Johanna Puurunen

ANDROGEN SECRETION AND CARDIOVASCULAR RISK FACTORS IN WOMEN WITH AND WITHOUT PCOS

STUDIES ON AGE-RELATED CHANGES AND MEDICAL INTERVENTION
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Studies on age-related changes and medical intervention

Academic Dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 4 of Oulu University Hospital, on 5 June 2015, at 12 noon

UNIVERSITY OF OULU, OULU 2015
Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. The main features of the syndrome include menstrual irregularities and hyperandrogenism. In addition to symptoms related to fertility, some women also suffer from an unfavourable metabolic profile including impaired glucose tolerance, dyslipidaemia and low-grade chronic inflammation.

In the present studies we aimed to investigate the role of age on adrenal and ovarian androgen secretion in 79 women with PCOS and 98 healthy women, with special focus on the menopause. Furthermore, we studied the effects of combined hormonal contraceptives (CHCs) administered orally, transdermally and vaginally (n=42, healthy women, 9 weeks) and atorvastatin treatment (n=28, women with PCOS, 6 months) on androgen levels and metabolic factors. Androgen secretion capacity was analysed by using adrenal and ovarian stimulation tests and glucose tolerance by using oral and intravenous glucose tolerance tests. Furthermore, chronic inflammation was assessed via assay of C-reactive protein and pentraxin-3.

Basal and stimulated adrenal and ovarian androgen production was elevated and levels remained higher in women with PCOS compared with healthy women even after the menopause. Furthermore, women with PCOS presented with enhanced insulin resistance and chronic inflammation, which persisted beyond menopausal transition. During CHC treatment, the route of administration was insignificant, and all treatments impaired insulin sensitivity and increased chronic inflammation. In women with PCOS, treatment with atorvastatin improved chronic inflammation and the lipid profile as expected, but worsened glucose tolerance and did not affect testosterone levels.

Regardless of strict exclusion criteria, where only relatively healthy women with PCOS were recruited, the results showed that enhanced androgen secretion and unfavourable metabolic alterations associated with PCOS persist through menopausal transition. The findings emphasize the importance of monitoring glucose metabolism during the use of CHCs, especially in women with known risks of type 2 diabetes. Atorvastatin treatment exacerbates insulin resistance in women with PCOS and therefore the treatment should only be considered after individual risk assessment of cardiovascular disease and not just because of PCOS.

Keywords: aging, androgens, cardiovascular diseases, combined hormonal contraceptives, CRP, cutaneous administration, hyperandrogenism, inflammation, insulin resistance, intravaginal administration, menopause, oral administration, polycystic ovary syndrome, statins
Puurunen, Johanna, Miessukuhormonieritys ja sydän- ja verisuonitautien riskitekijät monirakkulaisessa munasarjaoireyhymässä ja terveillä naisilla. Ikääntymisen ja lääkehoidon vaikutukset

Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Medical Research Center Oulu; Oulun yliopistollinen sairaala; Valtakunnallinen kliininen tutkijakoulu

Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

Tiivistelmä


Tutkimuksessa selvitettiin ikääntymisen ja vaihevuosien vaikutuksia lisämunuais- ja munasarjaperäiseen androgeenieritykseen 79 PCOS-naisella ja 98 terveellä naisella. Lisäksi tutkittiin eri yhdistelmäehkäisyvalmisteiden antoreittien (suu, iho, emätin) (n=42, terveet naiset, 9 viikkoa) ja atorvastatiinihoidon (n=28, PCOS-naiset, 6 kuukautta) vaikutuksia androgeenitasoihin ja aineenvaihdunnallisiin muutoksiin. Androgeenierityystä tutkittiin lisämunuaisten ja munasarjojen stimulaatiotestillä ja sokeriaineenvaihdunnan muutoksia suun kautta ja suonensäisesti tehtävillä sokerirasituskokeilla. Tulehduskellista tilaa mitattiin määrittämällä C-reaktivisen proteiinin ja pentraksiinin-3:n pitoisuksia.


Asiasonat: androgeenit, CRP, emättimen kautta annostelu, hormonaalinen yhdistelmäehkäisy, hyperandrojenismi, ihon kautta annostelu, ikääntyminen, insuliiniresistentti, monirakkulainen munasarjaoireyhymä, statiinit, suun kautta annostelu, sydän- ja verisuonitautit, tulehdus, vaihevuode
There are only two ways to live your life. One is as though nothing is a miracle. The other is as though everything is a miracle.

–Albert Einstein

To Olli and our lovely sons
Acknowledgements

The present work was carried out at the Department of Obstetrics and Gynecology, University of Oulu, and the Medical Research Center Oulu, Oulu University Hospital, during the years 2004–2015.

First and foremost, I wish to express my deepest gratitude to my supervisor professor Juha Tapanainen for introducing the fascinating field of medical research to me. I am grateful for his expert guidance, encouraging attitude and support, and also for his patience during my periods of parental leave. I have been privileged to work as a member of his research group in an excellent scientific environment.

I owe warm and grateful acknowledgement to my supervisor Terhi Piltonen, M.D., Ph.D., who has had an enormous role in this project. Her excellent guidance ever since I started as a medical student has been invaluable. I also want to thank Terhi for her warm and close friendship and those numerous hours around her dining table.

I wish to express my sincerest gratitude to docent Laure Morin-Papunen for her energetic and supportive attitude and her helpfulness in any difficulties I have had. Of course, I also thank her for many summer parties arranged in her lovely home. I thank professor Hannu Martikainen, docent Ilkka Järvelä and docent Tommi Vaskivuo for their valuable advice and supportive comments in meetings and elsewhere.

I wish to thank professor Aimo Ruokonen, professor Markku Savolainen, professor Karl-Heinz Herzig, docent Antti Perheentupa, Antti Nissinen, Shivaprapash Mutt, Katri Puukka and Pirjo Hedberg for their help and valuable contributions during the course of this project. I also want to thank Risto Bloigu for his guidance in the field of statistics.

I have had the privilege to work in an inspiring and cheerful atmosphere and I want to thank my colleagues Mervi Haapsamo, Minna Jääskeläinen, Sanna Koskela, Kristiina Mäkelä, Katriina Niemelä, Pekka Pinola, Anni Rantala, Reeta Törnälä, Outi Uimari and Zdravka Veleva for their support, encouragement and excellent company in the office and at evening gatherings. I also want to thank the new members of our research group, Anna-Maaria Auvinen, Marika Kangasniemi, Salla Karjula, Masuma Khatun, Shilpa Lingaiah, Maija Ollila, Henna Rossi and Saara Vuontisjärvi for many memorable moments.
I wish to thank Mirja Ahvensalmi and Anu Ojala for their friendly and skilful care of the subjects. I also want to thank our new research nurse Elina Huikari for her energetic and helpful attitude and pleasant conversations during coffee breaks.

I am grateful to laboratory manager Tiina Hurskainen for her help and for arranging a quiet room to write this thesis. I wish to thank all the members in other MRC Oulu research groups for the warm atmosphere in the coffee room. Special thanks are owed to my roommate Johanna Huusko for all the help.

The official reviewers of my thesis, docent Maritta Hippeläinen and professor Risto Kaaja, are gratefully acknowledged for their constructive comments and suggestions, which improved the quality of the thesis. I thank Nicholas Bolton for his skillful linguistic revision of the thesis and my manuscripts over the years.

I want to thank my dear friends and colleagues Marjo Dahlbacka, Minna Kivenmäki, Sanna Koskela, Marianne Rasi, Kati Retsu-Heikkilä, Saara Vuontisjärvi, Anne Vuontissalmi and Laura Ylikauma for their support in the field of medicine and in life in general. I also want to thank Päivi Haaranen, Anne Kivilahti, Jenni Krankka and Anna Mäkivuoti for their warm friendship and support. I wish to thank Jenni Rask for deep conversations and numerous evenings in the swimming pool. I have spent several enjoyable times with you all and I am so lucky to have you in my life.

I am grateful to my parents Eeva-Liisa and Kari Kesti for our lovely family and for their support and encouragement in life. I want to thank my father for those thousands of kilometres driven due to sailing, and my mother for those desperate hours teaching me English. I thank my little sisters Jutta Kesti and Jenni Lämsä for close friendship and unforgettable moments spent together. I have always felt loved. I also thank my brother-in-law Mikko Lämsä, my godson Eino and a newborn nephew for providing such enjoyable company in our family.

Above all, my deepest thanks are owed to my beloved husband Olli for believing in me. I am grateful for all his help with computers and for encouragement during the deepest moments of desperation. I thank my dear and precious sons, Joona and Peetu, for reminding me of the importance of life apart from work. You three are my everything and I am deeply grateful for having you beside me.

I want to express my sincere gratitude to all the study subjects who volunteered to participate in this project. Without those ladies my thesis would never have been possible.

This study was financially supported by the Academy of Finland, the Sigrid Jusélius Foundation, Oulu University Hospital, the National Graduate School of...
Clinical Investigation, the North Ostrobothnia Regional fund of the Finnish Cultural Foundation, Tyyni Tani Foundation of the University of Oulu, the Finnish-Norwegian Medical Foundation, the Finnish Society of Obstetrics and Gynecology, Oulu Medical Research Foundation, the Finnish Medical Foundation and the Paulo Foundation, who are gratefully acknowledged.

Oulu, April 2015

Johanna Puurunen
Abbreviations

17-OHP 17-hydroxyprogesterone
A androstenedione
ACTH adrenocorticotropic hormone
AMH anti-Müllerian hormone
BMI body mass index
CHC combined hormonal contraceptive
CHD coronary heart disease
CRH corticotrophin-releasing hormone
CRP C-reactive protein
CVD cardiovascular disease
DHEA dehydroepiandrosterone
DHEAS dehydroepiandrosterone sulphate
DHT dihydrotestosterone
E2 estradiol
EE ethinyl estradiol
ET-1 endothelin-1
FAI Free Androgen Index
FSH follicle-stimulating hormone
GDM gestational diabetes mellitus
GnRH gonadotrophin-releasing hormone
hCG human chorionic gonadotrophin
HDL high-density lipoprotein
HOMA homeostatic model assessment
IFG impaired fasting glucose
IGF insulin-like growth factor
IGT impaired glucose tolerance
IMT intima-media thickness
IR insulin receptor
IVGTT intravenous glucose tolerance test
LDL low-density lipoprotein
LH luteinising hormone
OGTT oral glucose tolerance test
OR odds ratio
PCO polycystic ovary
PCOS polycystic ovary syndrome
<table>
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<th>Full Form</th>
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<tr>
<td>PTX-3</td>
<td>pentraxin 3</td>
</tr>
<tr>
<td>SHBG</td>
<td>sex hormone-binding globulin</td>
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<tr>
<td>StAR</td>
<td>steroidogenic acute regulatory protein</td>
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<tr>
<td>T</td>
<td>testosterone</td>
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<td>T2DM</td>
<td>type 2 diabetes mellitus</td>
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<td>VLDL</td>
<td>very low density lipoprotein</td>
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<td>WHR</td>
<td>waist to hip ratio</td>
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List of original publications

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


*equal contribution
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1 Introduction

Polycystic ovary syndrome, PCOS, is the most common endocrine disorder in fertile-aged women, with a prevalence of 6–20% depending on the diagnostic criteria used (Yildiz et al. 2012). The main symptoms of the syndrome include irregularities in menstrual cycles and hyperandrogenism. Hyperandrogenism can be detected by clinical findings of male-type hair growth, hirsutism, or by the measurement of elevated androgen levels or their bioactivity. One of the typical signs of PCOS is anovulation, and often it leads to a finding of polycystic ovaries (PCO) in ultrasonography. Furthermore, anovulation is the most common cause of infertility in these women.

Hyperandrogenism has a prominent role in PCOS, and it can be of both ovarian and adrenal origin. Furthermore, especially in the presence of obesity, decreased concentrations of sex hormone-binding globulin (SHBG) also play a role in hyperandrogenism, resulting in increased levels of bioactive hormones. How androgen secretion changes with ageing in PCOS is not well described. It is an important question, as hyperandrogenism has been associated with increased risks of several adverse health effects such as cardiovascular diseases.

Women with PCOS present with increased risks of insulin resistance, metabolic disorders and development of glucose intolerance and type 2 diabetes mellitus (T2DM). Furthermore, obesity is common among these women. For a long time it was unclear if women with PCOS also had an increased risk of cardiovascular disease. However, today there are several studies supporting this perception.

As women with PCOS present with several health risks, different types of medication for the symptoms have been studied extensively. Insulin-sensitizing drugs have been shown to be useful for the treatment of glucose intolerance and in specific cases of menstrual irregularities and infertility. There has also been a growing interest in statin therapy, as it could potentially affect both dyslipidaemia and hyperandrogenism. However, up to now the most common and widely used forms of medication for menstrual irregularities and hyperandrogenism are combined contraceptives.

The aim of this study was to explore hyperandrogenism of adrenal and ovarian origin, especially age-related changes and the role of menopausal transition in PCOS. Secondly, we aimed to compare the metabolic effects of three different administration routes of combined contraceptives, which are commonly used to treat irregular cycles and hyperandrogenism in women with PCOS.
Thirdly, given that PCOS has a strong metabolic component, there was an interest to investigate the effects of atorvastatin treatment on metabolic factors in women with PCOS.
2 Review of the literature

2.1 Polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome was first described as early as 1935, when doctors Stein and Leventhal discovered an association between excessive hair growth, irregular menstrual cycles and polycystic ovaries (Stein & Leventhal 1935). Today it is widely accepted that PCOS is a hyperandrogenic anovulatory disorder combined with an unfavourable metabolic and inflammatory profile where the first two characteristics are considered as hallmarks of the diagnosis. PCOS is considered to be the most common endocrine dysfunction among fertile-aged women and one of the most common causes of female infertility worldwide. Despite the efforts of the scientific community to define the cause of PCOS, its aetiology and pathogenesis remain unclear. It might involve a genetic predisposition influenced by gestational environment, lifestyle factors or both (Norman et al. 2007). As the syndrome is related to infertility and several adverse health consequences, understanding the mechanisms that cause PCOS is an important goal in medical research. It has been estimated that more than 4 million women suffer from PCOS in the U.S. and the estimated costs for the treatment of different symptoms exceed 4 billion dollars annually, the largest proportion of the money consumed being connected to the treatment of PCOS-associated diabetes (Azziz et al. 2005).

2.1.1 Criteria for PCOS

There are three different diagnostic criteria for PCOS available today, and the definitions underline some findings in cases of PCOS differently. In 1990 the expert conference on PCOS was held in the U.S., sponsored by the National Institutes of Health (NIH). The diagnostic criteria for PCOS were defined by consensus among the participants and were published by Zawadski and Dunaif as follows: “the criteria for PCOS should include (in order of importance): 1) clinical or biochemical signs of hyperandrogenism, 2) chronic anovulation and 3) exclusion of other possible pathologies, such as hyperprolactinemia, thyroid disorders, and nonclassic adrenal hyperplasia” (Zawadski & Dunaif 1992). The NIH criteria represented an important step as regards standardization of the
diagnosis of PCOS, as those in the research field were able to publish studies on women fulfilling standardized diagnostic criteria for PCOS.

However, there was a need for broader diagnostic criteria, as it had been recognised that some women with PCOS displayed ovarian dysfunction without evidence of androgen excess. Therefore, revised diagnostic criteria were defined in 2003 at an expert conference in Rotterdam sponsored by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). The Rotterdam criteria were defined as follows: “the diagnosis of PCOS can be established if at least two of the following criteria are met: 1) oligo- and/or anovulation, 2) clinical and/or biochemical hyperandrogenism, or 3) PCO. Hyperandrogenism should be evaluated by assessing the degree of hirsutism or by biochemical measurement of free testosterone (T) or the free androgen index (FAI). Polycystic ovaries were defined as the presence of 12 or more follicles in one ovary measuring 2–9 mm in diameter, and/or increased ovarian volume (> 10 ml)” (Balen et al. 2003). In addition, other aetiologies such as congenital adrenal hyperplasia, androgen-secreting tumours, Cushing’s syndrome and hyperprolactinaemia should be excluded. The use of Rotterdam criteria has increased the prevalence of PCOS, as the criteria have broadened the spectrum of the clinical symptoms, thereby allowing inclusion of women with milder forms of the syndrome. Notably, recently it has been suggested that a follicle number threshold of more than 25 per ovary could be considered to be a more accurate measure of PCO when modern ultrasonographic technology is used (Dewailly et al. 2014), although in clinical practice ovarian volume measurements may be more useful.

