Metabolic Aspects in the Polycystic Ovary Syndrome

ÅSA MARIA LINDHOLM
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Abstract

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of childbearing age and is associated with a number of metabolic disturbances. It has been hypothesised these women carry an increased risk of developing cardiovascular diseases (CVD) with advancing age.

The first aim of this thesis was to establish the prevalence of PCOS-related symptoms in Northern Sweden. The Northern part of the WHO MONICA project was used for this purpose. Based on self-reported menstrual disturbances and hirsutism together with biochemical analyses of free androgen index, the estimated prevalence of PCOS in Northern Sweden was 4.8%, which corresponded with previous prevalence studies.

Disturbances in the fibrinolytic system are predictors of future cardiovascular events and measurements of plasminogen activator inhibitor 1 (PAI-1) activity and tissue plasminogen activator (tPA) mass concentration may be used to assess fibrinolytic activity in women with PCOS. From the findings, over-weight women with PCOS had impaired fibrinolysis, especially if they displayed objective biochemical markers of hyperandrogenism. Conversely, lean women with PCOS, displayed no signs of disturbed fibrinolysis.

The adipose tissue is an active endocrine organ that produces and releases hormones, pro-and anti-inflammatory cytokines, and chemoattractant cytokines. Proinflammatory molecules produced by adipose tissue can be active participants in the development of insulin resistance and the increased risk of cardiovascular disease associated with obesity. The findings suggested being overweight, rather than the PCOS diagnosis per se, was the main explanatory variable for elevated adipose tissue inflammation in PCOS patients.

Weight reduction is the primary target for intervention in overweight and obese women with PCOS. When this thesis was planned, no placebo-controlled trials on anti-obesity drugs in women with PCOS had been conducted. Sibutramine in combination with lifestyle intervention resulted in significant weight reduction in overweight women with PCOS. In addition to the weight loss, sibutramine appeared to have a beneficial effect on metabolic and cardiovascular risk factors.

*Keywords:* PCOS, prevalence, fibrinolysis, inflammatory markers, over weight

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List of Papers

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


II Lindholm A, Bixo M, Eliasson M, Hudcova M, Arnadottir J, Holte, J, Sundström Poromaa I. Tissue plasminogen activator and plasminogen activator inhibitor 1 in overweight and lean patients with polycystic ovary syndrome. *Submitted.*

III Lindholm A, Blomquist C, Bixo M, Dahlbom I, Hansson T, Sundström Poromaa I, Burén J. Adipose tissue inflammation in polycystic ovary syndrome (PCOS). *Submitted*


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*Cover illustration made by Liselott Andersson.*
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE-PCOS</td>
<td>The Androgen Excess and PCOS Society</td>
</tr>
<tr>
<td>ASRM</td>
<td>American Society for Reproductive Medicine</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CCL2</td>
<td>Chemokine (C-C motif) ligand 2 (also MCP-1)</td>
</tr>
<tr>
<td>CCR2</td>
<td>Chemokine receptor 2</td>
</tr>
<tr>
<td>CD14</td>
<td>Cluster of differentiation (cell surface molecules present on white blood cells) 14</td>
</tr>
<tr>
<td>CD163</td>
<td>Cluster of differentiation (cell surface molecules present on white blood cells) 163</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>COC</td>
<td>Combined oral contraceptives</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DHEAS</td>
<td>Dehydroepiandrosterone sulfate</td>
</tr>
<tr>
<td>ESHRE</td>
<td>European Society for Human Reproduction &amp; Embryology</td>
</tr>
<tr>
<td>EMA</td>
<td>European Medicines Agency</td>
</tr>
<tr>
<td>FAI</td>
<td>Free androgen index</td>
</tr>
<tr>
<td>FDP</td>
<td>Fibrin degradation products</td>
</tr>
<tr>
<td>FP</td>
<td>Fasting plasma</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>High sensitive c-reactive protein</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostasis model assessment of insulin resistance</td>
</tr>
<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
</tr>
<tr>
<td>IFG</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired glucose tolerance</td>
</tr>
<tr>
<td>IL-18</td>
<td>Interleukin-18</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>IVF</td>
<td><em>In vitro</em> fertilization</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>MBS</td>
<td>Metabolic Syndrome</td>
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</tbody>
</table>
MCP-1 Monocyte chemoattractant protein-1 (also CCL2)
MIF Macrophage migration inhibitory factor
MONICA Multinational MONItoring of trends and determinants in CArdiovascular disease
mRNA Messenger-RNA
NCEP National Cholesterol Education Program
NIH The US National Institute of Health
OGTT Oral glucose tolerance test
PAI-1 Plasminogen activator inhibitor 1
PCO Polycystic ovary
PCOS Polycystic ovary syndrome
PCR Polymerase chain reaction
PPIA Peptidylprolyl isomerase A
QoL Quality of Life
R² Coefficient of determination
RNA Ribonucleic acid
RPLP0 Ribosomal protein, large, P0
SCOUT Sibutramine Cardiovascular OUTcomes trial
SHBG Sexual hormone binding globulin
SPSS Statistical Package for the Social Sciences
T Testosterone
TG Triglycerides
TNF-α Tumor necrosis factor-α
tPA Tissue plasminogen activator
TSH Thyroid stimulating hormone
WHO World Health Organization
Introduction

Polycystic ovaries and the polycystic ovary syndrome

Polycystic ovary syndrome (PCOS), one of the most common endocrine disorders in women of childbearing age [1, 2], is a heterogeneous clinical condition characterized by hyperandrogenism and signs of chronic oligo/anovulation. Recent changes in diagnostic criteria may have further increased the heterogeneity of the patient population.

Polycystic ovaries (PCO) were first described by Stein and Leventhal in 1935, but were not included in the diagnostic criteria for polycystic ovary syndrome (PCOS) until 2003. Polycystic ovaries are characterized by stroma hyperplasia, increased numbers of follicles less than 8 mm and, often but not always enlarged ovaries [3]. Currently, polycystic ovaries are diagnosed by transvaginal ultrasound and defined as the presence of 12 or more follicles, measuring 2–9 mm in diameter, in each ovary and/or increased ovarian volume (>10 ml) [2]. However, the typical ultrasound feature is only one criterion for the whole syndrome, as polycystic ovaries also can appear in otherwise healthy women [4]. Hence, for PCOS diagnosis a further criterion has to be present.

Most women diagnosed with PCOS in the gynecologic setting present with symptoms of oligomenorrhea or amenorrhea and seek medical care primarily because they fear their menstrual disturbance may impair future or present fertility. Other reasons for seeking medical care include hirsutism, manifest infertility, and anovulatory menometrorrhagia. Most women are diagnosed after the age of 18-20 years, as hyperandrogenism and menstrual disturbances are considered common symptoms during normal adolescence [5]. However, because of the heterogeneity of the syndrome, women with PCOS may be encountered in various clinical settings such as the dermatological-, endocrinological-, and surgical departments.

A number of health risks are associated with PCOS, among which the most common are obesity, insulin resistance, type 2 diabetes [6-8], and changes in lipid profile [9-11]. Possible health risks include cardiovascular disease (CVD) [12] and associated risk factors [13-15]. However, whether all women with PCOS carry a similar increased risk for type 2 diabetes and cardiovascular disease remains unknown.
PCOS diagnosis

Different diagnostic criteria have been used for the PCOS diagnosis. In 1990, a conference sponsored by the U.S. National Institute of Health (NIH) put forward recommendations concerning diagnostic criteria for PCOS, which included clinical or biochemical hyperandrogenism together with chronic anovulation, provided other disorders such as late onset congenital adrenal hyperplasia, androgen secreting tumors, and Cushing’s syndrome had been excluded [16]. The NIH criteria are sometimes referred to as “classical PCOS”.

Since 1990, it has become apparent, especially in the infertility setting, many women present with ovarian dysfunction and polycystic ovaries on ultrasound but without clinical evidence of androgen excess. In 2003, a consensus group, with representatives from the European Society for Human Reproduction & Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), modified the criteria to include transvaginal ultrasound evaluation of the ovaries. The revised ESHRE/ASRM criteria (also referred to as the Rotterdam criteria) are the ones primarily used in this thesis. The criteria stipulate two of the three following features should be present: signs of chronic anovulation; clinical or biochemical hyperandrogenism; and typical ultrasonographic appearance of the ovaries, provided other disorders with similar clinical presentation are excluded [17]. With the addition of the ESHRE/ASRM criteria, two new PCOS phenotypes emerged: patients with oligomenorrhea and polycystic ovaries (sometimes referred to as “PCOS light”); and, patients with hyperandrogenism and polycystic ovaries.

