for BMI, birth period, country of birth and level of education.

	Non-PCOS aHR (95%CI)	NA-PCOS aHR (95%CI)	HA-PCOS aHR (95%CI)
	n=119 545	n=21 803	n=2 026
Hypertension	(ref)	1.77 (1.63-1.94)	5.91 (5.09-6.86)
Dyslipidaemia	(ref)	2.47 (2.04-3.00)	7.32 (5.28-10.16)
	BMI <25.0 aHR (95%CI)	BMI 25.0-29.9 aHR (95%CI)	BMI ≥30.0 aHR (95%CI)
Hypertension	(ref)	1.64 (1.49-1.79)	2.95 (2.69- 3.24)
Dyslipidaemia	(ref)	1.96 (1.58-2.42)	2.98 (2.38-3.73)

NA-PCOS=normoandrogenic PCOS phenotype, HA-PCOS=hyperandrogenic PCOS phenotype, BMI=Body Mass Index.

Table 6. Risk of hypertension and dyslipidaemia, Model 2, adjusted for obesity diagnosis, birth period, country of birth and level of education.

	Non-PCOS aHR (95%CI)	NA-PCOS aHR (95%CI)	HA-PCOS aHR (95%CI)
	n=242 014	n=44 723	n=5 709
Hypertension	(ref)	1.94 (1.83-2.07)	5.51 (4.97-6.10)
Dyslipidaemia	(ref)	2.62 (2.29-2.99)	7.82 (6.34-9.64)
	No obesity aHR (95%CI)	Obesity aHR (95%CI)	
Hypertension	(ref)	3.33 (3.12-3.56)	
Dyslipidaemia	(ref)	3.92 (3.41-4.49)	

NA-PCOS=normoandrogenic PCOS phenotype, HA-PCOS=hyperandrogenic PCOS phenotype, BMI=Body Mass Index.

When women were stratified according to their BMI, we were able to show that women with PCOS who were normal weight (BMI <25.0 kg/m²), had higher risk of hypertension than women without PCOS, aHR 1.75 (95% CI 1.52-2.02). The risk was even more increased in normal weight women with the HA phenotype, where the risk of hypertension was 6-fold higher than in non-PCOS women, aHR 5.77 (95%CI 4.47-7.45). Similarly, the risk of dyslipidaemia was increased in women with vs without PCOS even among normal weight individuals, aHR 2.51 (95% CI 1.82-3.46), with a slightly higher estimate among HA women, aHR 4.24 (95% CI 1.97-9.13).

Paper IV

In study IV, 124 women with PCOS and 74 controls were included. Participants had a median age of 50 years in the PCOS group and 51 years in the control group. Almost all women were born in Sweden or the other Nordic countries. Women with PCOS had a higher median BMI and were obese to a higher extent than controls ($p < 0.001$, Table 7). In the control group, more women were post-menopausal and used menopausal hormonal treatment (MHT) than women in the PCOS group.

Table 7. Demographic information of study population, paper IV.

	Controls n=74 n (%)	PCOS n=124 n (%)
Age , median, (min, max)	51 (45, 66)	50 (45, 66)
Marital status		
Married	50 (73.5)	79 (79.0)
Single household	16 (23.5)	20 (20.0)
Other	2 (3.0)	1 (1.0)
BMI , median (min, max)	24.9 (18.2, 41.4)	29.7 (21.2, 50.0)
<25.0 kg/m ²	37 (50.0)	22 (17.9)
25.0–29.9 kg/m ²	25 (33.8)	43 (35.0)
≥30.0 kg/m ²	12 (16.2)	58 (47.1)
Contraceptives		
No hormonal method	58 (79.4)	94 (75.8)
Combined oral contraception	1 (1.4)	2 (1.6)
Hormonal IUD	14 (19.2)	18 (14.5)
Oral progestins	0 (0)	10 (8.1)
MHT		
No	68 (93.2)	120 (96.8)
Yes	5 (6.8)	4 (3.2)
Menopausal status ¹		
Premenopausal	34 (45.9)	82 (66.1)
Postmenopausal	29 (39.2)	34 (27.4)
Unclassified	11 (14.9)	8 (6.5)
Country of birth		
Nordic countries	71 (95.9)	105 (84.7)
Other countries	3 (4.1)	5 (4.0)
Not reported	0 (0.0)	14 (11.3)

¹ Defined in the following way: Premenopausal = bleeding within the last 12 months and no hormonal treatment or hormonal treatment and AMH ≥ 0.1 pmol/L; Postmenopausal = no bleedings within the last 12 months, no hormonal treatment or hormonal treatment and AMH < 0.1 pmol/L; Unclassified = no bleedings within the last 12 months, hormonal treatment and AMH absent or bleedings within the last 12 months with hormonal treatment and AMH absent. IUD = intrauterine device; MHT = menopausal hormone therapy. Percentages are presented as % of subjects with a reported value.

