Pekka Pinola

HYPERANDROGENISM, MENSTRUAL IRREGULARITIES AND POLYCYSTIC OVARY SYNDROME

IMPACT ON FEMALE REPRODUCTIVE AND METABOLIC HEALTH FROM EARLY ADULTHOOD UNTIL MENOPAUSE
PEKKA PINOLA

HYPERANDROGENISM, MENSTRUAL IRREGULARITIES AND POLYCYSTIC OVARY SYNDROME
Impact on female reproductive and metabolic health from early adulthood until menopause

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Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women of reproductive age, affecting 5–18% of them. Menstrual irregularities, hyperandrogenemia and obesity are key features in PCOS and they are suggested to be the most important metabolic risks linked to PCOS, but their respective roles are still under debate. Anti-Müllerian hormone (AMH) is involved in sexual differentiation and follicle growth and its level is increased in women with PCOS.

The aims of this project were to clarify the significance of menstrual irregularities, hyperandrogenemia and serum levels of AMH in adolescence as predictive factors of the syndrome and to investigate the respective roles of obesity and hyperandrogenism as metabolic risk factors in women with PCOS from adolescence to late adulthood.

The study populations were the Northern Finland Birth Cohort 1986 (N=3373 women) and a Nordic population including 1553 women with PCOS and 448 controls.

At the age of 16 years, women with menstrual irregularities were more hyperandrogenic compared with women with normal menstrual cycles. Serum AMH levels correlated positively with those of testosterone at this age. They were higher in adolescents with menstrual irregularities compared with those with regular cycles and in women with hirsutism or PCOS at the age of 26 years. However, AMH was not a good marker of metabolic abnormalities in adolescence or a reliable tool to predict PCOS in later life. Androgen levels were higher in women with PCOS throughout life compared with controls. The parameters that best predicted PCOS at all ages were the free androgen index, and androstenedione. Women with PCOS exhibited increased abdominal obesity, altered insulin metabolism, worse lipid profiles and higher blood pressure from early adulthood until menopause compared with controls. The highest prevalence of metabolic syndrome was detected in obese and hyperandrogenic women with PCOS.

In conclusion, irregular menstrual cycles, identified by a simple question at adolescence, represent a good marker of hyperandrogenemia, later metabolic risks and development of PCOS. Due to the persistence of hyperandrogenism and metabolic alterations, the treatment of PCOS should be focused on prevention and treatment of these problems as early as in adolescence in order to decrease future morbidity.

Keywords: aging, androgens, cardiovascular diseases, hyperandrogenism, metabolism, polycystic ovary syndrome, reproductive health
Monirakkulainen munasarjaoireyhtymä (polycystic ovary syndrome, PCOS) on lisääntymisikäisten naisten tavallisin (5-18%) hormonaaisten häiriö. Kuukautishäiriöt, mieshormoniylimääriä eli hyperandrogenismi ja lihavuus kuuluvat oireyhtymään oleellisesti ja niiden ajatellaan olevan tärkeimmät PCOS:aan liittyvien metaboliisten riskitekijöiden tärkeimmät. Anti-Müllerian hormoni (AMH) vaikuttaa sukupuolijkenemiseen sekä munarakkuloiden kypsymiseen hedelmäisessä iässä ja sen pitoisuus on suurentunut PCOS-naisilla.

Tämän väitöskirjan tavoitteena oli selvittää kuukautishäiriöiden, hyperandrogenismin ja AMH-pitoisuuden ennustava PCOS-oireyhtymä, sekä arvioida lihavuuden ja hyperandrogenismin vaikutusta metaboliisiin riskitekijöihin PCOS-naisilla läpi elämän. Tutkimusaineistoina olivat Pohjois-Suomen syntymäkohortti 1986 (N=3373 naisa) sekä pohjoismaalaistuomion yhteisöaineisto, jossa oli 1553 PCOS-naisa ja 448 kontrollia.


Asiaanot: androgeenit, hyperandrogenismi, lisääntymisterveyds, metabolia, mieshormoniylimääriä, monirakkulainen munasarjaoireyhtymä, sydän- ja verisuonisairaudet
The secret of life, though, is to fall seven times and to get up eight times.

Paulo Coelho

To my family
Acknowledgements

The present study was carried out at the Department of Obstetrics and Gynecology, University of Oulu and the Medical Research Center Oulu, Oulu University Hospital, in collaboration with the National Institute for Health and Welfare. The first two papers were based on data from the Northern Finland Birth Cohort 1986 and without the contribution of two magnificent ladies, Professor Marjo Riitta-Järvelin and Professor Anna-Liisa Hartikainen, the project would not have even started. Their commitment to data collection and supporting young students is essential for research in Northern Finland.

The present study was also dependent on great Nordic collaborative work and I wish to thank our highly important collaborators, Professor Eszter Vanky, Professor Inger Sundström-Poromaa, Docent Elisabet Stener-Victorin, Professor Angelica Lindén Hirschberg, Docent Pernille Ravn, Professor Marianne Skovsager Andersen, Dr. Dorte Glintborg, M.D., Ph.D. and Dr. Jan Roar Mellembakken, M.D., Ph.D. Thank you for supporting young students in Finland.

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Oulu, July 2016

Pekka Pinola
Abbreviations

A4 Androstenedione
ACTH Adrenocorticotropic hormone
AE-PCOS Androgen Excess and Polycystic Ovary Syndrome
AFC Antral follicle count
AMH Anti-Müllerian hormone
ANCOVA Analysis of covariance
ANOVA Analysis of variance
BMI Body mass index
BP Blood pressure
cFT Calculated free testosterone
CRP C-reactive protein
CVD Cardiovascular disease
DHEA Dehydroepiandrosterone
DHEAS Dehydroepiandrosterone sulfate
DHT Dihydrotestosterone
e.g. exempli gratia, for example
F&G Ferriman and Gallwey
FAI Free androgen index
FSH Follicle-stimulating hormone
FT Free testosterone
GC-MS Gas chromatography-mass spectrometry
GnRH Gonadotropin-releasing hormone
HA Hyperandrogenism
HA-PCOS Hyperandrogenic (woman) with PCOS
hCG Human chorionic gonadotropin
HDL High-density lipoprotein
HOMA-IR Homeostasis model assessment of insulin resistance
HOMA-S Homeostasis model assessment of insulin sensitivity
HPO Hypothalamus–pituitary–ovary
hs-CRP High-sensitivity C-reactive protein
i.e. id est, that is
IGT Impaired glucose tolerance
IR Insulin resistance
LC-MS/MS Liquid chromatography-tandem mass spectrometry
LDL Low-density lipoprotein
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic syndrome</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease</td>
</tr>
<tr>
<td>NA-PCOS</td>
<td>Normoandrogenic (woman) with PCOS</td>
</tr>
<tr>
<td>NFBC-1986</td>
<td>Northern Finland Birth Cohort 1986</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>OA</td>
<td>Oligo-amenorrhea</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>OSA</td>
<td>Obstructive sleep apnea</td>
</tr>
<tr>
<td>P450c17</td>
<td>17-hydroxylase and 17,20 lyase activity</td>
</tr>
<tr>
<td>P450scc</td>
<td>Side-chain cleavage enzyme</td>
</tr>
<tr>
<td>PCOM</td>
<td>Polycystic ovary morphology</td>
</tr>
<tr>
<td>PCOS</td>
<td>Polycystic ovary syndrome</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristic</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SES</td>
<td>Socioeconomic status</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex hormone-binding globulin</td>
</tr>
<tr>
<td>StAR</td>
<td>Steroidogenic acute regulatory protein</td>
</tr>
<tr>
<td>T</td>
<td>Testosterone</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type II diabetes mellitus</td>
</tr>
<tr>
<td>TG</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>WHR</td>
<td>waist-to-hip ratio</td>
</tr>
</tbody>
</table>
List of original publications

This thesis is based on the following publications, which are referred to throughout the text by their Roman numerals:


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1 Introduction

Polycystic ovary syndrome (PCOS) is the most common gynecological endocrinopathy, affecting women of fertile age, and the most common cause of infertility in women. The prevalence of PCOS varies from 6% to 18% depending on the diagnostic criteria used (March et al. 2010). According to the Rotterdam consensus (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004) diagnosis of the syndrome requires at least two of the following features: polycystic ovaries detected by ultrasonography, oligo- or anovulation and/or either clinical (hirsutism) or biochemical (high serum testosterone or free androgen index [FAI]) hyperandrogenism.

PCOS usually has its onset early in life. Typically, the cardinal features of the syndrome are most severe during the reproductive years and the severity of hyperandrogenism and menstrual disorders decrease premenopaually (Welt & Carmina 2013). In addition, women with PCOS may present typical metabolic abnormalities such as insulin resistance (IR) and visceral obesity at a young age. Long-term exposure to these abnormalities throughout fertile life may exacerbate the adverse effects and expose these women to higher risks of metabolic syndrome (MetS), cardiovascular diseases (CVDs) and type II diabetes mellitus (T2DM) (Glintborg et al. 2012, Johnstone et al. 2012, Puurunen et al. 2011). However, the mechanisms leading to these increased metabolic risks, especially those regarding lifelong morbidity and mortality linked to the syndrome are still controversial, as the current evidence has come mostly from cross-sectional data sets with relatively small numbers of subjects and heterogeneous populations (Dumesic et al. 2015).

Anti-Müllerian hormone (AMH) is involved in sexual differentiation prenatally and later in ovarian follicle growth. It is produced by small ovarian follicles and its serum levels reflect the function of the ovary. It is well known that AMH levels are high in women with PCOS (Dewailly et al. 2010), but follow-up studies on the capacity of AMH to predict the development of PCOS are still lacking (La Marca et al. 2009, Piltonen et al. 2005).

Given the reproductive and metabolic risks linked to PCOS, it is important to identify these women early in order to prevent morbidity and even mortality linked to these risks throughout life. Improved knowledge concerning the prediction of PCOS would help clinicians to design tailored treatment to prevent late health disorders linked to the syndrome.

The main objective of the present study was to provide new information on the relationships between obesity, testosterone levels and symptoms of
hyperandrogenemia in cases of PCOS both in adolescence and in early adulthood. A second objective was to study the value of AMH in adolescence as a possible PCOS marker, and its association with reproductive and metabolic health later in early adulthood.

Lastly, we wanted to investigate the evolution of hormonal and metabolic parameters with age in both control women and women with PCOS and to find cut-off values of hormonal parameters in regard to the risk of PCOS at a population level.

Our hypothesis was that hyperandrogenemia and/or menstrual irregularities in adolescence have a major impact on women’s reproductive and general health both in adolescence and in later life. In addition, we hypothesized that hormonal and metabolic changes with age occur differently in women with PCOS compared with a control population.


2 Review of the literature

2.1 Androgen secretion in women

Approximately 25% of the testosterone in women is produced by the ovaries, another 25% by the adrenal glands and the remaining 50% through peripheral conversion of estrogens to androgens (Burger 2002, Longcope 1986). The major androgens in women, listed in decreasing order according to serum concentrations, are dehydroepiandrosterone sulfate (DHEAS), dehydroepiandrosterone (DHEA), androstenedione (A4), testosterone (T) and dihydrotestosterone (DHT). Of these, T and DHT are considered to induce most of the androgenic effects by binding to nuclear androgen receptors, while A4, DHEA and DHEAS are pro-androgens requiring conversion to T and DHT in order to have an androgenic effect (Burger 2002). Androgen secretion in women originates in response to their tropic hormones regulating it, i.e. luteinizing hormone (LH) acting on the ovary and adrenocorticotropic hormone (ACTH) on the adrenal gland (Figure 1). Virtually all of the circulating DHEAS in women is of adrenal origin (Longcope 1986, Stanczyk 2006). Both in the ovaries and in the adrenals, cholesterol is the precursor of pregnenolone, which is further converted to androgens through an enzymatic cascade described in Figure 2.

The rate-limiting step in androgen production is the movement of cholesterol inside the cell brought about by steroidogenic acute regulatory protein (StAR). The conversion of cholesterol to pregnenolone is regulated by tropic hormones and carried out by the side-chain cleavage enzyme (P450scc). As regards steroid hormone synthesis, the rate-limiting step is regulation of P450c17 gene (17-hydroxylase and 17,20 lyase activity) expression inducing DHEA and A4 production from pregnenolone and progesterone. Furthermore, the expression of P450c17 depends on the concentrations of tropic hormones in the ovaries and adrenal glands. Other important enzymes include 3β-hydroxysteroid dehydrogenase, inducing DHEA conversion to A4, and 17β-hydroxysteroid dehydrogenase catalyzing A4 conversion to T (Baptiste et al. 2010 and Figure 2).

The most potent endogenous circulating androgen is T and its biological activity depends on the amount of its free fraction in the blood. Most of the circulating T is bound to carrier proteins such as sex hormone-binding globulin (SHBG) and (partly) albumin, leaving only about 1–2% circulating unbound (Catteau-Jonard & Dewailly 2013). Therefore, changes occurring in SHBG levels
alter the severity of hyperandrogenism. As many factors (see 2.2.1) influence the levels of SHBG, measurement of total testosterone alone may not be reliable in estimating the degree of hyperandrogenism.

Fig. 1. The neuroendocrine axis regulating androgen biosynthesis in the ovaries and adrenal glands.
Fig. 2. Female steroidogenetic cascade in the ovaries and adrenal glands. In the adrenal gland steroidogenesis takes place in the inner parts of the adrenal cortex and in the ovary the production of androgens takes place in the theca cells and estrogen biosynthesis in the granulosa cells.
2.1.1 Ovarian androgen secretion

Gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus causes the pituitary to secrete LH and FSH, further controlling ovarian androgen and estrogen biosynthesis. Ovarian steroidogenesis works in a two-cell model such that LH stimulates the theca cells to produce androgens. Androgens then diffuse to the FSH-controlled granulosa cells where they are aromatized to estrogens that again have negative feedback on the hypothalamus, constituting a regulatory loop (Figure 1). Ovarian androgens are not part of a specific negative feedback system on hypothalamic GnRH and pituitary LH secretion, as ovarian androgens are by-products of estrogen biosynthesis. Therefore, the control of ovarian hyperandrogenism in connection with normal estrogen synthesis is weak.

Serum testosterone levels are at their lowest in the early follicular phase of the menstrual cycle. They rise to a peak at mid-cycle and decrease again during the luteal phase towards the early follicular phase (Abraham et al. 1974, Bui et al. 2013).

According to the results of previous studies, a decrease in androgen levels in women occurs during fertile life (Liang et al. 2011, Piltonen et al. 2003, Winters et al. 2000). Thereafter, even though estradiol levels clearly decrease during menopausal transition due to follicle depletion, little change is apparent in serum androgen levels (Davison et al. 2005). After menopause androgen production capacity in the ovaries diminishes progressively, but still persists for years (Couzinet et al. 2001, Hughes et al. 1991) and there is evidence that T concentrations in the ovarian vein are higher than those in the peripheral blood after menopause. Thus, the ovaries appear primarily to be androgen-secreting organs after the fertile period of life (Couzinet et al. 2001, Judd et al. 1974).

