

**METABOLIC AND CLINICAL
CHARACTERISTICS OF WOMEN
WITH SELF-REPORTED
SYMPTOMS OF POLYCYSTIC
OVARY SYNDROME**

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OULU 2004



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Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium of Kastelli Research Center (Aapistie 1), on April 16th, 2004, at 12 noon.

OULUN YLIOPISTO, OULU 2004

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ISBN 951-42-7316-8 (nid.)
ISBN 951-42-7317-6 (PDF) <http://herkules.oulu.fi/isbn9514273176/>
ISSN 0355-3221 <http://herkules.oulu.fi/issn03553221/>

OULU UNIVERSITY PRESS
OULU 2004

Taponen, Saara, Metabolic and clinical characteristics of women with self-reported symptoms of polycystic ovary syndrome

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2004

Oulu, Finland

Abstract

Oligomenorrhea (menstrual disturbances) and hirsutism (excessive growth of body hair) are typical symptoms of polycystic ovary syndrome, a common endocrine disorder with long-term health risks among fertile-age women.

Associations between body size development and polycystic ovary syndrome symptoms in a cohort design (528 symptomatic and 1479 asymptomatic women) and endocrine, metabolic and clinical characteristics of women with self-reported symptoms of oligomenorrhea or hirsutism in a nested case-control design (518 cases and 1036 controls) were investigated in this general population-based study. Gynecologic ultrasonographic examinations were performed in 196 cases and 67 controls to assess the morphology of the ovaries and its relationship to biochemical and clinical parameters. The study population was derived from the Northern Finland Birth Cohort 1966, which included all births with expected birth dates in 1966 in Northern Finland and is well representative of the general female population.

Polycystic ovary syndrome symptoms in adulthood were associated with obesity, particularly abdominal obesity, in adolescence and in adulthood, but not with birth weight or being small for gestational age. Hormonal changes typical of polycystic ovary syndrome, i.e. higher circulating concentrations of testosterone, luteinizing hormone (LH) and insulin and lower levels of sex hormone-binding globulin (SHBG), were detected in women with self-reported symptoms of oligomenorrhea and/or hirsutism compared with the controls. Less favorable metabolic cardiovascular disease risk factor profiles, higher body mass index (BMI), waist-hip ratio (WHR), and triglyceride and C-reactive protein (CRP) concentrations and lower high density lipoprotein cholesterol (HDL-C) levels, were detected in women with symptoms, being the most severe among women who reported both hirsutism and oligomenorrhea. Unfavorable characteristics were pronounced in the presence of overweight or obesity. Women with symptoms more often had features characteristic of polycystic ovarian morphology associated with an endocrine and clinical profile reflecting polycystic ovary syndrome.

This study shows that questioning in regard to symptoms of oligomenorrhea and hirsutism is useful in detecting women at risk of polycystic ovary syndrome and associated health risks. Avoidance of being overweight is important among young women to prevent the development of insulin resistance. Systematic follow-up of women with symptoms of oligomenorrhea and hirsutism is justified for prevention and early detection of long-term health risks.

Keywords: cardiovascular diseases, cohort studies, diabetes, hirsutism, non-insulin-dependent, oligomenorrhea, polycystic ovary syndrome, risk factors

Acknowledgements

This work was carried out at the Departments of Clinical Chemistry, Obstetrics and Gynecology, and Public Health Science and General Practice, University of Oulu, during the years 1998–2004. In this project, I had the opportunity to use data from the extraordinary Northern Finland Birth Cohort 1966, founded by Professor Paula Rantakallio, to whom I wish to express my appreciation and respect. I would like to thank Professor Pentti Jouppila, Head of the Department of Obstetrics and Gynecology, for his support during the course of this project. I am indebted to Professor Arto Pakarinen, Head of the Oulu University Hospital Laboratory, for providing excellent facilities.

I have had the privilege to work under the guidance of three distinguished supervisors. I want to express my sincere gratitude to Professor Aimo Ruokonen, for introducing me to the field of scientific research, for giving encouragement and for having faith in me. He always managed to find time for me and optimistically turned problems into nothing more than issues to be solved. I am grateful to Docent Hannu Martikainen, for his patient supervision, for new and fresh ideas for the project, and for his overall encouragement to become actively involved in the scientific world. I warmly thank Professor Marjo-Riitta Järvelin, for making this project possible. As the leader of the Cohort, she kept all the threads successfully in her hands, and broadened the research network into an international collaborative effort.

I have had the pleasure of working with a big and beautiful PCOS team. Our meetings have been delightful and scientifically intriguing. I want to thank Docent Jaana Laitinen, Riitta Koivunen, M.D., Ph.D., Docent Anneli Pouta and Professor Anna-Liisa Hartikainen, for their collaboration in this work and for occasionally reminding me of the truly important things in life. Special thanks go to Ulla Sovio, M.Sc., for providing the statistical expertise. It was an honor to work in collaboration with Professor Stephen Franks, Imperial College London, and Professor Mark McCarthy, University of Oxford, as well as Vanessa King, M.Sc., Imperial College London, who had an essential role in the functioning of the whole international team.

I am grateful to Professors Timo Laatikainen and Leo Niskanen for their constructive criticism of the thesis as the external reviewers. I want to thank Laure Morin-Papunen, M.D., Ph.D., for sharing her wide knowledge of the study field, and Nick Bolton, Ph.D., for revising the English language in this thesis.

I thank Sari Ahonkallio, M.D., Mika Paldanius, M.Sc., and Ms. Iona Millwood for their collaboration.

I am grateful to Ms. Anja Heikkinen and Ms. Salli Kämäräinen for excellent technical assistance, and to Ms. Tuula Ylitalo and to Ms. Anne Ollila for their valuable practical and secretarial help.

Thanks to my friends, sister and brother, I have found that one can have an enjoyable social life as a Ph.D. student. Thanks to my parents, I have been taken seriously with feelings of frustration as well as scientific grandeur. Owing to my dear husband Miikka and daughter Liisa, love, support and delight have been given to me incessantly.

This study has been financially supported by the Academy of Finland, the European Commission Quality of Life and Management of Living Sources Programme, Oulu University Hospital, the Foundation of the University of Oulu, the Oskar Öflund Foundation, the Finnish Gynaecological Association, the Lilly Foundation and the Aarne and Aili Turunen Foundation.

Oulu, March 2004

Saara Taponen

Abbreviations

ACTH	adrenocorticotropic hormone
BMI	body mass index
BP	blood pressure
CVD	cardiovascular disease
CI	confidence interval
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
FAI	free androgen index (calculation)
FFA	free fatty acid
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
hs-CRP	highly-sensitive C-reactive protein
HDL-C	high density lipoprotein cholesterol
IGF-1	insulin-like growth factor-1
IGFBP-1	insulin-like growth factor-binding protein-1
LDL-C	low density lipoprotein cholesterol
LH	luteinizing hormone
MBS	metabolic syndrome
NIH	National Institutes of Health
OC	oral contraceptive
PAI-1	plasminogen-activating inhibitor-1

PCO	polycystic ovaries
PCOS	polycystic ovary syndrome
SD	standard deviation
SGA	small for gestational age
SHBG	sex hormone-binding globulin
VLDL-C	very low density lipoprotein cholesterol
WHR	waist-hip ratio

List of original articles

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

- I Laitinen J, Taponen S, Martikainen H, Pouta A, Millwood I, Hartikainen A-L, Ruokonen A, Sovio U, McCarthy MI, Franks S, Järvelin M-R (2003) Body size from birth to adulthood as a predictor of self-reported polycystic ovary syndrome symptoms. *Int J Obes* 27(6):710-715.
- II Taponen S, Martikainen H, Järvelin M-R, Laitinen J, Pouta A, Hartikainen A-L, Sovio U, McCarthy MI, Franks S, Ruokonen A (2003) Hormonal profile of women with self-reported symptoms of oligomenorrhea and/or hirsutism: Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab* 88 (1): 141-7.
- III Taponen S, Martikainen H, Järvelin M-R, Sovio U, Laitinen J, Pouta A, Hartikainen A-L, McCarthy MI, Franks S, Paldanius M, Ruokonen A (2004) Metabolic cardiovascular disease risk factors in women with self-reported symptoms of oligomenorrhea and/or hirsutism: Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab*, in press.
- IV Taponen S, Ahonkallio S, Martikainen H, Koivunen R, Ruokonen A, Sovio U, Hartikainen A-L, Pouta A, Laitinen J, King V, Franks S, McCarthy MI, Järvelin M-R (2004) Prevalence of polycystic ovaries in women with self-reported symptoms of oligomenorrhea and/or hirsutism: Northern Finland Birth Cohort 1966 study. *Human Reproduction*, in press.

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1 Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among women of reproductive age, the prevalence being 5–10% (Franks 1995, Dunaif 1997). The etiology remains unknown in spite of increasing research activity. PCOS is characterized by anovulation and hyperandrogenism. Anovulation presents as menstrual disturbances, oligomenorrhea or amenorrhea. Hyperandrogenism may manifest itself as symptoms of hirsutism, acne or androgenic alopecia, or as biochemical hyperandrogenism, elevated serum testosterone and/or androstenedione levels (Franks 1995). The association between bilateral polycystic ovaries and amenorrhea, oligomenorrhea, infertility, hirsutism and obesity was first described in 1935 by Stein and Leventhal.

Obesity is a common feature of PCOS, as is insulin resistance, which is frequently found even in lean women with PCOS (Dunaif 1997). Typically, women with PCOS have increased levels of serum testosterone and luteinizing hormone (LH), and decreased levels of sex hormone-binding globulin (SHBG), which correlate with hyperinsulinemia and obesity (Holte *et al.* 1994b).

Increased cardiovascular risk factors, particularly higher body mass index (BMI), waist-hip ratio (WHR), insulin, total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides and blood pressure (BP) and lower high-density lipoprotein cholesterol (HDL-C) have been reported among PCOS patients compared with their controls (Wild *et al.* 1985, Conway *et al.* 1992, Talbott *et al.* 1995). The well-documented presence of increased risk factors has led to suggestions that women with PCOS are at higher risk of cardiovascular diseases. However, this was not observed in two large follow-up studies, as no difference in morbidity and mortality from circulatory diseases was found between PCOS women and controls (Pierpoint *et al.* 1998, Wild *et al.* 2000). Further prospective studies with representative populations are needed to clarify the age-related changes associated with PCOS.

Low-grade inflammation, reflected by elevated levels of circulating highly-sensitive C-reactive protein (hs-CRP), has been suggested to be related to insulin resistance and cardiovascular disease development (Kuller *et al.* 1996, Ridker *et al.* 2000). Studies on possible low-grade inflammation in PCOS as part of cardiovascular risk factor accumulation have suggested elevated CRP levels among PCOS patients, although it may

partly be explained by their higher BMI (Kelly *et al.* 2001, Fenkci *et al.* 2003, Morin-Papunen *et al.* 2003a).

Polycystic ovaries detected by ultrasonography are frequently noted in women with PCOS but also in about 20% of women who consider themselves healthy (Koivunen *et al.* 1999). In a hospital-based study, up to 90% of women with oligomenorrhea and/or hirsutism had polycystic ovaries (Adams *et al.* 1986). The detection of PCOS is not simple, as the syndrome is associated with multiple clinical presentations. The most recent recommendation for the diagnostic criteria of PCOS was agreed in Rotterdam (May 1–3, 2003) as two out of three of 1) oligo- and/or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, and 3) polycystic ovaries, when other etiologies such as congenital adrenal hyperplasia, androgen-secreting tumors and Cushing's syndrome have been excluded (Revised 2003 consensus on PCOS 2004).

In this study, the general population-based Northern Finland Birth Cohort 1966 was investigated to see whether women with self-reported symptoms of oligomenorrhea and/or hirsutism are distinguishable from asymptomatic controls in terms of metabolic and clinical characteristics. Body size from birth to adulthood, endocrine profiles, metabolic cardiovascular disease risk factor profiles and ultrasonographic findings in women with self-reported PCOS symptoms and in their asymptomatic controls were investigated.

2 Review of the literature

2.1 Definition of polycystic ovary syndrome (PCOS)

Stein and Leventhal were the first to report the association between bilateral polycystic ovaries and amenorrhea, oligomenorrhea, infertility, hirsutism and obesity in 1935. The term 'PCOS' came into use in the 1960's, when it was understood that clinical and histological diversity was typical of the syndrome (Goldzieher & Green 1962). The first reported biochemical disturbance in PCOS was disordered gonadotropin secretion, especially increased LH secretion (McArthur *et al.* 1958). The association between hyperandrogenism and insulin resistance was first reported in 1976 (Kahn *et al.* 1976) and in 1980, it was found that women with PCOS have elevated basal and post-glucose load insulin levels compared with weight-matched controls (Burghen *et al.* 1980).

In the 1960's, histological criteria were used to diagnose PCOS (Goldzieher & Green 1962). In the 1980's, transvaginal ultrasonographic imaging replaced ovarian biopsy as a non-invasive, simple technique to assess the size, shape and the internal structure of the ovaries (Dewailly 1997). In 1990, the National Institutes of Health – National Institute of Child Health and Human Development (NIH-NICHD) Conference was held and although no consensus was reached, the majority of participants agreed that PCOS should be defined by clinical and/or biochemical evidence of hyperandrogenism, chronic anovulation and exclusion of other known disorders (Zawadski & Dunaif 1992). These criteria, which do not include ultrasonographic examination of the ovaries, have been widely used in North America. On the other hand in Europe, polycystic ovaries detected by ultrasonography with one or more of the clinical symptoms and/or one or more of the recognized biochemical disturbances have been used to diagnose PCOS (Balen & Michelmore 2002). The differences of definitions of PCOS have resulted in varying populations of PCOS women in studies, which are then difficult to compare. In Europe, it is believed that PCOS manifests itself as various combinations of characteristics and there are women with polycystic ovaries and mild biochemical changes at one end and women with severe metabolic and endocrine disturbances at the other end of the syndrome's spectrum. The North American stringent definition includes only women with more severe changes, PCOS-diagnosed women being, for instance, more overweight than European PCOS patients. An American study on prevalence and predictors of

dyslipidemia involved investigation of 195 PCOS-diagnosed women, of whom as many as 79% were obese ($\text{BMI} \geq 27 \text{ kg/m}^2$) (Legro *et al.* 2001), while in a European study of 1741 PCOS-diagnosed women the prevalence of overweight was 38.4% ($\text{BMI} > 25 \text{ kg/m}^2$) (Balen *et al.* 1995). Therefore, the PCOS populations studied may have varied considerably.