Over 40% of women with PCOS had hirsutism (mFG score ≥ 8), the majority reported removal of facial hair during the past year, with 45.2% of HA and 19.4% of NA women with PCOS doing so on a daily basis. Androgenic hair loss was uncommon among controls, more common and more severe in HA than in NA women with PCOS. Acne was also more common among women with than without PCOS. In the control group, 4% had hirsutism.

Table 8. Androgenic symptoms, treatment and biochemical hyperandrogenism at the follow-up assessment in women with PCOS and controls.

	Controls n=74 n (%)	PCOS n=124 n (%)	p-value
Hair removal last 12 months			
No	59 (79.7)	43 (34.7)	<0.001
Less than daily	14 (18.9)	41(33.0)	
Daily	1 (1.4)	40 (32.3)	
Androgenic hair loss			
No	41 (95.3)	96 (77.4)	0.066
Ludwig grade I	2 (4.7)	21 (17.0)	
Ludwig grade II	0 (0)	6 (4.8)	
Ludwig grade III	0 (0)	1 (0.8)	
Acne, current problem			
No	64 (86.5)	86 (69.4)	0.006
Yes	10 (13.5)	38 (30.6)	
Acne treatment, ongoing			
No	71 (95.9)	120 (96.8)	0.375
Yes	3 (4.1)	4 (3.2)	
Ferriman–Gallwey score			
0–7	71 (95.9)	71 (57.7)	<0.001
≥ 8	3 (4.1)	52 (42.3)	
Biochemical hyperandrogenism ³			
Testosterone, ng/mL, median (min, max)	0.28 (0.13, 0.81)	0.34 (0.02, 1.29)	0.102
SHBG, nmol/L, median (min, max)	65.0 (27.0, 142.0)	41.0 (6.4, 110.0)	<0.001
FAI, median (min, max)	0.004 (0.00, 0.01)	0.008 (0.00, 0.06)	<0.001

Among women with PCOS, the quality of life was most negatively associated with weight concerns (median score 3.2) and hirsutism (median score 4.7), (Table 9). Not surprisingly, women with the HA phenotype had more concerns about hirsutism than those with the NA phenotype. Infertility was not considered a problem, while menstrual disturbances and emotional concerns due to

PCOS were still a problem, but to a lower extent than concerns regarding weight and hirsutism.

Table 9. Health-related quality of life in women with PCOS.

PCOSQ domain	All PCOS Median (Min, Max)	NA PCOS Median (Min, Max)	HA PCOS Median (Min, Max)	p- value
Emotional concerns	5.9 (2.8, 7.0)	6.1 (2.8, 7.0)	5.6 (2.8, 7.0)	0.095
Hirsutism	4.7 (1.0, 7.0)	5.8 (1.0, 7.0)	3.0 (1.0, 7.0)	<0.001
Weight concerns	3.2 (1.0, 7.0)	3.8 (1.0, 7.0)	3.0 (1.0, 7.0)	0.151
Infertility concerns	7.0 (1.5, 7.0)	7.0 (1.5, 7.0)	6.8 (2.5, 7.0)	0.175
Menstrual concerns	5.6 (2.0, 7.0)	5.8 (2.0, 7.0)	5.5 (3.0, 7.0)	0.634

Discussion

Main findings

The main findings of this thesis are that PCOS has a major impact on women's lives, also beyond the fertile period. While women with PCOS achieve (at least one) childbirth as often as non-PCOS women, they less often have additional children, and they have their first child at an older age. In addition, PCOS is an independent risk factor for T2D, hypertension and dyslipidaemia, irrespectively of body weight. The risk seems to be more pronounced in those with the hyperandrogenic phenotype. Finally, hyperandrogenism is persisting to a high extent in midlife women with PCOS, continuing to be a major source of concern, both by still being symptomatic and by having a negative impact on quality of life.