2.1.2 Adrenal androgen secretion

The three layers of the adrenal cortex each have specific enzymatic cascades resulting in the biosynthesis of steroid hormones. The zona glomerulosa has the capacity to produce mineralocorticoids, e.g. aldosterone. The inner layers, the zona fasciculata and the zona reticularis, synthesize androgens (DHEA and A4). The zona fasciculata also produces glucocorticoids such as cortisol, which is mainly responsible for controlling ACTH levels via negative feedback (Figure 1) (Baptiste et al. 2010). The adrenal medulla produces catecholamines (adrenaline and
noradrenaline), which are known as fight-or-flight hormones and are driven by the sympathetic nervous system.

The most important adrenal androgens are DHEA and its sulfate conjugate DHEAS. The ovaries produce approximately 20% of total circulating DHEA, but DHEAS originates from the adrenal glands and peripheral tissues (small intestine and liver) after conversion from DHEA (Goodarzi et al. 2014). DHEAS is commonly used as a marker of adrenal androgen production (Goodarzi et al. 2014, Lobo et al. 1981). The circulating (serum) concentration of DHEAS is about 1000-fold compared with the concentration of DHEA due to its high production rate and low metabolic clearance rate (Goodarzi et al. 2014). Depending on the phase of the menstrual cycle, one third to a half of the amount of A4 is produced in the ovaries.

Due to the aforementioned decline in ovarian androgen production during menopausal transition, the adrenal glands are the major source of circulating androgens after the menopause (Couzin et al. 2001). However, DHEAS levels also decrease linearly with age (Goodarzi et al. 2014).

2.1.3 Peripheral tissues

Clinically the most important peripheral tissues converting androgens are the liver, the lungs, adipose tissue and the skin (Ala-Fossi et al. 1998). These tissues present both 3β-HSD and 17β-HSD enzyme activity in order to convert DHEA and DHEAS into A4 and further to T (Payne & Hales 2004). Moreover, T is converted to DHT by 5α-reductase, present in these peripheral tissues. The aromatase activity of tissues such as the skin and adipose tissue enables the metabolism of androgens to estrogens (Nelson & Bulun 2001).

2.2 Hyperandrogenism in women

Hyperandrogenism or androgen excess is a common endocrine condition affecting approximately 5–10% of women of reproductive age (Azziz et al. 2004, Carmina et al. 2006). There are multiple causes of hyperandrogenism in women and the most common is PCOS, covering over 80% of women with androgen excess (Azziz et al. 2004). The next most important causes of androgen excess are presented in Table 1 (Azziz et al. 2004, Yildiz 2006). There are few well-characterized hyperandrogenic syndromes (Cushing’s syndrome, androgen-secreting tumors and non-classical adrenal enzymatic deficiencies) to be taken into account (Carmina et al. 2006). Another possible cause of hyperandrogenemia is androgen intake.

<table>
<thead>
<tr>
<th>Hyperandrogenic disorders</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCOS</td>
<td>72.1</td>
</tr>
<tr>
<td>Idiopathic hyperandrogenism</td>
<td>15.8</td>
</tr>
<tr>
<td>Idiopathic hirsutism</td>
<td>7.6</td>
</tr>
<tr>
<td>Non-classical congenital adrenal hyperplasia</td>
<td>4.3</td>
</tr>
<tr>
<td>Ovarian or adrenal androgen-secreting neoplasms</td>
<td>0.2</td>
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</table>

Clinically, hyperandrogenism in women presents as hirsutism, acne and androgenic alopecia. However, it is noteworthy that not all women with hirsutism have hyperandrogenism, and, vice versa, hyperandrogenic Asian patients with PCOS, for example, exhibit less hirsutism and acne (Carmina et al. 1992).

Biochemically, hyperandrogenism is defined as elevated levels of T and calculated indices of androgenicity, such as the free androgen index (FAI) and free testosterone (FT) exceeding the upper limit of the normal range (see 2.2.1).

2.2.1 Evaluation of hyperandrogenism in women

Clinical evaluation

Hirsutism is the most reliable symptom used in clinical practice for the evaluation of clinical hyperandrogenism, whereas acne and alopecia show poor correlation with it. Androgens determine the amount and distribution of hair-growth, clinically expressed as hirsutism, i.e. excess male-pattern terminal hair growth. Hirsutism is a clinical diagnosis and is generally evaluated by using the Ferriman–Gallwey (F&G) scale, which gives scores from 1 to 4 for the presence of hair growth in eleven areas of the body (Ferriman & Gallway 1961, Table 2). An F&G score of >7 defines hirsutism.
Table 2. Definition of hair growth grading according to Ferriman and Gallwey.

<table>
<thead>
<tr>
<th>Site</th>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Upper lip</td>
<td>1</td>
<td>A few hairs at outer margin.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>A small mustache at outer margin.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>A moustache extending halfway from outer margin.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>A moustache extending to mid-line</td>
</tr>
<tr>
<td>2. Chin</td>
<td>1</td>
<td>A few scattered hairs.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Scattered hairs with small concentrations.</td>
</tr>
<tr>
<td></td>
<td>3&amp;4</td>
<td>Complete cover, light and heavy.</td>
</tr>
<tr>
<td>3. Chest</td>
<td>1</td>
<td>Circumareolar hairs.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>With mid-line hair in addition.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Fusion of these areas, with three-quarter cover.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Complete cover</td>
</tr>
<tr>
<td>4. Upper back</td>
<td>1</td>
<td>A few scattered hairs.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Rather more, still scattered.</td>
</tr>
<tr>
<td></td>
<td>3&amp;4</td>
<td>Complete cover, light and heavy.</td>
</tr>
<tr>
<td>5. Lower back</td>
<td>1</td>
<td>A sacral tuft of hair.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>With some lateral extension.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Three-quarter cover.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Complete cover</td>
</tr>
<tr>
<td>6. Upper abdomen</td>
<td>1</td>
<td>A few mid-line hairs</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Rather more, still mid-line.</td>
</tr>
<tr>
<td></td>
<td>3&amp;4</td>
<td>Half and full cover.</td>
</tr>
<tr>
<td>7. Lower abdomen</td>
<td>1</td>
<td>A few mid-line hairs</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>A mid-line streak of hair.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>A mid-line band of hair.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>An inverted V-shaped growth</td>
</tr>
<tr>
<td>8. Arm</td>
<td>1</td>
<td>Sparse growth affecting not more than a quarter of the limb surface.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>More than this; cover still incomplete.</td>
</tr>
<tr>
<td></td>
<td>3&amp;4</td>
<td>Complete cover, light and heavy.</td>
</tr>
<tr>
<td>9. Forearm</td>
<td>1, 2, 3 &amp; 4</td>
<td>Complete cover of dorsal surface; 2 grades of light and 2 of heavy growth</td>
</tr>
<tr>
<td>10. Thigh</td>
<td>1, 2, 3 &amp; 4</td>
<td>As for arm</td>
</tr>
<tr>
<td>11. Leg</td>
<td>1, 2, 3 &amp; 4</td>
<td>As for arm</td>
</tr>
</tbody>
</table>

At present, other scoring systems to diagnose hirsutism are also used, such as modifications of the original F&G score, using five to nine of the body areas originally assessed by Ferriman and Gallwey (Yildiz 2006, Figure 3). Ethnic variations should be taken into account when assessing hirsutism, e.g. Asians have less dense hair than Caucasians (Ewing & Rouse 1978). The subjectivity of self-
reported hirsutism may cause unreliability in reporting clinical hyperandrogenism, even though self-reported hirsutism has been shown to identify most women with typical PCOS findings (Kazemi et al. 2015, Taponen et al. 2003, Taponen et al. 2004).

![Modified Ferriman & Gallwey scoring sheet used for clinical evaluation of hair growth. Scores of seven or more are defined as hirsutism. Modified from Hatch et al. 1981.](image)

Acne and alopecia are diagnosed according to a carefully recorded patient history and physical examination. Acne has a multifactorial etiology, but androgens play an important role in the development of this disorder. The androgenic profile should be examined in patients suffering from severe disease or additional symptoms of hyperandrogenism (Yildiz 2006). Alopecia, i.e. loss of scalp hair, is caused by increased androgen activity in the hair follicles. Most women suffering from it,
however, do not exhibit increased levels of circulating androgens and its significance in the diagnosis of PCOS is low (Rebora 2004, Schmidt & Shinkai 2015).

Virilization is a relatively uncommon finding, extensively including clinical features of hyperandrogenism such as hirsutism, acne, alopecia, clitoromegaly, deepening of the voice, loss of female body shape and usually amenorrhea. In women with PCOS mild virilization (mostly solely clitoromegaly) has been described as having an incidence of 21%, and other studies on the prevalence of this alteration are scarce (Goldzieher & Axelrod 1963). Women with virilization usually present markedly increased levels of androgens (Marshburn & Carr 1995) and androgen-secreting tumors should always be ruled out (Fraser & Kovacs 2004).

**Biochemical evaluation**

In order to complete the evaluation of hyperandrogenism the clinician needs to understand the methods used to measure androgens in the clinical laboratory. The methods in use vary as regards diagnostic accuracy, strengths and limitations. Especially when measuring the relatively low levels of androgens in women, the method must be relevant and highly reliable (Stanczyk 2006).

Direct immunoassay, the most commonly used measurement method for serum concentrations of T, is generally considered to be inaccurate, particularly when measuring the low levels found in women and children (Handelsman & Wartofsky 2013). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is available for use in T measurement, but initially it was too expensive for routine use. This has changed during the last decade and LC-MS/MS has become the gold-standard method for T measurement. Many journals in the field nowadays recommend this method in endocrine studies (Handelsman & Wartofsky 2013, Janse et al. 2011). The strengths of LC-MS/MS are enhanced analytical specificity, sensitivity and accuracy and it serves as a reference method for a number of analytes (Shea et al. 2014). However, a comparative study of two assays showed that in women with PCOS a select radioimmunoassay (RIA) method and LC-MS/MS had equal precision in measuring T and that correlation with the clinical parameters may be even stronger with RIA (Legro et al. 2010b).

DHEAS is present at high concentrations in female serum and direct immunoassay is considered to be reliable. Androstenedione and DHEA should preferably be measured by LC-MS/MS, unless the direct immunoassay methods used have been extensively validated (Stanczyk 2006).
Sex hormone-binding globulin

Sex hormone-binding globulin is a glycoprotein encoded by a single gene in chromosome 17 and it is produced in the liver by hepatocytes (Hammond & Bocchinfuso 1996). SHBG has a unique capacity to bind sex hormones with high affinity, which decreases their metabolic clearance and regulates their bioavailability (Joseph 1994). Serum levels of SHBG are regulated mainly by sex hormones, but thyroid hormones, cytokines and some dietary components have also been shown to be involved in the regulation of SHBG production (Simo et al. 2015, Thaler et al. 2015). Therefore, some disorders such as pituitary, thyroid or liver diseases, as well as breast cancer, may influence the production of SHBG. Changes in circulating levels of SHBG affect the amount of biologically active free androgens, thus affecting the clinical presentation linked to the severity of hypo- or hyperandrogenism. Hyperinsulinemia due to insulin resistance decreases the synthesis of SHBG and its levels in plasma, whereas weight loss or use of insulin sensitizers increase serum SHBG levels (Pasquali et al. 1995, Plymante et al. 1988). However, recent studies in vitro and in vivo could not confirm the mechanism of insulin-regulated SHBG production and further studies are required (Simo et al. 2015, Winters et al. 2014). Further, it has been shown that the strongest predictor of SHBG levels is the amount of liver fat and that there is an inverse correlation between the two parameters (Peter et al. 2010).

Free Androgen Index and Calculated Free Testosterone

To overcome the limitations of T measurement previously described, algorithms have been developed to estimate the amount of free T. The free androgen index (FAI) is calculated from T and SHBG concentrations (Table 3) and it is considered more informative than T alone as regards evaluation of androgenicity (Cho et al. 2008, Handelsman & Wartofsky 2013, Pinola et al. 2015). However, the FAI has been shown to be unreliable in men, but it seems to correlate well with the level of free testosterone in women (Miller et al. 2004, Rosner et al. 2007). The results of some studies suggest that calculated free testosterone (cFT, Table 3), which also takes into account albumin concentrations, could be more informative than the FAI (Rosner et al. 2007, Shea et al. 2014). Calculated FT has been shown to correlate well with free testosterone concentrations measured by equilibrium dialysis, which is considered to be the most sensitive and physiologically adequate form of analysis of T (Vermeulen et al. 1999). In the equation for cFT the albumin concentration is...
fixed within the normal physiological range (at 43 g/L) and it has shown good accuracy, except in pregnancy, when albumin concentrations are typically lower.

In the latest Rotterdam consensus the use of cFT or FAI instead of serum total T was recommended in order to detect hyperandrogenism in women with PCOS (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). The reference ranges for FAI and cFT have to be verified and validated in each laboratory in different populations as a result of the different methods used for T and SHBG analyses (Salameh et al. 2014, Shea et al. 2014).

### Table 3. Algorithms used for evaluation of androgen indices.

<table>
<thead>
<tr>
<th>Androgen indices</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Androgen Index (FAI)</td>
<td>FAI = 100 × (total T / SHBG)</td>
</tr>
<tr>
<td>Calculated Free Testosterone</td>
<td>cFT = [-b + √(b² + 4a[TT]) / 2a]</td>
</tr>
<tr>
<td></td>
<td>a = k_μ + k_t + (k_μ × k_t)[SHBG] + [albumin] – [T]</td>
</tr>
<tr>
<td></td>
<td>b = 1 × k[SHBG] × k[albumin] × (k_μ + k_t)[T]</td>
</tr>
</tbody>
</table>

_1Vermeulen et al. 1999_

#### 2.3 Anti-Müllerian hormone

Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance, is a dimeric glycoprotein and a member of the transforming growth factor-beta (TGF-β) superfamily (La Marca et al. 2009). The TGF-β family includes a large number of structurally related proteins with multiple roles in developmental patterning, tissue differentiation, and maintenance of homeostasis (Morikawa et al. 2016). In embryogenesis AMH plays a central role in sexual differentiation by inducing regression of the Müllerian ducts in male fetuses. In females, AMH is produced by the granulosa cells of the human ovary after midgestation in the fetus (Rajpert-De Meyts et al. 1999, Vigier et al. 1984). AMH is expressed in granulosa cells of growing follicles up to antral stage, suggesting an important role in early ovarian folliculogenesis (Stubbs et al. 2005, Weenen et al. 2004). AMH is able to inhibit the initiation of primordial follicle growth (Durlinger et al. 2002) and it may also decrease the sensitivity of antral follicles to follicle-stimulating hormone (FSH) (Durlinger et al. 1999, Durlinger et al. 2002, Gruijters et al. 2003).

In humans, the physiological relationship between circulating levels of androgens and AMH remains uncertain, and the exact function of AMH in follicular recruitment and its long-term effects are not well understood. Serum AMH levels
and the ovarian antral follicle count (AFC) correlate to each other both in healthy subjects and in women with PCOS (Pigny et al. 2003, Pigny et al. 2006, Weenen et al. 2004). In PCOS, serum concentrations of AMH correlate positively to those of serum testosterone and negatively to age (Piltonen et al. 2005). Furthermore, women with elevated serum levels of AMH and T have longer menstrual cycles compared with those with lower levels (Kristensen et al. 2012). AMH may also have an important role in regulation of the GnRH-dependent pulsatility of LH. AMH receptors have been discovered in GnRH neurons of mice and humans, and in murine studies AMH has been shown to activate GnRH neuron firing (Cimino et al. 2016). This is of importance, as it might clarify the extragonadal effects of AMH and add new information on the pathophysiology of PCOS in the future.