The debate over the diagnostic criteria of PCOS went on for almost 15 years after the first international conference on PCOS. In May 2003, a conference of the European Society for Human Reproduction (ESHRE) and the American Society for Reproductive Medicine (ASRM) was held in Rotterdam, the Netherlands, to revise and to reach a consensus on the criteria of PCOS. The new criteria take into account the fact that clinical expression of PCOS may be broader than that defined by NIH in 1990. The new criteria of PCOS are (two out of three) 1) oligo- and/or anovulation, 2) clinical (hirsutism, acne, androgenic alopecia) and/or biochemical signs of hyperandrogenism, 3) polycystic ovaries. Other etiologies such as congenital adrenal hyperplasia, androgen-secreting tumors and Cushing's syndrome have to be excluded (Revised 2003 consensus on PCOS 2004). In another, earlier proposal for a uniform protocol for the diagnosis of PCOS worldwide it was suggested that symptoms of menstrual disturbance, hirsutism, acne or anovulatory infertility should justify ultrasonographic examination. The diagnosis of PCOS in a symptomatic woman should be confirmed by a positive ultrasonographic result or a biochemical test result (Homburg 2002). Figure 1 shows the proposed protocol.

Symptom → (menstrual disturbance, hirsutism, acne or anovulatory infertility)	Ultrasonographic examination	Diagnosis confirmed
	Positive → Negative ↓	
	Biochemical examination →	If: 1) Elevated testosterone, 2) elevated LH, 3) elevated FAI or 4) glucose/insulin < 4.5, then diagnosis confirmed

Fig. 1. A proposal for a uniform protocol to diagnose PCOS (Homburg 2002).

2.2 Etiology of PCOS

The etiology of PCOS remains unknown and it is believed that there may be several different pathways leading to the syndrome. PCOS is a syndrome of ovarian dysfunction (Revised 2003 consensus on PCOS 2004). The secretion of LH is increased and disordered, which could be accounted for by an excessive hypothalamic gonadotropin-releasing hormone (GnRH) drive of unknown primary cause (Barontini *et al.* 2001). Overall increased androgen production is suggested to result from increased steroidogenesis in both ovarian and adrenal pathways (Holte 1998). Human theca cells

from polycystic ovaries produce 20 times more androstenedione than cells from normal ovaries (Gilling-Smith *et al.* 1994). Familial clustering of PCOS cases suggests that genetic factors are importantly involved in the pathogenesis. About 50% of sisters of PCOS women have hyperandrogenemia (Legro *et al.* 1998a), associated with markers of insulin resistance (Legro & Strauss 2002). It has been suggested that environmental factors, particularly nutrition and exercise, affect the expression of PCOS in those who have a genetic predisposition to the syndrome (Franks *et al.* 1997).

Barker's hypothesis suggests that poor intrauterine growth, reflected by low birth weight, can lead to metabolic abnormalities related to metabolic syndrome (MBS) in adulthood (Hales *et al.* 1991, Barker *et al.* 1993, Phillips *et al.* 1994). The close relationship between MBS and PCOS has brought up the possibility that PCOS could be programmed *in utero*. Cresswell *et al.* (1997) suggested that there are two common forms of PCOS having different origins in intrauterine life. Precocious pubarche, hyperinsulinism and ovarian hyperandrogenism in girls have been related to low birth weight (Ibanez *et al.* 1998).

Dysfunction in insulin secretion and action may be a key factor in the pathophysiology of PCOS. Insulin secretion is inappropriately low for the degree of insulin resistance in both obese and nonobese women with PCOS with no impaired glucose tolerance (IGT) or type 2 diabetes, suggesting β -cell dysfunction (Ehrmann *et al.* 1995b, Dunaif & Finegood 1996). Hyperinsulinemia, the compensatory result of insulin resistance, can affect hypothalamic control of gonadotropin secretion, and appetite, it can stimulate ovarian and adrenal androgen secretion and suppress SHBG, which leads to an increase in bioavailable androgens (Legro & Strauss 2002).

There is evidence that hyperandrogenism has a pivotal role in the pathogenesis of PCOS. It has been proposed that the clinical and biochemical features of PCOS are a consequence of genetically determined hypersecretion of androgens by the ovary during or long before puberty (Abbott *et al.* 2002). This hypothesis suggests that the resultant hyperandrogenism would affect the hypothalamic-pituitary unit to favor excess LH secretion and encourage abdominal adiposity that predisposes individuals to insulin resistance (Abbott *et al.* 2002).

The genetic background of the syndrome is not clear. Most investigators support the concept that a gene or several genes are linked to PCOS susceptibility (Legro & Strauss 2002). The earliest recognizable phenotype of PCOS may be premature pubarche. Affected girls display hyperinsulinemia and elevated levels of serum dehydroepiandrosterone sulfate (DHEAS) as well as oligomenorrhea after menarche (Ibanez *et al.* 1999). Candidate genetic factors that may be involved in PCOS include polymorphisms of the insulin gene region called the insulin VNTR (variable number of tandem repeats) (Waterworth *et al.* 1997), a region near the insulin receptor gene (Tucci *et al.* 2001, Siegel *et al.* 2002) and the key steroid synthesis gene, *CYP11A*, which encodes cytochrome P450_{scc} (cholesterol side chain cleavage enzyme) (Gharani *et al.* 1997, Urbanek *et al.* 1999, Diamanti-Kandarakis *et al.* 2000). In a recent study of a large population, however, the association between *CYP11A* and PCOS could not be confirmed (Gaasenbeek *et al.*, in press). Another candidate gene is that for peroxisome proliferator-activated receptor- γ (PPAR- γ), which promotes the differentiation of preadipocytes into adipocytes. Because the presence of the Ala isoform of the PPAR- γ gene has been associated with increased insulin sensitivity and lower BMI, a recent Finnish study was

carried out to investigate PPAR- γ genotype frequencies in women with PCOS. The frequency of the Ala isoform was significantly reduced in the PCOS group and thus the authors suggested that the presence of the Ala isoform is protective against the development of PCOS (Korhonen *et al.* 2003). More studies in different populations are needed to confirm the findings in genetic studies carried out so far and to understand the etiology of PCOS.

2.3 Prevalence of PCOS

The lack of an international consensus on diagnostic criteria of PCOS has made designing and comparing studies on the prevalence of PCOS complex. The prevalence of polycystic ovaries (PCO) in the general population has been studied widely but only a few investigators have taken up the challenge to study the prevalence of the syndrome. Table 1 shows the population-based studies on the prevalence of PCOS. In the United States, among 369 women examined at the time of a preemployment physical at the University of Alabama, Birmingham, a 4.0% prevalence of PCOS as defined by the 1990 NIH criteria was reported (Knochenhauer *et al.* 1998). In the Greek island of Lesbos, using the same diagnostic criteria, 6.8% of 192 clinically healthy women had PCOS (Diamanti-Kandarakis *et al.* 1999). There is a possible selection bias in this study, since the participants were recruited by way of an information campaign on television, radio and newspapers offering a free medical examination. In Spain, 154 blood donors were studied and a prevalence of PCOS of 6.5% was found (Asuncion *et al.* 2000). Using the strict criteria of the NIH in 1990, the prevalence of PCOS has been reported to be 4–7%. In contrast, Michelmore *et al.* (1999) studied 224 volunteers aged 18–25 and found a prevalence of 26% for PCOS diagnosed by ultrasonography and one or more symptom or sign typical of PCOS. In this study, the authors estimated that the prevalence of PCOS by NIH 1990 criteria would have been as low as 8%. The study also had a selection bias, though the authors emphasized that the participants were not able to select themselves according to ovarian morphology and they showed that menarcheal age and BMI of the participants were similar to those in other community-based studies of similar aged women (Michelmore *et al.* 1999). As can be seen in Table 1, the populations studied varied considerably by BMI, the American and Greek PCOS women diagnosed according to 1990 NIH criteria being notably heavier than the European women diagnosed by criteria used in Europe. It may be that when comparing studies on PCOS diagnosed by different criteria, one is actually comparing patients with two different conditions. However, the new, 2003 revised criteria take into account women who have not fitted the NIH 1990 criteria used in population studies earlier and thus the prevalence of PCOS may be even higher than the mentioned studies have suggested. The new criteria for PCOS come closer to the definition that has been used in Europe, taking into account the polycystic ovarian morphology as one of the three diagnostic features.

The age of the population being studied also affects the results. Menstrual irregularities and hyperandrogenemia tend to normalize as women with PCOS approach

their late 30s and early 40s (Winters *et al.* 2000, Elting *et al.* 2000). Polycystic ovaries were seen in about 20% of women less than 35 years of age and in only 7.9% of older women (≥ 36 years) in a study of women not treated for menstrual disturbances or hirsutism (Koivunen *et al.* 1999). In another study, within the age range of 18–25 years, the prevalence of PCO was 33% (Michelmores *et al.* 1999). It is possible that the ultrasonographic appearance of the ovaries changes with increasing age, particularly in those who do not gain weight or become predisposed to other risk factors of PCOS development. Age-related changes of PCOS are difficult to evaluate owing to a lack of prospective studies with adequately long follow-up times.

Table 1. Prevalence of PCOS.

Authors	Knochenhauer <i>et al.</i> 1998	Diamanti-Kandarakis <i>et al.</i> 1999	Michelmores <i>et al.</i> 1999	Asuncion <i>et al.</i> 2000
PCOS Criteria	1990 NIH	1990 NIH	PCO by ultrasound and a symptom and/or sign of PCOS	1990 NIH
Population	369 women examined at the time of preemployment physical at University of Alabama, Birmingham, USA	Women attending a free medical examination in the Greek island of Lesbos ($n=192$)	224 volunteers from two universities and two general practice surgeries in Oxford, UK	154 blood donors in Madrid, Spain
Prevalence of PCOS (%)	4.0	6.8	26	6.5
Mean BMI among PCOS women (kg/m^2)	30.3	28.6	23.7 (median)	25.1
Mean age among PCOS women (years)	22.7	24.6	Range 18-25	24.9

2.4 Clinical features of PCOS

2.4.1 Gynecological symptoms of PCOS

Oligomenorrhea and hirsutism are typical and frequently reported symptoms of PCOS. One study showed that 87% of women with oligomenorrhea and 92% with hirsutism and regular menses have polycystic ovaries (Adams *et al.* 1986). Consistent with these findings, in another study it was reported that 86% of women with irregular cycles have PCO and on the other hand, 76% of women who consider themselves normal and have

ultrasonographic evidence of PCO have irregular cycles (Polson *et al.* 1988). Further suggesting that PCOS is the leading cause of oligomenorrhea, 90% of women with oligomenorrhea and 73% of those with oligo- or amenorrhea had laboratory and/or clinical findings consistent with PCOS (Hull 1987).

Obesity, itself a common feature in PCOS, exacerbates symptoms of PCOS. In a study of 263 ultrasonographically diagnosed PCOS women, 35% of them were obese (Kiddy *et al.* 1990). Of the obese PCOS women, 73% were hirsute and of the lean, 56%. Irregular cycles were reported in 88% of the obese and 72% of the lean PCOS women (Kiddy *et al.* 1990). Hirsutism is generally thought to be the best primary clinical indicator of androgen excess (Diamanti-Kandarakis *et al.* 1999). However, hirsutism is a relatively subjective symptom and may be less prevalent in hyperandrogenic women of Asian origin (Carmina *et al.* 1992) or in adolescence (Ruutiainen *et al.* 1988). Acne and androgenic alopecia can also be manifestations of hyperandrogenism, but their relationship to androgen excess is not as clear as that of hirsutism (Futterweit & Mechanick 1988, Slayden *et al.* 2001).

Earlier studies on PCOS are mostly based on hospital populations. There are only a few studies in which a symptom-based approach has been used and linked with possible health consequences of PCOS, cardiovascular diseases and type 2 diabetes (Solomon *et al.* 2001, Solomon *et al.* 2002). In these studies, a history of menstrual disturbances was associated with an increased risk of cardiovascular diseases (CVDs) and type 2 diabetes at an older age, but information on hirsutism was lacking and no laboratory measures were assessed. In a study from the Greek island of Lesbos, women in the general population were investigated and the association between oligomenorrhea, hirsutism or both symptoms and hormonal and metabolic profiles showed that compared with asymptomatic women, these profiles were less favorable in those who had hirsutism, even less favorable in those with oligomenorrhea and least favorable in those with both symptoms (Diamanti-Kandarakis *et al.* 1999). Lipid profiles were not studied and a selection bias most likely affected the population studied.

2.4.2 Ovarian morphology

The definition of polycystic ovarian morphology by Adams *et al.* (1985) is the most widely used: presence of 10 or more cysts, 2–8 mm in diameter, arranged either peripherally around a dense core of stroma or scattered throughout an increased amount of stroma. This definition includes two main histological features of PCOS, multifollicularity and stromal hypertrophy. Ovarian volume and ovarian hypertrophy have been proposed instead of stromal hypertrophy because the former are less subjective and easier to define (Pache *et al.* 1992, Dewailly *et al.* 1994, van Santbrink *et al.* 1997). Multifollicular ovaries are also present in physiological and pathological situations other than PCOS, such as mid-late normal puberty, central precocious puberty, hypothalamic anovulation, hyperprolactinemia and the early normal follicular phase in adult women in one ovary, before one follicle becomes dominant. To avoid confusion with these situations, the sizes and number of follicles used for a specific PCO diagnosis have been questioned. It has been proposed that a definition of ≥ 12 follicles measuring 2–9 mm in

diameter (mean of both ovaries) would be more specific for PCO than the original Adams' definition (Jonard *et al.* 2003).