In 2006, a third definition of the diagnosis was suggested by the Androgen Excess and PCOS Society (AE-PCOS), formerly known as the Androgen Excess Society, emphasizing the hyperandrogenic features of the syndrome. The AE-PCOS proposed a PCOS diagnosis that includes the criteria: clinical and/or biochemical hyperandrogenism as obligate criteria, and/or, ovarian dysfunction expressed as oligo/anovulation, and/or polycystic ovaries, and exclusion of other androgenic disorders [18].

Table 1. Diagnostic criteria for PCOS.

<table>
<thead>
<tr>
<th></th>
<th>NIH</th>
<th>ESHRE/ASRM</th>
<th>AE-PCOS</th>
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<tbody>
<tr>
<td>Hyperandrogenism</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Olig/Anovulation</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>PCO ultrasound</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
</tbody>
</table>

Depending on which criteria are used, the prevalence of PCOS differs in the same population. For example, the diagnostic criteria according to AE-PCOS exclude women without hyperandrogenic symptoms, thus, entailing a lower
prevalence than the ESHRE/ASRM criteria. Conversely, the AE-PCOS criteria will result in a slightly higher prevalence than the NIH criteria, in which oligo/anovulation is only assessed by reports on menstrual disturbances and not by ultrasound features (Table 1).

In addition to the plausible differences in prevalence rates, it has been suggested that the different criteria may be associated with different metabolic disturbances [19]. For instance, the ESHRE/ASRM criteria will, in addition to women with classical PCOS (i.e. PCOS diagnosed according to NIH criteria), include the PCOS phenotype with oligomenorrhea and polycystic ovaries. This PCOS phenotype is associated with a slightly lower risk for obesity, insulin resistance, hypertension and (hypothetically), fewer cardiovascular complications [20-22].

Quality of life and mental health
PCOS is not only associated with metabolic consequences and reduced fertility but may also influence the quality of life and mental health of affected women. Although most research in the field of mental health and PCOS is devoted to the psychological consequences of infertility [23], other symptom of PCOS may, individually or in concert, negatively affect quality of life (QoL) and mental health of women with PCOS [24].

Independent of obesity/overweight, clinical depression and depressive symptoms appear to be more common among PCOS women than in weight matched controls [25-27]. Likewise, anxiety symptoms including phobic symptoms appear to be more common among PCOS patients [28]. Without adjusting for the confounding effect of overweight, women with PCOS have higher lifetime incidence of social phobia and eating disorders and more often report previous suicide attempts than healthy controls [29]. Current and lifetime use of antidepressants and anxiolytic drugs also appear to be more common in the PCOS group [29].

Needless to say, clinical depression is important to identify in PCOS patients, not only because of the specific disease burden associated with major depression, but also because reduced motivation is a common feature of depression. Reduced motivation, in turn, may negatively impact the success of weight-reducing therapies.

Prevalence Epidemiology
The estimated prevalence for PCOS is approximately 5-10% among fertile women, but a precise determination has not been obtained [30-32]. When this thesis was planned, only two population-based studies had been conducted and neither included the ultrasound criterion. These studies are criticized, as women on combined oral contraceptives (COC) were often excluded. It is thought that the exclusion of COC users will yield an underes-
timation of the true prevalence of PCOS, as the syndrome per se may pre-dispose patients to the use of hormonal therapy.

One of the prevalence studies with a population-based effort is a Greek study [30] from the island of Lesbos, which included 192 women (age 17 - 45 years) invited to a free medical examination through public advertisements in newspapers and on television. In this Greek island population, the prevalence of PCOS was 6.8% [30]. PCOS was diagnosed according to the NIH criteria and the definition of hyperandrogenism was a Ferriman-Gallwey score $\geq 6$ or free plasma testosterone higher than the 95th percentile: other possible causes for the symptom profile were excluded.

In addition, a Finnish population-based study on self-reported PCOS symptoms [33] diagnosed PCOS according to NIH, and assessed hyperandrogenism through self-report. Women on COC were excluded from the study. Overall, the prevalence of PCOS in the Finnish sample was estimated to be 3.4%, which may be an underestimation as there was no assessment of biochemical hyperandrogenism in addition to self-reported hirsutism.

Two other prevalence studies are also available, including a Spanish study of 154 female blood donors (18-45 years) [32]. PCOS was diagnosed according to NIH and hyperandrogenism was defined as Ferriman-Gallwey score $\geq 8$ and/or free plasma testosterone and/or dehydroepiandrosterone sulfate (DHEAS) and/or free androgen index (FAI) higher than the 95th percentile. The prevalence of PCOS in Spain is estimated as 6.5% [32]. In a U.S. study [31] of 400 fertile women (ages 18-45) attending a pre-employment physical examination at the University of Alabama, 56% of the women included were overweight or obese and 140 were COC users. PCOS was diagnosed according to NIH and hyperandrogenism defined as Ferriman-Gallwey score $\geq 6$ and/or total testosterone and/or androstendione and/or DHEAS level above the 95th percentile: related syndromes were excluded. The total prevalence of PCOS in this population was 6.6%, but prevalence rates differed between ethnic groups (8.0% of black women and 4.8% of white women).

According to NIH criteria, the prevalence rates of PCOS may differ between ethnic groups, with white American women and Scandinavian women displaying the lowest prevalence of PCOS. Due to the possible underestimation of PCOS in Scandinavian women, this work was an attempt to determine the PCOS prevalence rate in Northern Sweden.

Clinical manifestation

Hirsutism/hyperandrogenism

In clinical studies on PCOS, hirsutism is usually scored by the Ferriman-Gallwey score. However, besides inter-rater variability in scorings, hirsutism is, in reality, difficult to assess, as many women have already treated their
symptoms prior to clinical evaluation. Biochemical hyperandrogenism is readily assessed by calculating the free androgen index (FAI) with the formula \([\text{testosterone/sex hormone binding globulin (SHBG)}] \times 100\) [34]. Approximately 80% of testosterone is bound to SHBG, 19% to albumin, and only 1% of testosterone is free and biologically available and active in women. For this reason, total testosterone is insufficient for evaluation of androgens in women [35]. Free testosterone is possible to measure with a direct immunoassay method but the assay only retrieves 20-60% of the levels compared with the equilibrium dialysis method, the gold standard free testosterone analysis [36]. Free androgen index is affected by circulating levels of SHBG and testosterone [37], but correlates relatively well with the more costly equilibrium dialysis method. In this thesis, a cut-off for free androgen index > 5 was used, based on recommendations from the Gynecologic Endocrinology Section of the Swedish Society of Obstetricians and Gynecologists [38]. This cut-off depends on the reference intervals for testosterone (0.5–3 nmol/l) and SHBG (35–150 nmol/l) and may not be applicable in settings where other reference intervals are used. Other androgens often used for diagnostic purposes include DHEAS and androstendione [17, 18].

**Anovulation/menstrual disturbances**

The other diagnostic criterion – oligomenorrhea or amenorrhea – as signs of anovulation is usually the reason a patient visits the gynecologist. Although there is no clear-cut recommendation to what should be considered as oligomenorrhea, for research purposes, a definition of less than eight menstruations a year, or menstrual cycles longer than 35 days, is usually applied [39]. A definition of cycle length of less than 21 days or greater than a 4-day variation between cycles is sometimes proposed; however, the rationale for this definition is unclear [40, 41].

**Pathophysiology of PCOS**

The genetic predisposition for PCOS has been evaluated, and a familiar clustering is indicated [42, 43] with higher androgen levels, increased prevalence of insulin resistance [43] and more often CVD in first-degree relatives [44-46]. The genetic contribution for symptom development in individual subjects is difficult to ascertain although 35% of mothers and 40% of sisters of women with PCOS also display characteristics of the syndrome [42]. There is no established male phenotype, but male relatives to women with PCOS have higher rates of obesity and insulin resistance than male controls [47, 48].

Hyperandrogenism in women with PCOS is considered a consequence of abdominal obesity [49], insulin resistance and/or resulting hyperinsulinemia, but may also be a contributing factor to the insulin resistance and abdominal
adiposity displayed by patients [49]. Long-term administration of testosterone in female-to-male transsexuals induces abdominal adiposity and insulin resistance [50], and androgen excess during fetal life and infancy is associated with increased risk of developing abdominal adiposity later in life [51-53]. Thus, chronic androgen excess in PCOS patients could favor insulin resistance by determining a predominantly abdominal distribution of body fat (Figure 1).

**Figure 1.** Simplified theoretical model for the pathophysiology underlying PCOS.

The interplay between hyperandrogenism and insulin resistance has led to the suggestion that PCOS and its metabolic co-morbidities could be explained by the existence of a ‘vicious circle’, whereby, a chronic androgen excess of ovarian and/or adrenal origin results in abdominal adiposity in affected women [49]. Conversely, abdominal adiposity, through insulin resistance and hyperinsulinism, favors further hyperandrogenism through different pathways including the ovaries, hepatic SHBG synthesis and, possibly the pituitary and adrenals [54] (Figure 1).