As AMH levels strongly correlate to both biochemical hyperandrogenism (HA) and antral follicle count, it has been suggested that it could be used as a surrogate tool in the diagnosis of PCOS instead of polycystic ovary morphology (PCOM, see 2.4.4) (Casadei et al. 2013, Chen et al. 2008, Eilertsen et al. 2012, Fanchin et al. 2003, Nardo et al. 2009). A serum AMH concentration over 35 pmol/L has been suggested to be more reliable than antral follicle count (Dewailly et al. 2011). However, up to now, AMH is not listed in the validated criteria for PCOS diagnosis (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). There are few studies on the possible value of the AMH serum level as a diagnostic tool for the diagnosis of PCOS in adolescence or as a possible predictor of PCOS in later life, and these studies have involved relatively small numbers of subjects and have produced controversial results (Pawelczak et al. 2012, Sopher et al. 2014).

AMH has also been shown to be associated with IR and to reflect disturbances of gonadotropin release in women with PCOS. However, results regarding the relationship between metabolic risk factors and AMH remain controversial (de Kat et al. 2015) and further studies are needed to evaluate the importance of AMH as a cardiovascular risk factor (Skalba et al. 2011). The process of ovarian aging (expressed in terms of AMH decline) and the emergence of cardiovascular risk factors seem to occur simultaneously during perimenopausal transition, and further studies are needed to clarify the connective mechanism (de Kat et al. 2015).
2.4 Polycystic ovary syndrome

2.4.1 Definition and prevalence

The first international diagnostic criteria (Table 4) for PCOS were developed in 1990 by a group of scientists and clinicians attending a conference sponsored by the National Institutes of Health (NIH). PCOS was defined as hyperandrogenism and/or hyperandrogenemia together with oligo-anovulation after exclusion of other endocrinopathies (Zawadski & Dunaif 1992). In 2003, PCOM was added as one of the criteria and it was required that at least two criteria should be met for PCOS diagnosis (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004).

Thereafter, in 2012 at an NIH workshop it was recommended that clinicians use the Rotterdam criteria (NIH guidelines of PCOS 2012). In addition, the Androgen Excess and PCOS (AE-PCOS) Society emphasized the importance of HA in PCOS, in keeping with the NIH criteria from 1990 (Azziz et al. 2006).

PCOS therefore represents a multifaceted syndrome with different phenotypes (Table 4). The prevalence of PCOS varies according to the criteria used, being twofold according to the Rotterdam criteria (17.8%) compared with the NIH criteria (8.7%) and with a prevalence of 12% according to the AE-PCOS society criteria (March et al. 2010). However, the prevalence also varies depending on ethnicity and the population studied. The generally accepted prevalence is between 6 and 18% (Carmina et al. 2006, Franks 1995, March et al. 2010).

Table 4. Different diagnostic criteria used for defining PCOS.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Hyperandrogenism</th>
<th>Oligo-anovulation</th>
<th>PCOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotterdam A*/AE-PCOS</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rotterdam B*/AE-PCOS</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Rotterdam C*/AE-PCOS</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Rotterdam D*</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NIH</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*Different phenotypes of PCOS according to the Rotterdam criteria.
2.4.2 Hyperandrogenism in PCOS

It is generally accepted that hyperandrogenism is a central feature in the syndrome. From 58 to 82% of hyperandrogenic women have PCOS, depending on the diagnostic criteria used (Azziz et al. 2004, Carmina et al. 2006).

In PCOS, the ovaries cover up to 60% of the total production of androgens. After suppressing the ovarian production with a gonadotropin-releasing hormone (GnRH) agonist, women with PCOS also exhibit adrenal overproduction of androgens, and vice versa (Cedars et al. 1992). Combined with low levels of SHBG linked to increased insulin resistance and accumulation of liver fat in PCOS, the hypersecretion of androgens leads to excess levels of biologically active forms of androgens.

Women with PCOS show an altered hypothalamic–pituitary axis, leading to increased LH secretion as regards pulse frequency and amplitude, and causing ovarian theca cells to overproduce androgens (Morales et al. 1996). Women with PCOS also present a higher number of androgen-producing theca cells, with increased expression of LH receptors (Gilling-Smith et al. 1994, Jakimiuk et al. 2001). In addition, androgenic hyper-responsiveness in PCOS seems to be linked to insulin action, which possibly mimics the tropic action of LH on theca cells (Wu et al. 2014). Further, improvement of IR in cases of PCOS has been shown to decrease the level of HA (Baillargeon et al. 2004).

Adrenal hyperandrogenism is a frequent issue in PCOS. Additionally, several extra-adrenal mechanisms have also been identified; of these, hyperresponsiveness to ACTH stimulation and long-term effects of high LH levels seem to play an important role (Cinar et al. 2012, Pabon et al. 1996, Tock et al. 2014). In women with normal ACTH levels, increased LH secretion, as observed in PCOS, may explain higher DHEAS concentrations, as LH/hCG receptors have been found in the adrenals, resulting in increased steroidogenesis of the adrenal cortex (Pabon et al. 1996). However, opposite results have also been published (Piltonen et al. 2002). In a recent review it was also suggested that hyperinsulinemia and increased peripheral metabolism of cortisol in women with PCOS exacerbate ACTH synthesis in order to maintain sufficiently high cortisol levels, with the by-product of increased adrenal androgen synthesis (Baskind and Balen 2016). However, further studies on the relationships between adrenal function, PCOS and obesity are needed.

Hyperandrogenism eases with time in women and this decreasing trend with age has been observed in both healthy women and PCOS patients (Bili et al. 2001,
However, despite the steady decrease of androgens with age, hyperandrogenism seems to persist or even worsen after menopause in women with PCOS (Burger et al. 2000, Burger et al. 2007, Puurunen et al. 2009, Puurunen et al. 2011, Schmidt et al. 2011, Winters et al. 2000). This phenomenon may be a result of higher gonadotropin levels after the menopause, a decrease in SHBG levels or a co-gonadotropic action of insulin. Overall, the mechanisms of hyperandrogenism after menopause appear to be multiple and the correlation to morbidities and mortality remains unclear (Markopoulos et al. 2015).

2.4.3 Menstrual irregularities in PCOS

The majority of the PCOS classifications include oligo-amenorrhea (OA) as a component (Table 4). This is defined as a menstrual cycle longer than 35 days more than twice a year, or chronic anovulation (Azziz et al. 2009, Broekmans & Fauser 2006). Among women with PCOS, OA is a very common feature and its prevalence varies from 75 to 90% depending on the age of the women and the criteria used to define menstrual irregularities (Adams et al. 1986, Burgers et al. 2010, Hull 1987).

Menstrual irregularities in adolescence are a frequent finding and are present in 20–30% of girls. Importantly, primary amenorrhea (genetic abnormalities, malformations) and secondary amenorrhea (thyroid dysfunction, hyperprolactinemia and eating disorders) need to be excluded. It is important to understand that maturation of the hypothalamic–pituitary–gonadal axis has been evaluated to take up to five years (Apter & Vihko 1983). However, previous studies have shown that two thirds of girls suffering from menstrual irregularities two years after menarche still present menstrual disorders 10 years later and tend to have PCOM and hyperandrogenism (elevated levels of A4 and T) in early adulthood (van Hooff et al. 2000, Wiksten-Almstromer et al. 2008). However, menstrual irregularities seem to ease with age in women with PCOS (see p. 45).

As for metabolic features, previous studies have not shown any association between OA and insulin resistance in adolescence, but girls of identical age and fulfilling PCOS diagnosis criteria exhibited an approximately 50% reduction in insulin sensitivity (Lewy et al. 2001, van Hooff et al. 2000). In adulthood, however, the menstrual pattern has been proposed as a surrogate marker of the metabolic profile in women with PCOS, as amenorrhea seems to be associated with more pronounced IR and hyperandrogenemia (Panidis et al. 2013). All in all, the
relationship between menstrual irregularities, insulin resistance, metabolic disorders, hyperandrogenemia and later risk of PCOS remains to be clarified.

### 2.4.4 PCO morphology at ultrasonography in PCOS

Polycystic ovary morphology was originally defined as the presence of ten or more follicles with a diameter of two to eight millimeters and/or increased ovarian volume (over 10 milliliters) in at least one ovary as observed in transabdominal ultrasonography (Adams et al. 1986). The Rotterdam consensus defined PCOM as the presence of 12 or more follicles with a diameter of two to nine millimeters and/or increased ovarian volume (over 10 milliliters) in at least one ovary (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). With improving imaging systems it has been debated whether this criterion should be changed. In 2014 a panel of experts in the field recommended that PCOM should be defined as 25 or more follicles in one ovary (Dewailly et al. 2014). However, so far, the Rotterdam criteria are still in use in clinical practice.

It has to be noted that PCOM is a common finding among young healthy women and that its prevalence has been estimated to be three- to fourfold when compared with the prevalence of PCOS (Johnstone et al. 2010, Polson et al. 1988). PCOM is also frequently found in adolescents shortly after menarche. It has been suggested that in young women with a gynecological age over two years, the most reliable indicator of PCOM may be ovarian enlargement (> 10 cm³, Carmina et al. 2010).

### 2.4.5 Other clinical features of PCOS

**Obesity**

Obesity is an increasing problem in the world. It is common in women with PCOS but is probably not a cause of the syndrome, as PCOS is also seen among lean women. Obesity seems to exacerbate many aspects of the syndrome (Diamanti-Kandarakis & Panidis 2007, Hoeger & Oberfield 2012, Legro 2012). It has been proposed that lean women with PCOS have the most severe defect in ovarian steroidogenesis and thus exogenous factors may not further provoke development of PCOS, whereas among women with mild defects the contribution of obesity,
abdominal obesity or insulin resistance will lead to development of the full-blown syndrome (Homburg et al. 2009, Escobar-Morreale & San Millan 2007).

The prevalence of obesity among women with PCOS varies greatly between different populations, from about 80% in the United States to approximately 50% in other countries (Ehrmann et al. 1999, Legro et al. 1999, Legro 2012). Even though obesity is not a diagnostic criterion as regards PCOS, it exacerbates many aspects of the syndrome, especially risk factors linked to cardiovascular diseases, e.g. alterations in glucose and insulin metabolism, and dyslipidemia (Moran et al. 2010). The physiopathology linking obesity and PCOS is complex and one possible mechanism may be interaction between insulin resistance and the co-gonadotropic effects of insulin and increasing circulating androgen levels (Harrison et al. 2011, Moran et al. 2003). However, lifestyle modifications and weight reduction are essential parts of the treatment of the syndrome, as they improve the menstrual cycle and reduce IR and HA, independently of the type of exercise (Harrison et al. 2011). Among morbidly obese women, however, the prevalence of PCOS is only 13%, suggesting that there are other factors in addition to obesity explaining the development of the syndrome (Gosman et al. 2010, Legro 2012).

Insulin resistance (IR)

When tissues normally sensitive to glucose-stimulating effects (e.g. muscle, liver and adipose tissue) became resistant to these signals the state is called insulin resistance (Smith 1994). Though IR and related compensatory hyperinsulinemia are common findings in patients with PCOS, these are not included in the diagnostic criteria. The relationship between HA, IR and obesity (Figure 4) is complex and despite extensive studies is still not totally clarified (Dumesic et al. 2015). Insulin resistance leads to impaired glucose tolerance (IGT), which is present in up to 30% of women with PCOS at reproductive age (Legro et al. 1999). Up to 10% of women with PCOS will develop T2DM before the age of 40 years, and obesity substantially increases (doubles) this risk (Legro et al. 1999, Moran et al. 2010).

Altered insulin metabolism is present both in lean and obese women with PCOS, but it is most marked when obesity, especially the abdominal type, is present (Diamanti-Kandarakis et al. 1995, Dunaif et al. 1992, Legro et al. 1999, Morin-Papunen et al. 2000). In the past 20 years there has been growing evidence supporting the hypothesis that altered insulin action and insulin signaling pathways
play an important role in the pathogenesis of PCOS (Baptiste et al. 2010). Furthermore, the use of insulin sensitizers, such as metformin, has been associated with improvement of menstrual cyclicity and hyperandrogenemia (Morin-Papunen et al. 1998). Additionally, according to some (Morin-Papunen et al. 2012), but not all studies (Legro R et al. 2007), metformin may be useful as an adjuvant treatment to achieve ovulation and live birth in obese women with PCOS and anovulatory infertility. However, the nature and mechanism of the disorders of insulin action in PCOS are still not fully clarified and further research is needed.

Chronic hyperinsulinemia seems to play an important role in the modulation of CVD risk factors and it also exacerbates insulin resistance and directly contributes to β-cell failure and development of diabetes (Randeva et al. 2012). Importantly, insulin resistance is linked to CVD risk factors and general health problems, such as metabolic syndrome (MetS) (Ginsberg 2000).

The gold-standard method for evaluating insulin action and secretion is the euglycemic hyperinsulinemic clamp (DeFronzo et al. 1979). Another highly specific and sensitive method is the intravenous glucose tolerance test (Bergman et al. 1989). However, these methods are time-consuming and expensive and therefore oral glucose-stimulated assessments (e.g. homeostasis model assessment for insulin resistance [HOMA-IR, calculated as: fasting glucose × fasting insulin / 22.5] and the Matsuda index [calculated as: 10000 / (fasting glucose × fasting insulin × mean OGTT glucose × mean OGTT insulin)0.5]) for evaluating insulin resistance are generally used clinically (Ader et al. 2014, Diamanti-Kandarakis et al. 2004, Hucking et al. 2008).
Fig. 4. Interactions between abdominal obesity, insulin resistance and hyperandrogenemia in the pathogenesis and progression of PCOS. Modified after Rojas et al. 2014.

### 2.4.6 Metabolic risks in PCOS

Various metabolic risks have been reported in patients with PCOS. The most common metabolic alteration is dyslipidemia (Hoffman & Ehrmann 2008). Levels of (cardioprotective) high-density lipoprotein (HDL) are decreased whereas those of low-density lipoprotein (LDL) and triglycerides (TGs) are elevated in women with PCOS (Hoffman & Ehrmann 2008). Both lean and obese women with PCOS exhibit an unbeneficial lipid profile compared with healthy, age- and BMI-matched control women (Glueck et al. 2009, Yildirim et al. 2003). However, the ethnic background (lifestyle and nutrition) as well as genetic differences may have an effect on the characteristics of dyslipidemia, as, for example, it seems that hypertriglyceridemia is more prevalent in Caucasian populations (Essah et al. 2008, Hillman et al. 2014, Wild et al. 2010). Together with other metabolic disturbances, dyslipidemia seems to be already present in adolescent subjects with PCOS (Kent & Legro 2002). Moreover, HA seems to be a key factor associated with the most adverse lipid profile in women with PCOS at a young age (Fruzzetti et al. 2009).

Circulating levels of the most commonly used marker of chronic inflammation, C-reactive protein (CRP), are elevated in women with PCOS (Escobar-Morreale et al. 2011). However, a previous meta-analysis did not reveal any differences between women with PCOS and healthy women as regards other inflammatory
markers, such as interleukin-6 and tumor necrosis factor-alpha (Escobar-Morreale et al. 2011). According to a recent review, studies on other adipokines (adiponectin, resistin and visfatin) in PCOS are also inconclusive and more studies are needed (Spitzer et al. 2015). Inflammation markers have been shown to be related to an increased risk of cardiovascular events independently of other risk factors (Danesh et al. 2004). Whether or not alterations in the levels of inflammation markers play a causal role in the syndrome or are a consequence of other metabolic abnormalities needs to be further clarified.