Polycystic ovarian morphology is common but not universal in PCOS. Polycystic ovaries in ultrasonography are seen in 92% of women with idiopathic hirsutism and 87% of women with oligomenorrhea (Adams *et al.* 1986). The 2003 revised criteria of PCOS take into account polycystic ovaries as one of three out of four symptoms or signs that are used to diagnose PCOS (see definition of PCOS).

Polycystic ovaries are also common among women who consider themselves healthy. Studies on "normal" women have revealed consistent results of about a 20% prevalence of PCO. Polycystic ovaries are seen in 21–23 % of randomly selected women (Clayton *et al.* 1992, Farquhar *et al.* 1994), 14–23% of regularly menstruating healthy women (Polson *et al.* 1988, Koivunen *et al.* 1999) and in 17% of women participating in routine PAP smears (Botsis *et al.* 1995).

2.4.3 Overall and abdominal obesity

About 50% of women with PCOS are overweight or obese (Yen 1980). Abdominal obesity in particular, measured by waist to hip ratio, is typical of PCOS (Lefebvre *et al.* 1997). Obesity exacerbates the effects of PCOS as it is associated with an increased risk of hirsutism and menstrual cycle disturbance, elevated circulating testosterone concentrations and an increased rate of infertility (Franks 1989, Balen *et al.* 1995). Gaining weight may have an important role in the development of PCOS, as indicated by studies that show the improving effect of reducing weight by diet, drugs or their combination on hirsutism, fertility, and the hormonal and metabolic markers of PCOS (Kiddy *et al.* 1992, Pasquali *et al.* 1997, Morin-Papunen *et al.* 1998).

Abdominal fat comprises subcutaneous and intra-abdominal fat. It seems that the amount of intra-abdominal fat correlates with insulin resistance and MBS (Norman *et al.* 2002). In spite of similar WHR, women with PCOS have been observed to have more intra-abdominal fat than controls (Holte *et al.* 1994a). In another study, abdominal fat and insulin resistance correlated in both women with PCOS and control women throughout the BMI range, and the differences in insulin resistance between obese controls and PCOS women could be accounted for by the difference in abdominal fat (Holte *et al.* 1995). However, the link between increasing adiposity and disease is not clear. The current line of evidence suggests that the endocrine function of adipocytes and ectopic fat storage may explain the association between adiposity and insulin resistance (Ravussin & Smith 2002). Adipose tissue is an active secretory organ, releasing free fatty acids (FFAs), inflammatory mediators such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), and adiponectin. Elevated FFAs reduce the normal responses to insulin in skeletal muscle tissue and liver, disturbing glucose uptake and suppressing hepatic glucose output. The inflammatory mediators TNF- α and IL-6 have adverse effects on energy metabolism and insulin sensitivity in liver and muscle. Adiponectin, unlike the inflammatory mediators, enhances insulin action and improves insulin sensitivity. However, the levels of adiponectin are reduced in obese subjects. Ectopic fat, triglyceride

stored in target organs themselves, is an important source of intracellular acyl-CoA (acyl coenzyme A) molecules. Increased acyl-CoA derivatives lead to an increase in the activity of cellular signaling molecules that affect normal insulin signal transduction (Goldstein 2003).

Abdominal obesity is associated with increased estrone concentrations (Pasquali *et al.* 1994). In obese women, SHBG concentrations are decreased, favoring greater amounts of free estradiol to be delivered to target tissues, and the formation of inactivated estrogen metabolites is reduced. These conditions favor a hyperestrogenic state and exert positive feed-back regulation on gonadotropin release, increasing LH secretion and further increasing ovarian androgen secretion (Yen 1980, Pasquali *et al.* 1997).

2.4.4 Blood pressure

The results of studies on blood pressure and arterial hypertension in PCOS women are inconclusive. In two studies, no difference was found in blood pressures between PCOS women and controls, both groups at reproductive age (Zimmermann *et al.* 1992, Sampson *et al.* 1996), while in a large case-control study a higher systolic blood pressure was reported in women with PCOS (Talbot *et al.* 1995) and in a recent study conducted in Czech females the results indicated that arterial hypertension is indeed more prevalent in PCOS women than in controls (Vrbikova *et al.* 2003). Holte *et al.* (1996) reported that women with PCOS have an increased prevalence of labile blood pressure, which may indicate a pre-hypertensive state. Hypertension was uncommon in young women with PCOS, but the prevalence seemed to increase by the time of perimenopause in one long-term follow-up study. Perimenopausal women with ovarian histopathology typical of PCOS had increased morbidity as regards hypertension (Dahlgren *et al.* 1992b).

2.5 Endocrine features of PCOS

2.5.1 Gonadotropin secretion

Luteinizing hormone (LH) stimulates thecal cell androgen production. Elevated levels of LH are characteristic of PCOS, caused by accelerated frequency and/or higher amplitude of pulses, augmentation of LH secretory burst mass and more disorderly LH release (Barontini *et al.* 2001). LH is secreted by the pituitary in pulses controlled by gonadotropin-releasing hormone (GnRH) from the hypothalamus. Follicle-stimulating hormone (FSH), however, is secreted with a normal or suppressed pattern in PCOS, leading to an increased LH:FSH ratio (Waldstreicher *et al.* 1988, Marshall & Eagleson 1999). The gonadotropin secretory abnormalities in PCOS are probably the result of increased GnRH pulsatility of unknown primary cause (Barontini *et al.* 2001). Women

with PCOS also demonstrate decreased sensitivity to the feedback effects of gonadal steroids on GnRH secretion (Marshall & Eagleson 1999). The first study in which it was demonstrated that there was an increase in LH secretion in PCOS was conducted by McArthur *et al.* (1958). Further studies confirmed the increase in LH and suppression of FSH (Yen *et al.* 1970, Berger *et al.* 1975).

2.5.2 Androgens

Polycystic ovary syndrome is one the most important causes of female hyperandrogenemia, reflected by elevated circulating androgen levels, particularly those of testosterone. Figure 2 illustrates the two-gonadotropin, two-cell model of ovarian steroidogenesis. Androgens and androgen precursors are secreted by both the ovary and the adrenal gland. The ovary is mostly responsible for the production of testosterone in premenopausal women. The remaining testosterone originates from the adrenal gland and peripheral conversion of other steroids (Norman 2002). The adrenal gland, however, is responsible for most of the production of dehydroepiandrosterone (DHEA). The primary sites of androgen production in the ovary are in the theca and stromal areas that surround the follicle (Rosenfield 1999). Normally, some androgens are exported into the granulosa cell layer of the ovary where androgens are aromatized into estrogens. Excess production of androgens or failure of aromatization leads to higher circulating androgen levels (Rosenfield 1999). Excess production and thus higher circulating levels of androgens in women lead to male distribution patterns of hirsutism, acne and disturbances of the reproductive system (Norman 2002). The secretion of androgens is controlled via the steroid pathways by LH. Normal ovary shows a rise in 17-hydroxyprogesterone production in response to LH (following GnRH) while this response is much higher in hyperandrogenemic conditions such as PCOS (Rosenfield 1999). There seems to be an intrinsic abnormality of thecal cells of the polycystic ovary, which show ongoing excess production of androgens in culture (Gilling-Smith *et al.* 1994). Hypertrophy of the theca cell layer, and greater size and number of cells producing androgens also contribute to hyperandrogenemia in PCOS women (Norman 2002).

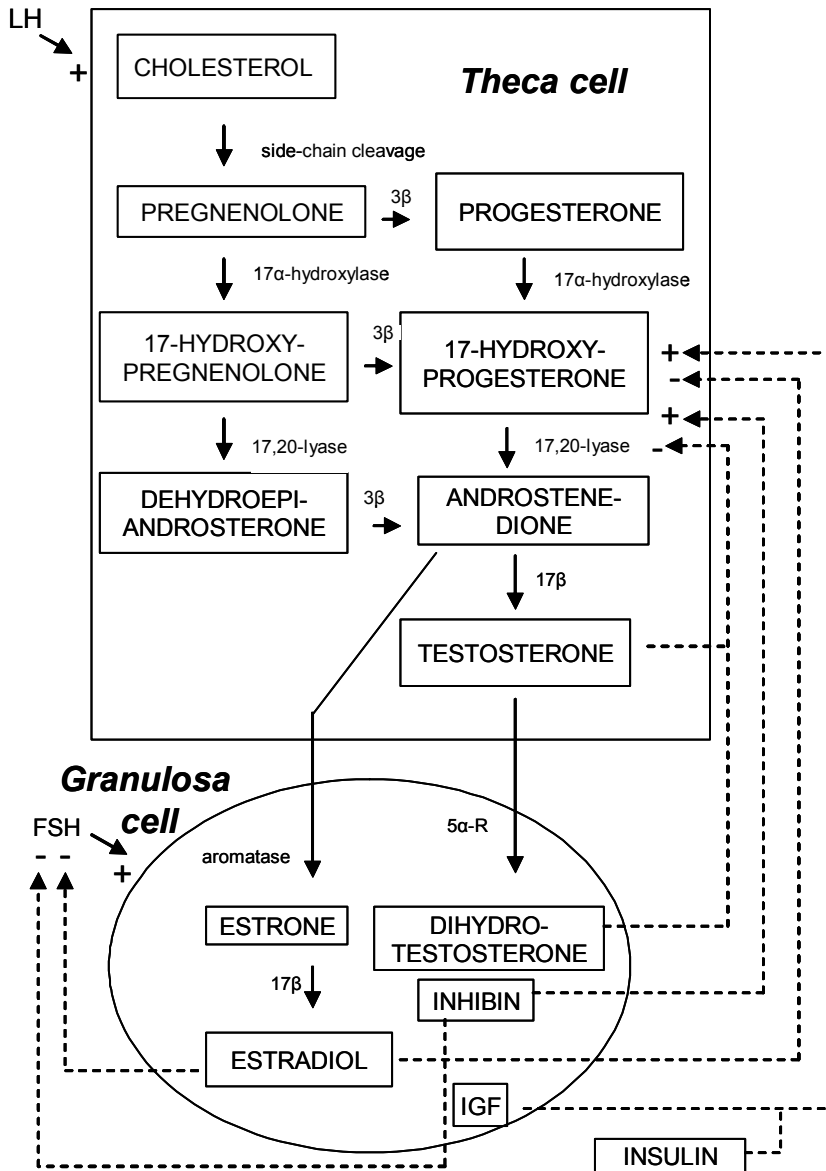


Fig. 2. The two-gonadotropin, two-cell model of ovarian steroidogenesis. LH stimulates (plus sign) androgen production in theca cells by the steroidogenic pathway common to the ovaries and the adrenal glands. FSH regulates estradiol biosynthesis from androgen by granulosa cells. Androgens and estradiol inhibit (minus signs) and inhibin, IGF-1 and insulin stimulate (plus signs) 17 α -hydroxylase and 17,20-lyase activities. 3 β = Δ 5-isomerase-3 β -hydroxysteroid dehydrogenase, 17 β = 17 β -hydroxysteroid dehydrogenase, 5 α -R = 5 α -reductase. (Modified from Ehrmann *et al.* 1995a).

2.5.3 Cortisol

The ovary and the adrenal share the main steps of steroid biosynthetic pathways. The major steroid biosynthesis pathways in the adrenal cortex are shown in Figure 3. Cortisol is a steroid hormone secreted by the adrenal gland. It is synthesized along the Δ^4 -steroid pathway from cholesterol. Cholesterol side-chain cleavage enzyme converts cholesterol to pregnenolone, which is converted to progesterone by Δ^5 -isomerase-3 β -hydroxysteroid dehydrogenase. Progesterone is converted to 17-hydroxyprogesterone by 17 α -hydroxylase and then to cortisol by 21-hydroxylase and 11 β -hydroxylase. 17 α -Hydroxylase and 17,20-lyase are activities of cytochrome P450c17, with the latter (17,20-lyase) having less activity in the Δ^4 -pathway.

Functional adrenal hyperandrogenism, often characterized by moderately increased secretion of the ketosteroid DHEA in response to adrenocorticotrophic hormone (ACTH), is found in about 50% of PCOS patients (Rosenfield 1999). The most likely cause of excessive androgen secretion is dysregulation of 17 α -hydroxylase and 17,20-lyase activities of P450c17, the rate-limiting step in androgen biosynthesis (Rosenfield 1999). Increased adrenal androgen and cortisol production in hyperandrogenic PCOS patients has been demonstrated (Martikainen *et al.* 1996, Tsilchorozidou *et al.* 2003). The cause of dysregulation of steroidogenesis in PCOS is unknown, but hyperinsulinemia seems to have a role, supported by a strong positive correlation between insulin concentrations and adrenal steroid secretion (Martikainen *et al.* 1996), though in a recent study no association was found between insulin and elevated cortisol production rate (Tsilchorozidou *et al.* 2003). In central obesity, circulating cortisol levels are normal or low, but total glucocorticoid turnover and production rates are increased (Walker *et al.* 2000). The enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) inactivates cortisol into cortisone, and type 1 (11 β -HSD1), in the other direction, locally regenerates active glucocorticoids (Seckl & Walker 2001). Enhanced 11 β -HSD1 activity in adipose tissue may be important in increasing local glucocorticoid action and promoting adverse metabolic effects (Seckl & Walker 2001).