General discussion

Fertility

The cumulative probability of childbirth in women with PCOS was not reduced compared with non-PCOS women in our study. Another smaller, Swedish study found that parity was similar between women with and without PCOS, implying that women with PCOS might reach the family size they aim for (143). Still, the finding that women with PCOS gave birth to fewer children than non-PCOS women implies that they might not have obtained the family size that they desired. However, we had no information on wished family size neither for women with PCOS nor without it. Similarly, we had no information on intention to conceive, or unsuccessful attempts to do so, and cannot therefore draw conclusions with certainty.

In addition, women with PCOS gave birth after use of ART to a higher extent than non-PCOS women, a finding that is expected (97, 99, 102). During IVF treatment, women with PCOS are more sensitive to exogenous hormones, with higher risk of the potentially serious side effect of ovarian hyperstimulation syndrome (OHSS) (144). In Sweden, assisted reproduction is offered within the general health insurance for one child, but if the parents wish for more than one child they need to cover the expenses by own resources. The latter may therefore affect negatively the total number of children that a woman with PCOS gives birth to. Assisted reproduction is associated with

costs, mental distress, side effects and risks, which may influence the readiness to have additional children beyond the first child (145-147).

Being born outside the Nordic countries, especially in Africa, or having high educational level was associated with lower fecundity in our population. Generally, immigrants from Africa have lower usage of health care, including cervical cancer screening and diabetes preventive health care (148). A similar low utilization may extend to fertility treatment. High educational level on the other hand is generally associated with childbearing later in life, when fertility is declining, potentially affecting the cumulative probability of childbirth (149).

Fecundity ratio was lower in women with PCOS than in non-PCOS women, which could have several explanations. In addition to dependence on ART, women with PCOS generally have a higher risk of miscarriage (144), potentially affecting the number of children born. Since we did not have reliable data on miscarriage rates we reckon that any conclusion on causality would be speculative.

Lastly, diagnosis of PCOS at an early age was associated with higher fecundity. The awareness of their PCOS diagnosis might influence fecundity by affecting family planning choices, lifestyle, knowledge about ART and avoidance of unnecessary delays of fertility treatment (150). Our findings support the claim that early diagnosis of PCOS gives women the best conditions to reach their family planning goals.

Type 2 Diabetes, hypertension and dyslipidaemia

The results of the studies included in this thesis suggest that PCOS is an independent risk factor for the development of the cardio-metabolic outcomes studied, i.e., T2D, hypertension and dyslipidaemia. The increased risk was independent of excess body weight, was seen even among normal-weight women and obesity exacerbated that risk further. In particular, normal weight (BMI <25 kg/m²) women with PCOS had a two-fold increased risk of T2D, hypertension and dyslipidaemia compared with non-PCOS women, with that risk increasing to six-fold among obese PCOS women.

The relation between visceral fat, obesity and increased metabolic risk has been established in the general population (114, 151). Previous Nordic studies suggested that the increased risk of T2D in women with PCOS is mainly due to obesity, a conclusion not completely supported by our results (108, 109). On the contrary, a Danish study with a well-defined PCOS cohort has similarly to us, found that women with PCOS were more likely to have a T2D diagnosis and anti-diabetic treatment reported in national registers than controls (152). Moreover, in a large questionnaire-based Australian study, a 3-fold higher risk to develop diabetes regardless of BMI was found in PCOS-women compared with controls, with the highest incidence ratio seen in normal weight women (110). To sum up, the risk of T2D in women with PCOS

increases with co-existing obesity, but many studies lack data on BMI, hindering the performance of meta-analysis and deduction of safe conclusions (153). Lastly, in several register-based studies with large cohorts of women with PCOS, the risk of T2D is proven to be elevated in normal weight women, but only few of the studies evaluated the impact of the HA phenotype (154).

Regarding hypertension, recent systematic reviews by Millan de Meer et al and Wekker et al showed higher systolic blood pressure in women with PCOS compared with controls (50, 155). However, unexpectedly, after menopause the risk of hypertension was no longer increased in PCOS women; whether this is due to amelioration of PCOS or worsening of metabolic function in control women is to be further studied (155, 156). In contrast to other studies, we showed that the increased risk of hypertension in women with PCOS was not only due to obesity but could be clearly seen even among normal-weight women with PCOS (157).

When exploring prevalence of dyslipidaemia as an established diagnosis between PCOS and non-PCOS women (and not comparing values of particular lipid parameters) no difference in the prevalence of the condition can be seen, probably since lipid values in the prior studies fall within the normal range despite differing between groups (155). A recent Dutch study investigating women ≥ 45 years of age confirmed that women with hyperandrogenism during the reproductive years continue to have an unfavourable cardio-metabolic profile with higher blood pressure and lower HDL cholesterol, even after adjusting for BMI, at later life stages, even despite becoming normoandrogenic (158).