A third set of diagnostic criteria was suggested by The Androgen Excess and PCOS Society (AE-PCOS Society) in 2006 (Azziz et al. 2006). This forum considered that hyperandrogenism was one of the main traits of PCOS and that the Rotterdam criteria were leading to phenotypes of PCOS that were too diverse. Thus, the AE-PCOS Society criteria were defined as follows: 1) hyperandrogenism and 2) ovarian dysfunction (oligo-anovulation and/or PCO) and 3) exclusion of other causes of androgen excess or related disorders.
Table 1. Different phenotypes of PCOS included in different sets of diagnostic criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Hyperandrogenism</th>
<th>Oligo-anovulation</th>
<th>PCO</th>
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<tr>
<td>NIH 1990</td>
<td>+</td>
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<td></td>
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<tr>
<td>Rotterdam 2003</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td></td>
<td>*</td>
<td>+</td>
<td>*</td>
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<tr>
<td>AE-PCOS Society 2006</td>
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The serum level of anti-Müllerian hormone (AMH) has been shown to correlate well with ovarian antral follicle count, and naturally the levels are higher in women with PCOS when compared with healthy women (Maas et al. 2015, Piltonen et al. 2005). Today, the possibility to use assay of AMH produced by the granulosa cells of ovarian follicles for the diagnosis of PCO has been of interest and the results seem promising (Lauritsen et al. 2014). However, the variability of the assays used for the measurement of AMH, has so far been an obstacle to inclusion of AMH levels in the criteria of PCO/PCOS. After the assays become more reliable, the diagnosis of PCO based on AMH measurement may spread into general practice.

Women with PCOS diagnosed by means of NIH criteria (hyperandrogenism with ovarian dysfunction) have a greater prevalence of menstrual irregularities and a greater degree of hyperandrogenism, total and abdominal obesity and insulin resistance and have more severe risk factors of T2DM and cardiovascular disease (CVD) than women diagnosed using Rotterdam or AE-PCOS Society criteria (Wild et al. 2010). Ovulatory women with PCOS have a lower body mass index (BMI) and less abdominal obesity, lesser degrees of hyperandrogenism and hyperinsulinaemia, a reduced prevalence of metabolic syndrome and milder forms of dyslipidaemia, whereas normoandrogenic women with PCOS have the most metabolically favourable profile, in some cases even comparable with that among women without PCOS (Wild et al. 2010).
2.1.2 Prevalence of PCOS

The prevalence of PCOS varies according to the diagnostic criteria used, and it has been estimated to be 0.9–10.7% based on NIH criteria (Asunción et al. 2000, Azziz et al. 2004b, Diamanti-Kandarakis et al. 1999, Knochenhauer et al. 1998, Lauritsen et al. 2014, March et al. 2010, Tehrani et al. 2011, Yildiz et al. 2012), 11.9–19.9% according to Rotterdam criteria (Lauritsen et al. 2014, March et al. 2010, Tehrani et al. 2011, Yildiz et al. 2012) and 10.2–15.3% with respect to AE-PCOS Society criteria (Lauritsen et al. 2014, March et al. 2010, Tehrani et al. 2011, Yildiz et al. 2012). The prevalence of diagnostic features of PCOS has been shown to decrease with age, as the prevalence of PCOS diagnosed by Rotterdam criteria in women under 30 years of age has been found to be 33.3%, but only 10.2% in women older than 35 years. Similarly, the prevalence of PCOS according to AE-PCOS Society criteria was 25.0% in women under 30 years and 9.0% in women more than 35 years (Lauritsen et al. 2014). Furthermore, the prevalence of PCO also declines with age (Koivunen et al. 1999).

2.2 Androgen synthesis and ovulatory dysfunction in PCOS

2.2.1 Hyperandrogenism

In women, biochemical hyperandrogenism is considered when serum levels of total T or free androgens are higher than the upper limit of the normal range. Clinical hyperandrogenism, on the other hand, is diagnosed when a woman presents with hirsutism, excessive male-type hair growth, alopecia or acne. Hirsutism is the most commonly studied clinical marker of hyperandrogenism, whereas acne has been shown to have a poor correlation with hyperandrogenism. Hirsutism is usually evaluated by using the scoring system introduced by Ferriman and Gallwey, where a score of 8 or more indicates hirsutism (Ferriman & Gallwey 1961). Today in clinical practice, the assessment of hirsutism is challenging, as women tend to shave their body-hair intensively.

The majority of women suffering from hyperandrogenism, even up to 82%, are diagnosed as having PCOS (Azziz et al. 2004a), and approximately 60% of women with PCOS have hirsutism, when the diagnosis has been defined by NIH criteria (Azziz et al. 2006, Legro et al. 2006). Hyperandrogenism in women with PCOS originates from both the ovaries and adrenals. In addition, low circulating levels of SHBG in women with PCOS increase the concentration of free
androgens (biologically active forms) and thereby worsen the level of hyperandrogenism. Lower SHBG levels are seen especially in overweight women, as obesity-induced hyperinsulinaemia inhibits the production of SHBG (Lim et al. 2013). Interestingly, hyperandrogenism in women with PCOS is at least partly of genetic origin, as mothers and sisters of women with PCOS present with elevated androgen levels when compared with control women (Yıldız et al. 2003).

Even though circulating levels of androgens are significantly lower in women than in men, androgens are also essential for women, as they serve as substrates for oestrogen biosynthesis. In women, the ovaries and adrenals are capable of synthesising cholesterol de novo from acetate, which serves as a precursor of androgen synthesis. Extracellular cholesterol is transported by circulating lipoproteins, in humans mostly by low-density lipoproteins (LDLs) and less by high-density lipoproteins (HDLs), representing the major source of substrate for steroidogenesis in the ovaries and adrenals. LDL and HDL cholesterol are transferred into cells via endocytosis, and the cholesterol is freed. If cholesterol uptake exceeds the rate of steroidogenesis, free cholesterol is transferred to storage directly as intracellular cholesteryl ester for future use, to ensure its availability (Gwynne & Strauss 1982, Wood & Strauss 2002). In the cell, cholesterol is trafficked to the outer membrane of the mitochondria and translocated to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR). The movement of cholesterol from the outer to the inner mitochondrial membrane by StAR is the rate-limiting step of steroidogenesis (Jamnongjit & Hammes 2006). After cholesterol is transferred to the inner mitochondrial membrane, the enzymatic chain of steroidogenesis can begin (Gwynne & Strauss 1982, Wood & Strauss 2002).

Androstenedione (A), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), T and dihydrotestosterone (DHT) are the main circulating androgens in women (Figure 1). DHT and T bind to nuclear androgen receptors, and thereby these two steroid hormones induce most of the androgenic effects, whereas the first three androgens are considered as pro-androgens, and require conversion to T and DHT to present their androgenic potential (Burger 2002). It has been estimated that at fertile age 25% of A and T production is of ovarian origin, 25% is of adrenal origin, and 50% is produced in peripheral tissues (Baptiste et al. 2010). After menopause the role of peripheral tissues on androgen secretion is emphasized, at adrenal and especially at ovarian expense.
In the circulation the majority of androgens are bound to various carrier proteins, mostly SHBG (carrying 65% of the total amount of circulating T) and albumin (carrying 33%) and only 1–2% of testosterone circulates unbound (Catteau-Jonard,S., Dewailly,D. 2013). As only free androgens have biological activity, calculation of the free androgen index (FAI, 100 × T/SHBG) is of importance when estimating androgenic potential and effects (Burger 2002). Even though the measurement of free androgens (mainly T) would be preferable to the measurement of total T, there are considerable weaknesses in the method and calculation of the FAI is considered to be a significantly more reliable measurement as regards active androgens.
Fig. 1. Androgen synthesis in the ovaries and adrenals.
**Ovarian androgen production**

Pulsatile secretion of pituitary follicle-stimulating hormone (FSH) and luteinising hormone (LH) are regulated by hypothalamic gonadotrophin releasing-hormone (GnRH) (Figure 2). Luteinising hormone stimulates ovarian theca cell androgen production by increasing the expression and activity of StAR (Jamnongjit & Hammes 2006), and the conversion of cholesterol to pregnenolone and further to androgens. This is mediated through an increase in synthesis of the steroidogenic enzymes CYP11A and CYP17 activity (Figure 1) (Payne & Hales 2004, Wood & Strauss 2002). Some of the androgens diffuse to granulosa cells, where they are converted into oestrogens by aromatase enzyme, which is under the control of FSH (Figures 1 and 2). As ovarian androgens are by-products of oestrogen synthesis, their excess is not under specific negative feedback regulation by pituitary LH secretion, and therefore the control of ovarian hyperandrogenism in connection with normal oestrogen synthesis is weak.

![Fig. 2. Ovarian steroid production.](image)

In cases of PCOS the ovaries are known to hyper-secrete androgens, and several mechanisms such as arrested follicular development have been suggested to play a role. Women with PCOS present with altered hypothalamic-pituitary axis
function, shown as increased LH secretion (Cook et al. 2002, Gilling-Smith et al. 1997, McCartney et al. 2004, Morales et al. 1996, Morin-Papunen et al. 2000) and elevated LH pulse frequency and amplitude when compared with healthy women (Eagleson et al. 2000, Morales et al. 1996). Thus these abnormalities promote androgen secretion in ovarian theca cells by enhancing the expression of StAR, CYP11A and CYP17 (Wood & Strauss 2002). The excess ovarian androgen secretion can be explained at least partly by greater LH and diminished FSH responses to GnRH stimulation especially in lean women with PCOS compared with healthy women (Barnes et al. 1989).

In addition to central mechanisms, the number of ovarian theca cells has been shown to be increased in women with PCOS, and they seem to hyper-respond to LH and human chorionic gonadotrophin (hCG) stimulation in vivo even at physiological concentrations (Barnes et al. 1989, Gilling-Smith et al. 1997, McCartney et al. 2004, Piltonen et al. 2004). In vitro, theca cells obtained from women with PCOS have been shown to exhibit increased expression of LH receptors when compared with theca cells obtained from healthy women (Jakimiuk et al. 2001). Furthermore, polymorphism in the LH receptor gene has been associated with an increased risk of PCOS (Mutharasan et al. 2013). These features may partly account for the increased responsiveness to LH and pronounced androgen secretion (Gilling-Smith et al. 1994, Gilling-Smith et al. 1994, McCartney et al. 2004, Nelson et al. 1999). Furthermore, theca cells have been shown to have enhanced gene expression and/or activities of StAR, CYP11A, CYP17, 3β-HSD and 17-HSD (Daneshmand et al. 2002, Jakimiuk et al. 2001, Nelson et al. 1999, Nelson-Degrave et al. 2005, Wickenheisser et al. 2000, Wickenheisser et al. 2004, Wickenheisser et al. 2005). It has also been suggested that women with PCOS can be divided into those with and without pronounced ovarian responses to hCG measured by 17-hydroxyprogesterone (17-OHP) levels (Maas et al. 2015).

Women with PCOS are often insulin resistant. Concomitant hyperinsulinaemia and increased insulin action mimics the gonadotrophic actions of LH, having trophic effects on theca cells, promoting proliferation and increased androgen secretion, as shown in in vitro studies (Nestler et al. 1998, Wu et al. 2014). Furthermore, theca cells obtained from women with PCOS have been suggested to be hyper-responsive and to have a lower stimulatory threshold to insulin when compared with controls (Nestler et al. 1998). An animal study has confirmed a causal pathway between hyperinsulinaemia and divergent ovarian androgen secretion and function, as depletion of insulin receptors in ovarian theca
cells protects mice with diet-induced obesity from these disturbances (Wu et al. 2014). Moreover, several studies have demonstrated that improvement of insulin resistance in women with PCOS results in lower androgen levels, improves ovulatory function and ameliorates the exaggerated steroidogenic response to LH stimulation (Baillargeon et al. 2004, Nestler & Jakubowicz 1996).

Women with PCOS have been found to have elevated serum levels of free insulin-like growth factor I (IGF-I) (Thierry van Dessel et al. 1999). IGFs, which exhibit high structural homology to insulin and signal through IGF-I receptors, have also been shown to promote ovarian steroidogenesis by enhancing the expression and activity of several steroidogenic enzymes such as CYP17 and CYP11A1 (Jamnongjit & Hammes 2006, Nahum et al. 1995, Nestler et al. 1998, Wood & Strauss 2002). Furthermore, IGF signalling has been shown to increase the expression of LH receptors, which in turn enhances LH-induced expression and activity of StAR (Jamnongjit & Hammes 2006).

All in all, the mechanisms that lie behind enhanced ovarian androgen secretion in women with PCOS are complex, with numerous abnormal actions and interactions taking place.

**Adrenal androgen production**

The adrenal cortex is composed of three layers where each layer has separate enzymatic cascades resulting in three different types of steroid hormones: glucocorticoids (zona fasciculata and reticularis), mineralocorticoids (zona glomerulosa) and androgens (zona fasciculata and reticularis). However, even though the zona fasciculata and reticularis mostly express the same enzymes, they differ in enzyme activities. The zona fasciculata produces mainly cortisol and the zona reticularis androgens (Miller & Auchus 2011). The zona glomerulosa synthesizes mineralocorticoids under regulation of the renin-angiotensin system. Steroid production by the adrenal zona reticularis and fasciculata is regulated by pituitary adrenocorticotropic hormone (ACTH), and ACTH is controlled by hypothalamic corticotrophin-releasing hormone (CRH) (Figure 3) (Miller & Auchus 2011). Androgens are used as substrates for mineralocorticoid and glucocorticoid synthesis (Figure 1). As previously mentioned, androgens do not exert significant feedback on the pituitary gland, and therefore they do not have a significant regulatory effect on ACTH production. DHEAS is almost uniquely secreted by the adrenal zona reticularis as a result of its sulphate activity. Thus, in clinical practice, DHEAS is often measured in order to evaluate the rate of
adrenal androgen production, even though the liver and small intestine also have the capability of converting DHEA to DHEAS (Goodarzi et al. 2015).

**Fig. 3. Adrenal steroid production.**

Around 20–65% of women with PCOS have been shown to have increased adrenal androgen production (Carmina et al. 1986, Carmina et al. 1992, Gallagher et al. 1958, Gallagher et al. 1958, Hoffman et al. 1984, Kumar et al. 2005). It has been postulated that women that are genetically prone to secrete more adrenal androgens would have a greater predisposition to develop PCOS (Azziz et al. 2001).

The possible mechanisms behind enhanced adrenal androgen secretion in PCOS have been of great interest. Interestingly, serum ACTH levels are comparable in women with PCOS and healthy women (Chang et al. 1982, Hoffman et al. 1984, Lanzone et al. 1995, Stewart et al. 1993). Furthermore, the pituitary response to CRH stimulation seems to be similar in women with PCOS with and without adrenal hyperandrogenism (Azziz et al. 1998), although contrasting findings have also been reported (Lanzone et al. 1995). It is possible that women with PCOS secrete above-normal levels of ACTH during stress or
other central nervous system stimulus, as these women have been shown to present with increased sympathetic nervous system activity (Sverrisdottir et al. 2008). Furthermore, increased peripheral metabolism or impaired reactivation of cortisol may result in decreased negative feedback suppression of ACTH secretion (Tsilchorozidou et al. 2003).

Women with PCOS have an increased capacity to secrete androgens during ACTH stimulation when compared with healthy controls (Azziz et al. 1998, Cinar et al. 2012, Glintborg et al. 2005). In particular, hyperandrogenic women with PCOS exhibit higher basal and ACTH-stimulated adrenal androgen secretion compared with non-hyperandrogenic women with PCOS, whose adrenal androgen secretion is comparable to that in controls (Cinar et al. 2012). Thus, the aetiology of adrenal hyperandrogenism in women with PCOS appears to be related to pronounced activity of the adrenal cortex and not to abnormal function of the hypothalamic-pituitary-axis or increased sensitivity of the adrenal cortex to ACTH stimulation. This is supported by the finding that women with PCOS and increased adrenal androgen secretion have increased 17α-hydroxylase activity of CYP17, although not all investigators agree (Azziz et al. 1995, Moran et al. 2004). Mutations in the CYP21 gene, which would increase steroid production towards the CYP17 route, do not seem to play a role in adrenal hyperandrogenism (Glintborg et al. 2005). In vivo, a strong correlation between the levels of adrenal androgens and insulin has been found in blood samples taken from adrenal veins in hyperandrogenic women (Martikainen et al. 1996). However, the relationship is incompletely understood and studies with contrasting results have been published (Goodarzi et al. 2015). Elevated levels of estradiol or increased peripheral sulphatase activity may also have a role in the increase of serum DHEAS levels (Carmina et al. 1999).