**Metabolic consequences of PCOS**

PCOS is associated with metabolic disturbances such as insulin resistance and hyperinsulinism, type 2 diabetes, dyslipidemia and, possibly cardiovascular disease [6]. Increased insulin resistance appears a crucial mechanism behind this association and is a common feature in PCOS and the metabolic syndrome (MBS). PCOS could be regarded as a variant of the metabolic syndrome that, for obvious reasons, only affects women. However, the correlation between PCOS and MBS is difficult to assess, as MBS has more
than one definition and numerous diagnostic criteria are suggested [55] (Ta-
ble 2). In this thesis, the term metabolic disturbance was used, as individual
criteria for MBS are associated with health risks.

Women with PCOS have multiple risk factors for type 2 diabetes includ-
ing obesity, a family history of type 2 diabetes and, abnormalities in insulin
action [12, 49, 56]. Studies over the past two decades indicated that insulin
resistance has been convincingly identified as an integral pathogenic feature
of PCOS, particularly in obese individuals [57-59]. Furthermore, insulin
resistance in PCOS patients is closely associated with abdominal obesity and
hyperandrogenism [60-63], and PCOS patients are generally insulin resistant
at lower BMI levels (27-28 kg/m²) than healthy controls [9, 58, 64, 65].

However, heterogeneity within the PCOS sample is reflected by more
conflicting results regarding insulin resistance in lean PCOS patients with
some studies [61, 66-69] indicating increased insulin resistance and other
[70-73] indicating no difference between lean PCOS patients and weight-
matched controls. In some studies [74, 75] PCOS patients without hyperan-
drogenic features are not insulin resistant.
Table 2. Different diagnostic criteria of the metabolic syndrome.

<table>
<thead>
<tr>
<th>WHO</th>
<th>NCEP</th>
<th>IDF</th>
<th>ESHRE/ASRM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes, IFG, IGT or insulin resistance and at least two of the below</td>
<td>At least three of the below</td>
<td>Waist ≥ 80 cm (depending on ethnicity) and at least two of the below</td>
<td>At least three of the below</td>
</tr>
<tr>
<td>Waist/hip ratio &gt; 0.85 and/or BMI &gt; 30 kg/m²</td>
<td>Waist &gt; 88 cm</td>
<td>Waist &gt; 88 cm</td>
<td>Waist &gt; 88 cm</td>
</tr>
<tr>
<td>Triglycerides ≥ 1.7 mmol/l and/or HDL ≤ 1 mmol/l</td>
<td>Triglycerides ≥ 1.7 mmol/l or treatment</td>
<td>Triglycerides ≥ 1.7 mmol/l or treatment</td>
<td>Triglycerides ≥ 1.7 mmol/l</td>
</tr>
<tr>
<td>Blood pressure ≥ 140/90 mmHg</td>
<td>Blood pressure ≥ 130/85 mmHg</td>
<td>Blood pressure ≥ 130/85 mmHg</td>
<td>Blood pressure ≥ 130/85 mmHg</td>
</tr>
</tbody>
</table>

Abbreviations: IFG = Increased fasting glucose; IGT = impaired glucose tolerance; fp = fasting plasma; OGTT = oral glucose tolerance test; HDL = high-density lipoprotein.

Cardiovascular consequences of PCOS

Even at young age, women with PCOS carry a number of independent risk factors for cardiovascular disease (CVD) such as obesity [13, 76-78], changes in lipid profile [9-11], decreased fibrinolytic capacity [14, 15], increased inflammatory markers [79-81] and, a labile control of blood pressure [82].

In addition, women with PCOS display signs of endothelial dysfunction, which is an early event in the development of atherosclerosis that precedes plaque formation and clinical disease. Endothelial function is evaluated in PCOS patients, mostly by measuring post-ischemic flow mediated dilatation of the brachial artery by high-resolution ultrasonography and by measuring arterial stiffness with pulse wave velocity [83-89]. Women with PCOS also display signs of sub-clinical atherosclerosis at young age [13, 76-78, 86, 90-92].

Two relatively small long-term follow-up studies of women with PCOS with prior history of ovarian wedge resection indicate that these patients suffer an
increased risk of myocardial infarction during the menopause [12, 93] and have an increased prevalence of coronary artery disease already in the peri-menopausal period [93]. Register-based long-term follow-up studies suggest an increased prevalence of stroke [94] but morbidity and mortality from coronary heart disease among women with PCOS is not as high as predicted [94, 95]. More recently, postmenopausal women with a retrospective diagnosis of PCOS are reported to have more angiographic coronary artery disease and lower cumulative five-year cardiovascular event-free survival than women without clinical features of PCOS [96] and postmenopausal mothers of PCOS patients have a higher prevalence of cardiovascular events than controls [44].

Fibrinolysis
Disturbances in the fibrinolytic system are known predictors for future CVD. Plasminogen activator inhibitor 1 (PAI-1) has a main role in the fibrinolytic system by inhibiting the tissue plasminogen activator (tPA). Tissue plasminogen activator (tPA) converts inactive plasminogen to plasmin and promotes degradation of fibrin, whereas, PAI-1 regulates fibrinolysis by inhibiting tPA [97] through forming an inactive complex (Figure 2).

Figure 2. Simplified schematic description of the fibrinolytic system. Thin arrows indicate inhibition and bold arrows indicate activation.

Increased mass concentration of tPA, as a marker of endothelial disturbances, is possibly a better predictor for coronary heart mortality [98] than PAI-1 activity and is specifically associated with myocardial infarction in young women [99].
The triad obesity, raised triglycerides and, raised insulin levels is the most consistent predictor of impaired fibrinolysis and is often found in PCOS women [15, 100-103]. Earlier studies on PCOS women suggest both increased and normal levels of PAI-1 activity [15, 100-103], while studies on tPA-mass in PCOS patients are less ambiguous with increased levels [104-106], although at least one study reported unchanged levels [107]. As most studies on fibrinolytic activity in women with PCOS are limited in size it is still unclear if lean women with PCOS have disturbances in the fibrinolytic system.

Obesity in PCOS patients

Overweight and obesity is common among PCOS patients. No precise determinations of the prevalence of overweight among women with PCOS have been made in Sweden but it is estimated approximately half of Swedish PCOS women are overweight. Worldwide, the prevalence of overweight among PCOS patients ranges between 38% and 88% depending on the study population and the diagnostic criteria used (105).

Obesity (BMI > 30 kg/m²) is reported to be prevalent in 10–38% of women with PCOS [30, 32, 108], and, the prevalence of PCOS is increased in obese women where 28% are reported to fulfill criteria for PCOS in contrast to approximately 5% in normal weight women [109].

As a result of the co-existing hyperandrogenism, overweight in PCOS patients is often of the abdominal type [49]. The PCOS phenotype with oligomenorrhea and PCO, according to the ESHRE/ASRM criteria, appears associated with a lower prevalence of overweight/obesity than PCOS phenotypes where hyperandrogenism is a prerequisite [20, 59].

However, whether women with PCOS are more prone to develop obesity or less able to lose weight than other women is unclear although findings indicate PCOS patients may be worse off in this respect [110]. Appetite is regulated by numerous factors, including the two gastrointestinal hormones ghrelin and cholecystokinin. Ghrelin stimulates appetite, whereas, cholecystokinin decrease appetite. Androgen levels play a role in this regulation, as high levels of testosterone are associated with decreased levels of cholecystokinin [111] and increased levels of ghrelin [112].

Inflammatory markers

Adipose tissue is an endocrine and autocrine organ [113] that produces and releases hormones, pro- and anti-inflammatory cytokines and chemoattractant cytokines (chemokines). Some of these proinflammatory molecules can be active participants in the development of insulin resistance and the increased risk of cardiovascular disease associated with obesity [114]. Central abdominal adiposity is a risk factor for metabolic disturbances and especially
visceral fat accumulation is associated with insulin resistance and CVD [115]. The link between central adiposity and subsequent risk of CVD may be mediated through increased production of inflammatory markers in adipose tissue.

Macrophages are an important source of inflammation and adipose tissue in obesity is characterized by macrophage infiltration [116]. Increased macrophage infiltration can be caused by different chemokines attracting inflammatory cells to adipose tissue. One such example is chemokine (C-C motif) ligand 2 (CCL2) (also known as monocyte chemoattractant protein-1, MCP-1) and its cognate receptor chemokine receptor 2 (CCR2), both of which contribute to systemic insulin resistance, macrophage infiltration and, maintenance of macrophages in the adipose tissue [116, 117].