The impact of hyperandrogenism

Our findings suggest that women with the metabolic less harmful NA phenotype of PCOS had at least two-fold increased rate of T2D, hypertension and dyslipidaemia when adjusting for BMI. The results remained unchanged when analysing women with a diagnosis of anovulatory infertility only, which is likely to be a group with high prevalence of PCOS.

Women with the HA phenotype on the other hand developed T2D, hypertension and dyslipidaemia more rapidly than those with the NA phenotype or non-PCOS women, a finding that persisted irrespectively of which HA definition was used in the T2D-study. The finding that T2D diabetes is more common in hyperandrogenic women is supported by a Danish register-based study, where relatively lean women with PCOS, in whom over 90% were hyperandrogenic, had a 4-fold increased risk of T2D compared with controls (159).

Potential underlying mechanisms of the increased risk of T2D in HA women with PCOS could be the combination of increased levels of insulin, contributing to hyperandrogenism, which in turn further aggravates insulin resistance (43, 44). The exact role of hyperandrogenism on the risk of hyperten-

sion and dyslipidaemia in women with PCOS remains unknown, it has however been demonstrated that androgen receptors are involved in lipid metabolism (160). Furthermore, elevated androgen levels may have a toxic effect on the pancreatic islet cells, inducing apoptosis in the pancreas, worsening insulin resistance and glucose intolerance (161, 162). Insulin resistance is in turn associated with decreased high-density lipoprotein cholesterol and increased low-density lipoprotein cholesterol and triglycerides and facilitates dyslipidaemia (163). The mechanism behind insulin resistance and hypertension is complex, but there seems to be an association, including low-grade chronic inflammation, oxidative stress and endothelial dysfunction contributing to future CVD (164, 165).

Persistence of symptoms

In our clinical study, we found that testosterone levels were similar between the PCOS group and the control group, but FAI and the prevalence of hyperandrogenic symptoms were higher in women with PCOS. (115, 141). It is advised to calculate the FAI, mirroring bioavailable testosterone, to evaluate biochemical HA (15). Previous studies evaluating biochemical hyperandrogenism used a disparity of cut off levels of androgens and measurement assays, hampering comparisons between studies. In several recent studies, levels of total testosterone were used to classify women as having NA or HA phenotype, with cut-offs ranging between 34 ng /dL (1.18 nmol/l) (166) and 70 ng/dL (2.43 nmol/l) (167).

We evaluated both premenopausal and postmenopausal women with median age of 50-51 years, which hinders us from drawing safe conclusions for postmenopausal women in general. However, our findings in combination with the knowledge that the endocrine transition during menopause into a relatively hyperandrogenic state with declining oestrogen levels and increasing incidence of CVD, point towards an association between HA-PCOS and risk of CVD. Still, large studies in postmenopausal women are needed, preferably with well-defined PCOS phenotypes, to further explore this topic. All our results point towards negative associations between PCOS in general and hyperandrogenism, in particular with the metabolic outcomes studied, as well as negative impact on quality of life.

Quality of life

Hirsutism persisted to a high degree with significant suffering in pre- and postmenopausal midlife women with PCOS. Similar results have been found in a Danish study, where higher mFG score and BMI were associated with an increased incidence of depression in women with PCOS (59). In a Finnish study, women with PCOS and women with hirsutism were more likely to suffer from anxiety and depression at age 46 than controls, but in contrast to our results, that study showed no association with obesity (58). Lastly, in the same Finnish

cohort, women with PCOS reported lower quality of life compared with controls (168).

The PCOSQ tool was used to measure quality of life, which is only validated in women with PCOS (169), and therefore quality of life in controls could not be assessed. However, our results, like the Finnish results, point towards a lower reported quality of life in women with hirsutism. In fertile aged women, concerns about weight, menstrual disturbances and fertility are the domains having the most negative impact on quality of life (57, 169). Many studies among fertile aged women recruited their participants in an infertility setting, where concerns regarding infertility are to be expected and the findings are therefore not applicable to the general population (57). This is however the first time that a sample of older women is assessed, demonstrating concerns about body weight; women in our study reported a negative impact on QoL while having a median BMI of almost 30 kg/m².