Elevated serum LH levels could participate in adrenal hyperandrogenism in women with PCOS, as the presence of LH receptors has been found in steroidogenic cells of the human zona reticularis and fasciculata (Pabon et al. 1996). It has been reported that transgenic female mice over-expressing LH develop aberrant adrenal gland function entailing altered morphology of the adrenals, increased LH receptor expression and elevated corticosterone production (Kero et al. 2000). Furthermore, in women with PCOS salivary gland cortisol levels positively correlate with LH levels (Tock et al. 2014), supporting a link between LH and adrenal function. However, in a clinical setting adrenal steroid secretion in healthy women does not seem to respond to short-term hCG/LH exposure (Piltonen et al. 2002). Nevertheless, the length of time of
exposure to LH may play an important role in this issue. All in all, several extra-adrenal factors may influence adrenal androgen secretion and participate in the pathogenesis of adrenal hyperandrogenism in PCOS.

**Peripheral tissues**

The most important organs that take part in peripheral androgen conversion are the skin, liver, lungs and adipose tissue. These tissues have 3β-HSD and 17β-HSD activity, and thereby they can convert DHEA and DHEAS to A and subsequently to T (Payne & Hales 2004). DHT, the biologically active form of T, is converted in the target tissue by 5α-reductase. Furthermore, peripheral tissues have an important role in regulating the levels of circulating androgens, as they have aromatase activity, which converts androgens to oestrogens.

**The effect of age on ovarian and adrenal androgen production**

It is estimated that approximately 400 ovulations occur during a woman’s lifespan. The rest of the follicles are depleted through apoptosis, programmed cell death (Vaskivuo et al. 2001). The number of follicles decreases gradually and by the age of 50 years a woman reaches the menopause, where the follicle pool has become exhausted and only a low number of follicles remains. As a result of follicle depletion, ovarian secretion of oestrogens decreases dramatically towards menopause. The secretion of androgens has also been shown to decrease towards menopause, and a change in circulating androgen levels can be detected as early as after the age of 20 years. The decrease seems to be steepest in the early reproductive years, with stabilisation before menopausal transition (Davison et al. 2005). In postmenopausal women only the adrenal cortex and ovarian stroma produce some androgens (Couzinet et al. 2001). The availability of free androgens remains stable or even increases as SHBG levels decrease during menopausal transition (Burger et al. 2000).

Ovarian androgen production decreases towards menopause both in healthy women and in women with PCOS. However, ovarian capacity to synthesise and release androgens remains high through the reproductive years in women with PCOS when compared with healthy women (Piltonen et al. 2003, Piltonen et al. 2004). Similarly, a cohort study performed in Sweden revealed significantly elevated T levels in middle-aged women with PCOS, but the difference had
disappeared after 21 years of follow-up (mean ages 49 and 70 years) (Schmidt et al. 2011).

Adrenal DHEAS levels increase markedly during the adolescent years in girls; they peak in early adulthood and decrease linearly with age thereafter (Orentreich et al. 1984). Similarly to ovarian aging, adrenal androgen production in hyperandrogenic women and women with PCOS decreases with age (Kumar et al. 2005, Moran et al. 1999). However, in a follow-up study of 3–5 years duration, serum DHEAS levels remained stable in the majority of women with PCOS (Yildiz et al. 2004).

2.2.2 Oligoamenorrhoea

Oligoamenorrhoea (cycle length more than 35 days) and amenorrhoea (absence of menstrual bleeding for 3 months or more) are typical signs of anovulatory cycles in PCOS. In fact, NIH criteria for PCOS require menstrual dysfunction for the diagnosis of PCOS. However, Rotterdam and AE-PCOS criteria are broader, as the diagnosis can be set without menstrual dysfunction. Among women diagnosed by using Rotterdam criteria, approximately 90% have oligo- or amenorrhoea (March et al. 2010). Interestingly, women with menstrual disturbances were diagnosed with PCOS with up to 90% probability in a study carried out in Sri Lanka (Kumarapeli et al. 2008), whereas the Northern Finland Birth Cohort Study revealed only a 62% probability of being diagnosed with PCOS (Rotterdam criteria), when the women reported having oligomenorrhoea (Taponen et al. 2004a). Interestingly only 30–40% of women with amenorrhoea have PCOS (Sirmans & Pate 2013).

2.2.3 Polycystic ovaries (PCO)

The recruitment of resting follicles and follicular maturation is a continuous process from fetal life throughout women’s reproductive years until menopause. The recruitment, growth and development of primordial follicles until the preantral stage takes 6 months or more and is gonadotrophin-independent, as these changes can occur even with minimal circulating FSH levels (Gougeon 1996) and in women with inhibiting FSH receptor mutations (Aittomaki et al. 1996). Menstrual cyclicity is based on changes in specific hormone levels. The increase in FSH levels as early as in the late luteal phase of the previous menstrual cycle, due to a decrease of serum E2 levels from the atrophic corpus
luteum, induces the growth of follicles towards ovulation and stimulates the production of \( E_2 \). One of the preovulatory follicles grows faster than the others and is selected as dominant follicle. During the late follicular phase granulosa cells in the dominant follicle produce high amounts of \( E_2 \), resulting in positive feedback, sensitizing the pituitary to GnRH and consequently sharply increased LH levels, leading to ovulation. Granulosa and theca cells from the ovulated follicle form a corpus luteum, which secretes progesterone under the regulation of LH. Regression of the corpus luteum in the late luteal phase enables follicle maturation for the next menstrual cycle (Messinis et al. 2014).

The microscopic and macroscopic ovarian architecture in cases of PCOS is abnormal, as these women have a greater density of primordial, primary, preantral and antral follicles when compared with normal ovaries (Dewailly et al. 2010, Hughesdon 1982, Maciel et al. 2004, Pigny et al. 2003, Webber et al. 2003). The follicles in PCO stop growing and developing, typically when they reach 4–7 mm in diameter (Franks et al. 1998), and hyperandrogenism seems to play a central role in this divergent early follicular growth (Abbott et al. 2005, Jonard et al. 2003, Vendola et al. 1998). The cessation of folliculogenesis at this stage results in the accumulation of multiple irregular-sized preantral and antral follicles typically situated in the ovarian cortex, with pearl-shaped manifestation, thereby generating the typical appearance of a PCO. However, it is important to notice that PCO criteria do not define “typical” PCO morphology, as only the number of follicles or ovarian volume are counted. Furthermore, a PCO finding in only one ovary is sufficient. Even though PCO are related to anovulation, this distinctive ovarian morphology is also observed in 6–16% of the female population with normal menstrual cycles (Abdel Gadir et al. 1992, Koivunen et al. 1999, Polson et al. 1988).

### 2.3 Metabolic alterations and health consequences of PCOS

Although infertility is common in cases of PCOS, irregular cycles, acne and hirsutism may be the most common reasons for seeking medical care during reproductive life. Obesity, dyslipidaemia, low-grade inflammation and hypertension together with insulin resistance are the most important determinants of long-term health in women with PCOS, and their roles increase with ageing.
2.3.1 Obesity

The World Health Organization defines overweight BMI as 25.0–29.9 kg/m² and obesity as BMI ≥ 30 kg/m². Not only weight but also the distribution of the fat tissue matters: abdominal obesity is considered more deleterious than female-type obesity, and the measurement of waist circumference and waist to hip ratio (WHR) are useful tools to assess the future risks of T2DM and CVD.

An overweight condition and obesity are common characteristics of women with PCOS, and the prevalence of these features varies between different countries (Table 2). It seems that in the U.S. women with PCOS are significantly more obese than the general population, whereas in Europe this is not the case. In one study, women with PCOS in the U.S. had a significantly higher BMI (35.1 kg/m²) than women in Italy (27.0 kg/m²). Despite the difference in BMI, WHRs were similar in these women. The difference in BMI was not explained by total calorie intake but by a higher amount of saturated fat in women living in the U.S. (Carmina et al. 2003).

Table 2. The prevalence of obesity among women with PCOS

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Criteria</th>
<th>N</th>
<th>PCOS women</th>
<th>General population</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diamanti-Kandarakis 1999</td>
<td>Greece</td>
<td>NIH</td>
<td>13</td>
<td>38%</td>
<td>Overweight</td>
<td>Obese</td>
<td></td>
</tr>
<tr>
<td>Asunción 2000</td>
<td>Spain</td>
<td>NIH</td>
<td>10</td>
<td>30%</td>
<td>Overweight</td>
<td>Obese</td>
<td>10%</td>
</tr>
<tr>
<td>Cibula 2000</td>
<td>Czech R</td>
<td>NIH</td>
<td>28/752</td>
<td>36%</td>
<td>Overweight</td>
<td>Obese</td>
<td>40%</td>
</tr>
<tr>
<td>Azziz 2004</td>
<td>U.S.</td>
<td>NIH</td>
<td>11/400</td>
<td>24%</td>
<td>Overweight</td>
<td>Obese</td>
<td>24%</td>
</tr>
<tr>
<td>Taponen 2004</td>
<td>Finland</td>
<td>Symptoms²</td>
<td>67/66³</td>
<td>BMI 25.9 kg/m²</td>
<td>Overweight</td>
<td>Obese</td>
<td>32%</td>
</tr>
<tr>
<td>Lo 2006</td>
<td>U.S.</td>
<td>PCOS⁴</td>
<td>12734</td>
<td>67.0%</td>
<td>Overweight</td>
<td>Obese</td>
<td>31.4%</td>
</tr>
<tr>
<td>Yildiz 2012</td>
<td>Turkey</td>
<td>NIH</td>
<td>24</td>
<td>25.0%</td>
<td>Overweight</td>
<td>Obese</td>
<td>10.2%</td>
</tr>
<tr>
<td></td>
<td>Rotterdam</td>
<td></td>
<td>78</td>
<td>15.4%</td>
<td>Overweight</td>
<td>Obese</td>
<td>10.2%</td>
</tr>
<tr>
<td></td>
<td>AE-PCOS</td>
<td></td>
<td>60</td>
<td>15.0%</td>
<td>Overweight</td>
<td>Obese</td>
<td>10.2%</td>
</tr>
<tr>
<td>Joham 2014</td>
<td>Australia</td>
<td>Self-reported</td>
<td>478</td>
<td>25.7%</td>
<td>Overweight</td>
<td>Obese</td>
<td>22.4%</td>
</tr>
<tr>
<td>Lauritsen 2014</td>
<td>Denmark</td>
<td>Rotterdam</td>
<td>74</td>
<td>16.2%</td>
<td>Overweight</td>
<td>Obese</td>
<td>13.1%</td>
</tr>
</tbody>
</table>

¹The definition of obesity BMI > 28.9 kg/m²
²The symptoms of PCOS are self-reported signs of hyperandrogenism and/or irregular menstruation and the finding of PCO in ultrasonography.
³The number of women with PCOS / the number of control women
⁴The diagnosis of PCOS was not further defined in the publication.

BMI has been shown to correlate with an increased rate of hirsutism, serum T concentrations, menstrual cycle disturbances and infertility in women with PCOS (Lim et al. 2013, Liou et al. 2009). Obesity further increases the risks of T2DM and CVD. Given that obesity significantly increases the severity of clinical features of PCOS (Table 3), weight loss is important for these women. Even 5–10% weight loss has been shown to improve PCOS symptoms and endocrine profiles (Moran et al. 2009).

Table 3. Detrimental effects of obesity in PCOS

<table>
<thead>
<tr>
<th>Endometrium &amp; pregnancy</th>
<th>Androgenic features</th>
<th>Glucose metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual irregularities†</td>
<td>Androgen secretion†</td>
<td>Insulin resistance†</td>
</tr>
<tr>
<td>Dysfunctional bleeding†</td>
<td>SHBG levels ↓</td>
<td>Hyperinsulinaemia↑</td>
</tr>
<tr>
<td>Infertility↑</td>
<td>Hirsutism↑</td>
<td>T2DM↑</td>
</tr>
<tr>
<td>Gestational diabetes↑</td>
<td>Acne↑</td>
<td>Dyslipidaemia↑</td>
</tr>
<tr>
<td>Pre-eclampsia↑</td>
<td>Preterm birth↑</td>
<td>Birth-weight of singletons↑</td>
</tr>
<tr>
<td>Caesarean sections↑</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(De Frène et al. 2014, Lim et al. 2013, Liou et al. 2009)

The association between obesity and the prevalence of PCOS has not been demonstrated in all studies (Table 4). However, the observations support the assumption that PCOS may be more prevalent among overweight and obese women.

Table 4. The prevalence of PCOS in different BMI groups

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Criteria</th>
<th>N</th>
<th>Underweight</th>
<th>Normal weight</th>
<th>Overweight</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alvarez-Blasco</td>
<td>Spain†</td>
<td>NIH</td>
<td>113</td>
<td>40.0%</td>
<td>25.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yildiz 2008</td>
<td>U.S.</td>
<td>NIH</td>
<td>675</td>
<td>8.2%</td>
<td>9.8%</td>
<td>9.9%</td>
<td>9.0%</td>
</tr>
<tr>
<td>Yildiz 2012</td>
<td>Turkey</td>
<td>NIH</td>
<td>392</td>
<td></td>
<td></td>
<td>5.1%</td>
<td>15.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rotterdam</td>
<td>392</td>
<td></td>
<td></td>
<td>18.8%</td>
<td>30.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AE-PCOS</td>
<td>392</td>
<td></td>
<td></td>
<td>14.5%</td>
<td>22.5%</td>
</tr>
</tbody>
</table>

†The prevalence of PCOS in the general population in the same city is 6.5% (Asunción et al. 2000).


Up to as many as 80–85% of women with PCOS diagnosed by NIH criteria have abdominal obesity (Ehrmann et al. 2006, Glueck et al. 2003). When waist circumference exceeds 88 cm, the risks of T2DM and CVD are significantly
increased. Most women with PCOS are thought to have an abdominal body fat distribution regardless of BMI. This assumption was supported by studies performed in Sweden and Ireland showing that women with PCOS have higher WHRs compared with BMI-matched controls (Mannerås-Holm et al. 2011, O'Connor et al. 2010). However, these results have not been confirmed in magnetic resonance or computed tomography imaging studies concerning fat distribution between visceral, abdominal, gluteal and mid-femoral subcutaneous regions (Barber et al. 2008, Ezeh et al. 2013, Mannerås-Holm et al. 2011, Yalamanchi et al. 2012). Abdominal subcutaneous and visceral fat accumulation in cases of PCOS has been reported in three other studies involving ultrasonography, total-body dual X-ray absorptiometry and subcutaneous adipose tissue topography (Carmina et al. 2007, Horejsi et al. 2004, Yildirim et al. 2003). Interestingly, a cohort study performed in Sweden revealed a significant difference in WHR between middle-aged women with PCOS and controls (mean age 49 years), but the difference had disappeared later in life (mean age 70 years) (Schmidt et al. 2011).

Today, the metabolic abnormalities seen in PCOS are not primarily thought to result from an increased amount of abdominal adipose tissue, but they may be linked to abnormal adipocyte function and morphology. Adipocytes aspirated from women with PCOS are significantly larger when compared with those from control women with a similar BMI (Dunaif et al. 1992, Faulds et al. 2003, Mannerås-Holm et al. 2011). This finding most likely contributes to altered adipose tissue and metabolic functions and the increased risk of T2DM in these women, as adipocyte size negatively correlates to adipose tissue insulin sensitivity (Salans et al. 1968), and enlarged adipocyte size has been shown to be a risk factor of T2DM (Lönn et al. 2010).

2.3.2 Insulin sensitivity and glucose tolerance

Insulin is secreted by the β-cells of the pancreatic islets of Langerhans in response to increased circulating levels of glucose and amino acids after a meal (Pessin & Saltiel 2000), and its role is to maintain whole body glucose homeostasis and promote efficient glucose utilization. The human INS gene encodes preproinsulin, which is converted to proinsulin. Insulin, a heterotetrameric protein consisting of two α-subunits and two β-subunits, is produced by cleavage of C-peptide from proinsulin.
In the liver, insulin inhibits the production of glucose by inhibiting gluconeogenesis and glycogenolysis and also promotes glycogen storage. In muscle and adipose tissue, insulin stimulates the uptake, storage and use of glucose (Moller & Flier 1991, Pessin & Saltiel 2000). Insulin also affects lipid metabolism by increasing lipid synthesis in the liver and adipocytes and it attenuates fatty acid release by suppressing lipolysis from triglycerides in adipose tissue and muscle, resulting in a decrease in circulating free fatty acid levels (Diamanti-Kandarakis & Dunaif 2012, Muniyappa et al. 2008, Pessin & Saltiel 2000).