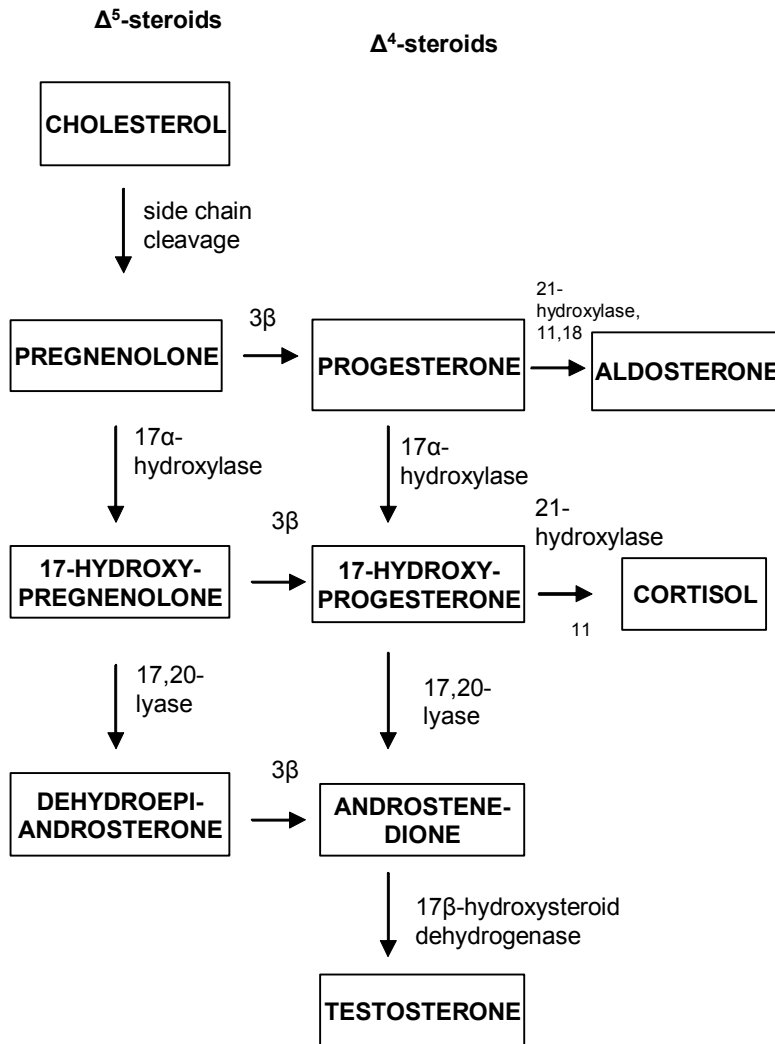


Fig. 3. Major steroid biosynthetic pathways in the adrenal cortex. 3β = Δ^5 -isomerase- 3β -hydroxysteroid dehydrogenase, 11 = 11β -hydroxylase, 18 = 18-hydroxylase-dehydrogenase. (Modified from Rosenfield 1999).

2.5.4 Sex hormone-binding globulin

In the circulation the principal sex steroids, testosterone and estradiol, are bound to the protein carrier protein SHBG. About 80% of testosterone is bound to SHBG, 19% loosely to albumin, and 1% is unbound. Testosterone is biologically active unbound, and a

decrease in serum SHBG concentration increases tissue availability of circulating testosterone. SHBG is a glycoprotein synthesized in the liver. Hyperthyroidism, pregnancy and estrogen increase, and corticoids, androgens, progestins, growth hormone, insulin and IGF-1 decrease circulating SHBG levels (Preziosi *et al.* 1993). Insulin directly suppresses SHBG production by cultured hepatoma cells (Plymate *et al.* 1988). Circulating SHBG levels are inversely related to weight (Lindstedt *et al.* 1991). Independently of serum androgens or degree of adiposity, an inverse correlation between serum levels of insulin and SHBG has been demonstrated (Haffner *et al.* 1988, Peiris *et al.* 1989). In obese women with PCOS, hyperinsulinemia directly reduces serum SHBG levels independently of any effect of sex steroids and thus it seems that insulin is the main regulator of SHBG levels (Nestler *et al.* 1991).

A low circulating SHBG concentration is a marker for the development of type 2 diabetes (Lindstedt *et al.* 1991). A decreased level of circulating SHBG can be used as a single predictor to identify those at an increased risk of insulin resistance among nonobese PCOS women, where a strong relationship between SHBG and insulin sensitivity has been demonstrated (Cibula *et al.* 2002). Low serum SHBG levels are associated with features of MBS, low HDL-C levels, and high levels of triglycerides and apolipoprotein B (Pugeat *et al.* 1995, Tchernof *et al.* 1997, Hergenc *et al.* 1999, Gascon *et al.* 2000). Indeed, SHBG was a more sensitive marker of the pathogenetic contribution of insulin resistance to the pathogenesis of atherosclerosis, determined by coronary angiography, than insulin in one study (Reinecke *et al.* 2002). These investigators found no association between coronary heart disease and a history of oligomenorrhea, hirsutism or unwanted childlessness but low plasma levels of SHBG were associated with coronary heart disease in women independently of insulin, obesity markers and hyperinsulinemia.

2.6 Type 2 diabetes and PCOS

2.6.1 Definitions of impaired glucose tolerance, type 2 diabetes and metabolic syndrome

Impaired glucose tolerance (IGT) is a metabolic state intermediate between normal glucose homeostasis and diabetes. Individuals with IGT may be euglycemic in their daily lives. The diagnostic criteria for IGT are a fasting concentration of plasma glucose < 7.0 mmol/l and a 2-hour post-glucose load glucose concentration ≥ 7.0 mmol/l and < 11.1 mmol/l (Alberti & Zimmet 1998).

Type 2 diabetes is characterized by disorders of insulin action and insulin secretion. Insulin resistance with relative insulin deficiency or an insulin secretory defect with or without insulin resistance can be predominant. The majority of patients are obese with abdominal fat accumulation. The diagnostic criteria for type 2 diabetes are a fasting concentration of plasma glucose ≥ 7.0 mmol/l or a 2-hour post-glucose load glucose concentration ≥ 11.1 mmol/l (Alberti & Zimmet 1998).

Metabolic syndrome is defined by the WHO as hyperinsulinemia with at least two of the following: abdominal obesity, dyslipidemia (serum triglycerides ≥ 1.70 mmol/l, for men: HDL-C < 0.9 mmol/l, for women: HDL-C < 1.1 mmol/l) and hypertension. According to the definition of the National Cholesterol Education Program (NCEP), at least three of the following are required: fasting plasma glucose ≥ 6.1 mmol/l, abdominal obesity, serum triglycerides ≥ 1.70 mmol/l, men's HDL-C < 1.0 mmol/l, women's HDL-C < 1.2 mmol/l, and blood pressure $\geq 130/85$ or medication for high blood pressure (Laaksonen *et al.*, in press).

2.6.2 Pathogenesis of type 2 diabetes

The pathogenesis of type 2 diabetes involves two main abnormalities: resistance to the actions of insulin in glucose and lipid metabolism, and inadequate insulin secretion from the pancreas. Type 2 diabetes often develops with obesity, particularly visceral or ectopic in distribution (Goldstein 2003).

2.6.2.1 Insulin resistance and/or hyperinsulinemia

Insulin is a polypeptide hormone secreted by the β -cells of the pancreas. Its main action is to regulate glucose metabolism. Insulin stimulates peripheral glucose uptake in fat and muscle tissues. It inhibits lipolysis and induces protein synthesis, cell growth and differentiation. In the liver, insulin inhibits gluconeogenesis and glycogenolysis and promotes glycogen storage (Kahn 1994).

Insulin acts on cells by binding to its cell surface receptor. The insulin receptor is a heterotetramer made up of two α,β dimers linked by disulfide bonds (Kasuga *et al.* 1982a). The α -subunit is extracellular, containing the ligand-binding domain, while the β -subunit spans the membrane and the cytoplasmic portion contains intrinsic tyrosine kinase activity (Kasuga *et al.* 1982b). Ligand binding induces autophosphorylation of insulin receptor on specific tyrosine residues and further activation of its intrinsic kinase activity. Activated insulin receptor tyrosine phosphorylates intracellular substrates to initiate signal transduction (Dunaif 1997).

The abnormal glucose metabolism in type 2 diabetes develops slowly. Insulin resistance with other features of MBS arise first. Hyperglycemia occurs later as pancreatic β -cells fail to secrete enough insulin to compensate for the insulin resistance. Hyperinsulinemia is thought to be the result of insulin resistance. Insulin resistance leads to decreased disposal of glucose into muscle, and postprandial hyperglycemia. Later, deficient insulin action results in increased hepatic output and fasting and all-day hyperglycemia. Insulin resistance is associated with obesity, a sedentary lifestyle and caloric excess. Adipose tissue is an active endocrine organ, secreting FFAs and inflammatory mediators that disturb insulin action in skeletal muscle and liver, and adiponectin, levels of which are decreased in obesity, which enhances insulin action.

Ectopic fat, being a source of intracellular acyl-CoA molecules, affects normal insulin signal transduction (Ravussin & Smith 2002, Goldstein 2003).

2.6.2.2 Beta-cell dysfunction

Insulin is secreted by pancreatic β -cells in two phases. The first phase is a rapid release of insulin occurring over minutes and the second phase is a release of insulin over hours following food intake or oral administration of glucose. To maintain glucose homeostasis, compensatory increases in insulin secretion from the β -cells are essential. Loss of the first phase of insulin secretion and abnormalities in the normal pulsatile fashion of insulin secretion are early signs of β -cell dysfunction in the development of diabetes. The mechanisms of disrupted β -cell function involve adverse effects of hyperglycemia and visceral lipid storage in persons with obesity and insulin resistance (Goldstein 2003).

2.6.3 Associations with PCOS

Glucose tolerance is often impaired in obese and occasionally in nonobese women with PCOS (Dunaif 1997). A prevalence of IGT of 31–35% and a prevalence of type 2 diabetes of 7.5–10% has been demonstrated in PCOS women (Ehrmann *et al.* 1999, Legro *et al.* 1999). The effect of obesity and PCOS on glucose tolerance is negatively synergistic (Dunaif 1997). The rate of conversion from IGT to type 2 diabetes is increased 5 to 10-fold in PCOS (Ehrmann *et al.* 1999, Norman *et al.* 2001). Regardless of age, PCOS is a major risk factor for type 2 diabetes. One study showed a 2-fold greater risk of conversion rate of IGT to type 2 diabetes among oligomenorrheic women compared with eumenorrheic women, regardless of obesity, thus indicating that oligomenorrhea is an independent predictor of type 2 diabetes (Solomon *et al.* 2001).

Insulin resistance is well-documented in PCOS patients (Dunaif 1997), though in nonobese women the results are controversial (Dunaif *et al.* 1989, Holte *et al.* 1994a, Acien *et al.* 1999, Morin-Papunen *et al.* 2000b). It has been suggested that nonobese women with PCOS have a form of insulin resistance that is not associated with obesity, and in obese women with PCOS, insulin resistance is partly related to excess adiposity (Diamanti-Kandarakis *et al.* 2003). Visceral and ectopic distribution of fat with adverse effects on energy metabolism and insulin sensitivity in the liver and muscle is probably an important link between PCOS and insulin resistance. Some studies (O'Meara *et al.* 1993, Ehrmann *et al.* 1995b, Dunaif & Finegood 1996), though not all (Morin-Papunen *et al.* 2000b) have demonstrated β -cell dysfunction in women with PCOS.

Fasting hyperinsulinemia can be considered to be a result of insulin resistance, derived from increased insulin secretion or decreased clearance of insulin, or a combination of these disturbances. Basally increased insulin secretion is present in at least obese PCOS women (O'Meara *et al.* 1993). In some studies, the insulin response to an oral glucose load was increased in lean and obese PCOS women, but first phase insulin secretion was

similar to that in weight-matched controls (Dunaif *et al.* 1987, Dunaif & Finegood 1996). In contrast, other studies have shown that indeed the first phase secretion of insulin is increased in PCOS, independently of BMI and body fat distribution (Holte *et al.* 1994a, Holte *et al.* 1995). These differences are possibly a result of the heterogeneity of the populations studied and the complexity of the syndrome. Hepatic insulin extraction has been shown to be decreased in some studies (O'Meara *et al.* 1993, Morin-Papunen *et al.* 2000b) but not all (Ciampelli *et al.* 1997).

Many of the abnormalities of the MBS are present in PCOS and thus it may be considered to be a component of the syndrome in women. Metabolic syndrome is a risk factor for CVD (Stern 1995). Insulin resistance is strongly implicated in the etiology of both MBS and PCOS (Dunaif 1997, Reaven 1999). However, not all women with PCOS have features of MBS and not all women with MBS have PCOS. Insulin resistance, hypertension and dyslipidemia are variably present in PCOS, suggesting that PCOS and MBS are not identical (Legro 2003). Overlapping of these conditions suggests that they may have been mixed in various studies. Populations of PCOS women vary from one study to another and depending on the criteria used, it could be that women studied as PCOS patients were in fact suffering from MBS or *vice versa*. The associations and differences between MBS and PCOS need further investigation.

2.6.4 Insulin and androgens

Hyperinsulinemia and hyperandrogenism are positively related in women. It is not clear which comes first, hyperinsulinemia or hyperandrogenism. To favor the former, it has been shown that weight loss decreases the circulating levels of insulin, IGF-1 and androgens and increases those of IGFBP-1 (Kiddy *et al.* 1989). Also, *in vitro*, insulin stimulates thecal cell androgen production (Barbieri *et al.* 1984). Experimental reduction of insulin in women reduces androgen levels in those with PCOS but not in normal women (Nestler *et al.* 1989). Insulin suppresses SHBG production in the liver, increasing the amount of bioavailable androgens (Plymate *et al.* 1988). Furthermore, after using GnRH agonist to normalize androgen levels, the hyperinsulinemic response to glucose tolerance testing remained in obese women with PCOS (Dunaif *et al.* 1990, Dale *et al.* 1992). On the other hand, there is evidence for the hypothesis that body fat distribution is a major determinant of insulin sensitivity in PCOS. Weight reduction in obese PCOS women improved insulin sensitivity, and reduction of abdominal adiposity normalized the post-diet insulin sensitivity index in PCOS women compared with weight-matched controls in one study (Holte *et al.* 1995). It has been proposed that hyperandrogenemia during fetal development and puberty affects body fat distribution and favors abdominal adiposity, which predisposes individuals to insulin resistance (Abbott *et al.* 2002).

2.6.5 IGF-1, IGFBP-1

Insulin-like growth factors (IGFs) are single chain polypeptides that resemble insulin in structure and function. In ovarian theca cells, IGF-I increases steroidogenesis, in granulosa cells, it affects the formation and increase in numbers of FSH and LH receptors, steroidogenesis, secretion of inhibin and oocyte maturation. Insulin-like growth factor-binding proteins (IGFBPs) modulate the biologic availability of IGFs. They bind IGFs and alter cellular responsiveness. Insulin can bind to IGF receptor and thus modulate ovarian cellular functions through its own or IGF-I receptor. Increasing insulin directly inhibits IGFBP-I production in the liver (Conover *et al.* 1992). In PCOS women with hyperinsulinemia, IGFBP-I levels are decreased (Pekonen *et al.* 1989, Buyalos *et al.* 1995). Lower IGFBP-I allows an increase in circulating IGF-I levels and thus greater local activity of IGF-I and/or IGF-II in the ovary.