The second issue of concern was hirsutism. Among women with the HA phenotype, hirsutism and body weight were equally rated problem areas. Hyperandrogenism and obesity are closely linked, both generally and specifically in PCOS, and obviously still bothering women with PCOS at ages beyond the fertile period. We were unable to compare quality of life between the control group and the PCOS group, but since more women with PCOS had hyperandrogenic symptoms and a higher median BMI, it is likely that these issues are not affecting quality of life to the same extent in the control group.

PCOS should be recognised as a condition associated with reduced quality of life, and increased risk of depression and anxiety. Women with PCOS should ideally be screened upon PCOS diagnosis as well as during clinical visits (15).

PCOS phenotyping

One of the challenges in research on PCOS is the subdivision of women into the different phenotypes. Therefore, within this thesis, we wanted to come up with a reasonable alternative definition of hyperandrogenism. This definition could be used in future epidemiologic research when information on clinical or biochemical hyperandrogenism is lacking. Being able to classify more women into their phenotypes will enhance knowledge on the impact of hyperandrogenism on long-term health in women with PCOS. The model needs further validation before being widely used.

Strengths of studies

The main strengths of the register studies were their large study population, long time-period for follow up and inclusion of women of different age groups and nationalities. The possibility to use register data to divide the PCOS population into NA and HA phenotypes was another strength. Lastly, since data was collected prospectively, there was no recall bias.

In the clinical study, the main strengths were the objective assessment of hyperandrogenic symptoms, access to medical records for historical medical information and a control group matched for age and area of residence. Since the participants were seen in person, we had the possibility to ask clarifying questions and we were able to assess the current health status after taking the PCOS phenotype at the time of diagnosis into consideration.

Limitations of studies

The limitations of paper I-III are mainly associated with the properties of the registers used for data collection. Firstly, by relying on register data for PCOS diagnosis, we capture only the more severe cases, who seek specialised medical care affecting the reported prevalence of the syndrome. In contrast, in a study with an unselected population, the prevalence of PCOS is expected to be higher, including also less severe cases.

Secondly, the NPR did not include information on which diagnostic criteria were fulfilled at PCOS diagnosis, and we could therefore not use the register to classify the PCOS phenotypes, except for cases who had a diagnosis of hyperandrogenism. Instead, we classified the hyperandrogenic phenotype based on prescription of anti-androgenic COCs and anti-androgenic drugs. This would underestimate the number of HA women, since it is unlikely that all women with hyperandrogenism received medical treatment. By using prescription of anti-androgenic drugs as a marker for hyperandrogenism, we were likely further selecting the most severe hyperandrogenic women. Our risk estimates for hyperandrogenic PCOS women therefore might be higher than it would be if all women were truly hyperandrogenic. Further, information on compliance with the prescribed treatment was lacking, even though we used at least two consecutive prescriptions to classify women as having the HA phenotype. The register also lacked information on indication for the prescription of drugs, which made it impossible to know if a COC was prescribed as a contraceptive only or for PCOS-related symptoms. For the same reason, we could not know if spironolactone was prescribed as an anti-androgenic treatment or against hypertension.

In paper II, the broad drug exposure was likely overestimating the prevalence of HA, as some women were probably prescribed contraceptives for birth control. In addition, the registers did not contain reliable information on

confounders like smoking, physical activity, alcohol consumption, family history or information on partner (i.e., age). Furthermore we lack BMI data on women who did not give birth during the study period. This lack of information on risk factors for diabetes, hypertension and dyslipidemia in the registers was an obvious limitation.

Lastly, reporting of smoking (and other life-style factors) during pregnancy in the MBR might not be representative of the general population or mirror the habits when non-pregnant.

One of the most obvious limitations when assessing fertility issues was the lack of information on intention to conceive and how many children women wished. Further lacking information included age and health status of partner, miscarriages and fertility treatments not resulting in childbirth. Data on assisted reproduction was available from 1995, so we lacked information for the first years of the study period.

Visits to general practitioners, as well as primary care contacts, are not registered in NPR, potentially affecting the reported diagnoses of T2D, hypertension and dyslipidaemia. By relying on the ICD codes E11 and E14 for T2D diagnosis, we were limited to women who, at some point, had their diabetes diagnosis registered in specialist health care. To capture women who were diagnosed by general practitioners, we chose to include prescription of anti-diabetic drugs as a proxy of T2D, which has been used in previous studies as well (159). However, metformin is widely used within the PCOS population as an off-label treatment. Prescription of metformin could therefore not be used as an outcome of T2D, thereby missing cases where metformin is prescribed as anti-diabetic treatment.