Obesity, body fat distribution and muscle mass all have important independent effects on insulin sensitivity. Abnormally low, i.e. impaired insulin sensitivity is called insulin resistance. It can be defined as a decreased ability of insulin to mediate its metabolic actions on glucose uptake, glucose production and lipolysis, resulting in a requirement for an increased amount of insulin to achieve euglycaemia (Diamanti-Kandarakis & Dunaif 2012). This leads to an increased release of insulin from the pancreas, and thereby compensatory hyperinsulinaemia, which is a characteristic of insulin resistance. As a consequence of insulin resistance, glucose concentrations rise slightly, although remaining within the normal range before reaching the diagnostic criteria for glucose intolerance (Smith 1994). The most important factors underlying insulin resistance are obesity, physical inactivity and genetic factors. Furthermore, insulin resistance is tightly associated with major public health problems – obesity, hypertension, dyslipidaemias, coronary artery disease and metabolic syndrome (Ginsberg 2000).

Impaired glucose regulation (impaired glucose tolerance [IGT] and impaired fasting glucose [IFG]) refers to a metabolic state intermediate between normal glucose homeostasis and diabetes. Furthermore, IGT and IFG are not clinical entities but risk categories as regards future T2DM and/or CVD (World Health Organization 2006). The development of T2DM, a state where euglycaemia is not reached, originates from insulin resistance occurring in skeletal muscle and adipose tissue, leading to failure in glucose uptake from the blood, with a simultaneous insufficient compensatory increase in insulin production from β-cells. Thus, it is a metabolic aetiology characterized by chronic hyperglycaemia resulting from defects in insulin secretion or action, or both (Alberti & Zimmet 1998). Impaired glucose tolerance, IFG and T2DM can be diagnosed from fasting blood samples or by way of oral glucose tolerance tests (OGTTs). In Table 5, the specific limits for diagnosing these disorders are listed.
Table 5. The World Health Organization criteria for diagnosing diabetes mellitus, impaired glucose tolerance and impaired fasting glycaemia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Glucose concentration in plasma (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>≥ 7.0</td>
</tr>
<tr>
<td>2-hour glucose</td>
<td>≥ 11.1</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>&lt; 7.0</td>
</tr>
<tr>
<td>2-hour glucose</td>
<td>7.8 ≤ glucose ≤ 11.0</td>
</tr>
<tr>
<td>Impaired fasting glycaemia</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>6.1 ≤ glucose ≤ 6.9</td>
</tr>
<tr>
<td>2-hour glucose</td>
<td>&lt; 7.8</td>
</tr>
</tbody>
</table>

(World Health Organization 2006)

Measuring glucose homeostasis

The gold standard for assessing insulin resistance in vivo is the hyperinsulinaemic, euglycaemic glucose clamp technique, which directly determines metabolic insulin sensitivity (Muniyappa et al. 2008). At steady state, the amount of glucose that is infused equals the amount of glucose taken up by the peripheral tissues, and it can be used as a measure of peripheral sensitivity to insulin – insulin-mediated glucose disposal (Diamanti-Kandarakis & Dunaif 2012, Dunaif 1997). Insulin sensitivity can also be evaluated indirectly by way of the intravenous glucose tolerance test (IVGTT). The minimal model provides a measurement of insulin sensitivity on the basis of glucose and insulin data obtained during IVGTTs (Muniyappa et al. 2008). This index reflects insulin action in stimulating glucose uptake and suppressing glucose production (Diamanti-Kandarakis & Dunaif 2012).

Because both the above-mentioned techniques are time-consuming, expensive and experimentally demanding methods to accomplish in practical use, the possibility to use fasting parameters as measurements of insulin resistance has been studied. Fasting levels of glucose or insulin are not suitable for insulin resistance measurements by themselves, as fasting glucose reflects endogenous glucose production (an index of hepatic insulin action) and fasting insulin reflects insulin secretion and metabolic clearance in addition to insulin sensitivity (Hücking et al. 2008). The indexes better describing insulin resistance are homeostatic model assessment data (Matthews et al. 1985), the fasting glucose to insulin ratio, and the quantitative insulin sensitivity check index (Katz et al. 2008).
The oral glucose tolerance test is widely used in clinical practice as a first-line measurement, and it reflects the efficiency of the body to dispose of glucose after an oral glucose load (Muniyappa et al. 2008). As an advantage, the OGTT mimics glucose and insulin dynamics under physiological conditions more closely than conditions of the glucose clamp or the IVGTT (Hücking et al. 2008). The OGTT provides useful information about glucose tolerance but not insulin sensitivity or resistance per se.

Biochemical screening of glucose tolerance is indicated for at least obese women with PCOS and those with increased visceral adiposity as measured by waist circumference. However, a current statement from the AE-PCOS Society recommends an OGTT with assessment of fasting and 2-hour glucose concentrations for all women diagnosed with PCOS (Salley et al. 2007, Wild et al. 2010), as fasting glucose levels are poorly associated with 2-h glucose concentrations in women with PCOS (Ehrmann et al. 2005). The Endocrine Society recommends rescreening by way of an OGTT every 3–5 years or even more often if a woman is obese, for example (Legro et al. 2013). In addition, if an OGTT is not possible, insulin resistance of women with PCOS may also be evaluated by measurement of fasting glucose and insulin concentrations and calculation of the glucose to insulin ratio. A ratio of < 4.5 is considered significant for insulin resistance in obese Caucasian women with PCOS, and the ratio has been shown to correlate well with insulin sensitivity obtained by way of IVGTTs in women with PCOS (Legro et al. 1998). Assay of glycated haemoglobin, HbA1c, the level of which reflects average plasma glucose concentrations over the previous month, has not been found to be a useful tool, as in women with PCOS it has low sensitivity to detect IGT and T2DM when compared with OGTTs (Velling Magnussen et al. 2011).

**Prevalence of glucose intolerance and related factors in PCOS**

Insulin resistance and compensatory hyperinsulinemia play a prominent role in the pathogenesis of PCOS, since as many as 60–80% of women with this syndrome suffer from these conditions, independently of obesity (Stepto et al. 2013, Wild et al. 2010). Insulin resistance has been recognised as a major risk factor as regards the development of T2DM (Lillioja et al. 1993), and PCOS has been associated with higher prevalence rates of IGT, gestational diabetes mellitus.
(GDM) and T2DM (Boomsma et al. 2006, Ehrmann et al. 1999, Kjerulff et al. 2011, Legro et al. 1999, Moran et al. 2010, Norman et al. 2001). Furthermore, insulin resistance has been shown to correlate positively with FAI and triglyceride and CRP levels, and negatively with SHBG concentrations (Karakas et al. 2010).

Table 6. Prevalence of IGT and T2DM in women with PCOS

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Criteria</th>
<th>PCOS N</th>
<th>Control women N</th>
<th>IGT</th>
<th>T2DM</th>
<th>IGT</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahlgren 1992</td>
<td>Sweden</td>
<td>PCO^2</td>
<td>33/132^1</td>
<td></td>
<td>15.0%</td>
<td>2.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ehrmann 1999</td>
<td>U.S.</td>
<td>NIH</td>
<td>122</td>
<td></td>
<td>35.2%</td>
<td>9.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legro 1999</td>
<td>U.S.</td>
<td>NIH</td>
<td>254/80^1</td>
<td></td>
<td>31.1%</td>
<td>7.5%</td>
<td>14.0%</td>
<td>0%</td>
</tr>
<tr>
<td>Cibula 2000</td>
<td>Czech R</td>
<td>NIH</td>
<td>287/52^1</td>
<td></td>
<td>32.1%</td>
<td>8.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ehrmann 2005</td>
<td>U.S.</td>
<td>NIH</td>
<td>408</td>
<td></td>
<td>23.0%</td>
<td>3.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lo 2006</td>
<td>U.S.</td>
<td>PCOS^3</td>
<td>11035/55175^1</td>
<td></td>
<td>9.0%</td>
<td>1.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hudecova 2011</td>
<td>Sweden</td>
<td>Rotterdam^4</td>
<td>84/87^1</td>
<td></td>
<td>9.5%</td>
<td>8.3%</td>
<td>2.3%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Joham 2014</td>
<td>Australia</td>
<td>Self-reported</td>
<td>478/8134^1</td>
<td></td>
<td>5.1%</td>
<td>0.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sirmans 2014</td>
<td>U.S.</td>
<td>ICD-9^5</td>
<td>1689/5067^1</td>
<td></td>
<td>17.6%</td>
<td>4.7%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^1Number of women with PCOS / number of control women
^2Histopathologically confirmed PCO
^3Diagnoses of PCOS not further defined in the publication
^4All women presented with PCO
^5ICD-9 diagnosis code for PCOS or oligomenorrhoea/amenorrhoea and hyperandrogenism


The prevalence of glucose intolerances are increased in women with PCOS (Table 6). A meta-analysis has revealed that the odds ratios (ORs) for IGT and T2DM are significantly increased in women with PCOS compared with BMI-matched controls, being 2.54 for IGT and 4.00 for T2DM (Moran et al. 2010). Therefore, it has been recommended that all women with PCOS should be screened for glucose intolerance by means of OGTTs (Legro et al. 2013, Salley et al. 2007).

Of women with PCOS, 15–67% are overweight or obese (Table 2). A recent study revealed that the impact of BMI on insulin resistance is even greater in cases of PCOS than in controls (Stepto et al. 2013). Even though the risk of glucose intolerance is further amplified by obesity (Boudreaux et al. 2006, Dunaif et al. 1992, Ehrmann et al. 1999, Legro et al. 1999), lean women with PCOS have also been shown to have increased rates of insulin resistance and glucose intolerance compared with BMI-matched healthy women (Dunaif et al. 1989, Legro et al. 1999, Morales et al. 1996, Stepto et al. 2013). The risk of glucose
intolerance varies among women with PCOS, as it may be highest in women with ovulatory dysfunction and hyperandrogenism, and lowest among women with anovulation and PCO without hyperandrogenism (Barber et al. 2007, Barber et al. 2007, Dewailly et al. 2006). Interestingly, irregular menstrual cycles have been shown to present as an independent risk factor of T2DM in women in general (Solomon et al. 2001).

Besides having an increased risk of T2DM, women with PCOS are at high risk of developing GDM. In a meta-analysis it was estimated that women with PCOS have a significantly higher risk of developing GDM (OR 2.94) compared with women without the syndrome (Boomsma et al. 2006). In addition, a large population-based study revealed a 2.4-fold increased risk of GDM in women with PCOS (Lo et al. 2006a). Moreover, women with previous GDM presented with PCO more often than controls (Koivunen et al. 2001).

Hyperinsulinaemia and defects in insulin secretion in PCOS

Pancreatic β-cell dysfunction lies behind the development of T2DM, as hyperglycaemia develops when insulin secretion is not sufficient for the increased requirements of insulin resistance. Therefore, the ability to dispose of carbohydrates depends on the responsiveness of pancreatic β-cells to glucose as well as the sensitivity of the glucose-utilizing tissues to secreted insulin (Bergman et al. 1981). The high prevalence of hyperglycaemia in women with PCOS suggests the presence of defects in insulin secretion in addition to peripheral insulin resistance.

It is well known that basal insulin secretion rates are elevated in women with PCOS, which results from the greater degree of insulin resistance compared with that in healthy women. However, previous studies have also suggested that women with PCOS present with a greater degree of compensatory insulin secretion for a given increment in insulin resistance, in a fasting state (Goodarzi et al. 2005). Even though first-phase insulin secretion has been shown to be normal in women with PCOS, decreased postprandial secretion of insulin in these women has been reported independently of obesity, especially when insulin release is proportioned to the degree of insulin resistance (Dunaif & Finegood 1996, Ehrmann et al. 1995, Ehrmann et al. 2004, O'Meara et al. 1993). However, studies with differing results have also been published (Ciampelli et al. 1997, Holte et al. 1994). The decreased postprandial insulin secretion resembles pancreatic β-cell dysfunction, which is also present in T2DM and may therefore
explain the high prevalence of glucose intolerance in women with PCOS. Interestingly, women with PCOS who present with a first-degree relative with T2DM are more likely to present these defects (Colilla et al. 2001, Ehrmann et al. 1995). Furthermore, daughters of women with PCOS as young as 8–12 years old present with postprandial β-cell dysfunction (Torchen et al. 2014). These findings suggest a genetic contribution to the disturbances in β-cell function in PCOS. One possible explanation for alterations in insulin secretion may be changes in the secretion of incretins, which augment insulin secretion after a meal. In lean women with PCOS, increased total glucose-dependent insulinotropic polypeptide and lower late-phase active glucagon-like peptide-1 concentrations during OGTTs have been observed when compared with BMI- and age-matched control subjects (Vrbikova et al. 2008). However, another study did not reveal an independent effect of PCOS on the incretin response (Svendsen et al. 2009). Furthermore, inflammation has been suggested to play a role in β-cell dysfunction in PCOS (Malin et al. 2015).

2.3.3 Cardiovascular risk factors and outcomes

Women with PCOS present with several risk factors of CVD. Both the Endocrine Society and The AE-PCOS Society have published recommendations that adolescents and women with PCOS should be screened for cardiovascular disease risk factors, as shown in Table 7 (Legro et al. 2013, Wild et al. 2010).
Table 7. Different cardiovascular risk categories of women with PCOS (Legro et al. 2013, Wild et al. 2010)

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Symptom / Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>At risk – women with any of the following</td>
<td>Obesity (especially increased abdominal obesity)</td>
</tr>
<tr>
<td></td>
<td>Cigarette smoking</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
</tr>
<tr>
<td></td>
<td>Dyslipidaemia</td>
</tr>
<tr>
<td></td>
<td>Subclinical vascular disease</td>
</tr>
<tr>
<td></td>
<td>Impaired glucose tolerance</td>
</tr>
<tr>
<td></td>
<td>Family history of premature cardiovascular disease</td>
</tr>
<tr>
<td>At high risk – women with</td>
<td>Metabolic syndrome</td>
</tr>
<tr>
<td></td>
<td>Type 2 diabetes</td>
</tr>
<tr>
<td></td>
<td>Vascular or renal disease, cardiovascular diseases</td>
</tr>
<tr>
<td></td>
<td>Obstructive sleep apnoea</td>
</tr>
</tbody>
</table>

Dyslipidaemia

Disturbances in lipid and lipoprotein metabolism are fairly common metabolic abnormalities in women with PCOS, diagnosed in approximately 70% of such women in the U.S. (Diamanti-Kandarakis et al. 2007, Legro et al. 2001, Randeva et al. 2012). Similarly, the incidence of dyslipidaemia in Brazil has been reported to be as high as 76% (Rocha et al. 2011). The diagnostic limits of dyslipidaemia can be seen in Table 8.

Table 8. Diagnostic limits of dyslipidaemia in women in Finland. An abnormal concentration can be found in one or more variable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL cholesterol</td>
<td>&gt; 3.0 mmol/l</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&gt; 1.7 mmol/l</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>&lt; 1.2 mmol/l</td>
</tr>
</tbody>
</table>

There are several studies in which lipid abnormalities in women with PCOS have been explored. In general, decreased levels of HDL cholesterol together with elevated levels of triglycerides and LDL cholesterol have been reported among these women (Berneis et al. 2007, Conway et al. 1992, Ehrmann et al. 2006, Glueck et al. 2003, Glueck et al. 2009, Legro et al. 2001, Rocha et al. 2011, Yildirim et al. 2003). Although the results concerning the levels of LDL cholesterol have been divergent, a meta-analysis revealed that women with PCOS present with significantly higher serum LDL cholesterol concentrations, even
when BMI has been taken into account (Wild et al. 2011). Furthermore, women with PCOS have elevated levels of small, dense LDL cholesterol particles compared with age- and BMI-matched controls (Berneis et al. 2007, Berneis et al. 2009, Dejager et al. 2001, Pirwany et al. 2001), which further exposes these women to CVD, as small dense LDL particles are taken up by arterial wall more easily and have decreased oxidative susceptibility compared with larger LDL particles (Rizzo & Berneis 2006). Furthermore, both normal-weight and obese women with PCOS have been shown to present with increased levels of oxidised LDL cholesterol when compared with age- and BMI-matched controls (Macut et al. 2006). All in all, the most common lipid profile in PCOS seems to be an atherogenic lipoprotein phenotype characterized by hypertriglyceridaemia, increased levels of small, dense LDL cholesterol and decreased HDL cholesterol levels, representing a risk factor of coronary heart disease (CHD) (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) 2002).

In PCOS, the main pathophysiological mechanisms of dyslipidaemia are probably central adiposity, hyperandrogenism and insulin resistance, but diet, exercise and genetics have their roles as well. However, overweight conditions and obesity have been suggested to be predominant factors affecting dyslipidaemia in cases of PCOS (Joharatnam et al. 2011). This hypothesis was supported by the results of a well-matched case-control study, which revealed differences only in the composition of LDL particles (Phelan et al. 2010).