In adipose tissue, the expression and release of proinflammatory cytokines, such as macrophage migration inhibitory factor (MIF), tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and, interleukin-18 (IL-18), is increased in obese adipose tissue [118-120]. Expanded adipose tissue mass of obese people is, directly or indirectly, correlated to an increased level of circulating inflammatory markers [121]. Circulating markers of inflammation, including CCL2, MIF and, high-sensitive C-reactive protein (hs-CRP), are elevated in overweight PCOS women in comparison with weight-matched controls [79-81]. However, no studies concerning inflammatory markers in adipose tissue have been conducted in PCOS patients.

**Weight-reducing treatment**

Weight reduction is the primary intervention target in overweight or obese women with PCOS and rapid weight loss may be mandatory before a planned pregnancy or before fertility treatment can be initiated. Weight loss of 5–10% of weight in overweight women with PCOS through energy restriction can reduce circulating insulin levels and hyperandrogenism [71, 122, 123] and improve menstrual cyclicity [124, 125], fertility and, cardiovascular disease risk factors [122].

Lifestyle modification focusing predominantly on diet and exercise behavior is considered the preferred first-line weight-reduction treatment for obese PCOS [124]. However, there is lack of consensus on the best nutritional management of obesity, in general, and for women with PCOS in particular [126] and diet recommendations for PCOS patients are most often based on local guidelines. Exercise appears equally effective as diet for reproductive outcomes in PCOS patients [127], as the addition of exercise to an energy-restricted diet improves body composition but has no additional effects on improvements in cardiometabolic, hormonal and, reproductive outcomes relative to diet alone [128].

When this thesis was planned, there was hope metformin would be the “all-in-one” drug for treatment of PCOS, including weight reduction in
obese subjects. Metformin, which is still one of the most widely used drugs for treating PCOS, has positive effects on a number of metabolic parameters such as insulin resistance, fasting insulin levels, blood pressure and, low-density lipoprotein levels (LDL) [129]. However, the effect of metformin as a weight-reducing agent in obese subjects with PCOS is discouraging [129-131]. The negative results from randomized clinical trials on metformin treatment in PCOS patients, together with the need for weight reduction in obese PCOS patients, prompted the evaluation of other anti-obesity drugs in this group of patients.

Two weight reducing drugs were available on the market at the time of the study: orlistat and sibutramine. Orlistat inhibits gastric and pancreatic lipases in the lumen of the gastrointestinal tract to decrease systemic absorption of dietary fat [132]. Studies on orlistat in combination with lifestyle intervention show better weight loss than placebo and lifestyle intervention [133, 134]. Sibutramine, on the other hand, acts by inhibiting the reuptake of serotonin and noradrenalin in the central nervous system appetite center, thus increasing satiety [135]. Compared to placebo, sibutramine induces more pronounced body weight reduction of between 5% and 10% in obese patients [136-139]. As with other anti-obesity drugs, the combined effect of lifestyle modification and sibutramine treatment is more effective than with just sibutramine treatment [138, 140]. Sibutramine had also been evaluated with promising results in two open-label studies on patients with PCOS [141, 142].

All weight reduction treatments rely on concomitant lifestyle interventions such as dietary recommendations and exercise [143] and as lifestyle interventions may be equally effective as only sibutramine treatment [138], a lifestyle modification treatment arm was incorporated into the clinical trial.

However, the results of this thesis must be interpreted in the light of current developments. The European Medicines Agency has recently recommended the suspension of the marketing authorization of sibutramine. This recommendation followed a safety review of preliminary data from the SCOUT-study [144]. The SCOUT study reported on 9800 obese or overweight patients with known cardiovascular disease who had been treated with sibutramine or placebo for a six-year period. Among patients treated with sibutramine, an increased risk of serious, non-fatal cardiovascular events, such as stroke or myocardial infarction was apparent: weight reduction, on the other hand, was modest with long-time treatment.
AIMS

To estimate the prevalence of symptoms related to PCOS in a population-based sample of Swedish women.

To evaluate if lean and over-weight/obese PCOS patients had disturbed fibrinolysis, as measured by PAI-1 activity and mass concentration of tPA in comparison with healthy control subjects.

To evaluate if lean and over-weight/obese PCOS patients displayed increased adipose tissue expression of inflammatory markers in comparison with healthy control subjects.

To examine the efficacy of sibutramine combined with brief lifestyle modification for weight reduction in over-weight and obese women with PCOS.
Materials and Methods

Patients and design

PCOS diagnosis

In Paper I, PCOS was defined according to NIH criteria [16], otherwise the Rotterdam criteria were used in the thesis [2]. The diagnostic procedures in Paper I are described in more detail below. In Papers II-IV, two out of three features had to be present for the PCOS diagnosis. The three features were: 1) oligomenorrhea with eight or fewer menstruations in the previous 12 months or amenorrhea; 2) clinical and/or biochemical signs of hyperandrogenism, such as testosterone >2.7 nmol/l, elevated DHEAS (according to reference range at participating clinics), free androgen index ≥ 5.0, or hirsutism (>7 on the Ferriman and Gallway scale); and, 3) polycystic ovaries on ultrasound examination (>12 follicles 2 to 9 mm in diameter and/or increased ovarian volume (>10 ml)). PCOS diagnosis implied no evidence of thyroid disease (normal thyroid-stimulating hormone (TSH)), adrenocortical dysfunction (normal 17-hydroxyprogesterone) or, hyperprolactinemia (prolactin <30 ug/ml) was present.

Paper I

Data from the Northern Sweden component of the WHO MONICA study was used [145]. The information was collected during a population-based survey in 2004: 1000 men and 1000 women were invited to participate, and among women, participation rate was 78%. The target population in the two most Northern counties of Sweden was 312,000 and subjects were randomly selected from population registers and stratified for age (25-74 years) and gender. For the purpose of the study, only females less than 40 years of age were included. Pregnant women and women who used hormonal contraceptives were excluded in the primary analysis. As the prevalence of PCOS may be increased among combined oral contraceptive users, these women were analyzed separately. A flow-chart for the study population from Paper I is presented in Figure 3.
To evaluate reproductive status and PCOS-related symptoms, the women were asked three questions:

1) Do you still have regular menses? Response alternatives were: a) yes, regularly; b) sometimes, but not as regular as before; c) I have not had any menses for the last six months; d) I’m pregnant.

2) Has your menstrual cycle length, at any time during the last two years, been more than 35 days (yes/no)?

3) Do you have excessive growth of body hair in comparison with other women of your age (yes/no)?

Biochemical hyperandrogenism was evaluated through testosterone and SHBG measurements.

For further evaluation of COC users, two additional questions were used:

4) What contraceptive method have you been using during the last year (multiple choices possible)? Response alternatives were: a) have not used any contraception; b) combined oral contraceptives (COC); c) oral progestagens; d) progestagenic intrauterine device; e) progestagen implants; f) progestagen injections; g) other methods.

5) If currently on combined oral contraceptives, how were your menstrual cycles during the year before you started with COC? Response alternatives were: a) regular; b) irregular; c) did not have any menses.

From the responses and the calculated free androgen index, subjects were grouped into four different categories: oligomenorrhoic subjects; hyperandrogenic subjects; oligomenorrhoic and hyperandrogenic subjects; and, control subjects.
Paper II

For this study, 135 patients with PCOS and 81 healthy controls were included. The PCOS patients were recruited from the Department of Obstetrics and Gynecology at Sunderby Hospital, Umeå University Hospital, Umeå, and Uppsala University Hospital, Uppsala, while seeking medical care for oligomenorrhea and/or hirsutism. PCOS was defined according to the Rotterdam criteria; however, due to the increasing controversy on possibly milder PCOS phenotypes, a separate analysis was conducted on women diagnosed according to the NIH criteria [16].

The control subjects were recruited through advertisements in local newspapers in the study regions or from population-based registers. Healthy control status was assessed by careful menstrual history, laboratory work-up and, transvaginal ultrasound. Control subjects had regular menstrual cycles, normal androgen levels and, no signs of polycystic ovaries on transvaginal ultrasound.

Paper III

Twenty overweight PCOS patients (BMI >27 kg/m²) [65], ten normal weight PCOS patients (BMI <25 kg/m²) and twenty overweight controls (BMI >27 kg/m²) were included in the study. The rationale for the BMI limits chosen was based on a previous study [65] indicating that insulin resistance is prevalent at lower BMI in PCOS patients than in healthy women. The PCOS patients were recruited from the outpatients ward at the Department of Obstetrics and Gynecology at Sunderby Hospital, Umeå University Hospital and Uppsala University Hospital. The overweight PCOS patients were included from Paper IV.