Women with PCOS have a higher prevalence and elevated risk of hypertension, early-onset atherosclerosis and cardiac dysfunction (Elting et al. 2001, Prelevic et al. 1995). The risk of developing arterial hypertension has been shown to be increased 2.5-fold at menopausal age compared with a healthy age-matched population, but it seems to be at least partly related to the obesity associated with the syndrome (Elting et al. 2001). In one study, young subjects with PCOS (aged 22 yr) already exhibited an increase in carotid intima-media thickness, a surrogate marker of atherosclerosis, suggesting early development of atherosclerosis in PCOS (Orio et al. 2004).

Despite the various CVD risks linked to PCOS, definitive data on CVD-related mortality in women with PCOS is lacking. In a follow-up study lasting 31 years, no significantly increased risk of CVD-related causes of death were found, even though nonfatal cerebrovascular diseases were more prevalent in women with PCOS (Wild et al. 2000). However, in a large prospective cohort, women reporting a history of menstrual irregularities had increased risks of both nonfatal and fatal CVDs (Solomon et al. 2002). Long-term prospective menopausal data have shown a higher incidence of hypertension and elevated triglyceride levels but no increased rates of cardiovascular events (Schmidt et al. 2011). Further, PCOS has been shown to be related to increased thrombin generation, which is considered to be a CVD risk marker, and the use of oral contraceptives in the treatment of the syndrome has even worsened this alteration (Glintborg et al. 2015a, Glintborg et al. 2015b). The 2012 Amsterdam Consensus Workshop Group opinion concerning women’s health in PCOS was that longitudinal studies are needed to clarify the associations between CVD risk markers and vascular events (Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group 2012).

The Rotterdam Consensus Group (Rotterdam 2004) has defined criteria for the diagnosis of MetS in PCOS populations (Table 5). The prevalence of MetS in PCOS patients varies from 33–47% in the U.S. to 8–25% in other countries, which represents a two- to threefold increase compared with age-matched controls (Wild
et al. 2010). Previous observations link hyperandrogenism to metabolic disturbances and a higher prevalence of MetS (Sung et al. 2014) and according to the Amsterdam consensus on the metabolic risks linked to PCOS, there is extensive evidence showing that MetS is most prevalent in the hyperandrogenic and anovulatory phenotype of PCOS. Thus, the consensus group recommends that patients in this clinical subset should be treated carefully, keeping in mind the possible long-term health risks (Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group 2012). On the other hand, the evidence in a large review supported the postulate that obesity, especially abdominal, is associated with a more adverse metabolic status (Moran & Teede 2009). Further, it is noteworthy that as early as in adolescence, women with PCOS and normal BMI exhibit a higher prevalence of MetS compared with control women, highlighting the importance of identifying young women at risk (Aydin et al. 2015). The Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society recommends lifestyle management for primary CVD prevention, targeting treatment of obesity, insulin resistance, elevated blood pressure and dyslipidemia (Wild et al. 2010).

Table 5. Criteria for metabolic syndrome in women with PCOS. Three out of five qualify for a diagnosis of metabolic syndrome. (Modified from the Rotterdam consensus statement for diagnosing PCOS [Rotterdam 2004]).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference</td>
<td>&gt; 88 centimeters</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>≥ 1.69 mmol/L</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>&lt; 1.29 mmol/L</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>≥ 130 / ≥ 85 mmHg</td>
</tr>
<tr>
<td>Fasting and 2-hour glucose from oral glucose</td>
<td>6.11–6.99 mmol/L and/or</td>
</tr>
<tr>
<td>tolerance test</td>
<td>2-h glucose 7.77–11.05 mmol/L</td>
</tr>
</tbody>
</table>

Overall, the roles and interactions of obesity, especially abdominal obesity, hyperandrogenism and the syndrome per se on the development of cardiovascular risks, events and mortality are still under debate.

2.4.7 Non-alcoholic fatty liver disease (NAFLD) and obstructive sleep apnea (OSA) in PCOS

Women with PCOS exhibit a high prevalence of non-alcoholic fatty liver disease (NAFLD) (Cerda et al. 2007). In NAFLD, insulin resistance has a key role and is
an independent contributor to progression of this condition (Mendez-Sanchez et al. 2007). NAFLD may develop at a young age, and liver function should be screened at an early age in women with PCOS, particularly in obese subjects and in women suffering from MetS (Randeva et al. 2012).

The prevalence of obstructive sleep apnea (OSA) has also been observed to be higher in women with PCOS compared with women without it (Vgontzas et al. 2001). Many physiopathological mechanisms have been proposed to explain the elevated risk of OSA in PCOS but no single validated mechanism has been identified (Randeva et al. 2012). Clinicians managing patients with PCOS should be aware of the high prevalence of OSA among these women and systematically screen them for the condition.

2.5 PCOS from adolescence to postmenopause

Polycystic ovary syndrome is a lifelong syndrome. Its characteristics are well known in the fertile, menopausal and even postmenopausal periods, but the developmental origins and the characteristics of the syndrome in adolescence are still to be clarified. The predominant features of the syndrome during life are presented in Figure 5.

![Fig. 5. Phenotypic features of women with PCOS during their lives. Modified from Welt and Carmina 2013.](image-url)
2.5.1 Prepubertal origins of PCOS

Increasing evidence shows that the intrauterine environment has an effect on genetic programming later in life. The first evidence of this was introduced by Barker et al. in 1989 and it is now known as the Barker hypothesis (Barker et al. 1989, Hanson & Gluckman 2008). Exposing a female fetus to high T levels prenatally causes dose-related masculine-type behavioral traits in young girls (Hines et al. 2002). Prenatally androgenized female Rhesus monkeys have been shown to express phenotypic characteristics similar to those typical of PCOS (Abbott et al. 1998, Abbott et al. 2002). In early gestation, androgenized female fetuses of Rhesus monkeys show hypersecretion of LH, indicating early programming of hypothalamic action. Later, they develop symptoms of PCOS (hyperandrogenism, menstrual irregularities, PCOM) as well as metabolic features of the syndrome in early life (Abbott et al. 2005). Many different hypotheses have been proposed to explain the epigenetic phenomenon of an early developmental origin of PCOS, but no validated data has yet been presented. In summary, studies show that exposure to excess androgens in utero encourage excess androgen production later in life. Some retrospective studies have also shown that girls born small for gestational age are at a higher risk of later development of early pubarche, early menarche and PCOS (Ibanez et al. 2007), but controversial results have also been published (Lahti et al. 2003, Legro et al. 2010a).

Early pubarche and adrenarche have been linked to later development of PCOS (Ibanez et al. 2000). Such girls may be at risk of PCOS-like symptoms and MetS, especially if they are obese (Ibanez et al. 1998, Zimmet et al. 2007). Prepubertal daughters of women with PCOS exhibit elevated AMH levels, which are considered to be a surrogate marker of the amount of antral follicles in the ovaries and, further, a correlative marker of hyperandrogenism (Dewailly et al. 2010, Sir-Petermann et al. 2006, Visser & Themmen 2005).

2.5.2 PCOS in adolescence

During adolescence and puberty the symptoms of PCOS overlap with normal physiological changes (Carmina et al. 2010). Menstrual irregularities are frequent in adolescents and the hypothalamus–pituitary–ovary (HPO) axis may need 2–5 years after menarche to reach full maturation (Apter 1980, Welt & Carmina 2013). In cases of PCOS ovarian volume and follicle number overlap with the volume and number observed in healthy subjects in adolescence and early adulthood.
The diagnosis of hyperandrogenism is difficult in adolescence, as acne is a common problem in young adults and hirsutism may take several years before development to its full expression. For this reason, the most useful diagnostic tool is to measure (elevated) androgen levels to define hyperandrogenism in adolescence (Blank et al. 2008). Reaching a diagnosis of PCOS in adolescence requires all three cardinal symptoms to be present. However, if ultrasonography is not available the diagnosis can be made in the presence of oligo-anovulation and biochemical hyperandrogenemia (Legro et al. 2013).

Age at menarche in girls with PCOS ranges from nine years upwards (Dramusic et al. 1997, Nduwayo et al. 1992, Rachmiel et al. 2008, Welt & Carmina 2013). Earlier menarche is strongly related to obesity and later menarche to higher androgen levels (Dramusic et al. 1997, Rosenfield et al. 2009). In a retrospective analysis, women with PCOS were more likely to report earlier or later menarche than age-matched controls (Carroll et al. 2012).

2.5.3 PCOS at reproductive age

Polycystic ovary syndrome generally remains stable in the early reproductive years between the ages of 18 to 35 (Davison et al. 2005). During this period women with PCOS may exhibit all the cardinal symptoms of the syndrome. Studies have shown that the hyperandrogenic phenotypes in early adult life exhibit the most severe disease, with not only anovulatory infertility and a higher risk of pregnancy complications such as gestational diabetes mellitus, hypertensive disorders of pregnancy and risk of pre-term birth, but also a worse metabolic profile (Barber et al. 2007, Boomsma et al. 2006, Franks 1995, Han et al. 2011). However, some data have shown that in overweight women with PCOS, serum concentrations of T are not independently associated with a higher prevalence of MetS and that the interaction between obesity, HA and PCOS is complex and still needs clarification (Moran et al. 2013).

During the reproductive years serum androgen levels decrease steadily in women with PCOS, with a decline in both ovarian and adrenal androgen production capacity (Davison et al. 2005). At later reproductive ages women with PCOS present lower F&G scores and lower T, A4 and DHEAS levels compared with younger women, but these parameters seem to remain higher than in healthy age-matched subjects (Bili et al. 2001, Carmina et al. 2012, Puurunen et al. 2009, Winters et al. 2000).
Ovulatory function appears to improve with age in women with PCOS. Studies have shown an increase of up to 30% in menstrual frequency with increasing age and it has been suggested that a relative increase in FSH with age might induce follicle development in later reproductive life in these women (Alsamarai et al. 2009, Carmina et al. 2012, Elting et al. 2000). The recovery of regular menstruation with aging in cases of PCOS could be predicted by measurement of serum AMH in a prospective dataset (5-year follow-up), showing that a serum AMH level lower than 0.56 pmol/L was correlated with recovery of ovulatory function in premenopausal women (Carmina et al. 2012).

Follicle count and ovarian volume exhibit a steady decline with age in all women, thus decreasing the prevalence of PCOM in women with PCOS (Alsamarai et al. 2009). Importantly, however, the number of follicles remains higher in women with PCOS compared with healthy controls at all ages (Aiyappan et al. 2016).

2.5.4 PCOS in premenopause and menopause

There are currently no diagnostic criteria for PCOS at menopausal age, but clinicians define it by a history of prior oligo-amenorrhea and hyperandrogenism (Azziz et al. 2006). Previous studies have shown that enhanced androgen secretion remains in perimenopausal and postmenopausal women with PCOS (Markopoulos et al. 2011, Puurunen et al. 2009, Puurunen et al. 2011). The enhanced secretion decreases with age and stabilizes, meeting the levels in healthy women at around the age of 70 years (Schmidt et al. 2011).

Abdominal obesity, IR, chronic inflammation and dyslipidemia have been shown to worsen throughout the menopausal transition in healthy women (Matthews et al. 2009). Longitudinal data have shown that a history of androgen excess and menstrual irregularity might not be associated with the increased incidence of MetS during menopausal transition in healthy women (Polotsky et al. 2014). There is strong evidence that women with PCOS have more components of MetS starting at an earlier age, therefore exposing these women for a longer period of life to adverse CVD risk factors (Polotsky et al. 2014, Welt & Carmina 2013). It is possible that the CVD risk factors become milder with age in women with PCOS. Further studies are needed to clarify whether the subgroup maintaining high androgen levels after menopause remains at a higher risk of cardiovascular morbidity and mortality (Welt & Carmina 2013).
3 Purpose of the present study

Polycystic ovary syndrome represents a major health issue among fertile-aged women, with adverse effects on women’s health integrity, affecting not only reproductive function, but also the emergence of the most frequent causes of morbidity and mortality in postmenopausal years, T2DM and cardiovascular diseases (Daan et al. 2014, Franks 1995, Johnstone et al. 2012, Morin-Papunen et al. 2000). Obesity and hyperandrogenism are part of the syndrome and confounding factors regarding the health impact of PCOS in the long run. The current epidemic of obesity suggests that the prevalence of PCOS and its adverse health effects may rise further in the future.

In adolescence, irregular menstrual cycles, acne and hirsutism are the principal symptoms of PCOS. Data on causality between clinical manifestations of hyperandrogenism and later metabolic risks is emerging. Studies on the metabolic significance of biochemical hyperandrogenism have shown controversial results, with only few studies involving the most accurate methods for testosterone determination.

Age has a significant effect on the syndrome as regards both hormonal and metabolic profiles. Ethnicity also has an impact on the clinical presentation of the syndrome. Up to now, there have been no studies describing the endocrine or metabolic profiles of women with PCOS in large Nordic populations at different ages.

Anti-Müllerian hormone is produced in the growing follicles of the human ovary and it is under intensive study, as women with PCOS have higher concentrations of serum AMH compared with healthy women. There are only a few studies on the predictive and diagnostic value of serum levels of AMH as regards PCOS and data on its relationship with metabolic risks in PCOS is still controversial.

There is a need to clarify the roles and interactions of obesity, weight gain, hyperandrogenism and the syndrome per se on the development of long-term health disorders, such as MetS, and on the risks of T2DM and CVD in PCOS. There is also a need to define the health impact of PCOS in Nordic populations, as there are important differences between ethnicities as regards clinical presentation and metabolic risks linked to the syndrome. Given the aforementioned metabolic risk factors associated with the syndrome, predicting PCOS early in life using simple, inexpensive and reliable tools is essential to decrease and prevent late morbidity.
and mortality linked to it. Lastly, the effect of menopause on the hormonal and metabolic hallmarks of the syndrome is still under debate.

The aims of the study were:

1. To evaluate the correlations between serum concentrations of testosterone and the free androgen index versus menstrual irregularities and metabolic parameters in PCOS in adolescence (at the age of 16 years).
2. To compare serum AMH concentrations in girls with menstrual irregularities and in controls at the age of 16 years and to evaluate the predictive value of AMH as regards menstrual irregularities and symptoms/diagnosis of PCOS over a follow-up period of 10 years in a population-based study.
3. To evaluate the association between serum levels of AMH and metabolic parameters in adolescence.
4. To investigate serum androgen levels at different ages both in women with PCOS and control women.
5. To determine cut-off values for serum levels of androgens to distinguish women with PCOS and healthy women.
6. At different ages, to investigate metabolic parameters both in women with PCOS and control women, to compare these parameters between the two populations and to clarify the respective roles of hyperandrogenism and obesity, especially abdominal obesity, as metabolic risk factors in PCOS.


4 Subjects and methods

4.1 Study populations and respective methods

A summary of the subjects, main study parameters and main results of the original studies is shown in Table 7.

4.1.1 Northern Finland Birth Cohort 1986 (Studies I and II)

The prospective Northern Finland Birth Cohort 1986 (NFBC-1986), which has been followed up since the fetal period, consists of 9362 mothers and their 9479 births (9432 children born alive), who had an expected date of birth between July 1, 1985 and June 30, 1986, from the two northernmost provinces of Finland. In total, 4567 girls were born. The earliest data consists of pregnancy and antenatal records and clinical data of the newborns and this has been supplemented by way of postal questionnaires and clinical examination at the ages of seven and eight years.