**Chronic inflammation**

Atherosclerosis has been shown to involve a chronic inflammatory process (Ross 1999). At present, C-reactive protein (CRP) appears to be the classic marker of inflammation and it is commonly measured by a highly sensitive method able to detect very low concentrations. However its use in clinical practice is still random. CRP is released by the liver in response to interleukin-6 and it acts both as a marker and a contributor to the vascular inflammatory process (Deanfield et al. 2005). Low-grade chronic inflammation is characterized by persistent moderately elevated CRP concentrations within the normal range. CRP independently predicts future coronary events such as myocardial infarction, stroke, coronary revascularization processes and cardiovascular death in asymptomatic individuals more strongly and more significantly than other inflammatory markers (Ridker et al. 1998, Ridker et al. 2000,
Rifai et al. 2002) or even LDL (Ridker et al. 2002). Furthermore, there is growing evidence that the lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can be described as an inflammatory disease (Ross 1999). In addition, inflammation plays a role in destabilizing the fibrous cap in coronary plaques, predisposing individuals to plaque rupture and thereby enhancing the risk of coronary thrombosis (van der Wal et al. 1994). Moreover, the results of an in vitro study suggested that CRP may play a direct role in promoting the inflammatory component of atherosclerosis (Pasceri et al. 2000). Obesity, metabolic syndrome and cigarette smoking have been shown to be associated with increased CRP levels (Tracy et al. 1997, Visser et al. 1999). Another marker of chronic inflammation, pentraxin 3 (PTX-3), is produced by peripheral tissues such as vascular endothelial cells, smooth muscle cells and leucocytes in response to proinflammatory signals. Therefore its secretion is not influenced by drug-induced hepatic protein synthesis (Mantovani et al. 2008).

It has been shown that women with PCOS present with increased levels of CRP when compared with control women, even after adjustment for age and BMI (Boulman et al. 2004, Diamanti-Kandarakis et al. 2006, Diamanti-Kandarakis et al. 2006, Kelly et al. 2001). Positive relationships between CRP levels and insulin resistance, body weight and adipose tissue mass have also been observed (Diamanti-Kandarakis et al. 2006, Diamanti-Kandarakis et al. 2006, Kelly et al. 2001, Morin-Papunen et al. 2003). In addition, levels of other low-grade chronic inflammation markers including cell adhesion molecules are increased in PCOS (Diamanti-Kandarakis et al. 2006, Diamanti-Kandarakis et al. 2006). Furthermore, levels of the proinflammatory cytokine interleukin-18, which promotes the synthesis of interleukin-6, are elevated in women with PCOS (Escobar-Morreale et al. 2004).

**Endothelial dysfunction**

The endothelium is a thin single-cell layer that covers the inner surface of the blood vessels and the heart. The endothelium plays a key role in the maintenance of a healthy vascular wall by regulating vascular tone and inhibiting several preatherogenic processes such as monocyte recruitment, platelet adhesion, LDL oxidation, synthesis of pro-inflammatory cytokines and smooth muscle cell proliferation (Cannon 1998). Endothelial dysfunction can be defined as a reduction of the bioavailability of vasodilators, such as nitric oxide, and an increase in vasoconstrictors, such as endothelin-1 (ET-1), produced by or acting
on endothelial cells (Deanfield et al. 2005), and it is an early marker of atherosclerosis (Brunner et al. 2005). In addition to nitric oxide, insulin has a physiological action in the vasodilation of skeletal muscle vasculature in humans (Cleland et al. 1998).

When obese women with PCOS were compared with weight-matched controls, women with PCOS exhibited significantly diminished endothelium-dependent vasodilation and ability of insulin to cause vasodilation (Diamanti-Kandarakis et al. 2006, Kelly et al. 2002, Paradisi et al. 2001). A diminished vasodilatory response seems to be at least in part due to impaired production and/or release of nitric oxide (Paradisi et al. 2001), and it can be normalized by way of metformin treatment (Diamanti-Kandarakis et al. 2005). Therefore, one could speculate that the insulin resistance mechanisms concerning impaired vasodilatation are the same as seen in adipocytes and skeletal muscle, as discussed above.

Increased serum levels of ET-1 have also been reported in women with PCOS when compared with BMI-matched controls (Diamanti-Kandarakis et al. 2001, Diamanti-Kandarakis et al. 2005, Diamanti-Kandarakis et al. 2006, Orio et al. 2004). Furthermore, the difference remained when BMI was used as a covariate (Diamanti-Kandarakis et al. 2001). A putative cause of the increased ET-1 levels could be insulin resistance, as insulin stimulates secretion of ET-1 in vivo and in vitro (Ferri et al. 1995). In line with this, metformin treatment reduces the level of ET-1 in PCOS (Diamanti-Kandarakis et al. 2001, Diamanti-Kandarakis et al. 2005).

In line with previous findings, reduced vascular compliance in the brachial and carotid arteries of women with PCOS has been reported, versus controls (Kelly et al. 2002, Lakhani et al. 2002). Decreased vascular compliance is a feature of subclinical atherosclerosis, as arterial elasticity is reduced during fatty-streak formation before other pathological changes occur (Hironaka et al. 1997), and it has a strong correlation with cardiovascular and all-cause mortality (Laurent et al. 2001).

The role of androgens as regards impaired endothelial function in cases of PCOS is an interesting question. Endothelial function has been found to be inversely correlated with serum free T levels (Paradisi et al. 2001). Furthermore, androgens have induced endothelial dysfunction in animal models (Hutchison et al. 1997). In line with this, in female to male transsexuals, androgen administration has been associated with impaired vascular reactivity, consistent with a deleterious effect of androgen excess on arterial physiology (McCredie et al. 2005).
The mechanism by which hyperandrogenism affects vascular reactivity is unknown. It is possible that T could have direct effects on the vessel wall, although an indirect effect via alteration of the lipoprotein profile cannot be excluded.

**Hypertension**

Essential hypertension is a highly prevalent pathological condition that is considered as one of the most relevant cardiovascular risk factors and is an important cause of morbidity and mortality around the world. Hypertension is considered, when systolic blood pressure is ≥ 140 mmHg or diastolic blood pressure is ≥ 90 mmHg (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) 2002).

Among women with PCOS who are of reproductive age, 21–48% develop elevated blood pressure or hypertension (≥ 130/85 mmHg) (Apridonidze et al. 2005, Dokras et al. 2005, Ehrmann et al. 2006, Glueck et al. 2003). A study performed in the U.S. revealed that 26.6% of women with PCOS aged from 15 to 44 years had diagnosed hypertension or elevated blood pressure compared with 11.7% of age-matched controls and women with PCOS were more likely to have elevated blood pressure or hypertension even after adjustment for BMI, age, diabetes and dyslipidaemia (Lo et al. 2006b). Furthermore, a recent cohort study revealed that women with PCOS have an OR of 2.85 for hypertension when compared with controls (Sirmans et al. 2014). Notably, a study performed in Sweden revealed that 39% of women with PCOS aged from 40 to 59 years were being treated for hypertension, whereas only 11% of age-matched control women used medication for this condition (Dahlgren et al. 1992). Twenty-one years later, 69% of the women with PCOS and 41% of the controls had hypertension and the difference was significant (Schmidt et al. 2011). However, some investigators have not found an increased prevalence of hypertension in cases of PCOS (Wild et al. 2000).

Obesity has been suggested as the primary cause of elevated blood pressure in women with PCOS (Luque-Ramírez et al. 2007). Furthermore, hyperandrogenism might have a role (Chen et al. 2007). In addition, increased activity of the sympathetic nervous system in PCOS results in vasoconstriction, the extent of which strongly correlates with hyperandrogenism (Sverrisdottir et al.
In summary, the development of hypertension in PCOS is multifactorial and the role of PCOS per se is not well understood.

**Subclinical atherosclerosis**

One of the first detectable stages of atherosclerosis is thickening of the arterial wall. Carotid intima-media thickness (IMT) is the thickness of two layers (the intima and media) of the walls of the carotid arteries, the largest conduits of blood going to the brain (Roger et al. 2012). Ultrasonographic measurement of common carotid IMT is a morphological marker used to assess precocious atherosclerosis (van den Oord et al. 2013) and a recent meta-analysis revealed that increased IMT is a predictor of future myocardial infarction and stroke (van den Oord et al. 2013). Increased IMT has been associated with general and abdominal obesity and increasing insulin resistance (De Michele et al. 2002, Rajala et al. 2002). It has also been reported in relatively young women with PCOS when compared with control women (Orio et al. 2004), as well as in women with PCOS older than 40 years (Guzick et al. 1996, Talbott et al. 2000). Furthermore, 7.2% of women with PCOS and 0.7% of control women have been reported to have a carotid plaque index of three or more (Talbott et al. 2000), indicating more intense carotid atherosclerosis in these cases.

Coronary artery calcification is a measure of the extent of atherosclerosis in the heart arteries. Detection of coronary calcium confirms the presence of coronary atherosclerotic plaque independently of symptoms (Roger et al. 2012). Women with PCOS have a significantly greater prevalence and extent of coronary artery and aortic calcification than age-matched ovulatory control women (Christian et al. 2003, Talbott et al. 2004). After adjustment for BMI, PCOS did not predict the presence of coronary calcium in one study (Christian et al. 2003), but it remained a significant predictor of coronary artery calcification in another (Talbott et al. 2004).

Diastolic dysfunction is considered to be an early sign of coronary artery disease. When women with PCOS were compared with BMI-matched but older control women, they presented several unfavourable changes in diastolic function and therefore they may be likely to develop diastolic dysfunction (Yarali et al. 2001).
Cardiovascular disease, cardiovascular events and mortality

As women with PCOS present with several risk factors of CVD, a question is whether they also have greater prevalence rates of CVDs and events, or deaths caused by CVD. It has been estimated that on the basis of specific risk factors, women with PCOS aged 40–49 yr present with a fourfold increased risk and women aged 50–60 yr even an 11-fold risk of developing CVD. However, divergent results concerning CVD diagnosis have been published. Symptoms related to PCOS such as hirsutism, irregular menstruation and PCO have been associated with coronary artery disease (Azevedo et al. 2006, Birdsall et al. 1997, Wild et al. 1990), but not all studies support this (Krentz et al. 2007).

Studies carried out to investigate the association between PCOS and CVD are few and the diagnosis of PCOS varies. Furthermore, the subjects are relatively young in some studies. Coronary artery disease has been reported to be more prevalent in women with PCOS in some (Cibula et al. 2000, Shaw et al. 2008) but not all (Wild et al. 2000) studies. Again, women with PCOS may have a higher prevalence of cerebrovascular disease (Wild et al. 2000). Cumulative cardiovascular event-free survival may be significantly shorter in women with PCOS and these women might even have a 3.3-fold greater risk of cardiovascular death or myocardial infarction compared with women without PCOS (Shaw et al. 2008), although differing results exist (Elting et al. 2001, Pierpoint et al. 1998, Schmidt et al. 2011). One meta-analysis of five studies revealed a twofold increased risk of CHD and stroke in women with PCOS when compared with women without the syndrome. When studies with results adjusted for BMI were investigated, the risk further increased by 55% (de Groot et al. 2011). In summary, the conclusions drawn from the results of clinical studies vary. When women with definitive diagnoses of PCOS are older, more data can be analysed and the answers will be found in the future.

2.4 Treatment of PCOS-related disorders

2.4.1 Combined hormonal contraceptives

Combined hormonal contraceptives (CHCs), including both oestrogens and progestogens, are the most widely used forms of contraception in the world. Besides contraception, these agents can be used in the treatment of menstrual irregularities,
hirsutism and acne, including women with PCOS. Furthermore, CHCs are used in connection with severe dysmenorrhea, dysfunctional uterine bleeding, endometriosis and scheduling the cycles for in vitro fertilization. Combined hormonal contraceptives can be administered orally, transdermally or vaginally.

The effects of CHCs are based on the effects of both oestrogens and progestogens. The most frequently used oestrogen is ethinyl estradiol (EE), but today more physiological oestrogens are available such as 17β-estradiol and estradiol valerate. Ethinyl estradiol exerts a stronger effect on hepatic metabolism than natural oestrogens. There are several different progestins available with various chemical structures, clinical effects and metabolic risks. The progestins are structurally related either to testosterone or progesterone. New 4th generation progestins have been designed to bind with high specificity to progesterone receptors and avoid interactions with androgen, oestrogen and glucocorticoid receptors, and they are promising in the treatment of hyperandrogenism. Unfortunately they are associated with a slightly increased risk of venous thrombosis when compared with 2nd generation preparations (Sitruk-Ware & Nath 2011). Progestins have been divided into different generations based on the occasions when they have been introduced to the market.

In addition to contraceptive effects, CHCs modify surrogate markers such as glucose tolerance, the lipid profile and coagulation factors, which have been associated with cardiovascular and venous risks. A cross-sectional study revealed 7.1-fold increased risk of IGT in current users and a 2.1-fold risk in former users of oral contraceptives compared with nonusers (Deleskog et al. 2011). However, this has not been confirmed as regards T2DM (Chasan-Taber et al. 1997, Rimm et al. 1992), and CHCs are not considered to cause clinically significant changes in glucose tolerance (Lopez et al. 2014). The oestrogen component is primarily responsible for the insulin resistance induced by CHCs, in a dose-dependent matter (Godsland et al. 1992, Kojima et al. 1993). When male to female transsexuals were treated with EE, peripheral glucose uptake was diminished, reflecting impaired insulin sensitivity (Polderman et al. 1994). Progesterone receptor expression has been observed in the pancreas (Pasanen et al. 1997), and progestins seem to increase insulin half-life, to varying degrees (Godsland et al. 1992, Kojima et al. 1993). In addition, the androgenic properties of progestins might play a role in the effects of CHCs on insulin sensitivity, as androgen supplementation in female to male transsexuals decreases insulin sensitivity (Polderman et al. 1994) and administration of anti-androgens improves it (Moghetti et al. 1996).
The effect of different CHCs on the lipid profile reflects the balance of oestrogen and progestin, both in terms of steroid dose and progestin type. Generally, oestrogen administration results in a favourable lipid profile and the magnitude is related to the potency of the oestrogen. On the other hand, progestins may counteract these effects and the detrimental effects are related to the androgenic properties of progestins (Sitruk-Ware & Nath 2013). Interestingly, CHCs may reduce LDL particle size (Crook & Godsland 1998), and they have been associated with a denser LDL subfraction pattern (de Graaf et al. 1993). Exposure to EE increases hepatic secretion of particles that are triglyceride-rich such as very low density lipoprotein (VLDL) and thereby the use of combined contraceptives leads to increased levels of triglycerides.

The Endocrine Society recommends the use of CHCs as first-line treatment for the menstrual irregularities, hirsutism and acne in women with PCOS. It does not suggest one formulation or administration route over another, and, naturally, it recommends screening for possible contraindications before the use of hormonal contraceptives (Legro et al. 2013). In a recent review, individualized risk stratification prior to prescribing combined hormonal contraceptives was suggested (Yildiz 2015).

2.4.2 Insulin sensitizers

Insulin sensitizers are drugs that improve insulin sensitivity in target cells. Insulin-lowering agents improve endocrine and reproductive abnormalities in women with PCOS by reducing insulin resistance and hyperinsulinaemia as these conditions are considered to be significant contributors to the pathophysiology of PCOS.

Metformin

Metformin is an orally administered drug that has been used extensively in the treatment of T2DM for decades. The beneficial effect of metformin on insulin sensitivity is complex (Hardie 2013). Furthermore, a small decrease in body weight might also contribute to its effects. The effects of metformin on glucose metabolism include a reduction of hepatic glucose production by inhibition of gluconeogenesis in the liver, an increase in glucose uptake by stimulation of GLUT4 expression and intracellular glucose transport, acceleration of glucose
utilization by enhancement of glucose metabolism, and activation of insulin receptors by stimulating their tyrosine kinase activity (De Leo et al. 2003).

Metformin is the most extensively studied insulin-lowering drug used in the treatment of PCOS. It improves insulin resistance and hyperandrogenism and increases the levels of SHBG in these women (Palomba et al. 2009)(Nestler & Jakubowicz 1996, Velazquez et al. 1994). It has also been widely reported that metformin improves menstrual irregularity and restores ovulatory function (De Leo et al. 2003, Fleming et al. 2002). The results of some randomized placebo-controlled studies (Kjøtrod et al. 2011, Morin-Papunen et al. 2012), but not all (Fleming et al. 2002), suggest that metformin treatment improves pregnancy and live-birth rates in women with PCOS. A meta-analysis revealed an improvement in clinical pregnancy rate but not in live-birth rate during metformin treatment versus placebo (Tang et al. 2012). In line with this, similar results were reported when metformin was used before or during assisted reproductive techniques (Tso et al. 2014). Moreover, metformin improves metabolic cardiovascular risk parameters in PCOS, such as dyslipidaemia (Fleming et al. 2002, Rautio et al. 2005) and chronic inflammation (Morin-Papunen et al. 2003). The advantage of metformin is that it is considered to be non-teratogenic drug (Cassina et al. 2014).