Paper IV

Fifty PCOS patients were screened for this investigator-initiated, multi-center, double-blind, randomized, parallel-group clinical trial comparing daily sibutramine 15 mg (Abbot Scandinavia, Sollentuna, Sweden) with placebo (Apoteket Production and Laboratories, Tillverkningsenheten, Stockholm, Sweden). Patients were recruited at the gynecological departments at Sunderby Hospital, Umeå University Hospital, Uppsala University Hospital, Läkarhuset Björnen, Piteå, and Lund University Hospital, Sweden. The study was conducted in 2005-2006. A flow-chart of the study population of Paper IV is presented in Figure 4.
Exclusion criteria for the study were ongoing or planned pregnancy, lactation and, use of hormonal treatment six months prior to study start. In addition, a number of contraindications for use of sibutramine were evaluated including hypertension (> 140/90 mmHg), for details see Paper IV.

Fifty patients were screened for the study. Among these, eight patients did not fulfill the inclusion criteria for participation in the study: 42 patients were included in the study.

The treatment period lasted 24 weeks. All patients received lifestyle modification in the form of dietary and physical activity advice. Subjects were advised to eat three regular meals every day and to reduce the intake of fat in favor for carbohydrates. All subjects were given a step-recorder and encouraged to walk a minimum of 10,000 steps a day, most days of the week. Homework assignments were also given, where food-intake, reason for food intake and physical activity were recorded.

The packing and randomization of treatment was done by Apoteket Production and Laboratories, Stockholm, Sweden. Randomization codes were kept secret at the Pharmacy at Uppsala University Hospital until completion of the study. Compliance was assessed by counting the remaining capsules at each visit. Study visits took place at baseline and at weeks 2, 6, 10, 14, 18, 22, and 24. At each visit, weight, blood pressure and, heart rate and adverse
effects from treatment and concomitant medication were assessed. Urinary pregnancy tests were taken from each subject before the start of the study, at week 14, and at the end of the study. Each woman was advised to use non-hormonal contraception throughout the study and to record vaginal bleeding on a bleeding chart, where absence of bleeding, spotting or, vaginal bleeding was recorded daily.

Methods

**Anthropometric measures, Papers I-IV**

Subjects were weighed on an electronic scale, in light clothes and no shoes: weight was measured to the nearest 0.2 kg. Height without shoes was measured to the nearest centimeter. Body mass index (BMI) was calculated as weight (kg) divided by height (m²). For all participants, the waist and hip were measured in a standing position with the feet fairly close together: waist circumference was measured midway between the lower rib margin and iliac crest and the hip was measured at the maximum circumference over the buttocks and to the nearest 0.0 or 0.5 cm. Blood pressure was measured twice in every person after a five-minute rest in a sitting position. The mean value of the two measurements was used in the study.

**Blood samples, Papers I-IV**

All blood samples in papers II – IV were drawn between 08.00 and 08.30 after overnight fasting and stored at -20°C until analyzed. In Paper I, blood samples were drawn after at least a 4-hour fast.

The blood samples for fibrinolytic activity were collected in vacuum tubes (Stabylite®, Biopool, Umeå, Sweden) and stored at -70°C until analysis. PAI-1 activity and tPA activity were measured by a chromogenic substrate assay. The tPA activity was reported in U/ml, by reference to the activity of the International tPA Standard, coded 86/670 (National Institute for Biological Standards and Control, Potters Bar, London). Similarly, the PAI-1 activity was given in arbitrary U/ml, where 1 U of PAI-1 was defined as the amount of PAI-1 inhibiting 1 U of the International tPA Standard (above). The plasma mass concentration of tPA and PAI-1 antigen were measured with an enzyme linked immunosorbent assay. The reagent kits for assay of mass tPA and PAI-1 antigen (TintElize®) and tPA and PAI-1 activity (Chromolize®) were purchased from Biopool AB, Umeå, Sweden.

Remaining analyses were performed at the department of Clinical Chemistry, Uppsala University Hospital with standardized techniques and are described in detail in each paper.
FAI was calculated as \[
\frac{\text{testosterone (mmol/L)}}{\text{SHBG (nmol/L)}} \times 100
\]
and HOMA-IR as \[
\frac{\text{fasting insulin (mU/L)}}{\text{fasting serum glucose (mmol/L)}} \times 22.5
\] [146].

**Fat biopsies**

Fat biopsies were taken in the skin area to the right of the umbilicus after a local anesthesia with prilocain (10 mg/ml): approximately 1.5 cm² of superficial subcutaneous adipose tissue was excised and snap frozen in liquid nitrogen and stored at -70°C until assayed.

**RNA extraction, reverse transcription, and real-time PCR**

Total RNA was extracted from adipose biopsies with RNeasyLipid Tissue Mini Kit (QIAGEN, Hilden, Germany). The yield and purity were determined by spectrophotometer (ND-1000 spectrophotometer, NanoDrop Technologies, Wilmington, DE9) and RNA integrity was analyzed by 1% agarose gel electrophoreses in the presence of ethidium bromide. One microgram of RNA was reverse transcribed with TaqMan RT reagents (High Capacity cDNA Reverse Transcription kit, Applied Biosystems, Foster City, CA) and RNase inhibitor (Applied Biosystems) at a final concentration of 1.0 U/ml. Subsequently, specific mRNAs were run in duplicate on an ABI Prism 7900HT sequence detection system (Applied Biosystems) with Taqman Universal PCR Master Mix (Applied Biosystems). Seven inflammation-related genes were included in the analyses, including the chemokine CCL2 and its receptor CCR2; the proinflammatory cytokines MIF, TNF-\(\alpha\), and IL-18; and, the monocyte/macrophage markers CD14 and CD163. The TaqMan gene expression assays (Applied Biosystems) used were; CCL2 (Hs00234140_ml), CCR2 (Hs00356601_m1), MIF (Hs00236988_g1), TNF-\(\alpha\) (Hs00174128_m1), IL-18 (Hs999999040_ml), CD14 (Hs00169122_g1), CD163 (Hs01016657_m1), LRP10 (Hs00204094_ml), PPIA (Hs999999904_ml), and RPLP0 (Hs99999902_ml). The parameter cycle threshold (Ct) was defined as the cycle number at which the fluorescence intensity exceeded a fixed threshold. Relative amounts of mRNA expression for target genes were calculated with the comparative Ct method (\(\Delta\Deltat\)). Reference genes were evaluated by running LRP10, PPIA and RPLP0 on the full study cohort, and the NormFinder [147] algorithm identified RPLP0 as the best normalization gene. Thus, the expression of RPLP0 was used to normalize samples for the amount of cDNA used per reaction.
Results

Paper I

Of the 267 women eligible for the study, 147 women less than 40 years without hormonal therapy or ongoing pregnancy were included.

Forty-four (29.9%) women were classified as oligomenorrhoic, of these, 13 women reported no menses during the last six months. Seventeen women (11.6%) were classified as hyperandrogenic; of these, nine women reported excessive body hair growth in the questionnaire, six had increased FAI only, and two women had increased FAI together with excessive hair growth. Seven (4.8%) women were classed as both oligomenorrhea and hyperandrogenism. No cases with increased levels of prolactin, follicle stimulating hormone (FSH) or TSH were identified among the women presenting with oligomenorrhea and hyperandrogenism.

Subjects with only hyperandrogenism had increased weight and BMI and increased waist circumference and waist:hip ratio, compared to controls. Furthermore, subjects with oligomenorrhea and hyperandrogenism had increased weight, BMI, waist and hip circumferences and, increased systolic and diastolic blood pressures compared to controls (Table 3). Subjects with hyperandrogenism and oligomenorrhea more often reported a history of hypertension and gestational diabetes than control subjects did.

Table 3. Clinical and biochemical parameters in healthy women, women with symptoms of oligomenorrhea, hyperandrogenism and both.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 86)</th>
<th>OM (n = 44)</th>
<th>HA (n = 10)</th>
<th>OM and HA (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>68.8 ± 13.0</td>
<td>68.0 ± 16.9</td>
<td>78.3 ± 15.2*</td>
<td>80.5 ± 14.6*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.8 ± 4.4</td>
<td>25.1 ± 6.9</td>
<td>28.0 ± 4.4*</td>
<td>29.5 ± 5.7*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>82 ± 11</td>
<td>82 ± 15</td>
<td>90 ± 11*</td>
<td>91 ± 16*</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>98 ± 9</td>
<td>99 ± 12</td>
<td>104 ± 9</td>
<td>106 ± 9</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.05*</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>110 ± 11</td>
<td>111 ± 10</td>
<td>115 ± 9</td>
<td>128 ± 14**</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>70 ± 8</td>
<td>70 ± 10</td>
<td>72 ± 8</td>
<td>82 ± 7**</td>
</tr>
</tbody>
</table>

OM=Oligomenorrhea, HA=hyperandrogenism, BP=blood pressure
* p < 0.05 compared to controls, Kruskal-Wallis test followed by Mann-Whitney U test
** p < 0.01 compared to controls, Kruskal-Wallis test followed by Mann-Whitney U test
Paper II

Although PCOS patients and control subjects did not differ in BMI, linear regression models were preferred for all relevant analyses in Paper II to exclude the possibility subtle weight differences would affect the results. Furthermore, as PCOS patients were younger than control subjects, adjustment for age was necessary.