In the clinical study, the most obvious limitations were the risk of recall bias and the risk that women with severe symptoms were more willing to participate in the study than milder cases. The relatively small study size and the difficulty to recruit, in particular controls during the pandemic, resulted in an even smaller sample size than we aimed for originally. In addition, we included women irrespectively of menopausal status, the study visits were not scheduled at any particular time of the menstrual cycle, and all medicines (hormones included) were allowed, which might influence biochemical testing.

In order to assess clinical hyperandrogenism and thus the HA-phenotype in our study (Paper IV) we used as cut-off value $mFG \geq 8$, which might differ from the cut-off used in other clinical studies.

Conclusions and clinical implication

Women with PCOS had lower fecundity ratio, gave birth to fewer children, were older at first childbirth and had longer time interval between childbirths than non-PCOS women. The fact that the cumulative probability of childbirth was similar to the general female population should be conveyed to the concerned women, who historically have been told that they might not be able to conceive naturally, both for reassurance and with the advice to use contraception when pregnancy is not desired.

Women with PCOS had a moderately increased risk to develop both T2D, hypertension and dyslipidaemia compared with non-PCOS women, even when accounting for body weight. Women who were prescribed anti-androgenic medical treatment developed T2D more rapidly than normoandrogenic women and had a six- to seven-fold increased risk to develop hypertension and dyslipidaemia compared with non-PCOS women. This risk is far greater than the risk due to obesity alone, which should be taken into account by health care professionals when assessing cardiovascular risk in women.

We propose that the PCOS diagnosis in general and the HA phenotype in particular should be included as independent risk factors when counselling women on the risk of CVD and T2D. With a cumulative incidence of 14 to 25%, we suggest that women with anti-androgenic treatment are considered at high risk of developing T2D. The current international guideline for PCOS management recommends assessment of glycaemic status at PCOS diagnosis and reassessment every three years based on individual risk factors, but does not mention the impact of the phenotypes (15). Continuing with hypertension and dyslipidaemia, the guideline states that all women with PCOS are considered at increased risk of CVD and should have annual measures of blood pressure and a lipid profile at diagnosis, thereafter based on the results and other risk markers (15). Development of recommendations specific to the NA/HA phenotypes could lighten the burden of screening in NA women if they are shown to be at lower risk than HA women.

In addition to the increased risks already mentioned, a high proportion of women with hyperandrogenic symptoms at the time of PCOS diagnosis had persisting symptoms in midlife. These women experienced a lower quality of life than normoandrogenic women with PCOS. Very few women had medical treatment for their hyperandrogenic symptoms and efforts should be made to tighten the gap between experienced suffering of symptoms and access to

treatment. Enhanced knowledge by physicians and extended information to the affected women is crucial to meet the medical needs.

The current international recommendation is to use COC as first line treatment for hyperandrogenism, which might be contraindicated in older women and not suitable post menopause (15). However, COCs and other anti-androgenic agents are used off-label in PCOS regardless of age, since no current approval with indication treatment of PCOS is present for any medical substance. Clinical trials should therefore include perimenopausal women with PCOS in order to assess the efficacy and safety of the existing pharmacological treatment regimens in this group.

Future directions

Further clinical trials are needed in order to assess whether early screening and/or prophylactic treatment of women with PCOS can prevent future cardiovascular events and to evaluate the effects of the different phenotypes on health outcomes. Similarly, even though a lot is known about PCOS and fertility, future studies should aim to evaluate if early detection and treatment of PCOS could improve fertility outcomes.

Our register-based studies imply that women with PCOS, especially women with anti-androgenic treatment, are at higher risk to develop diabetes, hypertension and dyslipidaemia, which in turn are risk factors for future cardiovascular events. Future research should therefore aim to evaluate clinical endpoints, i.e., myocardial infarction, stroke and cardiovascular death. This requires large cohorts and follow up to an older age than most existing studies since the endpoints above have lower incidence and occur at an older age than T2D, hypertension and dyslipidaemia. These studies should optimally be possible to adjust for body weight/BMI and lifestyle factors.

To enhance availability of treatment for women with PCOS, there is a need for clinical trials, market authorisation applications as well as approval by regulatory agencies with PCOS as indication for use of drug substances.