The Endocrine Society gave recommendations concerning metformin use in women with PCOS in 2013. Metformin use is recommended for women with PCOS and IGT or T2DM who fail to modify their lifestyles. The Endocrine Society recommends metformin as a second-line therapy for the treatment of menstrual irregularities in women who cannot take or do not tolerate hormonal contraceptives. However, in clinical practice, when treating obese women, metformin can also be considered as first-line treatment. Furthermore, the Endocrine Society suggests the use of metformin as adjuvant therapy during in vitro fertilization in order to prevent ovarian hyperstimulation syndrome. Notably, the Endocrine Society does not suggest the use of metformin in first-line treatment of cutaneous manifestations, for prevention of pregnancy complications, or for the treatment of obesity (Legro et al. 2013).

Thiazolidinediones

Thiazolidinediones, TZDs, were first introduced as promising drugs for the treatment of insulin resistance, as they enhance insulin action in skeletal muscle, liver and adipose tissue. However, the first TZD, troglitazone, was withdrawn from the market by the U.S. Food and Drug administration in 2000 as a result of
its hepatotoxicity. In Europe, only one TZD, pioglitazone, is currently available, as rosiglitazone has been withdrawn from the market because of an excess of cardiovascular events.

Thiazolidinediones have been shown to improve menstrual cyclicity, hyperandrogenism, insulin resistance and hyperinsulinaemia in women with PCOS (Rautio et al. 2006). However, as TZDs have been suspected of retarding fetal development in animal studies, their use is contraindicated for infertility treatment and during pregnancy. The Endocrine Society does not recommend the use of thiazolidinediones for the treatment of PCOS because of safety concerns (Legro et al. 2013).

2.4.3 Statins

Statins are widely used to reduce plasma cholesterol levels and the risk of cardiovascular events (Taylor et al. 2013). They reduce the conversion of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) to L-mevalonate by inhibiting the activity of HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. The reduction in the intracellular levels of cholesterol induces enhanced LDL receptor expression on hepatocyte cell surfaces, resulting in increased extraction of LDL cholesterol from the blood and thereby decreased circulating concentrations. In addition to decreased cholesterol levels, this results in inhibition of the biosynthesis of isoprenoids such as geranylgeranyl pyrophosphate and farnesyl pyrophosphate. These isoprenoid metabolites participate in the maintenance of cell shape, motility, factor secretion, differentiation and proliferation. Furthermore, cholesterol-independent beneficial effects of statins include, for example, improvement of endothelial function, decrease of vascular inflammation and oxidative stress, and stabilization of atherosclerotic plaques (Gazzerro et al. 2012). According to their pharmacokinetics, statins can be classified as lipophilic (atorvastatin, fluvastatin, lovastatin and simvastatin) and hydrophilic (pravastatin, rosuvastatin) (Gazzerro et al. 2012). Lipophilic statins show efficient activity at both hepatic and extra-hepatic sites, whereas hydrophilic statins are more hepato-selective (Gazzerro et al. 2012). The most important adverse effects associated with statins are myopathy and an asymptomatic increase in hepatic transaminases (Girardi 2014). It has been suggested that the risk of myopathy may be lowest with hydrophilic statins as a result of minor muscle penetration (Girardi 2014).
As up to 70% of women with PCOS present with dyslipidaemia and an increased risk of CVD (Wild et al. 2010), the use of statins is often indicated. As cholesterol serves as a substrate for androgen synthesis, the possible effects of statin use on hyperandrogenism besides cardiovascular health have also been studied. However, there is no good evidence available that statins would improve menstrual irregularities, ovulation rate, hirsutism or acne in cases of PCOS (Raval et al. 2011). Unfortunately, the use of statins cannot be extended to women who wish to become pregnant, as statin treatment during pregnancy is considered to be teratogenic. However, the results of some studies suggest that statins may not be major teratogens and animal studies suggest that statins might even be a good therapeutic option for the treatment of pre-eclampsia (Girardi 2014). The American Endocrine Society does not recommend the use of statins for the treatment of hyperandrogenism or anovulation until there are more studies that demonstrate more benefits than risks (Legro et al. 2013). The studies concerning statin therapy in women with PCOS are specified in Table 9.
Table 9. Studies on statin therapy in women with PCOS.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Design</th>
<th>No</th>
<th>Criteria</th>
<th>Treatment</th>
<th>Dose</th>
<th>Duration</th>
<th>BMI</th>
<th>Age T</th>
<th>DHEAS</th>
<th>f-insulin</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duleba 2006</td>
<td>Randomised</td>
<td>24</td>
<td>Rotterdam</td>
<td>simvastatin + OCP</td>
<td>20 mg</td>
<td>12 weeks</td>
<td>21.7</td>
<td>24.0</td>
<td>↓</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Banaszewska 2007</td>
<td>Randomised cross-over</td>
<td>45</td>
<td>Rotterdam</td>
<td>simvastatin + OCP</td>
<td>20 mg</td>
<td>12 weeks</td>
<td>22.3</td>
<td>24</td>
<td>↓</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Banaszewska 2009</td>
<td>Randomised</td>
<td>40</td>
<td>AE-PCOS</td>
<td>simvastatin</td>
<td>20 mg</td>
<td>3 months</td>
<td>23.1</td>
<td>26.1</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td>Rotterdam</td>
<td>simvastatin</td>
<td>20 mg</td>
<td>6 months</td>
<td>↓</td>
<td>↓</td>
<td>→</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Kaya 2009</td>
<td>Randomised</td>
<td>32</td>
<td>Rotterdam</td>
<td>atorvastatin</td>
<td>20 mg</td>
<td>12 weeks</td>
<td>24.7</td>
<td>23.4</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>Rotterdam</td>
<td>simvastatin</td>
<td>20 mg</td>
<td></td>
<td>25.2</td>
<td></td>
<td></td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Sathyapalan 2009</td>
<td>RDBPC</td>
<td>19</td>
<td>NIH criteria</td>
<td>atorvastatin</td>
<td>20 mg</td>
<td>12 weeks</td>
<td>33.2</td>
<td>26.6</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Kaya 2010</td>
<td>Randomised</td>
<td>32</td>
<td>Rotterdam</td>
<td>atorvastatin</td>
<td>20 mg</td>
<td>3 months</td>
<td>24.0</td>
<td>27.6</td>
<td>↓</td>
<td>→</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>Rotterdam</td>
<td>simvastatin</td>
<td>20 mg</td>
<td></td>
<td>24.2</td>
<td>26.8</td>
<td>↓</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Kazerooni 2010</td>
<td>Randomised placebo-controlled</td>
<td>42</td>
<td>Rotterdam</td>
<td>metformin +</td>
<td>500 mg</td>
<td>12 weeks</td>
<td>28.5</td>
<td>25.6</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>simvastatin</td>
<td></td>
<td>20 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raja-Khan 2011</td>
<td>RDBPC</td>
<td>9</td>
<td>NIH and LDL&gt;2.6</td>
<td>atorvastatin</td>
<td>40 mg</td>
<td>6 weeks</td>
<td>40.1</td>
<td>33.8</td>
<td>→</td>
<td>↓</td>
<td>→</td>
</tr>
<tr>
<td>Sathyapalan 2012</td>
<td>RDBPC</td>
<td>40</td>
<td>atorvastatin</td>
<td></td>
<td>20 mg</td>
<td>3 months</td>
<td></td>
<td></td>
<td>↓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No, Number of subjects; IS, insulin sensitivity; OCP, oral contraceptive pill; RDBPC, randomized double blind placebo-controlled

3 Purpose of the present study

The pathogenesis of PCOS has been studied intensively and not just a single factor but several mechanisms have been identified. Furthermore, for a long time the focus in PCOS was on infertility and hyperandrogenic symptoms such as acne and hirsutism and not much attention was paid to the long-term metabolic consequences of the syndrome. However, as the age-related health risks became obvious the diversity of the syndrome revealed itself.

There are only a few studies available on age-related androgen secretion in women with PCOS and hardly any data on the effect of menopausal transition on the hormonal and metabolic profiles in these women. As women with PCOS usually have oligomenorrhoea, CHCs are commonly used to control the menstrual cycle and improve androgenic symptoms. Moreover, these women often have an atherogenic lipid profile and an increased risk of T2DM and therefore they could benefit from statin therapy. At least theoretically, statins could also improve hyperandrogenism by decreasing cholesterol, which is a proximal substrate of androgens. However, both CHCs and statins have also been suggested to have unbeneﬁcial metabolic effects, and the use of statins in particular has been associated with disturbed carbohydrate metabolism and even an increased risk of T2DM. Thus, the specific aims were:

1. To evaluate age-related changes and the role of menopausal transition on adrenal and ovarian androgen secretion, glucose metabolism and inflammation in women with PCOS (Studies I and II)
2. To study the effect of different administration routes of CHCs on hormonal, metabolic and inflammatory parameters (Study IV)
3. To evaluate whether or not atorvastatin is useful in improving hyperandrogenism and metabolic and inflammatory alterations in women with PCOS (Study III)
4 Subjects and methods

All study protocols were approved by the Ethics Committee of Oulu University Hospital. Studies III and IV were also approved by the Finnish Medicines Agency. Studies III and IV were registered at the Clinical Trials Register (http://clinicaltrials.gov) with identifier codes NCT01072097 and NCT01087879 and the EU Clinical Trials Register (https://www.clinicaltrialsregister.eu) with identifier codes 2006-003584-31 and 2007-004984-23.

4.1 Subjects

All subjects signed a written informed consent document prior to participating in the study.

Table 10. Characteristics of the subjects in Studies I–IV.

<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 who completed the study</td>
<td>58 PCOS* 69 Controls</td>
<td>21 PCOS* 29 Controls</td>
<td>28 PCOS* 42 Controls</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>18–59 PCOS 19–62 Controls</td>
<td>43–59 PCOS 45–62 Controls</td>
<td>29–50 20–33</td>
<td>20–33</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.0–42.9 PCOS 19.2–35.0 Controls</td>
<td>25.9–36.9 PCOS 20.8–29.7 Controls</td>
<td>19.9–53.8 17.9–26.4</td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td>-</td>
<td>-</td>
<td>Atorvastatin or placebo</td>
<td>Oral, transdermal or vaginal CHC</td>
</tr>
<tr>
<td>Duration of treatment</td>
<td>-</td>
<td>-</td>
<td>6 months</td>
<td>9 weeks</td>
</tr>
</tbody>
</table>

*PCOS according to Rotterdam criteria
4.1.1 Women with PCOS (Studies I, II and III)

Women with PCOS were recruited from the Department of Obstetrics and Gynaecology, Oulu University Hospital. In addition, for Study III, subjects were also recruited from the central hospital of Kemi and the district hospital of Oulaskangas, and also by advertising in local newspapers and personnel handouts at Oulu University Hospital.

A diagnosis of PCOS was made if at least two of the following criteria were met: 1) oligomenorrhoea (intermenstrual interval > 35 d or irregular menstruation [menstrual interval > 7 d different from one period to another]), 2) hyperandrogenism (hirsutism score ≥ 8 [Ferriman-Gallwey score] (Ferriman & Gallwey 1961) or serum testosterone ≥ 2.7 nmol/L), or 3) PCO (observed in transvaginal ultrasonography; at least eight follicles of 3–8 mm in diameter in one plane, in at least one ovary). Thus, the criteria were based on the Rotterdam consensus (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004) except for the diagnosis of PCO which was determined according to the old PCO criteria presented by Adams (Adams et al. 1985). In addition, other disorders that can present with a PCOS-like phenotype, such as hyperprolactinaemia, Cushing’s syndrome, androgen-secreting tumours and 21-hydroxylase-deficient non-classic adrenal hyperplasia were excluded in the clinical investigation and by using assay of 17-OHP.

The PCOS diagnoses were made for all participants during their reproductive age and confirmed by the researchers from the medical records if the women were pre- or postmenopausal when they entered the study. For postmenopausal women with PCOS, the diagnosis was based on oligomenorrhoea or irregular menstruation combined with hyperandrogenism reported in their medical records, as ultrasonographic examinations were not available at the time of their diagnosis. A woman was considered postmenopausal if she had not had menstrual bleeding for at least one year or her serum FSH level was more than 30 U/l. In Study II, one premenopausal and one postmenopausal woman with PCOS had undergone hysterectomy. Subjects with previous ovarian drilling, wedge resection or oophorectomy were excluded.

4.1.2 Control (healthy) subjects (Studies I, II and IV)

Control women were recruited by advertising in personnel handouts at Oulu University Hospital and by sending advertisement emails to students at the
Faculty of Medicine, University of Oulu and the School of Health and Social Care, Oulu University of Applied Sciences.

Control subjects had regular menstrual cycles at fertile age, no signs of hyperandrogenism and normal-appearing ovaries in transvaginal ultrasonography when entering the study. Postmenopausal women had not had menstrual bleeding for at least a year and their serum FSH levels were over 30 U/l.

4.1.3 Exclusion criteria and medication

General exclusion criteria included pregnancy and lactation, cigarette smoking, abuse of alcohol, previous history of cancer, and medication for glucose tolerance or hyperlipidaemia. Further information on medication and wash-out periods are clarified in original publications I–IV.

4.2 Methods

4.2.1 Study visits

All study visits were performed at the research unit of the Department of Obstetrics and Gynaecology, Oulu University Hospital, with the exception of three-month blood samplings in Study III, which were performed at the nearest laboratory for subjects at a long distance from Oulu. In these cases, the blood samples were sent to the Oulu research unit on the day of collection. All visits were performed between 7:00–12:00h.

4.2.2 Intervention measures

Clinical measurements

Blood pressure was measured after a 15-minute rest in a sitting position. Weight was measured with underwear and height was measured without shoes. Body mass index was calculated as follows: weight (kg) / [height (m)]^2. Waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest and hip circumference at the maximum circumference over the buttocks. Thus, the measurements were performed according to the World Health Organization STEPS protocol (World 2005) with a
soft tape, and the waist to hip ratio was calculated as \[\frac{\text{waist circumference (cm)}}{\text{hip circumference (cm)}}\].

**Imaging examinations**

Vaginal ultrasonography was performed at the first study visit in all Studies (I–IV). The size of the ovaries was measured using three different dimensions (length, width, thickness) and the number of follicles in each ovary was calculated in one plane (Studies I–III) or in the whole ovary (Study IV).

Although the breasts of all subjects were clinically examined, one subject was diagnosed with breast cancer after entering Study II. Thereafter, mammography within one year before entering Study II was required. If this was not the case, mammography was performed prior to the study examinations.
Table 11. Interventions used in Studies I–IV

<table>
<thead>
<tr>
<th>Method / variable</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
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<tbody>
<tr>
<td><strong>Tests</strong></td>
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</tr>
<tr>
<td>ACTH test</td>
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<td></td>
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<tr>
<td>hCG test</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td>OGTT</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IVGTT</td>
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<tr>
<td><strong>Hormonal parameters</strong></td>
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</tr>
<tr>
<td>LH</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>17-OHP</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>X</td>
<td>X</td>
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<td>X</td>
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<td>T</td>
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<tr>
<td>Inhibin-B</td>
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</tr>
<tr>
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<td>X</td>
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<td>FAI</td>
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<tr>
<td><strong>Glucose tolerance</strong></td>
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<tr>
<td>Glucose</td>
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</tr>
<tr>
<td>Insulin</td>
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<td>X</td>
<td></td>
</tr>
<tr>
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<tr>
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<td>HDL cholesterol</td>
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<tr>
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<td></td>
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<td>X</td>
</tr>
</tbody>
</table>

*Atorvastatin*

In Study III the subjects were randomized to use atorvastatin (20 mg/day; Lipitor®, Pfizer Incorporated) or placebo for 6 months. The subjects were advised to take the medication in the evening before going to bed.

Randomization was conducted using a computer-generated randomization list in blocks of six. It was performed at the Pharmacy of Oulu University Hospital by personnel not involved in the study. They also repacked the medication into
envelopes, which were sequentially numbered and then closed. The subjects were advised not to change their physical activity or dietary habits during the study period.

**Combined contraceptives**

In Study IV the subjects were randomized to use oral contraceptive pills (EE 20 μg and desogestrel 150 μg; Mercilon®; Organon Ltd., Dublin, Ireland), a transdermal contraceptive patch (EE 20 μg/day and norelgestromin 150 μg/day; Ortho Evra®; Janssen Pharmaceutica N.V., Beerse Belgium) or a contraceptive vaginal ring (EE 15 μg/day and etonogestrel 120 μg/day; NuvaRing®; N.V. Organon, Oss, the Netherlands) continuously for 9 weeks.