Lean PCOS patients did not differ from control subjects in PAI-1 activity or tPA mass, regardless of how PCOS diagnosis was established (for details see Paper II).

The results of stepwise multiple regression analyses with PAI-1 as the dependent variable in relation to PCOS diagnosis (ESHRE/ASRM), age, BMI and HOMA-IR in overweight subjects are presented in Table 4. In the final model, 40% of the variation in PAI-1 activity was explained by age, BMI, and HOMA-IR; whereas, PCOS diagnosis did not remain a significant explanatory factor in the regression model when BMI was included.

Table 4. Results of a stepwise multivariate regression analysis of PAI-1 activity in relation to other variables in overweight subjects (n = 126, BMI >25).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>R²</td>
<td>0.05</td>
<td>0.12</td>
<td>0.28</td>
<td>0.40</td>
</tr>
<tr>
<td>PCOS diagnosis</td>
<td>0.23*</td>
<td>0.16</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Age</td>
<td>-0.27**</td>
<td>-0.18*</td>
<td>-0.18*</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td>0.42***</td>
<td>0.22*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td></td>
<td></td>
<td>0.38***</td>
</tr>
</tbody>
</table>

β = standardized regression coefficient
*p < 0.05
**p < 0.01
***p < 0.001

The results of a stepwise regression analysis with tPA mass as the dependent variable in overweight subjects are presented in Table 5. In this model, PCOS diagnosis remained a significant independent explanatory variable for the variation in tPA mass concentration among overweight subjects, with an explanation rate of 23% in the final model.
Table 5. Results of a stepwise multivariate regression analysis of tPA mass concentration in relation to other variables in overweight subjects (n = 126, BMI >25).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1 β</th>
<th>Model 2 β</th>
<th>Model 3 β</th>
<th>Model 4 β</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^2$</td>
<td>0.04</td>
<td>0.06</td>
<td>0.19</td>
<td>0.23</td>
</tr>
<tr>
<td>PCOS diagnosis</td>
<td>0.21*</td>
<td>0.24**</td>
<td>0.19*</td>
<td>0.17*</td>
</tr>
<tr>
<td>Age</td>
<td>0.14</td>
<td>0.22*</td>
<td>0.22*</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>0.38***</td>
<td>0.23*</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td></td>
<td></td>
<td>0.25*</td>
</tr>
</tbody>
</table>

$\beta$ = standardized regression coefficient.

*p < 0.05

**p < 0.01

*** p < 0.001

Similar regression models for PCOS patients diagnosed according to NIH criteria are presented in Tables 6 and 7. In these analyses, PCOS diagnosis remained an independent explanatory variable for both PAI-1 activity and tPA-mass in overweight women.

Table 6. A multivariate regression analysis of PAI-1 activity in 51 PCOS patients with hyperandrogenism (FAI > 5.0) and 81 controls across a wide range of BMI.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BMI &lt; 25 β</th>
<th>BMI &gt; 25 β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 53</td>
<td>n = 79</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.50</td>
<td>0.15</td>
</tr>
<tr>
<td>Age</td>
<td>-0.07</td>
<td>-0.20</td>
</tr>
<tr>
<td>BMI</td>
<td>0.18</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.29*</td>
<td>0.41**</td>
</tr>
<tr>
<td>PCOS diagnosis</td>
<td>0.17</td>
<td>0.29*</td>
</tr>
</tbody>
</table>

$\beta$ = standardized regression coefficient

*p < 0.05

**p < 0.01

*** p < 0.001
Table 7. A multivariate regression analysis of tPA mass concentration in 51 PCOS patients with hyperandrogenism (FAI > 5.0) and 81 controls across a wide range of BMI.

<table>
<thead>
<tr>
<th></th>
<th>BMI &lt; 25</th>
<th>BMI &gt; 25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 53</td>
<td>n = 79</td>
</tr>
<tr>
<td>R²</td>
<td>0.39</td>
<td>0.34</td>
</tr>
<tr>
<td>β</td>
<td>β</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.45**</td>
<td>0.33**</td>
</tr>
<tr>
<td>BMI</td>
<td>0.18</td>
<td>0.41*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.23</td>
<td>0.63</td>
</tr>
<tr>
<td>PCOS diagnosis</td>
<td>0.12</td>
<td>0.34*</td>
</tr>
</tbody>
</table>

β = standardized regression coefficient
*p < 0.05
**p < 0.01

Paper III

Relative amounts of adipose tissue expression of inflammatory markers in overweight and lean PCOS patients and in overweight controls are displayed in Figure 5. Overweight PCOS patients and overweight control subjects had more increased relative amounts of CCL2, CCR2, TNF-α, IL-18, CD14 and, CD163 adipose tissue expression than lean PCOS patients. There were no significant differences between overweight PCOS patients and overweight control subjects in relative adipose tissue expression of any of the inflammatory markers. MIF expression did not differ between groups.
Figure 5. Inflammatory gene expression (mean ± SD) in abdominal superficial subcutaneous adipose tissue in lean PCOS patients (white bars, n = 10), overweight PCOS patients (diagonal lined bars, n = 20), and overweight control subjects (black bars, n = 20). Expression levels are relative to the RPLP0 normalization gene. Abbreviations: chemokine ligand 2 (CCL2), chemokine receptor 2 (CCR2), macrophage migration inhibitory factor (MIF), tumor necrosis factor α (TNF-α), interleukin 18 (IL-18), * p < 0.05, ** p < 0.01 and, *** p < 0.001.

Adipose tissue markers of inflammation, except for MIF, were correlated with most metabolic risk markers within the PCOS group (Table 8). CCR2, IL-18, CD14, and CD163 expression was negatively correlated with serum concentrations of SHBG. Free androgen index correlated with CD 163 but not with any other inflammatory marker in adipose tissue.

BMI was the main explanatory variable for adipose tissue inflammatory markers in PCOS patients. On adjustment for age and BMI, only the positive correlations between adipose mRNA expression of CCR2 and fasting glucose levels and, CCL2, TNF-α, and CD14 with systolic blood pressure remained (for details see Paper IV).
Table 8. Bivariate correlations between adipose tissue expression of CCL2, CCR2, MIF, TNFα, IL-18, CD14 and CD163 and selected anthropometric/metabolic variables in women with PCOS (n = 30).

<table>
<thead>
<tr>
<th></th>
<th>BMI</th>
<th>Waist</th>
<th>Glucose</th>
<th>Insulin</th>
<th>HOMA-IR</th>
<th>TG</th>
<th>BP systolic</th>
<th>BP diastolic</th>
<th>FAI</th>
<th>SHBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL2</td>
<td>0.52**</td>
<td>0.42*</td>
<td>0.55**</td>
<td>0.34</td>
<td>0.35</td>
<td>0.33</td>
<td>0.52**</td>
<td>0.28</td>
<td>0.20</td>
<td>-0.24</td>
</tr>
<tr>
<td>CCR2</td>
<td>0.52**</td>
<td>0.42*</td>
<td>0.59**</td>
<td>0.50**</td>
<td>0.50**</td>
<td>0.50**</td>
<td>0.12</td>
<td>0.30</td>
<td>0.24</td>
<td>-0.42*</td>
</tr>
<tr>
<td>MIF</td>
<td>-0.08</td>
<td>-0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.06</td>
<td>-0.07</td>
<td>-0.18</td>
<td>-0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.55**</td>
<td>0.48**</td>
<td>0.58**</td>
<td>0.42*</td>
<td>0.41*</td>
<td>0.46*</td>
<td>0.65**</td>
<td>0.52**</td>
<td>0.07</td>
<td>-0.23</td>
</tr>
<tr>
<td>IL-18</td>
<td>0.76**</td>
<td>0.73**</td>
<td>0.67**</td>
<td>0.54**</td>
<td>0.53**</td>
<td>0.57*</td>
<td>0.47*</td>
<td>0.55**</td>
<td>0.32</td>
<td>-0.42*</td>
</tr>
<tr>
<td>CD14</td>
<td>0.78**</td>
<td>0.75**</td>
<td>0.67**</td>
<td>0.55**</td>
<td>0.54**</td>
<td>0.56*</td>
<td>0.53**</td>
<td>0.58**</td>
<td>0.34</td>
<td>-0.41*</td>
</tr>
<tr>
<td>CD163</td>
<td>0.77*</td>
<td>0.68**</td>
<td>0.61**</td>
<td>0.50**</td>
<td>0.50**</td>
<td>0.50**</td>
<td>0.52**</td>
<td>0.55**</td>
<td>0.38*</td>
<td>-0.45*</td>
</tr>
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*p < 0.05, and ** p < 0.01

Paper IV
Thirty-four women with PCOS completed the clinical trial. One subject on placebo was excluded prior to statistical analyses as she developed an eating disorder during the course of the study. Four patients dropped out during the study due to positive pregnancy tests: three of the pregnant women were treated with sibutramine and one with placebo. Four patients dropped out for personal reasons.
Figure 6. Mean ± SEM BMI reduction after 24 weeks of treatment with sibutramine 15 mg daily (n = 21) and placebo (n = 20), both together with brief life style intervention. Patients dropping out of the study are kept in the analysis as far as possible. Compared to placebo, sibutramine treatment resulted in reduced BMI (main effect of treatment: F(1,30) = 11.18; p < 0.01, treatment by time interaction F(1,30) = 6.00; p < 0.05)).