Sammanfattning på svenska – Summary in Swedish

Introduktion

Polycystiskt ovarialsyndrom (PCOS), är den vanligaste endokrina åkomman bland kvinnor i fertil ålder och drabbar ca 10% av dem. PCOS är associerat med lägre fertilitet och hälsoproblem som fetma, insulinresistens och diabetes, högt blodtryck, avvikande blodfetter och sämre livskvalitet. De flesta studier om PCOS är gjorda under de fertila åren, och väldigt lite fakta finns kring senare delen av livet och vad som händer efter menopaus. Dessutom saknas information kring vilken roll PCOS i sig spelar, och vad som beror på den ökade förekomsten av övervikt och fetma. Diagnosen PCOS ställs genom att minst 2 av 3 diagnoskriterier ska vara uppfyllda samtidigt som andra orsaker till besvären ska vara uteslutna. Diagnoskriterierna är följande: 1) överskott av androgener (hyperandrogenism) som kan mätas antingen via blodprov eller kliniska symtom (ökad kroppsbehåring, akne, håravfall av manlig typ), 2) ägglossningsstörning, som visar sig via oregelbundna/glesa menstruationer och 3) polycystiska ovarier vid ultraljudsundersökning.

Syfte

Övergripande syfte med denna avhandling var att undersöka hälsa på lång sikt hos kvinnor med PCOS jämfört med kvinnor utan PCOS avseende fertilitet, typ 2 diabetes, hypertoni, dyslipidemi, hyperandrogenism och livskvalitet. Särskilt fokus var betydelsen av kroppsvikt och den hyperandrogena fenotypen av PCOS.

Metod

Studie I-III genomfördes med liknande metod, genom att länka sex nationella register (Medicinska födelseregistret, Registret över totalbefolkningen, Registret över befolkningens utbildning, Patientregistret, Dödsorsaksregistret och Läkemedelsregistret) med hjälp av personnummer. Alla kvinnor som dia-

gnostiserats med PCOS under studieperioden inkluderades och upp till 5 kvinnor utan PCOS-diagnos valdes slumpmässigt ut som matchade kontroller, matchning skedde på födelseår och bostadsort. Från medicinska födelseregistret hämtades information om BMI (som alltså bara fanns tillgängligt för de kvinnor som fött barn under studieperioden). Data på förekomst av fetma hämtades från patientregistret. För att definiera den hyperandrogena fenotypen av PCOS användes data från läkemedelsregistret. För utfallen i studie II-III hämtades data för diagnoser (typ2 diabetes, hypertoni, dyslipidemi) från Patientregistret och information om förskrivna läkemedel från Läkemedelsregistret.

Studie IV var en klinisk studie där alla kvinnor som fått PCOS-diagnos identifierades via patientregistret vid ingående kliniker i Sundsvall, Uppsala, Falun, Stockholm (Karolinska institutet). För varje kvinna med PCOS bjöds en kontrollperson in, som matchades för ålder och bostadsort. På grund av svårigheter att rekrytera kontroller under COVID-19-pandemin ändrades studieprotokollet till att även inkludera kontroller från en tidigare studie med samma inklusionskriterier och undersökningar. Efter inhämtat muntligt och skriftligt samtycke genomfördes ett kliniskt besök med intervju och enkätfrågor om livskvalitet, klinisk undersökning och provtagning.

Kaplan-Meiers överlevnadsanalys användes för att beräkna kumulativ sannolikhet för förlossning, diabetes, hypertoni och dyslipidemi. Hazardkvoter beräknades med cox-regressioner med 95% konfidensintervall. I arbete IV användes chi-två test och Fishers exakt test för att jämföra kategoriska variabler och Mann-Whitneys U-test för att jämföra kontinuerliga variabler.

Studie I

Till studie I inkluderades 45 395 kvinnor med PCOS och 218 049 utan PCOS. De flesta var födda på 1980-talet i de nordiska länderna. De viktigaste resultaten var att kvinnor med PCOS födde barn i samma utsträckning som kvinnor utan PCOS [kumulativ sannolikhet för förlossning 80.2% (95% KI 79.5-80.9%) vs 78.2% (95% KI 77.9-78.5%)], men de födde färre barn totalt sett, hade längre tid mellan första och andra barnet, var äldre vid sin första förlossning och det var mer sannolikt att de behövde assisterad befruktning för att bli gravida.