Questionnaire and investigations at the age of 16 years

In 2001–2002, when the children were 15–16 years old (mean age 15.5 years, standard deviation [SD] 0.37 years), the adolescents and their parents each received a large postal questionnaire (80 and 76% response, respectively). The questionnaire included questions about puberty, smoking and use of alcohol, and one question about the regularity and length of the menstrual cycle: “Is your menstrual cycle (the interval from the beginning of one menstrual period to the beginning of the next period) often (more than twice a year) longer than 35 days?” The girls who answered “yes” to this question were considered to be suffering from menstrual irregularities and were classified as “symptomatic”. The girls who answered “no” were defined as “non-symptomatic”. In addition, 3373 girls (74%) underwent clinical examination (including weight, height and waist-hip measurements) and gave fasting blood samples.

After excluding twins and triplets, pregnant girls ($n = 20$), oral contraceptive users and users of other forms of hormonal contraception and treatment ($n = 377$), and subjects with incomplete data ($n = 824$), 2448 singleton females remained eligible at the age of 16 years (Figure 6). This population was used in Study I.
Questionnaire at the age of 26 years

At the age of 26 years, the young women ($n = 4567$) were sent another questionnaire with questions on socio-demographic and other (health) background factors mainly concerning reproduction, menstruation (including the same question on menstrual disorders as at the age of 16 years) and infertility. After combining data from the 16- and 26-year follow-up questionnaires, the final study sample included 2033 singleton females (West et al. 2014).
The incidence of hirsutism was self-assessed by using a modified F&G score sheet and a diagnosis of PCOS was enquired about via the questionnaire (answer to the question: “Have you been diagnosed with PCOS by a physician?”). The response rate to the 26-year-old’s questionnaire was 50.4%. The women with both hirsutism (F&G score >7) and oligo- or amenorrhea according to the questionnaire at the age of 26 years were considered as having PCOS according to both the NIH (Zawadski & Dunai 1992) and Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004).

For measurement of serum AMH in Study II, we selected 400 subjects who had responded to both questionnaires at the ages of 16 and 26 years. The study population was split into testosterone quartiles and the study subjects were randomly selected from each quartile (100 subjects from each testosterone quartile: 50 subjects with reported menstrual irregularities and 50 with normal menstrual cycles at the age of 16 years) using a validated statistical method (SPSS software).

4.1.2 Nordic multicenter data (Studies III and IV)

In Study III, the participants in five Nordic PCOS studies were included (two studies from Finland, two from Sweden and one from Norway), with a total of 681 women with PCOS (age range 18–59 years) and 230 healthy control women (age range 19–62 years) (Hudecova et al. 2011, Morin-Papunen et al. 2000, Piltonen et al. 2004, Piltonen et al. 2012, Puurunen et al. 2009, Stener-Victorin et al. 2010, Vanky et al. 2004).

In Study IV, three more study sites were included (one from each: Sweden, Norway and Denmark), resulting in a total number of 1550 PCOS subjects and 447 control women (Glintborg et al. 2012, Nybacka et al. 2011).

The diagnosis of PCOS was assessed according to the Rotterdam criteria. Diagnosis of PCOS in peri- and postmenopausal women was based on oligo-amenorrhea combined with hyperandrogenism (either biochemical or clinical) reported at reproductive age, thus retrospectively meeting the Rotterdam criteria. The control population consisted of women with normal ovaries in ultrasonography and absence of PCOS-related symptoms, e.g. oligo- or anovulation and/or hirsutism.
4.1.3 Methods

Anthropometric measurements

In all studies, body mass index (BMI) was calculated by dividing the body weight in kg by the squared height in meters. Waist and hip circumferences (measured to the nearest centimeter with a soft tape at the midway level between the lowest rib margin and the iliac crest and at the widest part of the gluteal region) were assessed and the waist-to-hip ratio (WHR) was calculated. In Study IV, only the waist circumference was used.

Laboratory methods

Androgens. In Studies I–III, serum samples for assay of testosterone were analyzed by using Agilent triple quadrupole 6410 LC/MS equipment with an electrospray ionization source operating in positive-ion mode (Agilent Technologies, Wilmington, DE, USA). Multiple reaction monitoring was used to quantify testosterone by using trideuterated testosterone (d3-testosterone), with the following transitions: m/z 289.2 to 97 and 289.2 to 109 for testosterone and 292.2 to 97 and 292.2 to 109 for d3-testosterone. The intra-assay CVs of the method were 5.3%, 1.6% and 1.2% for testosterone at 0.6, 6.6 and 27.7 nmol/L, respectively. The interassay CVs were 5.3%, 4.2% and 1.0% for the respective concentrations. The three supplement populations in Study IV (Glintborg et al. 2012, Nybacka et al. 2011) had T measured with accredited methods in their respective laboratories prior to inclusion in the study.

Androstenedione and DHEAS in Study III were assayed in the laboratories of the different study centers according to their routine methods (RIA, immunoassay and mass spectrometry, Table 7). The reference ranges were very similar in all laboratories, except for a lower upper limit in mass spectrometry-analyzed samples for A4 (Table 6).

Anti-Müllerian hormone. Serum AMH concentrations were assayed in the stored samples by AMH Gen II enzyme-linked immunosorbent assay (ELISA, Beckman Coulter Inc. 250 S. Kraemer Blvd. Brea, CA 92821 U.S.A.) as previously described (Kumar et al. 2010, Wallace et al. 2011). Intra- and interassay coefficients of variation were 4.6 and 8.0% at a concentration of 0.05 ng/mL. An AMH level of 1 ng/mL equals 7.14 pmol/L.
Glucose, insulin, lipids and oral glucose tolerance test. In Studies I and II plasma glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol) and triglycerides were determined by using an automatic chemical analyzer (Cobas Integra 700, Roche Diagnostics, Switzerland). Serum insulin was analyzed by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden), high sensitivity C-reactive protein (hs-CRP) by an immunoenzymometric assay (Medix Biochemica, Espoo, Finland) and SHBG by time-resolved fluoroenzymoassay (AutoDelfia, PerkinElmer, Turku, Finland).


Oral glucose tolerance tests (OGTTs) were carried out via the routine protocol recommended for women with PCOS, with a fasting component and a 75-gram glucose intake (Bozdag & Yildiz 2013). Mean OGTT glucose and serum insulin levels were calculated as the means of concentrations at different time points [(basal + 2-hour) / 2].

FAI and cFT. The methods used to obtain the free androgen index and calculated free testosterone are described above (Table 3).

To quantify the degree of insulin sensitivity and insulin resistance, homeostasis model assessment (HOMA-S and HOMA-IR) values were calculated using the validated calculator available at http://www.dtu.ox.ac.uk.
Table 6. Analysis methods for androstenedione and dehydroepiandrosterone sulfate in Study III.

<table>
<thead>
<tr>
<th>Population</th>
<th>Androstenedione</th>
<th>Dehydroepiandrosterone sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method</td>
<td>Reference ranges</td>
</tr>
<tr>
<td>Population 1 (n = 321)</td>
<td>RIA²</td>
<td>1.4–14.3 nmol/L</td>
</tr>
<tr>
<td>Population 2 (n = 228)</td>
<td>Chemiluminometric Immunoassay / RIA¹</td>
<td>1.4–14.3 nmol/L</td>
</tr>
<tr>
<td>Population 3 (n = 128)</td>
<td>GC-MS³</td>
<td>1.6–5.7 nmol/L</td>
</tr>
<tr>
<td>Population 4 (n = 90)</td>
<td>RIA²</td>
<td>0.7–11.0 nmol/L</td>
</tr>
<tr>
<td>Population 5 (n = 145)</td>
<td>Not analyzed</td>
<td>-</td>
</tr>
</tbody>
</table>


4.2 Data analysis and statistical methods

The Chi squared test was used to compare categorical variables between the study groups. Comparisons of continuous variables were conducted by using Student’s t-test or the (nonparametric) Mann–Whitney test, depending on the distribution of the variable.

Trends in biochemical variables were assessed by means of analysis of variance (ANOVA) or the Kruskal–Wallis test when having more than two study groups. In order to be able to adjust for confounding variables, ANOVA and analysis of covariance (ANCOVA) were adopted when comparing, for example, AMH levels between two groups.

If the distribution of a laboratory measurement was skewed, it was logarithmically transformed to achieve normality. If the parameter was further skewed, the (nonparametric) Kruskal–Wallis test was used.

Correlations were tested using Pearson's and Spearman's correlation analyses. Partial correlation analysis was used to control for confounding variables. Linear regression analysis was used to calculate percentage differences in biochemical characteristics between the study groups and for adjustments. The results were adjusted, if needed, for smoking, socioeconomic status (SES), alcohol consumption, BMI, WHR and age at menarche.
**Specific methods in different studies**

*Study I.* The whole study population was stratified into FAI and BMI quartiles and analysis of variance was used to assess the trends in biochemical variables across these quartiles. Confidence intervals were calculated using a CIA computer program (Gardner & Altman 1988).

Statistical analyses were performed by using SPSS 17.0 software (IBM Corp., Armonk, NY. IBM SPSS Statistics for Windows, Version 17.0. Released 2010.).

*Study II.* The study population of 400 girls was constituted from four quartiles of 100 women according to their testosterone levels (described previously, see 4.1). Confidence intervals for proportions were calculated using a CIA computer program (Gardner & Altman 1988). Bias-corrected and accelerated 95% confidence intervals of the correlation coefficients were calculated with 1000 bootstrap re-samples. We constructed receiver operating characteristic (ROC) curves with AMH and testosterone concentrations at the age of 16 years, and for women with PCOS at the age of 26 years. The cut-off values of AMH and testosterone levels were selected to identify PCOS subjects with the best sensitivity and specificity.

Statistical analyses were performed by using SPSS 18.0 software (IBM Corp., Armonk, NY. IBM SPSS Statistics for Windows, Version 18.0. Released 2010.).

*Study III.* The PCOS and control groups were divided into seven age groups: 18–24, 25–29, 30–34, 35–39, 40–44, 45–49 and over 50 years. Risk ratios were estimated by using logistic regression analysis in which the dichotomy of the variables was created with a specific cut-off concentration (with the best combination of sensitivity and specificity) analyzed from the ROC curve for each variable, if needed.

Statistical analyses were performed by using SPSS 19.0 software (IBM Corp., Armonk, NY. IBM SPSS Statistics for Windows, Version 19.0. Released 2010.).

*Study IV.* The PCOS population was divided into hyperandrogenic PCOS (with biochemical and/or clinical hyperandrogenism, HA-PCOS) and normoandrogenic PCOS (women with both oligo-amenorrhea and polycystic ovaries in ultrasonography, but no hyperandrogenism, NA-PCOS) groups. The control population consisted of women with normal ovaries in ultrasonography and absence of PCOS-related symptoms, e.g. oligo- or anovulation and/or hirsutism and/or elevated T levels. The PCOS and control populations were grouped according to age as follows: < 30 years old, 30–39 years old and > 39 years old.
Statistical analyses were performed by using SPSS 21.0 and 22.0 software (IBM Corp., Armonk, NY. IBM SPSS Statistics for Windows, Version 21.0/22.0. Released 2010.).
<table>
<thead>
<tr>
<th>Study details</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>Symptomatic girls, N=709 Non-symptomatic girls, N=739</td>
<td>Menstrual irregularity, N=200</td>
<td>Controls, N=230</td>
<td>Controls, N=447</td>
</tr>
<tr>
<td>Age</td>
<td>~16 years</td>
<td>~16 years and 26 years</td>
<td>Controls: 19-62 years</td>
<td>Controls: 18-62 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PCOS: 18-59 years</td>
<td>PCOS: 14-59 years</td>
</tr>
<tr>
<td>Main study parameters</td>
<td>Menstrual cycle</td>
<td>Hormone assays, AMH BMI and metabolic parameters at age 16</td>
<td>Hormone assays</td>
<td>Hormone assays</td>
</tr>
<tr>
<td></td>
<td>Hormone assays</td>
<td>Hirsutism and PCOS at age 26</td>
<td>Estimated parameters for free androgens Age and BMI</td>
<td>BMI and metabolic parameters</td>
</tr>
<tr>
<td></td>
<td>BMI and metabolic parameters</td>
<td></td>
<td></td>
<td>Metabolic syndrome and its components</td>
</tr>
<tr>
<td>Main results</td>
<td>Menstrual irregularity is a good marker for hyperandrogenaemia</td>
<td>AMH levels at age 16 correlate with hyperandrogenaemia and</td>
<td>Women with PCOS exhibit</td>
<td>Women with PCOS have</td>
</tr>
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<td></td>
<td>and associates with subclinical</td>
<td>menstrual irregularities, but not with metabolic risk factors. AMH</td>
<td>elevated both ovarian and adrenal androgens through life</td>
<td>unfavorable metabolic profile</td>
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<tr>
<td></td>
<td>metabolic alterations in</td>
<td>at age 16 correlates with</td>
<td>compared with controls. The best predictors for PCOS are</td>
<td>from early adulthood until</td>
</tr>
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<td></td>
<td>adolescence.</td>
<td>hirsutism and PCOS at age 26</td>
<td>cFT, A4 and FAI,</td>
<td>menopause compared with</td>
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<td>controls. Abdominal obesity</td>
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<td></td>
<td></td>
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<td></td>
<td>seems to play a more adverse role than HA as regards</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>metabolic alterations in PCOS.</td>
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</tbody>
</table>

*PCOS was defined by Rotterdam criteria.
5 Results

5.1 Menstrual disorders and hyperandrogenemia in adolescence and early adulthood (Studies I and II)

5.1.1 Menstrual disorders

According to the questionnaire, 709 (29%) girls had menstrual disorders (symptomatic subjects) and 1739 had regular periods (non-symptomatic subjects) at the age of 16 years. The symptomatic girls were comparable to their non-symptomatic counterparts as regards alcohol consumption, smoking habits and SES.

Menarcheal age was slightly higher in symptomatic (13.1 years) than in non-symptomatic girls (12.9 years, $P < 0.001$). Gynecological age, defined as the number of years since menarche, was calculated (based on the date given in the questionnaire) to be 2.34 (SD 1.1) years in the symptomatic girls and 2.59 (SD 1.09) years in the non-symptomatic girls ($P < 0.001$). The results were therefore adjusted for gynecological age.

Symptomatic and non-symptomatic girls were similar as regards to BMI, serum levels of insulin, hs-CRP and lipids (serum levels of total cholesterol, HDL, LDL and triglycerides), plasma glucose and HOMA-S. Symptomatic girls exhibited significantly higher serum concentrations of testosterone, lower serum levels of SHBG and higher FAI values (Table 8).

Table 8. Hormone parameters in the girls with self-reported menstrual disorders.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-symptomatic girls</th>
<th>Symptomatic girls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N: Geometric mean (IQR)</td>
<td>N: Geometric mean (IQR)</td>
<td></td>
</tr>
<tr>
<td>Testosterone [nmol/L]</td>
<td>1739: 1.59 (1.28, 2.02)</td>
<td>709: 1.65 (1.31, 2.07)</td>
<td>0.010</td>
</tr>
<tr>
<td>SHBG [nmol/L]</td>
<td>1739: 51.49 (36.60, 68.90)</td>
<td>709: 48.99 (35.28, 65.85)</td>
<td>0.042</td>
</tr>
<tr>
<td>FAI</td>
<td>1739: 3.08 (2.15, 4.74)</td>
<td>709: 3.38 (2.27, 5.18)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

IQR: interquartile range from 25th to 75th percentile.