2.7 Cardiovascular diseases and PCOS

2.7.1 Lipoprotein lipids and triglycerides

The circulating concentrations of total cholesterol, LDL-cholesterol (LDL-C) and triglycerides are frequently reported to be higher and those of HDL-cholesterol (HDL-C) to be lower among women with PCOS than in controls. Tens of studies have involved assessment of lipid profiles in PCOS women, mostly in case-control settings. In metabolic syndrome, LDL-C and total cholesterol are typically not affected whereas elevated triglyceride levels and low HDL-C levels are associated with insulin resistance and are also required for the diagnosis of MBS (Laaksonen *et al.*, in press). The study by Wild *et al.* (1985) was one of the earliest to involve lipid profiles in PCOS. Although the study sample was small, the results indicated higher levels of triglycerides and VLDL-C, higher BMI and lower HDL-C levels among PCOS patients than controls. Table 2 summarizes the findings of case-control studies (involving at least 100 cases) on cardiovascular disease risk factors among PCOS women. Conway *et al.* (1992) studied lean and obese women separately and Talbott *et al.* (1995, 1998) had large study samples. Legro *et al.* (2001) matched the study population ethnically.

2.7.2 Risk factors

Dyslipidemia, obesity, insulin resistance and elevated blood pressure are frequently reported manifestations of increased cardiovascular disease risk factors clustering in PCOS.

Table 2. Cardiovascular disease risk factors in women with PCOS in studies with at least 100 PCOS patients.

Authors	Country	Age (mean)	<i>n</i> PCOS Ctrls	PCOS ↑	PCOS ↓
Conway <i>et al.</i> 1992	UK	-	102	Lean: insulin, obese: BP, triglycerides, glucose	HDL
Talbott <i>et al.</i> 1995	USA	35.9, 37.2	206	BMI, insulin, triglycerides, total cholesterol, LDL, WHR, BP	HDL
Talbott <i>et al.</i> 1998	USA	35.3, 36.7	244	Total cholesterol, LDL-C, insulin, BMI, WHR, triglycerides, BP	HDL
Legro <i>et al.</i> 2001	USA	Adjusted for age	195 62	LDL, total cholesterol; in obese, HDL and triglycerides	

2.7.3 Low-grade inflammation

There is strong evidence suggesting that low-grade inflammation, reflected by elevated highly-sensitive C-reactive protein (hs-CRP) levels in the circulation, may be a part of the development of atherosclerosis (Kuller *et al.* 1996, Festa *et al.* 2000, Ridker *et al.* 2000). CRP is a pentameric protein synthesized in the liver. Its main action is to activate complement and to counteract infections. Hs-CRP reflects low-grade inflammation apart from infections. The central mediator of CRP production is the cytokine IL-6. Circulating levels of CRP are positively related to obesity, smoking, serum fibrinogen, heart rate, blood pressure, serum triglycerides, fasting blood glucose, and apolipoprotein B and inversely related to HDL-C (Lind 2003). CRP independently seems to predict coronary heart disease (Ridker *et al.* 1998, Ridker *et al.* 2000) and has been shown to be associated with insulin resistance (Yudkin *et al.* 1999, Festa *et al.* 2000). The presence of low-grade inflammation in PCOS women has been demonstrated on the basis of elevated hs-CRP levels (Kelly *et al.* 2001, Fenkci *et al.* 2003, Morin-Papunen *et al.* 2003a), which may contribute to the increased risk of CVD and type 2 diabetes in women with PCOS.

2.7.4 Morbidity and mortality

The results of several studies suggest that women with PCOS have signs of increased morbidity as regards circulatory diseases. The mechanism explaining the link between PCOS and this increased risk is not clear. Polycystic ovaries were related to more extensive coronary artery disease in one study (Birdsall *et al.* 1997) in which women who had undergone coronary angiography were screened for PCO. A risk factor model, established from independent risk factors for myocardial infarction in a prospective population study of 33 PCOS women and 132 controls, gave a relative risk of 7.4 of

developing myocardial infarction for women with PCOS (Dahlgren *et al.* 1992a). Increased oxidative stress and decreased antioxidative capacity in PCOS women were shown in a case-control study with small sample sizes (PCOS, $n=30$; controls, $n=31$), as contributing factors to the increased risk of CVD (Fenkeci *et al.* 2003). Premature carotid atherosclerosis in PCOS women has been suggested on the basis of the presence of carotid plaque (Talbot *et al.* 2000), greater intima-media thickness (Guzick *et al.* 1996) and more prevalent coronary artery calcium (Christian *et al.* 2003), though these results did not prevail after adjustment for BMI. Furthermore, impaired vascular function, which is related to insulin resistance, has been demonstrated in women with PCOS (Paradisi *et al.* 2001, Kelly *et al.* 2002). Increased levels of plasminogen-activating inhibitor-1 (PAI-1) and endothelin-1 are surrogate markers of early atherosclerosis in PCOS women (Velazquez *et al.* 1997, Diamanti-Kandarakis *et al.* 2001). Menstrual cycle irregularity, the most frequent cause of which is PCOS (Hull 1987), is associated with an increased risk of CVD (Solomon *et al.* 2002).

While the evidence for cardiovascular disease risk factors among PCOS women accumulates, follow-up studies have not been able to show that women with PCOS have increased morbidity or mortality resulting from circulatory diseases (Pierpoint *et al.* 1998, Wild *et al.* 2000). In one study, the hospital records of 786 women diagnosed with PCOS between 1930 and 1979 were investigated but the results did not demonstrate that mortality from circulatory diseases in PCOS women was higher than that in the general population (Pierpoint *et al.* 1998). Another UK study included 319 PCOS women and 1060 age-matched control women. Morbidity and mortality from CVD were not higher in the PCOS women than in the controls, although a history of nonfatal cerebrovascular disease and cardiovascular risk factors including diabetes was more prevalent in PCOS women (Wild *et al.* 2000). The authors suggested that possible explanations for the discrepancy between the prevalence of risk factors and of CVD could include inaccuracy of predictive models for CVD in women or the presence of some protective factor against CVD in women with PCOS, such as prolonged exposure to unopposed estrogen or elevated levels of vascular endothelial growth factor. However, the mean age of the women at follow-up was less than 60 years and the overall prevalence of CVD among women at this age is low. To establish the effect of PCOS on morbidity and mortality in CVD, prospective studies with longer follow-up times and representative study samples are needed.

2.8 Treatment of PCOS

Weight reduction is beneficial in PCOS. Weight loss reduces total and visceral fat, improves menstrual cycles and fertility rate, reduces androgen and insulin concentrations and improves insulin sensitivity in women with PCOS (Pasquali *et al.* 1997).

Hirsutism can be treated by a combination of an estrogen-progestin contraceptive plus, if needed owing to the severity of hirsutism, an antiandrogen. Infertility caused by PCOS is treated currently by weight loss and clomiphene citrate (Barbieri 2003). Oral contraceptives (OCs) have long been a standard therapy for PCOS. Their advantages are

regularization of menses, amelioration of hirsutism and acne and protection from the development of endometrial carcinoma. On the other hand, OCs have adverse metabolic effects. In obese PCOS women, OC administration made glucose tolerance worse without a change in plasma insulin levels, suggesting that OCs decreased insulin sensitivity (Morin-Papunen *et al.* 2000a). In nonobese women, OC administration did not change glucose tolerance or insulin sensitivity, suggesting that metabolic effects differ according to body phenotype (Elter *et al.* 2002, Morin-Papunen *et al.* 2003b). The use of OCs may also enhance the risk of cardiovascular disease (WHO 1997, Tanis *et al.* 2001).

Interventions to improve insulin sensitivity by means of a combination of diet and exercise in individuals at high risk of type 2 diabetes reduced progression to type 2 diabetes by 58% (Tuomilehto *et al.* 2001, Knowler *et al.* 2002). Hence, in PCOS, it would likewise be useful to improve insulin sensitivity. Weight loss and exercise have beneficial effects on insulin sensitivity (Norman *et al.* 2002). Metformin is a biguanide antihyperglycemic drug used to treat type 2 diabetes. It lowers blood glucose levels by increasing intestinal uptake of glucose, enhancing peripheral glucose uptake and inhibiting hepatic glucose production. It also enhances insulin sensitivity at postreceptor level and stimulates insulin-mediated glucose disposal without stimulating insulin secretion (Williams 1994). Metformin improves insulin sensitivity, hyperandrogenism, menstrual cyclicity and ovulatory function in women with PCOS (Velazquez *et al.* 1994, Nestler & Jakubowicz 1997, Morin-Papunen *et al.* 1998, Elter *et al.* 2002, Morin-Papunen *et al.* 2003b), regardless of obesity. These effects are probably the result of a decrease in abdominal obesity (Pasquali *et al.* 2000, Morin-Papunen *et al.* 2000a, Morin-Papunen *et al.* 2000b). Metformin also reduces circulating CRP levels in accordance with other known beneficial effects of the drug, suggesting that CRP could be used as a marker of the efficiency of treatment in women with PCOS (Morin-Papunen *et al.* 2003a).

2.9 Summary of the literature review

It is well known that PCOS is a common, complex disorder, associated with major metabolic health issues such as type 2 diabetes and cardiovascular disease risk factors. However, it is not known why morbidity and mortality in regard to cardiovascular diseases in PCOS women could not be shown to be increased in large follow-up studies. Most studies have involved case-control settings, often modest sample sizes and varying criteria for the diagnosis of PCOS. The roles of oligomenorrhea and hirsutism, well-documented symptoms of PCOS, in detecting women with endocrine and metabolic profiles similar to that in PCOS is not well established. These symptoms were associated with hormonal features mimicking PCOS in a study of 192 women from the Greek island of Lesbos, but the study population was likely to have been biased and lipid profiles were not studied (Diamanti-Kandarakis *et al.* 1999). Two large studies approaching PCOS from the symptom-view on the association between menstrual disturbances and risk of cardiovascular diseases and type 2 diabetes have been conducted, and they showed that menstrual cycle irregularity indeed implies a risk of these diseases (Solomon *et al.* 2001, Solomon *et al.* 2002). These investigations of the Nurses' Health Study, with 121 700

female nurses, lacked information on hirsutism, and although disease outcomes were assessed, no biochemical measures as predictors of these diseases were carried out. Health risks solely underlying the symptoms of oligomenorrhea and hirsutism in young women have not been clarified.

3 Purpose of the present study

While PCOS is known to indicate major health risks, the detection of women with PCOS or hormonal and metabolic changes implying a risk of developing PCOS is not simple. The overall aim of this study was to clarify the significance of symptoms of oligomenorrhea and hirsutism in a sample representing the general female population. Since there is a strong link between obesity and PCOS, the association between body size from birth to adulthood, adult abdominal obesity and self-reported hirsutism and oligomenorrhea were studied. The specific aims of this study were to investigate

1. whether low birth weight, pre-term birth, being small for gestational age, obesity in adolescence and adulthood and abdominal obesity in adulthood are associated with increased risk of PCOS symptoms.
2. whether simple, symptom-based questions would be useful to trace women with an endocrine profile typical of PCOS.
3. whether disadvantageous metabolic alterations linked with PCOS, CVD and type 2 diabetes could be seen in women with oligomenorrhea and/or hirsutism compared with women with no symptoms.
4. whether women with self-reported symptoms suggesting PCOS more often have polycystic ovaries as detected in ultrasonography and whether women with PCO differ from controls in biochemical measures.

4 Subjects and methods

4.1 Study population

The Northern Finland birth cohort 1966 (NFBC 1966) consists of 12 068 unselected births in Finland's two Northernmost provinces, Oulu and Lapland (Rantakallio 1969, Rantakallio 1988). All mothers in this district with calculated term falling between January 1–December 31, 1966, were recruited. The study covered all live born and stillborn infants with birth weight of 600 grams or more (Rantakallio 1969). A total of 12 231 children (5964 females) were born, representing 96.3% of all births in the area. The number of those born alive was 12 058 (5889 females). At 14 years of age, the teenagers (or parents in a case of non-response) answered a postal inquiry (97% response in total). Figure 4 illustrates the formation of the study populations in studies I–IV. In 1997–8, at the age of 31 years, 5731 women were alive, of whom 5687 women were traced for the 31-year follow-up study when a postal questionnaire was sent to all women. Those still living in the North or in the capital city area ($n=4074$) were invited to a clinical examination. Of these subjects, 3077 answered the postal questionnaire, gave a blood sample in a clinical examination and did not refuse the use of their data for research purposes. The postal inquiry included questions on the regularity and length of the menstrual cycle and on the presence of excessive and psychologically harmful body hair. The questions were 1) Is your menstrual cycle often more than (over twice a year) 35 days? and 2) Do you have excessive growth of body hair? Those women who used hormonal contraception ($n=859$) or were pregnant ($n=211$) were excluded from the data. In a cohort design for paper I there were 1479 women without symptoms and 528 women with symptoms. The nested case-control design was used (in papers II–IV) when hormonal and other metabolic profiles were analyzed as well as ultrasonographic findings, and included two random controls ($n=1036$) for each case ($n=518$). Those who used medication for diabetes ($n=13$) were excluded from the endocrine and metabolic analyses and those who had reported eating or drinking during the fasting time were excluded from the analyses of glucose, insulin and triglycerides.

All cases who were traced ($n=417$) and a random sample of 163 traced controls were invited to a gynecological ultrasonographic examination in four phases between 1998 and

2002 (IV). All cases who had not attended this examination after the first invitation, were re-invited ($n=344$). A total of 196 cases and 67 controls attended the ultrasound examination. Of these, pregnant women ($n=12$) were excluded. Those who attended the ultrasonographic examination filled in an additional questionnaire with specific questions about reproductive health.