After six months, the sibutramine group had a mean weight loss of 7.8 ± 5.1 kg (range 5.7 to -14.3 kg, -9.0%) and the placebo group a mean loss of 2.8 ± 6.2 kg (range 7.0 to -15.7 kg, -1.7%) (Figure 6). Sibutramine treatment resulted in significant decreases in ApoB (p <0.01), ApoB/ApoA ratio (p <0.05), triglycerides (p < 0.05), cystatin C levels (p <0.05) and, free androgen index (p <0.05); whereas, SHBG levels increased (p <0.05). The reduction in triglycerides, cystatin C, FAI and the increase of SHBG were greater among subjects treated with sibutramine than in those treated with placebo (Table 9).
Table 9. Metabolic and hormonal parameters before and after sibutramine and placebo treatment.

<table>
<thead>
<tr>
<th></th>
<th>Sibutramine mean ± SD</th>
<th>Placebo mean ± SD</th>
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<tbody>
<tr>
<td></td>
<td>Baseline (n = 21)</td>
<td>Baseline (n = 20)</td>
</tr>
<tr>
<td></td>
<td>6 month (n = 17)</td>
<td>6 month (n = 16)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.68 ± 0.87</td>
<td>1.66 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>1.32 ± 0.62&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>1.80 ± 1.09</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.36 ± 0.14</td>
<td>1.43 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>1.42 ± 0.22</td>
<td>1.47 ± 0.16</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.01 ± 0.26</td>
<td>0.99 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>0.94 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96 ± 0.33</td>
</tr>
<tr>
<td>ApoB/ApoA</td>
<td>0.75 ± 0.20</td>
<td>0.69 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>0.68 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67 ± 0.25</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.87 ± 0.51</td>
<td>4.97 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>4.74 ± 0.52</td>
<td>5.00 ± 0.54</td>
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<tr>
<td>Insulin, mU/L</td>
<td>12.88 ± 6.65</td>
<td>16.83 ± 12.33</td>
</tr>
<tr>
<td></td>
<td>13.07 ± 9.09</td>
<td>19.42 ± 15.03</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.81 ± 1.63</td>
<td>3.86 ± 3.21</td>
</tr>
<tr>
<td></td>
<td>2.75 ± 1.72</td>
<td>4.60 ± 4.13</td>
</tr>
<tr>
<td>Cystatin C, mg/L</td>
<td>0.77 ± 0.09</td>
<td>0.72 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>0.74 ± 0.10&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>0.75 ± 0.08</td>
</tr>
<tr>
<td>Hs-CRP, mg/L</td>
<td>3.3 ± 2.5</td>
<td>4.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>4.1 ± 6.1</td>
<td>6.0 ± 6.6</td>
</tr>
<tr>
<td>Testosterone, nmol/L</td>
<td>2.08 ± 0.87</td>
<td>2.68 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>1.80 ± 0.65</td>
<td>2.56 ± 0.87</td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>37.1 ± 15.8</td>
<td>32.6 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>44.9 ± 19.2&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>30.7 ± 13.2</td>
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</table>

<sup>a</sup> p < 0.05 compared with baseline values of the corresponding group, paired t-test.
<sup>b</sup> p < 0.01 compared with baseline values of the corresponding group, paired t-test.
<sup>c</sup> change from baseline significantly greater than in the placebo group, ANOVA with repeated measures (time by drug interaction), p < 0.05.
Discussion

Prevalence of PCOS

In Northern Sweden, an estimated 4.8% of women of fertile age fulfill the criteria for PCOS, according to NIH criteria: this prevalence rate corresponded with other studies in Northern Europe [33, 148] and white women in the U.S. [31]. However, the prevalence rate for the Swedish population was lower than reported for Southern European women [30, 32], black American women [31] and Australian women [40], where prevalence rates for PCOS, according to NIH criteria, varies between 6.5 – 8.7%. Less data is available from Asian populations but in a hospital-based sample [149], 2.2% of Chinese women are reported to fulfill the NIH criteria for PCOS.

An Australian community-based study compares prevalence rates according to different PCOS criteria [40]. As only 39% of the women in the cohort consented to the transvaginal ultrasound examination, prevalence rates for the Rotterdam and AE-PCOS criteria are reported with and without approximations of expected PCO features. The estimated prevalence of PCOS was 8.7% according to NIH criteria, 11.9% according to the Rotterdam criteria and 10.2% according to AE-PCOS criteria: with the inclusion of imputed data, the prevalence rate increased to 17.8% for Rotterdam and to 12.0% for the AE-PCOS criteria. However, the approximation of ultrasound features for women declining the transvaginal ultrasound examination may be biased, as it can be assumed the majority of symptomatic women are more concerned about their health and, therefore, more inclined to be investigated. Furthermore, a recent community-based study from Sri Lanka [150] reports 6.3% of women of childbearing age have PCOS, according to the 2003 Rotterdam criteria

Hirsutism was not assessed by physicians nor was an ultrasonographic examination of the ovaries performed in this prevalence study: this could be considered a weakness of the study. Besides the inter-rater variability in hirsutism scorings, the feasibility of the Ferriman-Gallwey score is impaired as many women treat the symptoms prior to clinical evaluation; thus, it is unlikely an objective assessment of hirsutism would have yielded a more accurate measure of clinical hyperandrogenism. Furthermore, a simple symptom-based questionnaire may be useful for identifying women with an endocrine profile typical for PCOS [33, 150]. In Sri Lanka, more than 90% of women with self-reported symptoms of oligo/amenorrhea and/or hirsutism
were later shown to have PCOS [150]. A Finnish study reports polycystic ovaries were later detected in 18% of women reporting only hirsutism, in 48% of women reporting only oligomenorrhea, and in 70% of women reporting both symptoms [148]. As indicated in the Australian prevalence study it is difficult to include the transvaginal ultrasound in prevalence studies of PCOS as less than half of women are willing to undergo the examination [40].

Based on the few studies including transvaginal ultrasound for PCOS diagnosis, it is plausible more women in both the oligomenorrhea-only group and the hyperandrogenism-only group would be diagnosed as PCOS, if the ESHRE criteria were used [40, 150]. However, the ESHRE criteria would probably not have yielded more subjects at risk of metabolic syndrome, as findings of the metabolic syndrome are more frequently encountered in the PCOS patients defined according to the NIH criteria [18, 19].

Another limitation to the study was the causes for oligomenorrhea and hirsutism such as hypothyroidism and premature ovarian failure not being evaluated in all subjects, at least not in the published paper. However, no cases with increased levels of prolactin, FSH or, TSH were found among the women who presented with oligomenorrhea and hyperandrogenism.

The relevance of the PCOS diagnosis suggested in the present study was strengthened by the population-based sample and the metabolic changes identified in the group with both oligomenorrhea and hyperandrogenism. This group had increased weight, increased BMI, increased waist and hip circumferences and, higher systolic and diastolic blood pressure, compared to controls, and more frequently reported a history of hypertension and gestational diabetes. Metabolic derangements were identified in the hyperandrogenism-only and the oligomenorrhea and hyperandrogenism groups but not in the oligomenorrhea-only group. Increased free testosterone is consistently associated with increased systolic and diastolic blood pressures [151-158]. Animal studies indicate the prohypertensive effect of androgens are mediated through increased vascular tone via upregulation of thromboxane A2 expression, norepinephrine, angiotensin II and endothelin-1 synthesis [159]: androgens may also influence blood pressure by affecting the renin-angiotensin-aldosterone system [159].