Resultatet ger viktig information till kvinnor med PCOS att de har goda chanser att få barn och att de bör använda preventivmedel när de vill undvika graviditet. En bidragande faktor till att kvinnor med PCOS föder färre barn kan vara att de i större utsträckning behöver hjälp att uppnå graviditet. Fertilitetsbehandling är allmänt påfrestande både mentalt och fysiskt, dessutom har kvinnor med PCOS större risk för allvarliga komplikationer (överstimuleringsyndrom, OHSS) vid IVF-behandling. I Sverige subventioneras fertili-

tetsbehandling för att få ett barn, men par som önskar fler barn får själva bekosta behandlingen, något som kan påverka möjligheten att få det antal barn som önskas.

Studie II och III

I studie II och III inkluderades 52 535 respektive 50 969 kvinnor med PCOS och 254 624 respektive 246 246 kvinnor utan PCOS. Kvinnor med PCOS hade 2 gånger ökad risk att utveckla typ 2 diabetes jämfört med kontrollgruppen, [aHR 2.52 (95% KI 2.15 -2.96)], medan de med hyperandrogen fenotyp hade ännu högre risk [aHR 3.86 (95% KI 3.16-4.72)]; analyserna var justerade för BMI, födelseperiod, födelseland och utbildningsnivå.

Resultatet för hypertoni och dyslipidemi var liknande, med aHR 2.08 (95% KI 1.92-2.25) för kvinnor med PCOS och aHR 5.91 (95% KI 5.09-6.86) för den hyperandrogena gruppen avseende hypertoni och för dyslipidemi var aHR 2.84 (95% KI 2.36-3.40) för kvinnor med PCOS och aHR 7.32 (95% KI 5.28-10.16) för den hyperandrogena gruppen.

Våra resultat visar att fetma inte är den enda faktorn som gör att kvinnor med PCOS har en högre metabol risk, eftersom risken för alla våra utfall kvarstod även efter justering för BMI. Däremot verkar hyperandrogenism ge en påtagligt ökad risk för alla studerade utfall, vilket bör tas i beaktande när läkare träffar kvinnor med PCOS och bedömer deras kardiovaskulära risk, samt vid utveckling av riktlinjer för screening av kvinnor med PCOS. Idag görs ingen skillnad på fenotyper, utan screening för diabetes och kardiovaskulära sjukdomar ser lika ut oavsett om hyperandrogenism finns eller inte.

Studie IV

Totalt inkluderades 124 kvinnor med PCOS och 74 kontroller. Medianåldern var 51 år i PCOS-gruppen och 50 år i kontrollgruppen. Kvinnorna med PCOS hade högre BMI, färre hade passerat menopaus och färre hade hormonbehandling för klimakteriebesvär.

Mer än 40% av kvinnorna med PCOS hade hirsutism (mFG ≥ 8 poäng), de flesta hade behandlat oönskad hårväxt i ansiktet, 45% av de med hyperandrogen fenotyp gjorde det dagligen.

När livskvalitet skattades av kvinnorna med PCOS var det viktproblem och hirsutism som hade störst negativ påverkan på livskvaliteten. Eftersom livskvalitet mättes med ett instrument framtaget specifikt för kvinnor med PCOS så kunde vi inte jämföra med kontrollgruppen.

De flesta tidigare studier om livskvalitet och PCOS är utförda under fertil ålder, dessutom har många rekryterat deltagare från fertilitetskliniker, och där har man sett att infertilitet och mensrubbingar är de vanligaste besvären med

negativ påverkan på livskvalitet. Det finns även en generell koppling mellan fetma och ökad risk för depression. Kvinnor med PCOS bör ses som en patientgrupp med ökad risk för negativ påverkan på livskvalitet och ökad risk för depression och ångestillstånd, de bör screenas och erbjudas adekvat behandling i de fall där det behövs.

Slutsatser

Kvinnor med PCOS har lika stora chanser som andra kvinnor att föda minst ett barn, vilket är positivt och bör förmedlas till patienterna.

Våra resultat som visar att kvinnor med PCOS har en ökad risk för diabetes typ 2, högt blodtryck och dyslipidemi oavsett BMI är viktig information för vårdpersonal som möter dessa kvinnor. PCOS-diagnosen, och särskilt förekomst av hyperandrogenism, bör tas i beaktande när kvinnor screenas och behandlas för diabetes och kardiovaskulär sjukdom.

Fortfarande behövs fler studier inom området som följer upp kvinnor till en högre ålder, efter menopaus. Optimalt vore att dessa studier justeras för kroppsvikt och andra livsstilsfaktorer som påverkar riskerna att utveckla diabetes och kardiovaskulär sjukdom.

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