This study protocol was approved by the Ethics Committee of the University of Oulu.

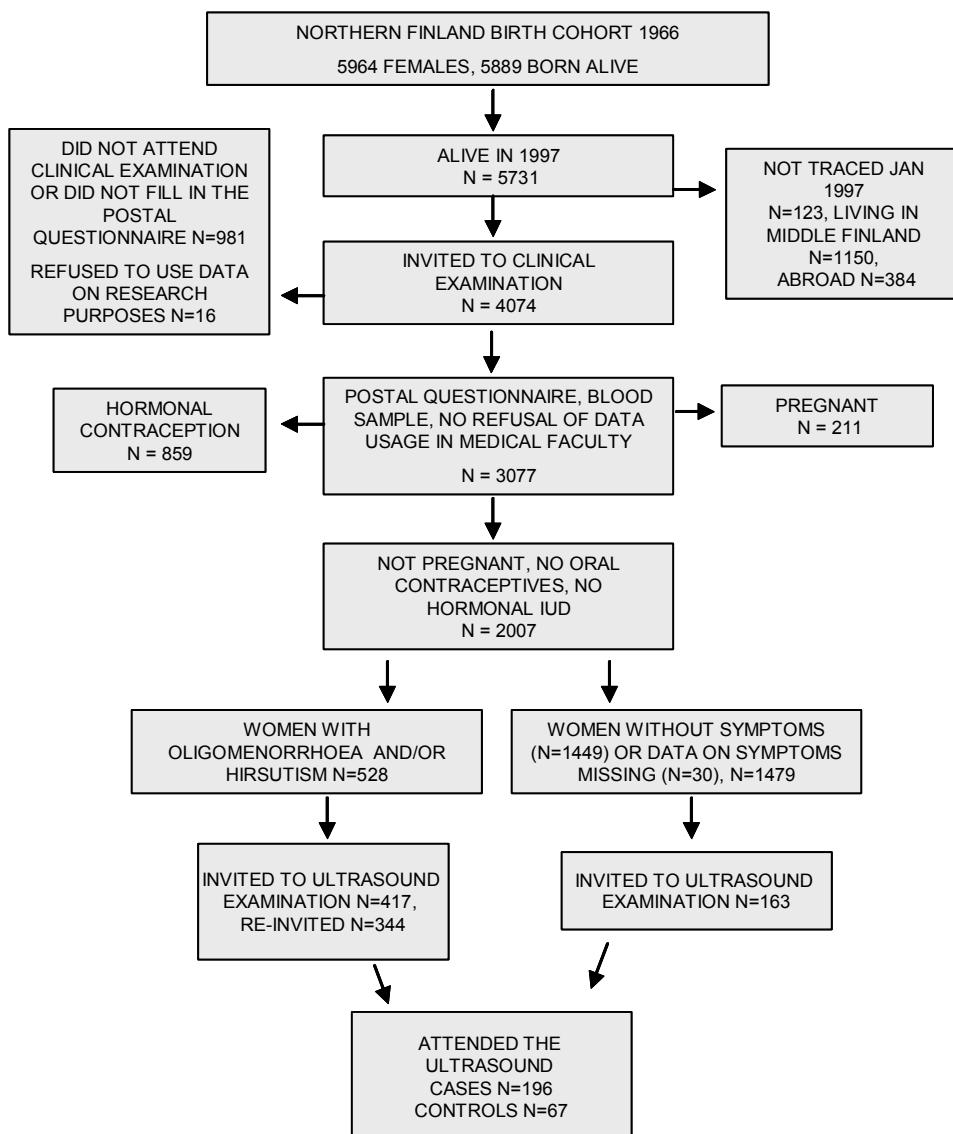


Fig. 4. Flowchart of the cohort study and the nested case-control study on PCOS symptoms and associated characteristics in the Northern Finland Birth Cohort 1966.

4.2 Clinical measures

Data on body size were collected prospectively since birth until the age of 31 years. Body weight and height were measured at birth, self-reported at 14 years and measured at the 31-year examination. Birth weight (within 5 g either way) and height were recorded from the hospital files. Those with a birth weight < 2500 g were classified in a low-birth weight group. Body Mass Index (BMI) (kg/m^2) was calculated at ages 14 and 31 years. An overweight condition and obesity at 14 years were defined as a BMI at or above the 85th or 95th percentile, respectively. Adult BMI at 31 years was classified as follows: underweight (< 18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese (> 30.0 kg/m^2) (WHO 1998). The subjects were also divided into four subgroups according to their weight development from adolescence to adulthood: 1) normal weight at 14 and 31 yr, 2) normal weight at 14 yr and overweight or obese at 31 yr, 3) overweight or obese at 14 yr and overweight or obese at 31 yr, 4) overweight or obese at 14 yr and normal weight at 31 yr. The waist-hip ratio (WHR) was measured at 31 years, as the ratio between the circumferences of the waist (at the level midway between the lowest rib margin and the iliac crest) and the hip (at the widest trochanters). Abdominal obesity was defined as a WHR at the 85th percentile or greater. Gestational age was calculated to the nearest week from the first day of the last menstrual period. Pre-term births were those < 37 gestational weeks. Social class in childhood was based on the father's occupation and its prestige. Small for gestational age (SGA) was defined as a birth weight relative to gestational age below the 10th percentile. Systolic and diastolic BP were measured twice with a mercury sphygmomanometer in a sitting position from the right arm after a 15 minute rest, by trained nurses using a standardized procedure and ongoing quality control (Vartiainen *et al.* 2000). The averages of the two measurements of systolic and diastolic BP were used.

4.3 Blood samples and laboratory methods

Blood samples were drawn after overnight fasting from 22:00 until 8:00–11:00). Samples for blood glucose analysis were stored at +4° C until analyzed later the same day. Serum samples for insulin assay were stored at -20 °C and were analyzed within 7 days of sampling. Samples for assay of testosterone, SHBG, LH, cortisol, IGFBP-1, triglycerides, lipoproteins, and CRP were stored at -80°C until analyzed. The FAI (free androgen index) was calculated by testosterone (nmol/l) x 100 divided by SHBG (nmol/l).

The concentrations of blood glucose were analyzed by a glucose dehydrogenase method (Granutest 250; Diagnostica Merck, Darmstadt, Germany), those of serum insulin by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden), those of SHBG and LH by fluoroimmunoassay (Wallac Ltd., Turku, Finland), those of serum testosterone by an automated chemiluminescence system (Ciba-Corning ACS-180, Medfield, MA, USA) those of serum cortisol by radioimmunoassay (Orion Diagnostica, Oulunsalo, Finland) and those of serum IGFBP-1 by immunoenzymometric assay (Medix Biochemica, Kauniainen, Finland). Fasting serum total cholesterol and triglyceride levels were

determined using a Hitachi 911 automatic analyzer and commercial reagents (Boehringer Mannheim, Germany). Serum levels of HDL- and LDL-cholesterol were also determined using the same analyzer and methods published before (Wieland & Seidel 1983, Sugiuchi *et al.* 1995). Serum C-reactive protein (CRP) concentrations were determined by immunoenzymometric assay (Medix Biochemica, Espoo, Finland). The intra- and inter-assay coefficients of variation respectively were 1.5% and 2.3% for blood glucose, 5.3% and 7.6% for insulin, 1.3% and 5.1% for SHBG, 4.9% and 6.5% for LH, 4.0% and 5.6% for testosterone, 4.0% and 4.3% for cortisol, 3.4% and 7.4% for IGFBP-1, 0.7% and 1.5% for total cholesterol, 0.5% and 3.2% for HDL-C, 1.6% and 2.6% for LDL-C, 0.9% and 2.4% for triglycerides and 4.2% and 5.2% for CRP. The sensitivity of the insulin assay was 14.35 pmol/l, that of the testosterone assay was 0.35 nmol/l and that of the CRP assay was 0.08 mg/l.

4.4 Transvaginal ultrasonography

Transvaginal ultrasonography of the ovaries was performed to measure ovarian volumes and the number of follicles. The instrument (Toshiba SSA-270A, Toshiba Co., Tokyo) was equipped with a 6 MHz curvilinear transvaginal probe, PVF-651VT, with a scanning angle of 120°. The examinations were performed by two investigators (S.A and R.K) on period day 3–5 and if amenorrhea was present, at any time. Polycystic ovaries were defined as 10 or more follicles of 2–8 mm in diameter in one plane of each ovary in association with increased and/or hyperechogenic ovarian stroma, evaluated visually (Adams *et al.* 1986). Ovarian volumes were determined by using the formula for the volume of an ellipsoid: $0.523 \times \text{length} \times \text{width} \times \text{thickness}$.

4.5 Statistical analyses

In paper I, cross-tabulation was used to evaluate the relationship between categorical explanatory variables and PCOS symptoms. Statistical significance was assessed by Pearson's Chi square test for independence. The relative risk (RR) and 95% confidence intervals (95% CIs) were reported. Assuming a causal relationship between obesity, abdominal obesity and symptoms of PCOS, the population-attributable risk was calculated [an estimate of the percentage of cases with PCOS symptoms in this population that would theoretically not have occurred if all women had been in the low-risk group (normal weight at 31 years)]. In papers II–IV, means and 95% confidence intervals or standard deviations, or medians and interquartile ranges (where the distribution remained skewed after log-transformation) for continuous variables were reported. Log-transformation was used, if needed, to normalize distributions before statistical testing by means of Student's two-tailed t-test. Where distributions continued to be skewed after log-transformation, the non-parametric Mann-Whitney *U* test was used. For categorical variables, the results were expressed as percentages. Statistical

significances in frequency differences between groups were evaluated by using Pearson's Chi-square test for independence. The analyses were stratified by BMI; the results are given separately for normal weight, overweight and obese women. In paper IV, the overall significance of differences between three groups (cases with PCO, cases with normal ovaries, controls) by one-way ANOVA or the Kruskal-Wallis test was first analyzed. Owing to many significant differences and our hypothesis, this was followed by paired tests between cases with PCO and controls, cases with normal ovaries and controls, and cases with PCO and cases with normal ovaries. Positive and negative predictive values of symptoms of oligomenorrhea and hirsutism were calculated. The positive predictive value is the probability that a person actually has polycystic ovaries given that she has symptoms of PCOS (oligomenorrhea and hirsutism). The negative predictive value is the probability that an individual truly has normal ovaries given that she has no symptoms of PCOS. Statistical significance was taken to be $p < 0.05$. Statistical analyses were performed using SPSS (Statistical Package for Social Sciences, Inc., Chicago, IL, USA) versions 10.1 and 11.5 for Windows.

4.6 Representativeness of the study participants

The study participants, cases with symptoms of polycystic ovary syndrome and controls with no symptoms, were compared with the remaining females of the cohort in order to assess the representativeness of the study sample. There were no statistically significant differences in maternal BMI, birth weight, basic education, unemployment and social class at 31 years between the study participants and the remaining female population in the cohort. This suggests that our study sample is well representative of the whole female cohort.

The representativeness of the individuals attending the ultrasound examination was assessed among both the cases and the controls. There were no statistically significant differences in BMI, WHR, glucose, insulin, testosterone and IGFBP-1 values between the subjects who attended the gynecological ultrasonographic examination and those who did not attend. Those who participated tended to have lower serum SHBG concentrations than non-participants, the result being borderline significant in controls (controls; median 55.00 nmol/l for participants vs. 63.45 nmol/l for non-participants, Wilcoxon test $p=0.051$; cases; 53.10 nmol/l for participants vs. 55.25 nmol/l for non-participants, $p=0.16$).

5 Results

Of the women who returned the questionnaire ($n=4523$), 24% reported symptoms of hirsutism and/or oligomenorrhea (including subjects with amenorrhea), 10.4% reported hirsutism alone, 10.2% reported oligomenorrhea alone and 3.4% reported both symptoms.

5.1 Body size development and PCOS symptoms (I)

Table 3 shows characteristics that were associated with increased risk of PCOS symptoms in adulthood and those that were not associated with PCOS symptoms.

Table 3. Characteristics associated significantly positively or not with PCOS symptoms in adulthood.

Associated with increased risk of PCOS symptoms in adulthood	Not associated with PCOS symptoms in adulthood
Abdominal obesity at 31 years	Birth weight
overweight gained after 14 years of age	Gestational age
overweight or obese at 14 years of age	Being small for gestational age

BMI at 14 and at 31 years were relatively strongly correlated (Pearson's correlation coefficient being 0.59 for continuous variables). Abdominal obesity had a strong association with PCOS symptoms; the proportion of women with symptoms of PCOS was about 30% higher among women with abdominal obesity (WHR > 85th percentile) compared with those without abdominal obesity.

The influence of abdominal obesity status on the effect of weight status from 14 to 31 years on PCOS was assessed. The analyses showed an increased risk of PCOS symptoms among women with abdominal obesity, who had gained weight after adolescence, being overweight or obese at 31 years [relative risk (95% CI) 1.44 (1.10-1.89)], and among

women with abdominal obesity and who were overweight or obese at both 14 and 31 y [1.71 (1.30-2.24)]. The attributable risks, estimates of the percentages of cases with PCOS symptoms in this population that would theoretically not have occurred if all women had been in the low-risk group (normal weight), are shown in Table 4. Women with abdominal obesity and with overweight or obesity since adolescence had the highest attributable risk suggesting that about 40% of symptomatic cases in this population could have theoretically been prevented if they had had normal body weight without abdominal fat accumulation.

Table 4. Attributable risks of PCOS symptoms according to abdominal obesity status.

Weight category	Attributable risk	Attributable risk by abdominal obesity	
		No (WHR < 85th pct)	Yes (WHR > 85th pct)
Normal weight at 14 years, but overweight or obese at 31 years	22%	17%	31%
Overweight or obese at 14 and 31 years	31%	19%	41%

5.2 Hormonal, metabolic and clinical characteristics of the cases and controls (II, III)

The hormonal, metabolic and clinical characteristics of asymptomatic control women, those who reported both oligomenorrhea and hirsutism, those who reported oligomenorrhea only, hirsutism only, and all cases (hirsutism and/or oligomenorrhea) are presented in Table 5. The greatest differences in all parameters were seen between the controls and those who reported both symptoms. Statistically significant differences could, however, also be seen between the controls and all cases (hirsutism and/or oligomenorrhea). Values of testosterone, LH, FAI, insulin, triglycerides, CRP, BMI and WHR were higher and those of glucose/insulin, SHBG and HDL-C lower among the cases than among the controls. The group with only oligomenorrhea looked like the all cases group and the group with only hirsutism looked like the control group with respect to the assessed measures.