Randomization was carried out with an allocation ratio of 1:1:1 and a block size of six. The randomization list was computer-generated and constructed by a pharmacist at Oulu University Hospital. The research nurse controlled the randomization list and assigned participants to their groups at their first study visit.

The subjects started the treatment after the first study visit on cycle days 2–4, and they were advised to use barrier contraception during the first 7 days. Advice on use of the different contraceptive methods was given by the research nurse and the researcher at study-entry. The subjects were told not to change their dietary habits or physical activity during the study.

**4.2.3 Laboratory analyses**

Blood samples were centrifuged (3000 rpm, 10 minutes), and serum and EDTA plasma were stored at -20 °C (Studies I and II) or at -80 °C (Studies III and IV) for later analyses. However, serum glucose and plasma creatinine and ALAT measurements were performed immediately. All assays were performed according to the instructions of the reagent manufacturers, and details are given in Table 12.
Table 12. Characteristics of the laboratory assays used in Studies I–IV.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method</th>
<th>Typical sensitivity</th>
<th>Coefficient of intra-assay variation (%)</th>
<th>Coefficient of inter assay variation (%)</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-OHP</td>
<td>RIA</td>
<td>0.2 nmol/l</td>
<td>5.0</td>
<td>5.4</td>
<td>0.6–8.8 nmol/l</td>
</tr>
<tr>
<td>A</td>
<td>RIA/CIA</td>
<td>0.14/1.0 nmol/l</td>
<td>5.0/6.3</td>
<td>7.0/8.6</td>
<td>1.4–14.3 nmol/l</td>
</tr>
<tr>
<td>T</td>
<td>ACLS/LC-MS/MS</td>
<td>0.35/0.03 nmol/l</td>
<td>4.0/5.3</td>
<td>5.6/5.3</td>
<td>0.4–2.3 nmol/l</td>
</tr>
<tr>
<td>DHEA</td>
<td>RIA</td>
<td>0.1 nmol/l</td>
<td>7.9</td>
<td>11.9</td>
<td>9–38 nmol/l</td>
</tr>
<tr>
<td>DHEAS</td>
<td>RIA/CIA</td>
<td>0.03/0.08 µmol/l</td>
<td>5.3/6.5</td>
<td>7.0/9.3</td>
<td>1–14 µmol/l</td>
</tr>
<tr>
<td>Cortisol</td>
<td>ACLS</td>
<td>5.0 nmol/l</td>
<td>4.0</td>
<td>4.3</td>
<td>150–650 nmol/l</td>
</tr>
<tr>
<td>E₂</td>
<td>RIA</td>
<td>5 pmol/l</td>
<td>4.6</td>
<td>5.8</td>
<td>0.07–0.53 nmol/l</td>
</tr>
<tr>
<td>LH</td>
<td>ACLS</td>
<td>0.07 U/l</td>
<td>2.9</td>
<td>3.8</td>
<td>2–10 U/l</td>
</tr>
<tr>
<td>FSH</td>
<td>ACLS</td>
<td>0.3 U/l</td>
<td>2.1</td>
<td>2.8</td>
<td>2–12 U/l</td>
</tr>
<tr>
<td>Inhibin-B</td>
<td>ELISA</td>
<td>&lt;15 ng/l</td>
<td>&lt;7</td>
<td>&lt;7</td>
<td>30–90 ng/l</td>
</tr>
<tr>
<td>SHBG</td>
<td>TRFIA/CIA</td>
<td>0.02/0.5 nmol/l</td>
<td>3.9/2.7</td>
<td>4.6/5.2</td>
<td>20–140 nmol/l</td>
</tr>
<tr>
<td>CRP</td>
<td>immunonephelometry</td>
<td>0.18 mg/l</td>
<td>0.9</td>
<td>3.5</td>
<td>&lt;3 mg/l</td>
</tr>
<tr>
<td>PTX-3</td>
<td>ELISA</td>
<td>0.025 ng/ml</td>
<td>3.8</td>
<td>6.1</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>Advia 1800</td>
<td>1.2</td>
<td>2.2</td>
<td>4.2–6.3</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Insulin</td>
<td>ACLS</td>
<td>0.5 mIU/l</td>
<td>4.6</td>
<td>7.5</td>
<td>3–25 mIU/l</td>
</tr>
<tr>
<td>C-peptide</td>
<td>ACLS</td>
<td>0.02 nmol/l</td>
<td>3.2</td>
<td>7.1</td>
<td>0.26–1.2 nmol/l</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Advia 1800</td>
<td>1.7</td>
<td>3.1</td>
<td>&lt;5 mmol/l (desirable)</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>Advia 1800</td>
<td>0.8</td>
<td>2.8</td>
<td>≤3 mmol/l (desirable)</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>Advia 1800</td>
<td>2.3</td>
<td>2.6</td>
<td>≥1.2 mmol/l (desirable)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Advia 1800</td>
<td>1.6</td>
<td>2.4</td>
<td>≤1.7 mmol/l (desirable)</td>
<td></td>
</tr>
</tbody>
</table>

RIA = radioimmunoassay, CIA = chemilumimetric immunoassay, ACLS = automated chemiluminescence system, LC-MS/MS = liquid chromatography-tandem mass spectrometry, ELISA = enzyme-linked immunosorbent assay, TRFIA = time-resolved fluoroimmunoassay

4.2.4 Power and statistical analyses

Power analyses were conducted for Studies III and IV, and specific justifications are explained in original publications.
All variables with skewed distribution were logarithmically transformed prior to statistical analysis. Statistical analyses were conducted by using SPSS (the Statistical Package for the Social Sciences software, SPSS Inc. Released 2006. SPSS for Windows, Version 15.0. Chicago, SPSS Inc.). The limit of statistical significance was set at $p \leq 0.05$.

To compare the results between two different study groups, the independent-samples $t$ test was used for normally distributed variables and the Mann–Whitney $U$ test for variables with a skewed distribution (Studies I–IV). Pearson’s correlation coefficient ($r$) was calculated to correlate age with the hormone levels measured (Study I). One-way analysis of variance (ANOVA) was used to assess the possible changes between different age or treatment groups (Studies I and IV). Differences in hormone responses between two groups were analysed and the impact of BMI was controlled by using multiple linear regression analysis (Study I) and ANOVA (Study II). To compare changes during treatment at two different time points, the paired samples $t$-test was used for normally distributed variables and Wilcoxon’s non-parametric test for variables with skewed distribution (Studies III and IV). To explore changes during treatment at three different time points, repeated measures ANOVA was performed for normally distributed variables and Friedman’s test for variables with a skewed distribution (Studies III and IV). The baseline level of T (Study III) and the change in BMI (Study IV) were used as covariates to adjust for changes in glucose metabolism in repeated measures ANOVA.
5 Results and Discussion

Table 13. Main results of Studies I–IV

<table>
<thead>
<tr>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal steroid production remains</td>
<td>Insulin resistance,</td>
<td>Atorvastatin therapy improves lipid profile</td>
<td>CHCs impair insulin sensitivity and increase</td>
</tr>
<tr>
<td>enhanced up to menopause in women</td>
<td>enhanced androgen secretion and systemic inflammation</td>
<td>but worsens systemic inflammation in women</td>
<td>healthy women regardless of administration route</td>
</tr>
<tr>
<td>with PCOS</td>
<td>inflammation persist after menopausal transition in PCOS</td>
<td>insulin sensitivity in women with PCOS</td>
<td>regardless of administration route</td>
</tr>
</tbody>
</table>

5.1 Hyperandrogenism

Even though hyperandrogenism is a crucial feature of PCOS and the significance of hyperandrogenism during fertile life in these women is well established, the pattern of androgen secretion during ageing is not widely described. To clarify this phenomenon further, ovarian and adrenal androgen secretion were investigated in women with PCOS and in healthy women in Studies I and II, and the changes in androgen levels were explored during the use of CHCs in healthy women (Study IV) and atorvastatin in women with PCOS (Study III).

5.1.1 Androgen secretion

Women with PCOS had significantly elevated levels of A and/or T when compared with control women younger than 31 and older than 41 years up to the postmenopausal period (Figure 4) (Studies I and II).
Serum levels of DHEAS decreased significantly during ageing in healthy women. In women with PCOS, a decrease was also observed, but it did not reach statistical significance (Study I). The adrenal androgen response (A, T, DHEAS) during ACTH tests was elevated in women with PCOS and the responses remained unchanged up to menopausal transition, whereas adrenal androgen secretion correlated negatively with age in control women (Study I). In Study II we compared premenopausal and postmenopausal women and found that the capacity of the ovaries to secrete 17-OHP and A in response to hCG decreased in both controls and women with PCOS during menopausal transition. Notably, the secretory capacity of these hormones remained significantly higher in women with PCOS even after menopause (Figure 5).
Although women with PCOS presented with significantly elevated BMI in Studies I and II, it did not explain the differences between the groups and proved that PCOS per se was the determining factor.

In our previous studies we have found that the ovarian capacity to synthesise and release androgens remains enhanced throughout the reproductive years in women with PCOS when compared with healthy women (Piltonen et al. 2003, Piltonen et al. 2004). In the present studies we further explored these observations as regards adrenal androgen secretion and ovarian androgen activity at the time of menopausal transition.

The enhanced androgen secretion in women with PCOS after menopause has also been reported in other studies (Markopoulos et al. 2011). Despite the fact that serum T levels were higher in women with PCOS compared with controls, testosterone levels decreased among women with PCOS up to premenopausal age (Study I), which is also supported by the results of a previous study (Winters et al. 2000). Furthermore, a cohort study revealed significantly elevated T levels in middle-aged women with PCOS when compared with control women, but the difference had disappeared by a mean age of 70 (Schmidt et al. 2011). The finding shows that enhanced androgen secretion decreases with age in women.
with PCOS and at some point it reaches the levels of control women. However, it does not occur at menopause.

Approximately 20–30% of women with PCOS present with adrenal hyperandrogenism (Goodarzi et al. 2015). The finding in Study 1 showing that adrenal androgen secretion decreases with age in women with PCOS is supported by the results of other studies in hyperandrogenic women and women with PCOS (Kumar et al. 2005, Moran et al. 1999). On the other hand, in a follow-up study of 3–5 years, serum DHEAS levels remained unchanged in the majority of middle-aged women with PCOS, which reflects the fact that the decrease in adrenal androgen secretion is a slow process (Yildiz et al. 2004).

In summary, enhanced androgen secretion in women with PCOS remains after menopause. This finding is important, as increased testosterone levels in older women have been found to be associated with an increase in metabolic disturbances and cardiovascular diseases (Patel et al. 2009).

5.1.2 The effects of combined contraceptives and statin therapy on androgen secretion

Combined contraceptives

According to the results of Study IV, orally, transdermally and vaginally administered combined contraceptives equally decreased the levels of 17-OHP and A. However, T concentrations remained unchanged in the pill and vaginal ring study groups and even an increase was observed in the patch group. As a result of a more than 200% increase in serum SHBG levels in all study groups, the FAI significantly decreased. The increase in SHBG level was significantly lower in women using a vaginal ring than in those using transdermal patches or oral pills (Study IV).

The increase in SHBG concentrations during the use of combined contraceptives originates from the hepatic impact of EE. It is related to the 17α-ethinyl group of the molecule, which prevents the inactivation of EE and results in slow metabolism, long tissue retention and a long half-life, and thereby even 500-fold more potent hepatic effects when compared with estradiol (Sitruk-Ware & Nath 2013). The hepatic effects of EE appear during both oral and vaginal administration (Goebelsmann et al. 1985). In contrast to our results, two studies on oral (20 µg/30 µg EE) and vaginal (15 µg) combined contraceptives have revealed that vaginal administration increased the
levels of SHBG more significantly than oral administration (Elkind-Hirsch et al. 2007, Tuppurainen et al. 2004) and thereby induced a significantly greater reduction of the FAI (Elkind-Hirsch et al. 2007). This difference may result from the different progestin components used in the pill (desogestrel vs. levonogestrel), as the progestins modify the hepatic effect of EE depending on their dose and type (Odlind et al. 2002). The production of SHBG is highly oestrogen-sensitive and levonorgestrel, which is less oestrogenic than desogestrel, does not enhance the synthesis of SHBG as much (Hammond et al. 1984, Odlind et al. 2002, Wiegratz et al. 2003). Interestingly, use of the patch (EE, 20 µg/day and norelgestromin 150 µg/day) has been reported to result in a greater increase of SHBG than that associated with an oral combined contraceptive containing 35 µg EE and 250 µg norgestimate, despite the fact that norelgestromin is the metabolic product of norgestimate (White et al. 2005, White et al. 2006). However, it has been shown that exposure to EE during patch use is 1.7 times higher when compared with an orally administered combined contraceptive (van den Heuvel et al. 2005), which may account for the results. This finding may also explain the differences in SHBG levels in Study IV, as the progestins used in the preparations are fairly similar as regards their oestrogenic features.

**Atorvastatin**

In Study III, atorvastatin treatment decreased serum levels of DHEAS, but the levels of LH, FSH, A, T and SHBG remained unchanged.

In line with our results, one earlier study revealed no change in serum testosterone levels during atorvastatin treatment (Raja-Khan et al. 2011). However, all other studies on atorvastatin have shown a decrease in serum levels of T (Kaya et al. 2009, Kaya et al. 2010, Sathyapalan et al. 2009). Studies exploring the effects of simvastatin in women with PCOS have also shown a decrease in T levels (Banaszewska et al. 2007, Banaszewska et al. 2011, Duleba et al. 2006, Kaya et al. 2009, Kaya et al. 2010). One significant difference between our study and several other studies is the androgenic state of the study subjects. In Study III, PCOS was diagnosed on the basis of Rotterdam criteria and not on NIH criteria, in which hyperandrogenism is one required criterion. Moreover, our subjects were older (late fertile age) and had lower androgen levels as a result of an age-related decrease of both ovarian and adrenal androgen secretion.

The idea that statins could theoretically affect androgen levels originated from the fact that cholesterol acts as a substrate for androgen synthesis. Later,
statins were found to directly inhibit rat theca cell proliferation in vitro (Izquierdo et al. 2004, Kwintkiewicz et al. 2006), and this has also been confirmed in human theca cells (Sokalska et al. 2010). Furthermore, statins have been shown to inhibit steroidogenesis (Izquierdo et al. 2004, Ortega et al. 2012, Sokalska et al. 2014), where inhibition of CYP17A1 mRNA expression has been thought to be a principal mechanism (Ortega et al. 2012). Simvastatin seems to be the most effective statin in the inhibition of growth and steroidogenesis of rat theca cells, whereas atorvastatin and pravastatin present with lower effectiveness and lovastatin has an effect between simvastatin and atorvastatin (Sokalska et al. 2014). Interestingly, resveratrol, a type of natural phenol, potentiates the effects of simvastatin on androgen secretion from the ovaries (Ortega et al. 2014).

Serum levels of DHEAS significantly decreased in Study III. This has also been reported in other studies on atorvastatin (Raja-Khan et al. 2011, Sathyapalan et al. 2012) and simvastatin (Banaszewska et al. 2009, Banaszewska et al. 2011, Duleba et al. 2006, Kazerooni et al. 2010), although not all studies agree (Banaszewska et al. 2007, Kaya et al. 2010). Because DHEAS serves as a precursor for testosterone synthesis, its decrease during atorvastatin treatment may reflect a compensatory mechanism to maintain prior testosterone levels. However, potentially statins may have direct inhibitory effects on androgen synthesis in the adrenal glands, similarly to the ovaries (Sathyapalan et al. 2012).

### 5.2 Metabolic alterations in women with and without PCOS

#### 5.2.1 Glucose tolerance

In Study II, fasting glucose levels were comparable in women with PCOS and controls in both premenopausal and postmenopausal study groups, but fasting insulin levels were elevated twofold in cases of PCOS. However, the difference reached statistical significance only between the postmenopausal groups, as did the AUC of glucose. Fasting and 2-hour insulin sensitivity indexes were significantly lower in postmenopausal women with PCOS when compared with control women. In addition, HOMA-IR results were increased and Matsuda indexes decreased significantly among both premenopausal and postmenopausal women with PCOS when compared with control women. When the differences between the groups were analysed using BMI as a covariate, higher BMI levels in
cases of PCOS explained differences in several glucose tolerance parameters, but not the AUC of insulin or the insulin sensitivity index at 120 min. It is well known that most women with PCOS present with insulin resistance. However, the possible changes during menopausal transition are not well understood. The results of Study II strongly suggest that impaired glucose metabolism in women with PCOS persists after the menopause. There are only a few studies available on metabolic parameters in older women with the syndrome. However, premenopausal and postmenopausal women with a history of PCOS have been found to present with T2DM more often than controls (Hudecova et al. 2011, Shaw et al. 2008). A study performed in Greece did not reveal any differences in fasting insulin and glucose, or insulin sensitivity indexes between postmenopausal women with PCOS and healthy women, but women with PCOS had significantly higher insulin levels during OGGTs without changes in glucose levels, reflecting increased insulin resistance (Markopoulos et al. 2012). These findings are of importance, as impaired glucose metabolism and increased prevalence of T2DM further predisposes these women to CVD.