In the group of symptomatic girls 7.8% (55/709) exhibited testosterone levels above the upper normal limit (95th percentile), i.e. 2.68 nmol/L in this population,
which was significantly higher than in non-symptomatic girls (4.9%, 86/1739, \(P = 0.007\)).

Adjustments for BMI or WHR (data not shown) did not alter the aforementioned associations. After adjusting for BMI the percentage differences between the symptomatic and non-symptomatic groups increased as regards the FAI (from 9.5% to 11.3%), testosterone (from 4.2% to 4.5%) and SHBG (-4.8% to -6.1%). Significant differences remained after additional adjustments for smoking, SES and alcohol consumption. Further adjustment for menarcheal or gynecological age did not change the results (data not shown). With a threshold level of 2.68 nmol/L for T, the sensitivity and specificity of being "symptomatic" as regards prediction of hyperandrogenemia were 39% and 72%, respectively.

In the whole study population, 3.5% (86/2448) of the girls exhibited both menstrual disorders and elevated serum testosterone levels fulfilling the Rotterdam criteria for PCOS.

**5.1.2 Effect of hyperandrogenemia**

In the whole study population, after stratifying the FAI values into quartiles, there was a significant linear trend in the higher FAI quartiles towards lower serum HDL-cholesterol levels (from 1.48, 95% CI [1.46–1.51] to 1.41 [1.39–1.43] mmol/L, \(P < 0.001\)). Moreover, serum triglyceride levels increased significantly from the second (0.72 [0.70–0.74] mmol/L) and third FAI quartiles (0.72 [0.70–0.75] mmol/L) to the fourth (0.77 [0.75–0.80 mmol/L], \(P = 0.009\) and \(P = 0.032\), respectively).

**5.1.3 Effect of weight**

In the whole population and both study groups, there were significant correlations between BMI (and WHR) and hyperandrogenemia (especially FAI) and metabolic parameters (hs-CRP, lipids and indicators of insulin sensitivity).

After stratifying the population into BMI quartiles, there was a significant trend towards higher FAI values in the higher BMI quartiles in the symptomatic as well as in the non-symptomatic girls. The differences in FAI values between the symptomatic and non-symptomatic girls were significant in the two highest BMI quartiles. In the highest BMI quartile (25.4 kg/m², range 22.5–46.9 kg/m²), symptomatic girls had significantly higher BMI (\(P = 0.005\)) and FAI values (\(P = 0.002\)), lower serum levels of SHBG (\(P = 0.016\)) and HDL-cholesterol (\(P = 0.002\),
higher serum levels of insulin (P = 0.031) and lower HOMA-S values (P = 0.027) than the non-symptomatic girls. Levels of hs-CRP did not differ between the two groups. There was a significant linear trend towards higher CRP levels from the lowest to the highest BMI quartiles in the whole study population (from 0.16 [95% CI 0.14–0.18] to 0.48 mg/L [0.43–0.54], P < 0.001) and in both the symptomatic (from 0.15 [0.12–0.18] to 0.52 mg/L [0.42–0.65], P < 0.001) and non-symptomatic girls (from 0.17 [0.14–0.19] to 0.47 mg/L [0.41–0.53], P < 0.001).

5.1.4 Anti-Müllerian hormone

There was a significant correlation between AMH and testosterone levels at the age of 16 years (r = 0.36, P < 0.001). After stratifying into T quartiles, the correlation was significant only in the highest T quartile (r = 0.33, 95% CI 0.01–0.56, P = 0.001) and AMH levels increased significantly from the lowest towards the highest T quartile (P-value for trend < 0.001).

AMH levels at the age of 16 years were significantly higher among girls with menstrual disorders compared with girls with normal menstrual cycles. At the age of 26 years, women with menstrual disorders and women without such disorders did not differ as regards their levels of AMH at the age of 16 years. Within the highest testosterone quartile, the women with menstrual disorders at the age of 26 years had higher AMH levels at the age of 16 years, compared with women with normal menstrual cycles at the age of 26 years, even after adjusting for T and BMI (Figure 7A).

Levels of AMH at the age of 16 years were higher in women with hirsutism at the age of 26 years compared with non-hirsute women and higher in women with PCOS at the age of 26 years compared with subjects without PCOS. (Figure 7B).

The sensitivity of serum AMH (cut-off level 22.5 pmol/L, ROC-curve analysis) for predicting PCOS at the age of 26 years was 85.7% and specificity was 37.5%. The addition of T into the model did not significantly improve the accuracy of the test.

In the whole study population, there was a significant but weak correlation between serum AMH levels and BMI at the age of 16 (r = 0.124, P = 0.013), but the significance disappeared after adjusting for T. There were no significant correlations between AMH levels and metabolic indices (WHR, plasma fasting glucose, serum insulin, HOMA-S, hs-CRP, serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides).
Fig. 7. A: Anti-Müllerian hormone levels at the age of 16 years according to the menstrual pattern in the whole study population at the age of 16 years and in the highest T-quartile at the age of 26 years in women with menstrual irregularities and women with normal menstruation. Fig. 7. B: AMH levels at the age of 16 years in women with hirsutism or PCOS at the age of 26 years, and controls. The 95% confidence intervals are shown as error bars.

5.2 Endocrine features of PCOS throughout life (Study III)

Serum androgen levels in women with PCOS decreased from the age of 18 towards menopause and then increased again after the age of 50. In the control group, serum levels of androgens and SHBG were more stable during ageing.

Correlations between age and endocrine parameters are shown in Table 9. After adjustment for BMI, all correlations except that for SHBG remained significant in women with PCOS, whereas the correlations between age and SHBG, cFT and FAI disappeared in the control population.
Table 9. Correlations between age and hormone parameters in the women with PCOS and the controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>P-value</th>
<th>PCOS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>N/A</td>
<td>NS</td>
<td>r = -0.237</td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td>SHBG</td>
<td>r = 0.141</td>
<td>P = 0.035</td>
<td>r = -0.100</td>
<td>P = 0.012</td>
</tr>
<tr>
<td>A4</td>
<td>r = -0.328</td>
<td>P &lt; 0.001*</td>
<td>r = -0.241</td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td>DHEAS</td>
<td>r = -0.258</td>
<td>P = 0.010*</td>
<td>r = -0.233</td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td>FAI</td>
<td>r = 0.205</td>
<td>P = 0.006</td>
<td>r = -0.113</td>
<td>P = 0.004*</td>
</tr>
<tr>
<td>cFT</td>
<td>r = 0.192</td>
<td>P = 0.010</td>
<td>r = -0.169</td>
<td>P &lt; 0.001*</td>
</tr>
</tbody>
</table>

* Statistical significance remained after BMI-adjustment

5.2.1 Testosterone, FAI and cFT

Serum testosterone concentrations were significantly higher in women with PCOS compared with control women in the first four age groups (from 18 to 39 yrs, P < 0.001) and the significance remained after adjusting for BMI (P < 0.001). Both cFT and FAI were higher in women with PCOS at ages between 18–44 years and > 50 yrs and the significance remained in the first five age groups after adjustment for BMI, except for FAI in the age group of 30–34 years (Figure 8).
Fig. 8. Mean levels of androgen parameters in different age groups. Error bars represent the standard deviation and only P-values after adjustment for BMI are shown in the figure.
5.2.2 Adrenal androgens

Serum levels of A4 were significantly higher in women with PCOS compared with controls at the age of 18–34 and in women aged 50 years or more, and the differences remained after adjustment for BMI. Serum DHEAS levels were significantly higher in women with PCOS between the ages 25 to 29 and 50 years or more after adjustment for BMI. Levels of DHEAS were higher in the control women than in the PCOS population between the ages 40 to 44, but the difference became non-significant after adjustment for BMI (Figure 9).

Fig. 9. Mean concentrations of adrenal androgens in different age groups. Error bars represent the standard deviation and only P-values after adjustment for BMI are shown in the figure.
5.2.3 Sex hormone-binding globulin

Serum concentrations of SHBG were lower in women with PCOS in all age groups except for that of 45–49 years. After adjusting for BMI the significance remained at ages 25–29, 40–44 and 50 years or more (Figure 10).

![Fig. 10. Mean concentrations of sex hormone-binding globulin in different age groups. Error bars represent the standard deviation and only P-values after adjustment for BMI are shown in the figure.](image)

5.2.4 Menstrual pattern

The occurrence of oligo-amenorrhea decreased with age in the PCOS population (odds ratio [OR] = 0.96, 95% confidence interval 0.94–0.98, P = 0.001), whereas it increased in the control population (OR = 1.10, 95% confidence interval 1.03–1.18, P = 0.007).

5.2.5 Evaluation of the risk of PCOS

According to the results of logistic regression analysis, the best predictive parameters regarding the risk of PCOS were cFT, A4 and FAI (Table 10). Calculated free testosterone exhibited the best estimated risk ratio, and the best sensitivity and specificity values according to the ROC curve in the subpopulation of women of less than 40 years. The results remained unchanged after exclusion of
subjects whose serum A4 levels were measured by GC-MS (N = 128). Adjustment for age and BMI strengthened the estimated risk ratio for A4 and to a lesser degree for T in the subpopulation of women less than 40 years of age (Table 10).

**Table 10. Estimated risk ratios (odds ratios) for PCOS as regards hormone parameters with specific cut-off values.**

<table>
<thead>
<tr>
<th>Parameters in subpopulations</th>
<th>Crude (OR, 95% CI) (^a)</th>
<th>Adjusted (^b) (OR, 95% CI) (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole study population, N = 856 (cut-off)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (≥1.1 nmol/L)</td>
<td>5.05 (3.65–7.00)***</td>
<td>4.67 (3.27–6.66)***</td>
</tr>
<tr>
<td>SHBG (&lt;46.0 nmol/L)</td>
<td>2.62 (1.91–3.59)***</td>
<td>1.53 (1.06–2.22)***</td>
</tr>
<tr>
<td>FAI (≥2.39)</td>
<td>6.71 (4.76–9.46)***</td>
<td>4.35 (3.01–6.29)***</td>
</tr>
<tr>
<td>cFT (≥0.014 nmol/L)</td>
<td>7.90 (5.41–11.55)***</td>
<td>5.10 (3.40–7.64)***</td>
</tr>
<tr>
<td>DHEAS (≥3.8 µmol/L)</td>
<td>3.80 (2.44–6.90)***</td>
<td>2.57 (1.59–4.14)***</td>
</tr>
<tr>
<td>A4 (≥29.7 nmol/L)</td>
<td>6.16 (4.20–9.05)***</td>
<td>5.75 (3.74–8.84)***</td>
</tr>
<tr>
<td><strong>Population under 40 years old, N = 678 (cut-off)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (≥1.08 nmol/L)</td>
<td>6.34 (4.24–9.47)***</td>
<td>7.00 (4.54–10.78)***</td>
</tr>
<tr>
<td>SHBG (&lt;46.8 nmol/L)</td>
<td>3.02 (2.04–4.46)***</td>
<td>1.59 (1.02–2.46)***</td>
</tr>
<tr>
<td>FAI (≥2.34)</td>
<td>9.22 (5.98–14.22)***</td>
<td>6.64 (4.18–10.56)***</td>
</tr>
<tr>
<td>cFT (≥0.014 nmol/L)</td>
<td>12.97 (7.75–21.70)***</td>
<td>10.18 (5.87–17.64)***</td>
</tr>
<tr>
<td>DHEAS (≥3.7 µmol/L)</td>
<td>3.57 (2.08–6.09)***</td>
<td>3.35 (1.92–5.87)***</td>
</tr>
<tr>
<td>A4 (≥10.7 nmol/L)</td>
<td>6.63 (4.27–10.30)***</td>
<td>7.95 (4.83–13.09)***</td>
</tr>
</tbody>
</table>

\(^a\) Confidence interval of 95%, \(^b\) Adjustment for BMI and age, * P-value < 0.05, ** P-value < 0.01, *** P-value < 0.001

5.3 **Metabolic features of PCOS throughout life (Study IV)**

5.3.1 **Comparison of women with PCOS and control women in the whole population**

The women with PCOS had higher BMI, serum levels of insulin (fasting and OGTT), total cholesterol, LDL, triglycerides (TGs) and blood pressure, and lower serum levels of HDL compared with the control population after adjustment for BMI (Table 11).
<table>
<thead>
<tr>
<th>Metabolic parameters</th>
<th>N</th>
<th>Control population</th>
<th>N</th>
<th>PCOS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [yr]</td>
<td>447</td>
<td>33.5 (9.9)</td>
<td>1550</td>
<td>30.0 (7.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>447</td>
<td>25.9 (5.4)</td>
<td>1497</td>
<td>29.2 (6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist [cm]</td>
<td>312</td>
<td>87.6 (14.6)</td>
<td>1204</td>
<td>92.9 (17.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting glucose [mmol/L]</td>
<td>376</td>
<td>5.1 (0.9)</td>
<td>1104</td>
<td>5.1 (0.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin [mU/L]</td>
<td>372</td>
<td>7.4 (6.0)</td>
<td>1093</td>
<td>12.3 (11.1)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total cholesterol [mmol/L]</td>
<td>364</td>
<td>4.6 (0.9)</td>
<td>982</td>
<td>4.8 (1.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HDL [mmol/L]</td>
<td>346</td>
<td>1.5 (0.3)</td>
<td>960</td>
<td>1.4 (0.4)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LDL [mmol/L]</td>
<td>347</td>
<td>2.6 (0.8)</td>
<td>963</td>
<td>2.9 (0.9)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Triglycerides [mmol/L]</td>
<td>366</td>
<td>0.9 (0.5)</td>
<td>974</td>
<td>1.3 (0.8)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>OGGT glucose 2h [mmol/L]</td>
<td>140</td>
<td>5.0 (1.3)</td>
<td>681</td>
<td>5.8 (1.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OGGT mean glucose [mmol/L]</td>
<td>140</td>
<td>5.0 (0.8)</td>
<td>681</td>
<td>5.5 (1.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OGGT insulin 2h [mU/L]</td>
<td>152</td>
<td>27.4 (20.5)</td>
<td>860</td>
<td>71.7 (73.9)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>OGGT mean insulin [mU/L]</td>
<td>152</td>
<td>17.2 (12.0)</td>
<td>840</td>
<td>42.6 (41.6)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Systolic blood pressure [mmHg]</td>
<td>318</td>
<td>118.2 (15.6)</td>
<td>1276</td>
<td>123.43 (16.2)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Diastolic blood pressure [mmHg]</td>
<td>318</td>
<td>74.3 (12.1)</td>
<td>1276</td>
<td>78.5 (12.1)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>hsCRP [mg/l]</td>
<td>159</td>
<td>1.5 (3.0)</td>
<td>761</td>
<td>2.8 (3.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*P-value < 0.05 after BMI-adjustment.
Table 12. Anthropometric and metabolic parameters as mean values in hyperandrogenic and normoandrogenic PCOS populations and in the control population. Standard deviation in parentheses.