Table 5. Hormonal, metabolic and clinical measures of controls and women with symptoms of PCOS. The values are expressed as means (95% CI) or medians (interquartile range).

	Controls n=1000-1036	Both symptoms n=72-79	Only oligomenorrhea n=207-222	Only hirsutism n=200-211	All cases n=500-518
BMI (kg/m ²)	24.2 (23.9, 24.4)*	27.9 (26.2, 29.6)	25.0 (24.3, 25.8)	24.1 (23.6, 24.7)	25.1 (24.6, 25.6)
WHR	0.81 (0.80, 0.81)*	0.85 (0.83, 0.87)	0.82 (0.81, 0.83)	0.81 (0.80, 0.82)	0.82 (0.82, 0.83)
Systolic BP (mmHg)	120 (119, 121)	126 (123, 130)	119 (117, 120)	120 (118, 121)	120 (119, 121)
Diastolic BP (mmHg)	75.0 (74.3, 75.6)	80.3 (77.5, 83.2)	75.1 (73.7, 76.5)	75.2 (73.8, 76.6)	75.9 (75.0, 76.8)
Testosterone (nmol/l)	1.90 (1.40, 2.40)*	2.50 (1.90, 3.23)	2.10 (1.50, 2.70)	2.00 (1.50, 2.50)	2.10 (1.60, 2.70)
SHBG (nmol/l)	60.7 (44.2, 81.0)*	38.4 (27.4, 61.5)	52.4 (35.8, 69.4)	56.3 (41.3, 77.6)	52.4 (36.3, 72.3)
LH (U/l)	4.85 (3.30, 7.13)*	6.45 (4.38, 11.6)	6.20 (3.93, 8.68)	4.20 (2.90, 6.90)	5.40 (3.40, 8.40)
Cortisol (µmol/l)	0.36 (0.27, 0.47)	0.39 (0.26, 0.53)	0.35 (0.25, 0.46)	0.36 (0.28, 0.46)	0.36 (0.27, 0.47)
FAI (free androgen index)	3.03 (2.06, 4.54)*	5.90 (3.92, 9.87)	3.95 (2.48, 5.77)	3.53 (2.26, 5.80)	4.01 (2.47, 6.29)
Insulin (pmol/l)	51.7 (43.1, 64.1)*	59.2 (45.9, 86.8)	53.1 (42.3, 68.9)	53.1 (43.8, 65.9)	53.8 (42.3, 68.2)
Glucose (mmol/l)	4.90 (4.70, 5.20)	5.00 (4.80, 5.20)	4.90 (4.60, 5.20)	4.90 (4.70, 5.20)	4.90 (4.70, 5.20)
Glucose/ insulin (x10 ⁸)	0.95 (0.77, 1.11)*	0.84 (0.56, 1.13)	0.92 (0.74, 1.16)	0.92 (0.78, 1.13)	0.91 (0.74, 1.14)
Total cholesterol (mmol/l)	4.84 (4.79, 4.90)	4.95 (4.75, 5.15)	4.93 (4.81, 5.05)	4.85 (4.73, 4.97)	4.90 (4.82, 4.98)
HDL-C mmol/l)	1.66 (1.63, 1.68)*	1.47 (1.40, 1.55)	1.61 (1.56, 1.66)	1.62 (1.57, 1.66)	1.60 (1.57, 1.63)
LDL-C (mmol/l)	2.80 (2.75, 2.84)	2.96 (2.78, 3.13)	2.87 (2.76, 2.99)	2.82 (2.71, 2.93)	2.87 (2.79, 2.94)
Triglycerides (mmol/l)	0.91 (0.88, 0.94)*	1.17 (1.00, 1.33)	0.96 (0.90, 1.02)	0.91 (0.85, 0.96)	0.97 (0.92, 1.01)
Hs-CRP (mg/l)	0.60 (0.30, 1.40)*	0.90 (0.40, 4.30)	0.80 (0.40, 1.70)	0.60 (0.30, 1.35)	0.70 (0.30, 1.70)

*Difference between controls and all cases statistically significant ($p < 0.05$).

5.3 Glucose to insulin ratio, SHBG and fasting insulin (II)

The glucose to insulin ratio, the concentrations of SHBG and fasting insulin in the symptomatic cases and the controls in three BMI groups are shown in Figure 5. The differences between the cases and the controls were statistically significant concerning SHBG in all BMI categories.

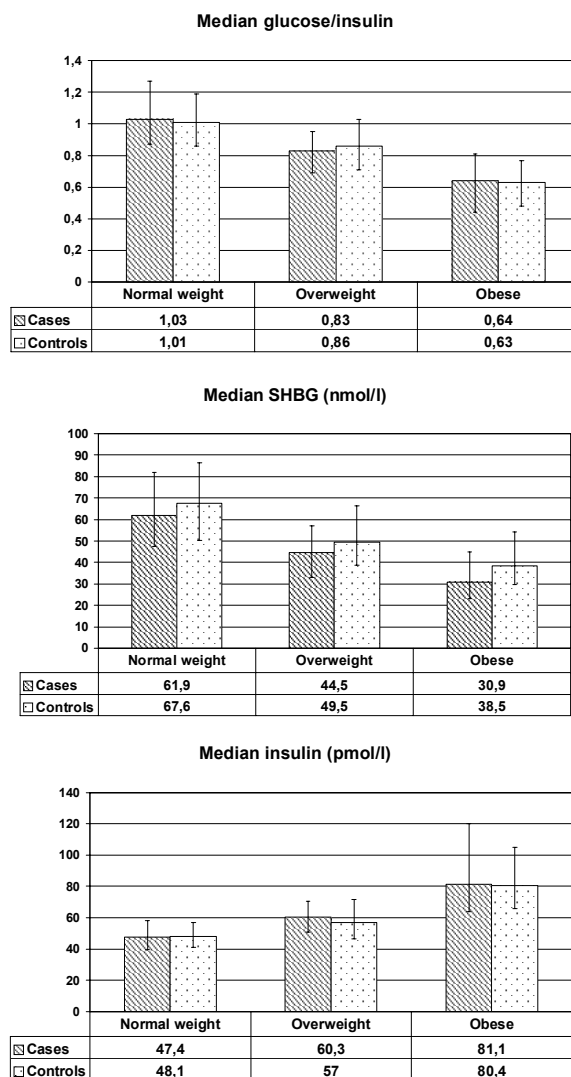


Fig. 5. Glucose to insulin ratio, SHBG and fasting insulin in normal weight, overweight and obese women with symptoms, and controls. Values are medians; error bars represent 25th and 75th quartiles.

5.4 Hormonal, metabolic and clinical characteristics of the cases and the controls according to BMI (II, III)

Body mass index is associated with PCOS symptoms and many of the hormonal, metabolic and clinical features assessed. The analyses were therefore stratified by BMI. Table 6 shows the statistically significant differences between the cases and the controls that persisted after BMI stratification, indicating that these differences are independent of body size.

Table 6. Statistically significant ($p < 0.05$) differences between the cases and the controls persisting after BMI (kg/m^2) stratification.

Normal weight BMI < 25	Overweight $25 \leq \text{BMI} < 30$	Obese BMI ≥ 30
Testosterone higher among cases	Testosterone higher among cases	Testosterone higher among cases
SHBG lower among cases	SHBG lower among cases	SHBG lower among cases
LH higher among cases	FAI higher among cases	FAI higher among cases
FAI higher among cases	WHR higher among cases	HDL-C lower among cases

5.5 Ovarian morphology (IV)

The prevalence of PCO was significantly higher in the cases than in the controls (69/185, 37.3% vs. 12/66, 18.2%; $p=0.004$). Figure 6 shows the prevalence of PCO in ultrasonographic examination of controls, all cases, those cases who reported hirsutism only (16/87, 18.4%), oligomenorrhea only (34/71, 47.9%), and both symptoms (19/27, 70.4%). The latter figure, 70.4%, i.e. positive predictive value, is the probability that a person actually has polycystic ovaries given that she has symptoms of PCOS (oligomenorrhea *and* hirsutism). The negative predictive value, the probability that an individual truly has normal ovaries given that she has no symptoms of PCOS, was 81.8%.

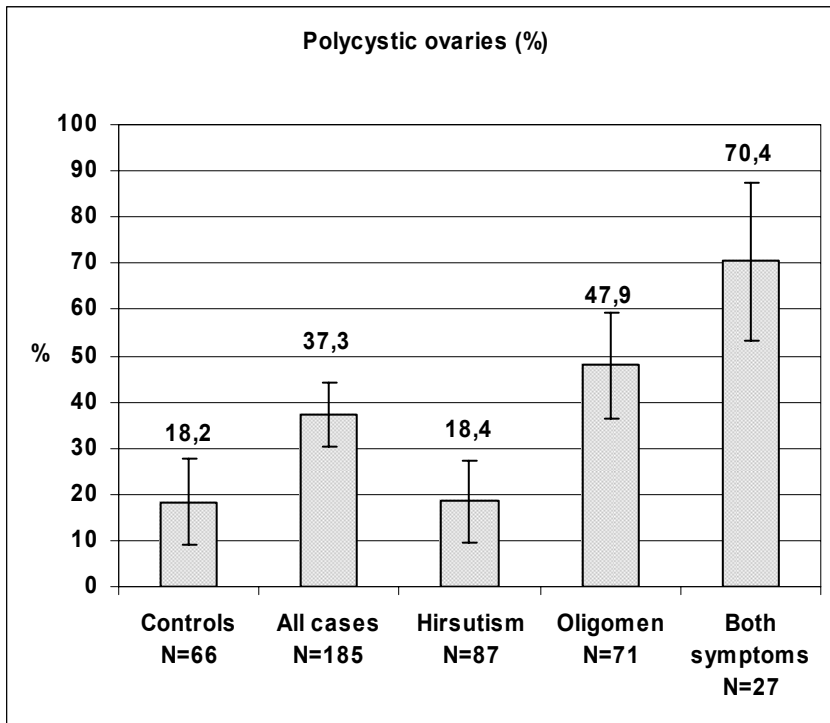


Fig. 6. Prevalence of PCO in controls, all cases, those women who reported only hirsutism, only oligomenorrhea, and both symptoms.

5.6 Clinical and biochemical characteristics in cases with PCO, cases with normal ovaries, and in controls (IV)

Clinical and biochemical characteristics in symptomatic cases with PCO according to ultrasonography, those symptomatic cases with normal ovaries and in asymptomatic controls are presented in Table 7. Cases with polycystic ovaries had significantly higher BMI, WHR, infertility rate, ovarian volume (right), FAI, insulin and glucose concentrations, and lower SHBG and IGFBP-1 levels than the controls. Circulating testosterone concentrations tended to be higher ($p=0.063$) and the glucose to insulin ratio lower ($p=0.060$) in the cases with PCO than in the controls. Cases with normal ovaries did not differ from the controls except in infertility rate, which was significantly higher in symptomatic cases with normal ovaries ($p=0.010$) than among the controls.

Table 7. Clinical and biochemical characteristics in cases with PCO, cases with normal ovaries and controls. The values are expressed as means and 95% CI or percentages.

	Oligomenorrhea and/or hirsutism and polycystic ovaries (Case+PCO) <i>n</i> =59-68	Oligomenorrhea and/or hirsutism and normal ovaries (Case+NO) <i>n</i> =78-117	Controls <i>n</i> =55-66
BMI (kg/m ²)	25.9 (24.6, 27.1)*	24.1 (23.2, 24.9)	24.1 (23.2, 25.0)
WHR	0.81 (0.79, 0.83)*	0.79 (0.78, 0.81)	0.78 (0.76, 0.79)
Infertility (%)	29.7*	26.4	10.0
Acne (%)	16.4	10.3	18.2
Miscarriages (%)	19.4	23.9	28.8
Ovarian volume (cm ³)			
right	7.78 (6.72, 8.84)*	5.40 (4.70, 6.09)	5.53 (4.77, 6.28)
left	6.62 (5.83, 7.40)	5.49 (4.91, 6.08)	5.92 (5.16, 6.69)
Testosterone (nmol/l)	2.21 (1.96, 2.47)	1.87 (1.74, 1.99)	1.88 (1.70, 2.06)
SHBG (nmol/l)	46.5 (32.0, 60.5)*	51.0 (36.0, 66.7)	51.1 (37.5, 72.0)
FAI (free androgen index)	5.88 (4.74, 7.01)*	4.44 (3.76, 5.11)	4.02 (3.38, 4.67)
LH (U/l)	6.05 (3.68, 9.70)	4.80 (3.25, 8.55)	5.40 (3.73, 7.55)
IGFBP-1 (µg/l)	3.77 (3.03, 4.51)*	4.70 (3.92, 5.47)	5.06 (4.18, 5.94)
Insulin (mU/l)	9.61 (7.98, 11.2)*	7.84 (7.22, 8.46)	7.65 (6.99, 8.31)
Glucose (mmol/l)	4.98 (4.87, 5.09)*	4.91 (4.78, 5.04)	4.83 (4.72, 4.94)
Glucose/insulin	0.63 (0.57, 0.69)	0.70 (0.66, 0.73)	0.70 (0.64, 0.75)

*Difference between case+PCO and controls statistically significant ($p < 0.05$).

6 Discussion

6.1 Sample representativeness

Oral contraceptives are generally used to treat symptoms of PCOS. All women using oral contraceptives or hormonal intrauterine devices were excluded from this study because of their effect on several metabolic parameters. Also, if the women receiving hormonal treatment had been included in the study, it would not have been clear whether they would have answered the question about symptoms according to the situation during medication or the situation prior to hormonal treatment. These women might therefore have been in the asymptomatic group as well as in the PCOS symptom group according to their interpretation of the question.