**The effects of combined contraceptives on glucose tolerance**

Fasting glucose levels remained unchanged during the use of oral, transdermal and vaginal contraceptives. However, the insulin sensitivity index during OGGTs and the Matsuda index decreased significantly in all study groups, reflecting deterioration of insulin sensitivity (Figure 6). This was accompanied by compensatory increases of fasting and glucose-stimulated insulin levels. A significant increase was also found in the levels of fasting C-peptide during use of the transdermal preparation. All the parameters reflecting insulin sensitivity were fairly similar among the study groups (Study IV).
Several previous investigators have reported similar results concerning alterations in insulin sensitivity in healthy women in prospective (Cagnacci et al. 2009a, Cagnacci et al. 2009b, Deleskog et al. 2011, Elkind-Hirsch et al. 2007) as well as cross-sectional studies (Frempong et al. 2008, Morin-Papunen et al. 2008). However, there are also data showing no change in glucose tolerance during combined contraceptive use (Cagnacci et al. 2009a, Gaspard et al. 2003). In women with PCOS, an oral contraceptive pill containing EE and drospirenone caused deterioration of insulin sensitivity (Battaglia et al. 2010, Duijkers et al. 2004), whereas treatment with EE and cyproterone acetate did not affect glucose tolerance (Gode et al. 2011). Interestingly, a new preparation containing estradiol valerate with dienogest has been reported not to worsen insulin sensitivity but to improve it (De Leo et al. 2013). Furthermore, favourable effects on glucose tolerance have been reported among hirsute women after the use of EE and desogestrel (Escobar-Morreale et al. 2000). The discrepancies between the studies may be explained in part by different doses of oestrogen and different progestins used in the formulations.

In Study IV, significant impairment in glucose metabolism was also found in subjects using transdermal and vaginal preparations as increases in the AUC of glucose and decreases in insulin sensitivity indexes were found in both groups. However, earlier investigators have published divergent results. One study on
transdermal patches revealed a decrease in fasting glucose levels (Kiriwat & Petyim 2010), whereas previous studies on vaginal rings have not shown any changes in insulin sensitivity (Cagnacci et al. 2009b, Elkind-Hirsch et al. 2007). Moreover, in women with type 1 diabetes, glycosylated haemoglobin concentrations and average daily insulin requirements remained stable (Grodnitskaya et al. 2010). In women with PCOS, the use of vaginal rings has even been reported to result in improvement of variables reflecting glucose tolerance (Battaglia et al. 2010). The discrepancies between our study and earlier studies can be partly explained by the different dosing patterns, in other words longer exposure to hormonal preparations due to continuous administration for nine weeks in Study IV vs. traditional cyclic dosing.

The effect of atorvastatin on glucose tolerance

In Study III, atorvastatin treatment resulted in a significant increase in fasting levels of insulin and AUCs of insulin and C-peptide, and a deterioration of insulin sensitivity indexes (Figure 7). Serum levels of glucose remained unchanged, reflecting a sufficient compensatory increase in insulin secretion (Figure 7).
Fig. 7. Glucose and insulin responses during OGTTs in the atorvastatin group (Study III). *Specific time points during OGTTs where the concentration was significantly higher after 6 months’ use of atorvastatin. Open circles, before treatment; closed circles, after 6 months of treatment.

The findings are interesting, as other studies concerning the use of atorvastatin in women with PCOS have revealed a decrease in fasting insulin levels and improvement of insulin resistance (Kaya et al. 2009, Kaya et al. 2010, Sathyapalan et al. 2009), or no change (Raja-Khan et al. 2011). Simvastatin treatment has not had any effect on values reflecting glucose tolerance (Banaszewska et al. 2007, Banaszewska et al. 2011, Duleba et al. 2006, Kaya et al. 2009).
The reported unaltered or improved glucose tolerance during simvastatin and atorvastatin treatment in women with PCOS in other studies may be explained by the fact that these studies have included women presenting with biochemical hyperandrogenism and improvement of this feature may result in improvement of insulin resistance by decreased release of free fatty acids (Xu et al. 1991).

In the general population a slightly increased risk of the development of diabetes during statin therapy has been observed (OR 1.09; 95% CI 1.02–1.17) (Sattar et al. 2010). However, a recent study revealed an increased risk of T2DM during atorvastatin and simvastatin use of up to 46% and the risk seemed to be dose-dependent (Cederberg et al. 2015). Furthermore, on the basis of the results of clinical trials, the mechanisms underlying statin-induced diabetes have been suggested to include an increase in insulin resistance and a decrease in insulin sensitivity-corrected insulin secretion (Cederberg et al. 2015). Similar alterations have been found in in vitro studies, as statins have been suggested to inhibit the uptake of glucose by human liver, adipose tissue and skeletal muscle cells by inducing conformational changes in glucose transporters (Nowis et al. 2014) and reducing glucose-induced insulin secretion by blocking Ca^{2+} channels (Yada et al. 1999). The divergent effects on glucose tolerance between different statins may be explained by the degree of lipophilicity. Lipophilic statins may have more adverse metabolic consequences than hydrophilic statins, such as impairment of insulin secretion and promotion of insulin resistance, by affecting other tissues besides the liver, while hydrophilic statins show greater hepatoselectivity (Schachter 2005). This has also been seen in clinical trials, in which hydrophilic pravastatin even improved insulin sensitivity and lipophilic simvastatin impaired it (Baker et al. 2010).

5.2.2 Lipid profile

The effects of combined contraceptives on the lipid profile

In Study IV, the effects on the lipid profile were fairly similar in all study groups. Levels of total cholesterol remained stable and levels of HDL cholesterol significantly increased. However, the change in HDL cholesterol was not significant in the oral contraceptive group when baseline and nine-week treatment were compared. Serum levels of LDL cholesterol were significantly increased
temporarily at five weeks during the use of oral contraceptives but remained unchanged in the other study groups. As expected, the levels of triglycerides significantly increased in all study groups during the total treatment period (oral group +78%, transdermal group +60% and vaginal group +53%) and the changes did not differ between the groups.

The results concerning total cholesterol are in line with that of a cross-sectional study showing comparable levels of total cholesterol between non-users and oral, transdermal and vaginal contraceptive users (Palan et al. 2010). However, an increase in the levels of total cholesterol has also been reported among the users of oral (Cagnacci et al. 2009a, Cagnacci et al. 2009b, Cau et al. 2008, Morin-Papunen et al. 2008), transdermal (Creasy et al. 2003, Kiriwat & Petyim 2010) and vaginal (Barreiros et al. 2011) contraceptives. In line with our results, there are also studies available that have not revealed any changes in total cholesterol during the use of vaginal ring (Cagnacci et al. 2009b, Elkind-Hirsch et al. 2007, Tuppurainen et al. 2004).

The improvement in HDL cholesterol might be of notable significance, as HDL cholesterol plays a role in the prevention of atherosclerosis (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) 2002). An increase in HDL cholesterol has also been found in other studies on oral (Cagnacci et al. 2009a, Cagnacci et al. 2009b, Cau et al. 2008, Frempong et al. 2008), transdermal (Creasy et al. 2003, Kiriwat & Petyim 2010) and vaginal (Barreiros et al. 2011) contraceptives.

The increment in triglyceride levels in Study IV is in line with the results of other studies (Barreiros et al. 2011, Cagnacci et al. 2009a, Cagnacci et al. 2009b, Cau et al. 2008, Creasy et al. 2003, Frempong et al. 2008, Kiriwat & Petyim 2010, Krintus et al. 2010, Morin-Papunen et al. 2008). This may have a significant impact, as elevated levels of triglycerides emerge as strong predictors of coronary atherosclerosis and CHD (Harchaoui et al. 2009). On the other hand, the increase in triglyceride levels may be caused by increased levels of larger VLDL particles, which may be less atherogenic than small VLDL particles (Crook & Godsland 1998).
The effects of atorvastatin on the lipid profile

As expected, levels of total and LDL cholesterol and triglycerides decreased during atorvastatin treatment in Study III, while the level of HDL cholesterol remained unchanged.

As LDL cholesterol is the major atherogenic lipoprotein, the beneficial effect of atorvastatin medication in reducing the risk of cardiovascular diseases is mediated through this effect. There are two other studies in which decreases in the levels of triglycerides during the use of atorvastatin in women with PCOS have been reported (Raja-Khan et al. 2011, Sathyapalan et al. 2009). This effect is also of an importance, as triglyceride levels have been identified as a risk factor of coronary atherosclerosis and CHD (Tenenbaum et al. 2014). However, one study revealed no change in triglyceride levels (Kaya et al. 2009). Studies on simvastatin have not shown any changes in triglyceride levels (Banaszewska et al. 2009, Banaszewska et al. 2011, Kaya et al. 2009). Decreases in total and LDL cholesterol levels, however, are well documented (Banaszewska et al. 2009, Banaszewska et al. 2011, Kaya et al. 2009, Raja-Khan et al. 2011, Sathyapalan et al. 2009).

5.2.3 Chronic inflammation

In Study II, premenopausal women with PCOS presented with serum levels of CRP almost twice as high as in premenopausal control women. In addition, postmenopausal women with a history of PCOS had significantly increased levels of CRP when compared with control women, and the differences between the groups remained after adjustment for BMI. Interestingly, menopause did not affect the level of chronic inflammation in either the PCOS or the control group.

Chronic inflammation (with high CRP levels) predisposes individuals to CVD by taking part in atherosclerosis plaque formation (Ridker et al. 2000). Notably, the detrimental effects of chronic inflammation are not limited to CVD, as CRP levels have also been shown to predict the incidence of type II diabetes (Pradhan et al. 2001). It is well known that women with PCOS present with increased systemic inflammation when compared with control women of fertile age (Diamanti-Kandarakis et al. 2006). This was also confirmed in Study II, where it was shown that chronic inflammation persists in women with PCOS after menopause and predisposes these women to unbenefficial health consequences after their reproductive years.
Inflammatory changes during combined contraceptive treatment

Serum concentrations of CRP rose significantly in all three groups in Study IV during combined contraceptive treatment (Figure 8). Even though subjects with CRP concentrations of more than 10 mg/l were excluded from the analysis, the increase remained significant in all study groups. Plasma levels of another marker of inflammation, PTX-3, increased significantly during the use of oral and transdermal contraceptives, and a similar trend was seen in the vaginal ring group (Study IV).

Fig. 8. Changes in serum CRP levels during the use of combined contraceptives (Study IV). Open circles, vaginal ring group; open squares, patch group; solid circles, pill group. Values of $p$ show the significance of changes within each study group.

Increased levels of CRP have been reported previously during the use of transdermal (White et al. 2006) and oral combined contraceptives (Morin-Papunen et al. 2008, White et al. 2006). Study IV was the first one in which an increase was also found during the use of vaginal contraception.

As combined contraceptives stimulate the synthesis of proteins such as CRP in hepatocytes through a mechanism which is not known, we wanted to examine another inflammation marker to investigate whether or not the elevated levels of CRP also represented extrahepatic systemic inflammation. We analysed the levels of PTX-3, which is produced by peripheral tissues such as vascular endothelial cells, smooth muscle cells and leucocytes in response to proinflammatory signals,
and thereby the secretion of PTX-3 is not influenced by drug-induced hepatic protein synthesis (Mantovani et al. 2008). Hence its assay gives more precise information on the actual inflammation. The finding of increased PTX-3 levels in the pill and patch groups supports the idea that the increase in CRP levels may not be only a consequence of liver induction but also a reflection of a true increase in systemic inflammation. However, the increase in the levels of CRP regardless of the exact mechanism might be of significance, as the presence of CRP molecules might lead to increased foam-cell formation in atherosclerotic plaques (Zwaka et al. 2001). Furthermore, CRP can accelerate atherosclerotic processes, because it can induce the expression of adhesion molecules (Pasceri et al. 2000) and inhibit nitric oxide expression in endothelial cells, causing endothelial dysfunction (Verma et al. 2002). Interestingly, progestin-only contraceptives do not stimulate the production of CRP, whether administered orally or in utero (Morin-Papunen et al. 2008).

As there seems to be a pronounced inflammatory milieu in users of combined contraceptives, it may be of some clinical significance in the long term through worsening endothelial function and promoting accumulation of asymptomatic atherosclerotic changes in women. However, the clinical importance warrants further investigations.

**Inflammatory changes during atorvastatin treatment**

Atorvastatin therapy significantly decreased serum levels of CRP in women with PCOS after excluding women with infection (CRP > 10 mg/l) (Study III).

Several studies have shown that statin therapy reduces chronic inflammation, usually assessed by the measurement of CRP levels (Girardi 2014). Similar findings have also been reported in women with PCOS by other investigators exploring the effects of atorvastatin (Kaya et al. 2010, Raja-Khan et al. 2011, Sathyapalan et al. 2009) and simvastatin (Banaszewska et al. 2007, Banaszew ska et al. 2009, Banaszewska et al. 2011, Kaya et al. 2010). As CRP levels independently predict the future risk of coronary events (Ridker et al. 2000), statin therapy in women with PCOS probably decreases the risk of CVD. The reduction of inflammation by way of statin therapy is of great importance, as statin therapy is able to reduce the risk of CVD in men and women without hyperlipidaemia only by reducing inflammation (Ridker et al. 2008).
5.3 Limitations of the study

The limitations of Studies I–IV are related to the limited numbers of study subjects, although for the clinical trials (Studies III and IV) appropriate power calculations were performed. In Studies I and II, women with PCOS had a significantly higher BMI when compared with control women. However, this difference was controlled by using BMI as a covariate in statistical analyses. As the study subjects were healthy in Study IV, the changes observed during the use of combined contraceptives may not be generalised to women with PCOS. Healthy women were selected for the study population, since the purpose was to explore the changes in metabolic parameters without confounding factors such as insulin resistance, which is commonly found in PCOS.

Further limitations are related to the methods used. During the ACTH test, for example, tetracosactide hexacetate might have had a more powerful effect on the adrenals if it had been injected intravenously. Furthermore, the IVGTTs may have benefitted from more frequent blood sampling. In principle, Rotterdam criteria were used for the diagnosis of PCOS, although the diagnosis of PCO was based on the older criteria, where eight or more follicles in one plane are needed for the diagnosis (Adams et al. 1985). The Rotterdam criteria require 12 follicles in the whole ovary (2004). Nevertheless, if there are eight follicles in one plane it is very obvious that there are twelve or more follicles in the whole ovary, and therefore the risk of over-diagnosing PCOS is small.
6 Conclusions

The present data support the fact that unfavourable changes in androgen secretion, glucose metabolism and chronic inflammation associated with PCOS persist beyond menopause, when the prevalent symptoms/findings related to PCOS itself have already disappeared, and this potentially has long-term health effects (Studies I and II). In Study IV we found that the use of combined contraceptives, often used to treat hyperandrogenism in women with PCOS, leads to impairment of insulin sensitivity in young healthy women regardless of administration route. As a result of the disadvantageous health consequences in PCOS, statin therapy has been suggested in order to improve lipid and androgen profiles in these women. However, on the basis of the results of Study III, despite the improvement in lipid profile, atorvastatin treatment did not have an effect on androgen secretion and it seemed to worsen glucose tolerance. Therefore, the treatment of women with PCOS should be based on a comprehensive individual risk evaluation in order to avoid adverse health effects related to treatments themselves.

**Symptoms/disorders**

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<tr>
<th>acné</th>
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<tr>
<td>oligoamenorrhée</td>
<td>glucose intolerance</td>
<td>métabolique syndrome</td>
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<td>hirsutisme</td>
<td>infertilité</td>
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**Medical treatments**

- metformine
- contraceptifs combinés
- statines
- traitements d'infertilité

*Fig. 9. The timeline of PCOS*
References


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van den Oord SC, Sijbrands EJ, ten Kate GL, van Klaveren D, van Domburg RT, van der Steen AF & Metab 86(7): 3421–3429.


Original publications


*equal contribution

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


1286. Rautiainen, Jari (2015) Novel magnetic resonance imaging techniques for articular cartilage and subchondral bone: studies on MRI Relaxometry and short echo time imaging


1288. Karsikas, Sara (2015) Hypoxia-inducible factor prolyl 4-hydroxylase-2 in cardiac and skeletal muscle ischemia and metabolism

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