<table>
<thead>
<tr>
<th>Metabolic parameters</th>
<th>N</th>
<th>Controls</th>
<th>N</th>
<th>NA-PCOS</th>
<th>N</th>
<th>HA-PCOS</th>
<th>P-valuea</th>
<th>P-valueb</th>
<th>P-valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [year]</td>
<td>447</td>
<td>33.5</td>
<td>684</td>
<td>29.9</td>
<td>842</td>
<td>30.0</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>447</td>
<td>25.9</td>
<td>666</td>
<td>28.8</td>
<td>811</td>
<td>29.4</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.027</td>
</tr>
<tr>
<td>Waist [cm]</td>
<td>312</td>
<td>87.6</td>
<td>590</td>
<td>92.1</td>
<td>604</td>
<td>93.6</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NSd</td>
</tr>
<tr>
<td>Fasting glucose [mmol/l]</td>
<td>376</td>
<td>5.1</td>
<td>542</td>
<td>5.1</td>
<td>552</td>
<td>5.1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin [mU/l]</td>
<td>372</td>
<td>7.4</td>
<td>544</td>
<td>12.0</td>
<td>537</td>
<td>12.4</td>
<td>&lt;0.001d</td>
<td>&lt;0.001d</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol [mmol/l]</td>
<td>364</td>
<td>4.6</td>
<td>368</td>
<td>4.7</td>
<td>603</td>
<td>4.8</td>
<td>0.041</td>
<td>0.004d</td>
<td>NS</td>
</tr>
<tr>
<td>HDL [mmol/l]</td>
<td>346</td>
<td>1.5</td>
<td>364</td>
<td>1.3</td>
<td>586</td>
<td>1.4</td>
<td>&lt;0.001d</td>
<td>&lt;0.001d</td>
<td>0.013</td>
</tr>
<tr>
<td>LDL [mmol/l]</td>
<td>347</td>
<td>2.6</td>
<td>349</td>
<td>2.8</td>
<td>504</td>
<td>2.9</td>
<td>&lt;0.001d</td>
<td>&lt;0.001d</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides [mmol/l]</td>
<td>366</td>
<td>0.9</td>
<td>367</td>
<td>1.2</td>
<td>596</td>
<td>1.2</td>
<td>&lt;0.001d</td>
<td>&lt;0.001d</td>
<td>NS</td>
</tr>
<tr>
<td>OGTT Glucose 2h [mmol/l]</td>
<td>140</td>
<td>5.0</td>
<td>442</td>
<td>5.9</td>
<td>238</td>
<td>6.0</td>
<td>&lt;0.001d</td>
<td>&lt;0.001d</td>
<td>NS</td>
</tr>
<tr>
<td>OGTT mean glucose [mmol/l]</td>
<td>140</td>
<td>5.0</td>
<td>442</td>
<td>5.4</td>
<td>238</td>
<td>5.5</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>OGTT Insulin 2h [mU/l]</td>
<td>152</td>
<td>27.4</td>
<td>476</td>
<td>67.8</td>
<td>376</td>
<td>76.0</td>
<td>&lt;0.001d</td>
<td>&lt;0.001d</td>
<td>NS</td>
</tr>
<tr>
<td>OGTT mean insulin [mU/l]</td>
<td>152</td>
<td>17.2</td>
<td>465</td>
<td>40.9</td>
<td>367</td>
<td>44.5</td>
<td>&lt;0.001d</td>
<td>&lt;0.001d</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure [mmHg]</td>
<td>318</td>
<td>118</td>
<td>605</td>
<td>123</td>
<td>657</td>
<td>124</td>
<td>&lt;0.001d</td>
<td>&lt;0.001d</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure [mmHg]</td>
<td>318</td>
<td>74</td>
<td>605</td>
<td>78</td>
<td>657</td>
<td>79</td>
<td>&lt;0.001d</td>
<td>&lt;0.001d</td>
<td>NS</td>
</tr>
<tr>
<td>hsCRP [mg/L]</td>
<td>159</td>
<td>1.5</td>
<td>488</td>
<td>2.8</td>
<td>291</td>
<td>2.9</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

*aP-value between control group and NA-PCOS group, bP-value between control group and HA-PCOS group, cP-value between NA- and HA-PCOS groups. *P-value < 0.05 after adjustment for BMI and age.
5.3.2 Comparisons between HA-PCOS, NA-PCOS and control women and the different age groups

5.3.3 Body mass index and waist circumference

The HA-PCOS group presented higher BMIs and waist circumferences compared with both the NA-PCOS and control populations, and the NA-PCOS group had higher BMIs compared with the control women (Table 12).

In age group analyses, both PCOS populations had higher BMIs and waist circumferences compared with the control women in all age groups. In addition, women in the HA-PCOS group had higher BMIs and waist circumferences compared with those in the NA-PCOS group in the groups aged 30 years or more (Figure 11).

![Fig. 11. Mean body mass indices and waist circumferences in the different age groups and in the different subpopulations of the study. Error bars represent standard deviation.](image)
5.3.4 Glucose and insulin metabolism

In the whole population, both PCOS populations were more hyperinsulinemic compared with control women, and women in the NA-PCOS group were more glucose intolerant, with higher 2-h glucose levels compared with the control population (Table 12).

Women in the NA-PCOS group under the age of 40 and those in the HA-PCOS group over the age of 39 exhibited significantly higher fasting insulin levels compared with the control population. In the groups older than 30 years, the 2-hour insulin levels and mean insulin levels in OGTTs were higher in both PCOS populations compared with control women (Figure 12). The 2-hour glucose and mean glucose levels in OGTTs were statistically higher in both the HA-PCOS group (6.1 mmol/L [SD 2.0] and 5.7 mmol/L [SD 1.2] respectively) and the NA-PCOS group (6.0 mmol/L [SD 1.6] and 5.6 mmol/L [SD 1.0]) compared with control subjects (4.5 mmol/L [SD 0.5] and 4.6 mmol/L [SD 0.4]) between the ages of 30 to 39 years.

Fig. 12. Mean OGTT insulin levels in the different age groups and in the different subpopulations of the study. Error bars represent standard deviation. Results are adjusted for BMI.
5.3.5 Lipids

In the whole population, serum levels of total cholesterol were significantly higher in the HA-PCOS group compared with controls. In addition, serum HDL levels were lower, and LDL and TG levels were higher in both PCOS populations compared with the control population (Table 12).

In those under 30 years of age, both PCOS subpopulations showed worse lipid profiles compared with the control women. At ages over 39 years, women in the HA-PCOS group had higher triglyceride levels compared with control women (Figure 13).

Fig. 13. Mean total cholesterol and triglyceride levels in the different age groups and in the different subpopulations of the study. Error bars represent standard deviation. Results are adjusted for BMI.
Serum levels of HDL were lower and levels of LDL higher in both PCOS populations when compared with the control women in the age group under 30 years. Serum levels of LDL were higher in the HA-PCOS group compared with both NA-PCOS and control women in the age group over 39 years (Figure 14).

![Graph showing HDL and LDL levels in different age groups and subpopulations.](image)

**Fig. 14.** Mean HDL and LDL levels in the different age groups and in the different subpopulations of the study. Error bars represent standard deviation. Results are adjusted for BMI.
5.3.6 Blood pressure

Both systolic and diastolic blood pressures were significantly higher in both PCOS subpopulations compared with the control women (Table 12). In the age group analyses, HA-PCOS-group women had higher systolic blood pressures when over 30 years of age and NA-PCOS-group women in all age groups compared with the control women. Diastolic blood pressure was higher in HA-PCOS-group women compared with control women in all age groups, whereas in NA-PCOS-group women diastolic blood pressure was higher only in those under 39 years of age compared with controls (Figure 15).

Fig. 15. Mean systolic and diastolic blood pressures in the different age groups and in the different subpopulations of the study. Error bars represent standard deviation. Results are adjusted for BMI.
5.3.7 High-sensitivity C-reactive protein

Levels of high-sensitivity CRP were higher in all women with PCOS as well as in both PCOS subpopulations compared with the control women, but the difference became nonsignificant after adjustment for BMI (Table 12).

5.3.8 Prevalence of metabolic syndrome

The prevalence of MetS (Rotterdam criteria) was two- to fivefold higher in the HA-PCOS group compared with its prevalence in the control women in all age groups and higher in the NA-PCOS group than in control women under 40 years of age. The prevalence of MetS was similar in the control and NA-PCOS populations compared with an approximately twofold higher prevalence in the HA-PCOS group when over 39 years of age (Figure 16). The results were similar when using the criteria of the International Diabetes Federation to define metabolic syndrome (Alberti et al. 2005).
Fig. 16. The prevalence of metabolic syndrome and its components in the different age groups and in the different subpopulations of the study. Results are adjusted for BMI.
6 Discussion

In the present study, the associations between early menstrual irregularities, determined by asking a simple question in adolescence, serum AMH levels, hyperandrogenism and other metabolic parameters in adolescence were evaluated. Moreover, we wanted to clarify the value of early menstrual irregularities and the reliability of serum levels of AMH in predicting the emergence of PCOS in later life. Further, we investigated the value of serum androgen levels from early until late adulthood in diagnosis of the syndrome in a large Nordic population of women with PCOS. Lastly, we investigated the evolution of the syndrome with age and the relationships between the typical hormonal and metabolic abnormalities related to the syndrome, such as abdominal obesity, hyperandrogenism, insulin resistance, impaired glucose tolerance, dyslipidemia and chronic inflammation as well as the prevalence of metabolic syndrome.

6.1 Early prediction of PCOS

6.1.1 Menstrual irregularities

In our study population of Finnish adolescent girls, one third of the subjects exhibited menstrual irregularities, confirming that these are common gynecological problems in adolescents.

Irregular menstruation during reproductive life has been suggested to be associated with metabolic risks, such as increased risks of CVD, CVD events and even increased mortality postmenopausally (Shaw et al. 2008). Menstrual irregularities in adolescence have also been linked to hyperandrogenism (Lewy et al. 2001). We were able to confirm these findings, as menstrual irregularities at the age of 16 years were associated with an increased prevalence of hyperandrogenism as well as with a lower level of SHBG, despite the fact that the symptomatic girls were leaner than their asymptomatic controls. Even though there were no other significant associations between menstrual irregularities and metabolic disorders at that age, the presence of lower SHBG levels in symptomatic girls is of interest, as SHBG has been shown to be a good surrogate marker of insulin resistance (Kajaia et al. 2007). Low serum SHBG levels have also been associated with an increased risk of T2DM in later life (Wallace et al. 2013). Our finding may therefore allow us to suggest that menstrual irregularities in adolescence may be linked to
metabolic risks later in life. The symptomatic girls, however, did not exhibit other metabolic risks linked to PCOS, possibly because they were very young and clinical manifestation of insulin resistance and metabolic disorders had not yet developed. Of note, the symptomatic group most probably also included girls with mild cycle irregularities, which may have decreased the differences between the two groups. Nevertheless, the symptomatic girls in the highest BMI quartile were more hyperandrogenic and insulin resistant compared with their leaner counterparts, and the symptomatic girls were more hyperandrogenic and insulin resistant compared with non-symptomatic girls. The most hyperandrogenic girls in the whole study population already presented a more adverse lipid profile at a young age. Lastly, adjustment for BMI even increased the differences between the two groups.

In our study, the mean gynecological age in the symptomatic girls with menstrual irregularities was only 2.5 years, suggesting that evaluation of the menstrual cycle might have taken place too soon after menarche. However, the results did not change after adjusting for gynecological age, which may suggest that the presence of menstrual disorders as early as two to three years after menarche could be a hallmark of hyperandrogenism and hyperinsulinemia, both typical disorders in cases of PCOS. Moreover, our results are consistent with earlier findings showing that 62% of adolescent girls suffer from irregular menstruation and 59% of such girls fulfilled PCOS criteria at the end of a 6-year follow-up period (Wiksten-Almstromer et al. 2008). Of interest is the fact that the girls with menstrual irregularities had entered menarche three months later than girls with regular menses, which could indicate that menstrual irregularities already existed at menarche, but this hypothesis remains to be confirmed. Inversely, in our study, the presence of regular menses at the age of 16 years diminished the risk of HA with a specificity of 72% and could be a predictor of a normal hormonal status later in life.

Overall, our results confirm that the association between obesity, hyperandrogenism and metabolic risks is already present in adolescence. More specifically, we could show that menstrual irregularities, identified via a simple question at the time of adolescence, represent a good marker of hyperandrogenemia and may be a risk factor as regards later metabolic risks and development of PCOS in adulthood. Inversely, the presence of regular menses at the age of 16 years could predict a normal hormonal status later in life.
6.1.2 Anti-Müllerian hormone

In Study II we aimed to clarify whether the relationship between AMH, hyperandrogenism, and menstrual and metabolic disorders already exists in adolescence and what could be the value of AMH as a predictive tool regarding the development of oligo-amenorrhea, hirsutism and PCOS later in early adulthood.

We observed a significant positive correlation between serum concentrations of T and AMH as early as in adolescence, as well as higher AMH levels in girls with menstrual irregularities at the age of 16 years, in line with previous results showing a positive association between the length of menstrual cycles and serum levels of AMH at the age of 20 years (Kristensen et al. 2012). Our results also showed also that AMH levels were associated significantly with oligo- or amenorrhea and were a good indicator of these conditions in adolescence. Moreover, retrospectively, the women reporting hirsutism at the age of 26 years had higher levels of AMH at the age of 16 years compared with non-hirsute women at the age of 26 years, and women with PCOS (either diagnosed or self-reported symptoms according to the questionnaire) at the age of 26 years had higher serum AMH levels in adolescence compared with healthy women. Serum AMH levels at the age of 16 years, however, did not differ significantly between women with oligo- or amenorrhea and women without these conditions at the age of 26 years.

We calculated the specificity and sensitivity of serum levels of AMH in relation to the risk of developing PCOS in later life, and serum AMH levels over 22.5 pmol/L could identify adolescent girls at risk of PCOS in early adulthood with good sensitivity (85.7%). However, the specificity of the test remained very weak. It improved substantially when using a higher cut-off value of 42.8 pmol/L, but the sensitivity dropped and the combination of levels of AMH and T did not improve the accuracy of the test, in line with the results of some (Casadei et al. 2013), but not all studies (Eilertsen et al. 2012). Previous studies have indicated that AMH alone may not be good enough as a single screening or diagnostic tool for PCOS (Singh & Singh 2015). Our study indicates that serum AMH levels in adolescence do not represent a reliable tool to predict PCOS in later life.

In adolescence no association between AMH and metabolic risks has been shown (Anderson et al. 2013, Lin et al. 2011), but contradictory results later in life have also been published (Nardo et al. 2009, Park et al. 2010, Skalba et al. 2011). We found a weak correlation between AMH levels and BMI at the age of 16 years, but after adjustment for T the result became nonsignificant. In addition, there was
no significant correlation between serum levels of AMH and metabolic indices, indicating that AMH is a poor marker of an adverse metabolic profile in adolescence, in line with previous results (Anderson et al. 2013, Cui et al. 2016).

In conclusion, girls with oligo- or amenorrhea at the age of 16 years or PCOS/isolated hirsutism at the age of 26 years already exhibited higher levels of AMH in adolescence, suggesting that the association between elevated AMH levels and symptoms of PCOS in adolescence persists into early adulthood. As a result of good sensitivity, use of a sufficiently high cut-off value of AMH could predict which adolescents are likely to develop PCOS in adulthood, enabling management of this condition from an early age. However, because of its low specificity, it is not an ideal diagnostic marker, and its routine use in clinical practice cannot at present be recommended. Lastly, AMH is not a good marker of cardiovascular risk factors in adolescence.