This exclusion means that probably those women with the most severe symptoms and biochemical changes were excluded owing to the use of hormonal medication. The included women most likely had milder symptoms than the excluded women owing to the fact that they did not receive medication for their symptoms. In addition, hirsutism is a relatively subjective symptom and may, as self-reported, be overestimated. The symptomatic group probably included some women who would on a clinical scale be considered asymptomatic, further diluting the differences between the case group and the control group. These arguments may lead to underestimation of differences between the cases and the controls. Nevertheless, despite the diluting effect of exclusions and self-reporting of symptoms, the symptomatic case group was distinguishable from the asymptomatic control group in several parameters.

The study sample was well representative of all females in the birth cohort, since no significant differences were found in maternal BMI, birth weight, unemployment and social class at 31 years between the study participants and the remaining females of the cohort.

6.2 Strengths of the study

The greatest strength of this study is the large and stable general female cohort longitudinally followed since the fetal period (Rantakallio 1988). The general population-based data have been collected prospectively since childhood, and thus there is no evident selection bias. It was determined whether the subjects who were obese at 14 years were less willing to participate in the 31-year examination, which might have diluted the results. The participation rate of obese subjects was not different from that of subjects with normal body weight at 14 years. Data on body weight from birth to adulthood, on gestational age, and also on distribution of fat in adulthood was available.

The study population base consisted of women born to the 1966 birth cohort who were living in the original target area in Northern Finland or in the Helsinki area. This introduces a small hypothetical risk of selection bias but, for example with regard to their circumstances earlier in life, they are well representative of the general female population.

Earlier studies are mostly hospital-based case-control studies on relatively small sample sizes. They also concentrate on PCOS-diagnosed patients compared with controls, using varying criteria to diagnose PCOS, thus resulting in heterogeneous groups of PCOS women. There have been three studies involving a symptom-based approach to investigate characteristics and health risks associated with PCOS. In these studies, disease outcomes associated with a history of menstrual disturbances have been assessed (Solomon *et al.* 2001, Solomon *et al.* 2002), and biochemical measures have been investigated, excluding lipid profiles in a possibly biased study sample of clinically healthy women reporting symptoms of oligomenorrhea or hirsutism (Diamanti-Kandarakis *et al.* 1999). The present study is by far the largest general population-based study in which a symptom-based approach with information on both oligomenorrhea and hirsutism has been used to investigate the association between self-reported symptoms of PCOS, body size development, and clinical and biochemical characteristics. In tens of studies, cardiovascular disease risk factor profiles in women with diagnosed PCOS have been assessed, but with lipid profiles in 31-year-old women, the present kind of setting has not been previously used. While the population in the present study represents the Finnish female population well, caution is needed in generalizing the results in other populations.

6.3 Body size and PCOS symptoms (I)

This study shows that an overweight condition and obesity from adolescence into adulthood, and weight gain after adolescence, particularly in the presence of abdominal obesity, increase the risk of hirsutism and menstrual disturbances in adulthood. In contrast to earlier studies (Cresswell *et al.* 1997), birth weight and gestational age were not associated with PCOS symptoms in adulthood in the present study. This discordance may be derived from smaller sample sizes in earlier studies and differences in definitions, as Cresswell *et al.* reported that 24% of their cases were symptomless.

Insulin resistance is one of the common features of the MBS and PCOS (Holte 1996, Holte 1998). It has been suggested that poor intrauterine growth, characterized by low birth weight, could lead to metabolic abnormalities commonly related to MBS in adulthood (Barker *et al.* 1993, Phillips *et al.* 1994). Nevertheless, the findings in the present study do not support an association between PCOS and poor intrauterine growth or between PCOS and long gestation (over 40 weeks).

Obesity in childhood, adolescence and early adulthood has been associated with menstrual problems and anovulatory infertility (Rich-Edwards *et al.* 1994, Lake *et al.* 1997). In concordance, menstrual disturbances and hirsutism at 31 years were associated with higher BMI in adolescence and in adulthood and with a higher waist to hip ratio in adulthood in this study. Abdominal adiposity had synergistic effects with overweight and obesity in adolescence and in adulthood and weight gain after adolescence, on the risk of PCOS symptoms at 31 years. About 30% of those who gained weight after adolescence being overweight or obese and with abdominal obesity at 31 years, and about 40% of those who were already overweight or obese at 14 years and had abdominal adiposity at 31 years had PCOS symptoms that could be accounted for by their body size and shape. In other words, theoretically, these symptoms could have been avoided by successful weight management in a remarkable proportion of women.

Weight gain, obesity and abdominal obesity are associated with a risk of insulin resistance syndrome (Everson *et al.* 1998). Insulin increases androgen production in the ovary, alone and synergistically with LH (Dunaif & Thomas 2001). Abdominal fat is metabolically active and influences androgen and estrogen metabolism (Norman & Clark 1998). This results in a hyperestrogenic state (Pasquali *et al.* 1994), which favors LH secretion and further increases ovarian androgen secretion (Pasquali *et al.* 1997), leading to the clinical symptoms. These results indicate that weight management, especially avoidance of abdominal adiposity, is important in the prevention of PCOS.

6.4 Hormonal, metabolic and clinical characteristics (II, III)

Women who reported symptoms of oligomenorrhea and/or hirsutism were distinguishable from their asymptomatic controls in a general population. Symptomatic cases had higher values of testosterone, FAI, LH, insulin, BMI, WHR, triglycerides, and CRP and lower glucose to insulin ratio, and SHBG and HDL-C concentrations than the asymptomatic controls. After stratification by BMI, the differences between levels of testosterone, SHBG and FAI, and LH in the normal weight group, WHR in the overweight group and HDL-C in the obese group remained statistically significant, suggesting that these differences were associated with the PCOS symptom effect, not body size. Clearly the most severe changes in hormonal and metabolic profiles were seen in those women who reported both hirsutism and oligomenorrhea. This finding indicates that the presence of both symptoms should be considered a sign of belonging to a high-risk group of women developing long-term health consequences and that this group should be addressed with special attention and counselling.

The clinical significance of these changes needs to be further studied. However, it has been suggested that at group level a BP difference of 1.5 to 2 mmHg could have a large impact on population CVD risk (Rose 1981). Therefore, the difference of 6 mmHg in systolic BP means between those who reported hirsutism *and* oligomenorrhea and the controls in our study suggests clinical relevance.

One study showed that the negative effects of PCOS and obesity on SHBG levels were independent of each other, whereas testosterone and the FAI were affected by obesity only in women with PCOS (Holte *et al.* 1994b). The present study indicates that the serum testosterone level, as well as the FAI, is affected by both PCOS symptoms and obesity. Testosterone and FAI levels were higher in symptomatic women compared with controls in all BMI strata and they increased with increasing BMI both among the cases and controls. In accordance with the results of Holte *et al.*, the levels of SHBG were significantly lower among the cases than the controls throughout all BMI categories in the present study, which suggests that the symptomatic cases have decreased insulin sensitivity and higher insulin secretion than the controls (Dunaif *et al.* 1989). Insulin secretion is increased even in lean PCOS subjects (Morales *et al.* 1996, Morin-Papunen *et al.* 2000b) and hyperinsulinemia directly reduces serum SHBG levels in PCOS women (Nestler *et al.* 1991). Thus, SHBG is a useful marker of hyperinsulinemia and/or insulin resistance (Nestler 1993). In line with the results of this study, one epidemiological study showed that the serum SHBG level is highly effective as a single marker for detecting women with PCOS (Escobar-Morreale *et al.* 2001). Furthermore, the glucose to insulin ratio, considered to be another marker of insulin resistance (Legro *et al.* 1998b), was also lower among the cases than the controls in this study. However, the differences between SHBG levels between the cases and the controls were more obvious than the differences between glucose to insulin ratios, which lost statistical significance after stratification by BMI unlike the differences in SHBG levels in this study. The lower SHBG concentrations among the cases may reflect disturbances in insulin secretion and insulin sensitivity. It can be disputed whether insulin measurements are needed to determine insulin resistance, since there is increasing evidence that SHBG is more reliable for this purpose. A limitation to the use of SHBG is that the levels are affected by OC use and thus it is not useful in a substantial proportion of fertile aged women.

In the present study, cortisol levels were not increased among the symptomatic cases compared with the controls. Earlier studies have indicated that cortisol and adrenal androgen secretion are increased in women with PCOS (Martikainen *et al.* 1996).

The levels of total cholesterol and LDL-C were not different between the cases and the controls in the present study in contrast to earlier studies on cardiovascular risk factors in PCOS (Talbot *et al.* 1995, Talbot *et al.* 1998, Legro *et al.* 2001). However, total cholesterol and LDL-C are not essential factors in the pathogenesis of MBS. Elevated triglycerides and lower HDL-C levels, which were seen in symptomatic cases compared with controls in the present study, are in line with the results of previous studies on dyslipidemia in PCOS (Wild *et al.* 1985, Talbot *et al.* 1995, Talbot *et al.* 1998)

An indicator of low-grade inflammation, hs-CRP, independently seems to predict coronary heart disease (Ridker *et al.* 1998, Ridker *et al.* 2000) and is associated with insulin resistance (Yudkin *et al.* 1999, Festa *et al.* 2000). The production of hs-CRP is mainly mediated by the cytokine IL-6 and the levels of hs-CRP are affected by well-known cardiovascular risk factors such as obesity, smoking, serum fibrinogen, heart rate,

blood pressure, serum triglycerides, fasting blood glucose, apolipoprotein B and HDL-C levels (Lind 2003). In women with PCOS, CRP levels have been shown to be higher than in controls (Kelly *et al.* 2001, Fenkci *et al.* 2003, Morin-Papunen *et al.* 2003a). In the present study, hs-CRP levels were higher among the cases than the controls but the difference disappeared when stratified by BMI, indicating that higher hs-CRP levels among the cases could be accounted for by their greater body size.

Diamanti-Kandarakis *et al.* (1999) reported higher type 2 diabetes risk factors among women with both hirsutism and oligomenorrhea compared with controls, but did not find any difference in blood pressure and did not report lipid profiles. The present study revealed that an increase in metabolic cardiovascular risk factors including lipid profiles may be observed in women with self-reported symptoms of PCOS and especially in those with both hirsutism and oligomenorrhea.

6.5 Ultrasonographic findings and associated measures (IV)

Polycystic ovaries were seen in 37% of symptomatic women and 18% of women with no symptoms of PCOS. In women with both oligomenorrhea and hirsutism, the prevalence of PCO was 70%. Eighteen percent of women who reported only hirsutism, and 47% of those with only oligomenorrhea, had PCO according to ultrasonography. The 18% prevalence of PCO in asymptomatic women is in concordance with the results of earlier studies on ovarian morphology of women with no menstrual problems or hirsutism (Polson *et al.* 1988, Koivunen *et al.* 1999). The 18% prevalence of PCO among those with only hirsutism may be explained by the fact that the symptom of hirsutism is subjective and may be overestimated, leading to a mildly symptomatic group of women with no actual hyperandrogenism. Oligomenorrhea, on the other hand, seems to be a specific symptom of PCO, since almost 50% of women with only oligomenorrhea had PCO morphology. Combining these symptoms seems to be most effective in detecting women with PCO since of those who reported the presence of both symptoms, 70% had PCO. This supports the results acquired from earlier studies on PCO in clinic-based patients with complaints of hirsutism and menstrual disturbances (about 70%) (Adams *et al.* 1986).

Women with symptoms and PCO in ultrasonography had higher levels of BMI, WHR, infertility rate, ovarian volume, LH, FAI, insulin and glucose and lower serum SHBG and IGFBP-1 concentrations than the controls. Similar to SHBG, the principal source of IGFBP-1 is the liver, where its production is mainly regulated by insulin (Buyalos *et al.* 1995). The lower SHBG and IGFBP-1 levels among women with symptoms and PCO are therefore most probably explained by the higher insulin levels of these individuals. The measures were also more unfavorable in symptomatic women with PCO than in symptomatic women with normal ovaries. These findings support the hypothesis that women with symptoms and PCO have more unfavorable biochemical changes than symptomatic women with normal ovaries or controls. Women with symptoms and PCO seem to have more marked risk factors (signs of insulin resistance and increased BMI) as regards the development of type 2 diabetes. Our study population was 31 years old and as

the health consequences usually become manifest at a later age, surveillance should be on-going into middle age. Further studies are required to assess the benefits of lifestyle counselling.

7 Conclusions

- I Obesity in adolescence and in adulthood, and also weight gain after adolescence, especially when abdominal obesity is present, was associated with higher frequency of self-reported PCOS symptoms in adulthood. Birth weight, gestational age and being small for gestational age were not associated with PCOS symptoms in adulthood. It is conceivable that prevention of obesity is important among young women.
- II Women with self-reported symptoms of oligomenorrhea and hirsutism had more marked hormonal characteristics of PCOS, signs of decreased insulin sensitivity and more unfavorable cardiovascular risk factor profiles than the asymptomatic controls. The most severe changes were noticed in those who reported both oligomenorrhea and hirsutism. Of the biochemical markers, SHBG seemed to be the most useful as an additional screening tool. The unfavorable changes in the metabolic parameters, also recorded among the overweight and obese controls, were magnified in the presence of PCOS symptoms, and they may be considered as risk factors for health in later life.
- III The prevalence of PCO in ultrasonography among women with self-reported symptoms of hirsutism and/or oligomenorrhea was significantly higher, double (37% vs. 18%) compared with women who did not report these symptoms. The prevalence of PCO was even higher (70%) in women who reported both hirsutism and oligomenorrhea. The women with symptoms and PCO had more disadvantageous biochemical profiles than the women with symptoms and normal ovaries, or the controls.
- IV Using self-reported symptoms of oligomenorrhea and hirsutism as screening tools to find a group of women with metabolic and clinical risk factor indications associated with PCOS is feasible at a population level. The most profound changes were noticed among women who reported both oligomenorrhea and hirsutism, meaning that the presence of both symptoms should be a sign to a physician to initiate further evaluation. Special attention – weight reduction and possibly medical treatment – should be paid to symptomatic overweight or obese women, in whom the most profound changes were observed.

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