6.2 Lifelong hormonal and metabolic parameters

6.2.1 Androgen parameters in PCOS across life

The data from this multicenter collaborative work carried out in Nordic countries showed that women with PCOS exhibited significantly elevated serum androgen levels, of both ovarian and adrenal origin. The differences were especially significant in early adulthood and in the pre- and postmenopausal periods, as shown previously in other ethnic populations (Liang et al. 2011, Winters et al. 2000). This finding confirms that young women suffer more often from adverse features related to hyperandrogenism (hirsutism and acne) than they do in later life. Additionally, mood disorders and decreased quality of life seem to be worse in early adulthood in women with PCOS (Dokras 2012, Johnstone et al. 2012, Livadas et al. 2011). These findings strengthen the importance of implementing active therapeutic intervention among young women in order to improve these adverse manifestations of hyperandrogenism, as well as quality of life.

Women with PCOS remained more hyperandrogenic until the age of 44 years when compared with the control population. After the steady decrease in androgen levels up to menopause, hyperandrogenism persisted or even worsened after menopause in women with PCOS, in line with the results of previous studies (Burger et al. 2000, Puurunen et al. 2009, Schmidt et al. 2011). Increases in the FAI and cFT after menopause were most probably related to the decrease of SHBG
levels, which has been linked to increased weight, and impaired glucose and insulin metabolism (Cao et al. 2013, Markopoulos et al. 2011, Puurunen et al. 2009, Puurunen et al. 2011). Supporting this hypothesis, after adjustment for BMI, the differences in FAI and cFT between women with PCOS and non-PCOS subjects became nonsignificant. We observed a similar result regarding A4 and DHEAS, thus further supporting the strong correlation between hyperandrogenism and obesity after menopause.

When evaluating the value of different parameters for assessing androgenicity, cFT, the FAI and A4 were found to be the best risk factors as regards the prediction of PCOS, thus supporting previous postulations that the FAI or cFT rather than T should be used when defining hyperandrogenism in clinical practice (Cho et al. 2008, Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004, Vermeulen et al. 1999). Some previous investigators have suggested that serum A4 levels are independent of BMI and that A4 is the most consistently elevated androgen in women with PCOS (Overlie et al. 1999, Puurunen et al. 2011). After adjusting for BMI, A4 showed the best estimated risk ratio in the whole population, but in the subgroup of women under 40 years, cFT exhibited the best predictive value.

In conclusion, this large Nordic multicenter collaborative study showed that obesity and hyperandrogenemia are worse throughout reproductive life in women with PCOS compared with healthy control women. Hyperandrogenemia improves toward menopause in women with PCOS, but remains elevated after menopause. According to the results of logistic regression analysis, cFT, the FAI, and serum A4 are the best discriminators of PCOS at all ages and may be used as additional tools for diagnosing PCOS in large populations. Lastly, in this population, the use of cFT instead of the FAI as a discriminator of PCOS seems to be more accurate in women younger than 40 years of age, at least when using LC-MS/MS for the assay of T.

### 6.2.2 Metabolic risk factors in PCOS across life

The main finding in this large Nordic collaborative study was that the unfavorable metabolic profile in women with PCOS, independently of hyperandrogenism, was already present in early adulthood and extended until menopause.

Studies carried out to evaluate metabolic abnormalities in women with PCOS have mostly involved small numbers of subjects of relatively narrow age range, and the role of HA as a metabolic risk factor in PCOS is still under debate (Moran &
Some data suggest that the metabolic risks linked to PCOS are mainly related to obesity and insulin resistance (Moran & Teede 2009, Tzeng et al. 2014), whereas others support the hypothesis that the syndrome per se and especially HA, independently of BMI, are the most important cardiovascular risks (Nisenblat & Norman 2009, Park et al. 2010, Piouka et al. 2009). It was therefore interesting to compare women in the NA- and HA-PCOS groups as regards their metabolic profiles in our large Nordic population, from early adulthood until menopause.

The results of some previous studies have suggested that the subpopulation of normoandrogenic oligo-amenorrheic women with PCOS does not differ from healthy BMI-matched controls as regards metabolic risks and that this subgroup of women may present a more favorable metabolic phenotype compared with their hyperandrogenic counterparts (Barber et al. 2007). More specifically, differences between non-hyperandrogenic women with PCOS and healthy women have been suggested to be mostly associated with increased abdominal obesity (Moran & Teede 2009). Our present results support this postulation, as the NA-PCOS population presented as a distinguishable subgroup as regards metabolic risks; such women were more abdominally obese, and, even after adjusting for BMI, more glucose intolerant, insulin resistant, hyperinsulinemic and dyslipidemic, especially in early adulthood, compared with control women.

As for the role of HA as a metabolic risk in PCOS, women in both the NA- and HA-PCOS groups exhibited more adverse metabolic alterations throughout their fertile lives compared with control women, without significant differences between the two PCOS subpopulations, except for significantly greater BMIs and waist circumferences among those in the HA-PCOS group over 30 years of age. Again, our results confirm a predominant role of obesity as regards the severity of the syndrome (Moran & Teede 2009). On the other hand, HA may have some significance as a late metabolic risk, as the prevalence of MetS was significantly greater in women in the HA-PCOS group compared with the NA-PCOS group and the control population after the age of 40 years, which may be partly due to the relatively low numbers of women in these groups. Indeed, women in the NA-PCOS group and control women exhibited similar waist circumferences at this age, which may have biased the results regarding the prevalence of MetS, but again supporting the postulation that abdominal obesity is the primary determinant of metabolic abnormalities in PCOS (Moran & Teede 2009). This finding, however, is also consistent with previous observations linking hyperandrogenism to metabolic disturbances and an elevated prevalence of MetS (Sung et al. 2014). In keeping
with this postulation, in our study population, serum levels of LDL were slightly higher in the HA-PCOS versus the NA-PCOS group in those over 40 years of age. Even though the isolated parameters of the metabolic risk profile did not considerably differ between PCOS subpopulations, lifelong exposure to hyperandrogenism may end up being an additional risk as regards MetS later in life. Hyperandrogenism may also directly or indirectly influence metabolic abnormalities and contribute to abdominal obesity.

Women in both the HA- and NA-PCOS groups already exhibited adverse changes in their metabolic profiles and blood pressure (BP) at early ages, independently of obesity. In particular, high levels of triglycerides have been shown to represent an independent predictor of future risk of myocardial infarction, and low levels of HDL reflect cardiovascular morbidity (Stampfer et al. 1996) and are associated with elevated cardiovascular risks in general, especially in women (Ulmer et al. 2005). The difference in BP levels (4–5mm Hg between the PCOS and the control groups), although relatively modest, may have clinical significance, since it was already present in the younger age group (under 30 years). Moreover, it has been shown that an increase of BP of 1.5–2 mm Hg could have a large impact on CVD risk at a population level, and reduction of mildly and/or moderately elevated BP to normal levels has been associated with a reduced risk of CVD in large placebo-controlled studies (Staessen et al. 1997). All these observations fit with recent epidemiologic data indicating that women with PCOS may have an increased risk of ischemic heart and cerebrovascular diseases (Hart & Doherty 2015).

In summary, the results highlight the importance of screening for PCOS and overweight/obesity in early adulthood in order to tailor treatment and intervention protocols and reduce the risks of future CVD and T2DM. However, more research is still needed to provide a greater understanding of the interaction of hyperandrogenism, insulin resistance and abdominal adiposity in PCOS (Tzeng et al. 2014), and only long-term follow-up will reveal whether the metabolic risk factors linked to PCOS, and more specifically to HA, translate into later cardiovascular morbidity and mortality.
6.3 Strengths and limitations of the studies

6.3.1 Studies I and II (NFBC-1986)

The strength of Studies I and II is the large, stable, population-based birth cohort. The answer and participation rates were good, so that generalization of the results to the Finnish population is appropriate. In addition, the questionnaires and clinical evaluations were standardized, which further increases the reliability of our findings.

In these studies serum levels of testosterone were determined by LC-MS/MS, the most modern and accurate method for T analysis. The method for the determination of AMH levels has been challenged recently, but the Gen II assay used in the present study has been considered to be the most reliable method (Singh & Singh 2015). Moreover, the determinations in our study were carried out at the same time and with the same assay, thus decreasing the risks of inaccuracy of the results.

One limitation in the studies is the questionnaire-based data for some parameters (self-reported menstrual irregularities and hirsutism). As the study was not designed to diagnose PCOS, neither ultrasonography nor clinical evaluations of hirsutism were carried out, which can be considered as a limitation. However, in a similar Northern Finland cohort, the Northern Finland Birth Cohort 1966, it has previously been shown that self-reported menstrual irregularities and hirsutism correlate with an endocrine profile typical of PCOS and that two simple questions on menstrual irregularities and hirsutism are sufficient to detect women with PCOS in the general population (Taponen et al. 2003).

The girls were not screened for late-onset adrenal hyperplasia, but the prevalence of this disorder (under 1/15 000) in our country is considerably lower than that reported for other populations (Jaaskelainen et al. 1997).

An important pitfall is that at the time of performance of our study, AMH assays lacked an agreed international standard and therefore the concentrations and cut-off points were method-dependent. The different immunoassays in use have been compared and the results have been comparable, but different threshold values should be used for automatic and manual assays (Pigny et al. 2016). According to a recent review on the diagnostic significance of AMH in PCOS (Singh & Singh 2015), the Gen II assay used in the present study appears to be the most reliable method for AMH measurement. The development of new methods for the measurement of AMH will reveal whether the observed associations can be
confirmed. Importantly, the development of an international standard is needed in the future.

6.3.2 Studies III and IV (Nordic population)

The strength of this Nordic collaborative study is the large number of subjects from a homogeneous Caucasian population. Moreover, the study population includes both women with PCOS and control women with a wide age range from adolescence until the postmenopausal period. As in the NFBC-1986 study, the gold standard method for measuring T in women, LC-MS/MS, was used in Study III and in 52% of the subjects in Study IV.

One important limitation is the cross-sectional study design, which can cause bias due to heterogeneity in the characteristics of the participants in the different age groups. Furthermore, the control group was smaller than the PCOS group, as not all trials involved in this collaborative study included a control population. The methods for laboratory analyses varied between subpopulations at different study sites. However, all were carried out according to accredited methods, and specific reference ranges in the laboratories were used in the definition of biochemical hyperandrogenism. The upper limit for T to define biochemical hyperandrogenism did not take into account the physiological decline in T serum levels observed with age. Thus, some hyperandrogenic women older than 39 years may have been classified as normoandrogenic, which could narrow the differences between the HA- and NA-PCOS groups. In Study IV, we could not clearly distinguish premenopausal, menopausal and postmenopausal women, as the number of women over 50 years of age was too small to obtain reliable results when comparing different age groups.
7 Conclusions and future plans

1. Menstrual irregularities, enquired about in adolescence by means of one simple question, represent a good marker of hyperandrogenemia and may predict the development of PCOS. The simultaneous presence of menstrual irregularities and overweight is associated with the worst metabolic risk profile at a young age. Menstrual irregularities should be noted early, as an association with hyperandrogenism, obesity and adverse metabolic risk factors is already present in adolescence.

2. At the age of 16 years serum AMH levels correlate with those of T, and menstrual irregularities. In addition, elevated AMH levels in adolescence are associated with hirsutism and PCOS in early adulthood. However, AMH levels in adolescence do not represent a reliable tool to predict PCOS in later life.

3. AMH is not a good marker of metabolic abnormalities in adolescence. In adolescence, establishing the presence of menstrual irregularities by means of a simple question, and measuring AMH levels, especially in hyperandrogenic girls, could help to predict the later development of PCOS and, further, allow the possibility to take preventive actions to avoid its development, which would decrease the burden of the syndrome at both an individual level and among the general public.

4. Women with PCOS have elevated androgen levels and are more obese compared with control women throughout their fertile lives. Hyperandrogenism seems to ease with age in women with PCOS, but androgen levels remain elevated after menopause.

5. The FAI, cFT, and serum A4 are the best discriminators of PCOS at all ages and may be used as additional tools for diagnosing PCOS in large populations.

6. Nordic women with PCOS present a worsened metabolic profile throughout their fertile lives. Abdominal obesity seems to exert a more predominant role than hyperandrogenism as regards the severity of the syndrome during the reproductive period. However, lifelong exposure to elevated androgen levels might result in a worsened metabolic burden in women with PCOS aged 40 years or more.

In order to tailor preventive actions and treatment of PCOS, age, androgenic status, obesity and clinical features of the disorder should all be taken into account.
7.1.1 Future plans

A follow-up investigation including a new questionnaire as well as clinical examinations is planned among the NFBC-1986 in 2018–2019, when the women will be 31–32 years of age. One of the most important challenges will be to check reproductive function as well as the hormonal and metabolic indices in these women and to investigate the relationships with their hormonal and metabolic indices at the age of 16 years. More specifically, we will investigate how menstrual irregularities, metabolic and hormonal parameters and serum levels of AMH at the age of 16 years correlate with reproductive and metabolic health at the age of 31–32 years. Determination of serum levels of AMH at the age of 31–32 years will also clarify the role and significance of AMH as a diagnostic tool for PCOS and as a hypothetical indicator of hormonal and metabolic disorders in this population. This follow-up study could help to clarify how the endocrine and metabolic profiles in adolescence translate into reproductive and metabolic health in adulthood.

In the Nordic collaboration project, we are planning to carry out studies focusing more specifically on chronic inflammation, and screening of dyslipidemia and glucose metabolism disorders in women with PCOS. It would also be of interest to investigate, in this same population, the prevalence of hirsutism and its relationship with hyperandrogenemia. In addition, it would be of great interest to measure serum AMH levels at different ages in this large population, and to evaluate the correlations between AMH, and hormonal and metabolic parameters. Further, in the future extended collaboration with other researchers in the field could be considered, and a large European cohort of women with PCOS would make it possible to reach deeper understanding of the syndrome.
References


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Original publications


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Original publications are not included in the electronic version of the dissertation.

1365. Aro, Jani (2016) Novel load-inducible factors in cardiac hypertrophy

1366. Myllymäki, Mikko (2016) Hypoxia-inducible factor prolyl 4-hydroxylase-2 in Tibetan high-altitude adaptation, extramedullary erythropoiesis and skeletal muscle ischemia


1368. Krökki, Olga (2016) Multiple sclerosis in Northern Finland: epidemiological characteristics and comorbidities

1369. Mosorin, Matti-Aleksi (2016) Prognostic impact of preoperative and postoperative critical conditions on the outcome of coronary artery bypass surgery


1372. Heikkala, Anne (2016) Ketoacidosis at diagnosis of type 1 diabetes in children under 15 years of age


1375. Lehtonen, Ville (2016) Dental and otologic problems in cleft lip and palate patients from Northern Finland: cleft associated problems

1376. Koivukangas, Jenni (2016) Brain white matter structure, body mass index and physical activity in individuals at risk for psychosis: The Northern Finland Birth Cohort 1986 Study

1377. Väyrynen, Sara (2016) Histological and molecular features of serrated colorectal adenocarcinoma and its precursor lesions


1379. Törmälä, Reeta-Maria (2016) Human zona pellucida abnormalities—a genetic approach to the understanding of fertilization failure

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