Unpublished findings from the entire premenopausal cohort of women in the WHO-Monica sample indicate many women with PCOS may go undiagnosed and among women with free androgen index > 5, less than 10% report excessive growth of body hair (Andersson et al., submitted). PCOS women are at risk for developing metabolic disturbances [6], thus, it would be a health benefit to identify women with PCOS. Early identification may be beneficial, as lifestyle interventions can be implemented before metabolic disturbances develop. A simple symptom-based questionnaire can assist in detecting women with PCOS, but the addition of biochemical markers for hyperandrogenism yields additional information.
Fibrinolytic activity in women with PCOS

The main finding of Paper II was that overweight women with PCOS displayed increased tPA mass in comparison with controls: this finding was consistent irrespective of diagnosis with Rotterdam criteria or NIH criteria. In addition, when biochemical NIH criteria were used for PCOS diagnosis, overweight women with PCOS displayed increased PAI-1 activity. No signs of derangement in fibrinolytic variables were detected in lean women with PCOS, irrespective of how the PCOS diagnosis was established.

These findings might elucidate some discrepancies regarding fibrinolytic activity in PCOS, as earlier studies indicate both increased PAI-1 activity [15, 100,101,160] and normal PAI-1 activity in PCOS patients [102, 103] [159]. Thus, it is plausible studies indicating no difference in PAI-1 activity have included a more heterogeneous population of PCOS patients with regard to hyperandrogenism. For example, Atiomo et al [102] do not assess biochemical hyperandrogenism and, in two studies [103, 159], only high testosterone is used for the assessment of hyperandrogenism. One prior study [100] uses increased FAI as the hyperandrogenism criterion in PCOS patients, while adjusting for BMI, and report increased PAI-1 activity in the PCOS group: this finding is in accordance with the findings of this study. The importance of free androgen index for PAI-1 activity is further strengthened by the finding that FAI, together with BMI and insulin resistance, was an independent explanatory variable for PAI-1 activity in women with PCOS.

Increased tPA mass concentration in overweight PCOS patients confirmed previous findings [104-107]. Among PCOS patients, tPA mass was influenced by age, BMI and, free androgen index. Mass concentration of tPA is considered a better predictor of cardiovascular mortality than PAI-1 activity after myocardial infarction [98], and tPA mass is associated with myocardial infarction in young women [99]. Thus, increased tPA mass concentration in overweight PCOS patients indicates that this group of women are at risk for cardiovascular disease. Among overweight PCOS patients, tPA mass was increased, independently of whether free androgen levels were included in the PCOS diagnosis or not: this finding suggested impaired fibrinolysis, as a marker for endothelial dysfunction, could also exist in patients with less severe hyperandrogenism (i.e. PCOS light).

Lean PCOS patients had similar tPA mass levels and PAI-1 activity as controls, and this finding remained when NIH-criteria were also applied, and was in accordance with another study on lean PCOS patients [107]; although others report increased tPA mass and increased PAI-1 activity in lean PCOS patients [15, 100]. A possible explanation for the discrepancies between the findings of this study and previous findings regarding lean PCOS patients is the ethnic differences between study populations. For example, Greek PCOS patients are more frequently carriers of the 4G allele in the...
4G5G polymorphism of the PAI-1 gene, and the presence of the 4G allele in the PAI-1 promoter region of the gene further increases PAI-1 levels [160].

In conclusion, overweight women with PCOS have impaired fibrinolysis, in particular if they also display objective biochemical markers of hyperandrogenism. However, there was no evidence lean PCOS patients carry this specific risk factor for cardiovascular disease. As PCOS is common in the population, identifying subjects at risk of future cardiovascular disease would be a health benefit for both the patient and the society.

Another important conclusion is that the Rotterdam criteria for PCOS diagnosis may be an improvement in the fertility setting, but is less valuable in the pursuit of long-term health risk and consequences of PCOS. Hyperandrogenism assessed by use of free androgen index could be included as a diagnostic criterion for PCOS when evaluating risk factors for cardiovascular disease in this specific group of patients. Independent of PCOS diagnosis, increased testosterone and decreased SHBG are strongly associated with a number of adverse cardiovascular disease risk factors in postmenopausal women, such as central adiposity, decreased HDL cholesterol levels and, increased systolic and diastolic blood pressures [151-154]. A recent nested case-control study of postmenopausal women [161] also suggests higher free androgen index is associated with CVD events such as first occurrence of nonfatal myocardial infarction, coronary revascularization, nonfatal stroke, coronary disease, or stroke death; although, this association is not independent of BMI and other cardiovascular risk factors. Besides a possible role of androgen hormones and SHBG in cardiovascular disease, low levels of SHBG are consistently linked to higher rates of diabetes [162, 163] and might contribute to a more adverse cardiovascular risk profile in women with diabetes [164, 165].

Inflammatory markers in adipose tissue of PCOS women

The gene expression pattern of inflammatory markers in abdominal superficial subcutaneous tissue was similar in overweight PCOS patients and overweight control subjects, whereas lean PCOS patients displayed lower expression of adipose inflammatory markers (Paper III). In accordance with this finding, most associations between markers of adipose tissue inflammation and variables of the metabolic syndrome in PCOS patients were lost when adjusted for age and BMI. Therefore, overweight or obesity, rather than the PCOS diagnosis per se, appeared to be the main explanatory variable for increased adipose inflammation in PCOS patients.

Serum concentrations of inflammatory markers such as CCL2 and CRP are increased in overweight PCOS patients, compared with weight-matched controls [81], and the inflammatory status is correlated with elevated androgen levels [80, 81]. However, other studies have failed to display any differences in circulating TNF-α and IL-6 between overweight controls and over-
weight PCOS patients [166]. In contrast to previous studies of peripheral inflammatory markers in PCOS patients, increased circulating levels of hs-CRP, CCL2, and IL-18 in overweight PCOS patients were not confirmed, probably due to a type II error. Similarly, the correlation between adipose expression of inflammatory markers and FAI or testosterone could not be demonstrated; although, as in other studies [80, 81], SHBG (as an indirect measure of hyperandrogenism) was negatively correlated with most of the inflammatory markers.

Support for a possible causal relationship between obesity-associated low-grade inflammation and metabolic impairment is gained from epidemiological data indicating an association of markers of systemic inflammation (i.e., CRP and IL-6) with insulin resistance and future risk of developing type 2 diabetes [167]. In agreement with other studies [118, 168-170], a pronounced effect of increased body mass on adipose expression of chemokines, pro-inflammatory cytokines, and inflammatory markers was found, although overweight PCOS patients did not differ from overweight controls. MIF expression was not influenced by fat mass, which was in agreement with unpublished data demonstrating MIF expression in subcutaneous fat depots is unaffected by fat mass, although apparently conflicting data on MIF from isolated human subcutaneous adipocytes correlating with BMI is reported [171]. The majority of adipose-secreted MIF originates from non-fat cells in obese women [34] and it is probable that the adipocyte isolation and culturing procedure per se influences the secretion pattern [172].

Preclinical studies provide evidence that ablation of the CCL2/CCR2 pathway improves insulin sensitivity in conjunction with reduced adipose tissue inflammation [173], and inhibition of CCR2 in obese mice improves insulin sensitivity and reduces macrophage content in adipose tissue [173]. Moreover, pharmacological inhibition of CCR2 in the early stage of obesity in db/db mice ameliorates insulin resistance and hepatic steatosis [174]. A positive correlation between adipose tissue CCR2 gene expression and fasting serum glucose levels was determined, which was independent of age and BMI in the PCOS group. In diabetic patients, expression levels of CCR2, CD36 and, CD68 on monocytes are increased, and poor glycemic control is associated with high levels of serum CCL2 and circulating monocyte CCR2 [175]. However, the possible contribution of elevated adipose CCR2 to adipose tissue, as well as whole body, metabolic dysregulation in humans remains to be determined.

Adipokine secretion patterns may influence blood pressure parameters. In this respect, most focus is on endocrine factors such as leptin, which may activate the sympathetic nervous system, and adiponectin, which may and inhibit the sympathetic nervous system (reviewed in [176]). Among the adipose inflammation-related factors with endocrine (and paracrine) effects (i.e., CCL2, MIF, and IL-18,), CCL2 correlated with systolic blood pressure after adjustment for age and BMI. Similarly, adipose TNF-α (mainly
autocrine/paracrine action) correlated with systolic blood pressure. As these molecules act on immune cells inducing local and systemic inflammation, endothelial and vascular function might be altered. Although systemic TNF-\(\alpha\) is suggested as an independent risk factor for elevated blood pressure in apparently healthy subjects [177], approximately one-third of the TNF-\(\alpha\) values were below the limit of detection and the study was cross-sectional [177]. The adipose tissue contribution of TNF-\(\alpha\) to the circulation has not been fully resolved and there is only a modest elevation of serum concentrations in obese patients [178].

The limited number of participants and the absence of a lean control group could be considered a limitation in Paper III. As the study did not include a lean control group, we could not determine whether lean PCOS patients deviated from weight-matched controls in terms of expression patterns of adipose tissue inflammatory markers. The inclusion of a lean control group would have enhanced understanding of how adipose tissue inflammation is influenced by hyperandrogenism and PCOS. Obtaining adipose tissue biopsies from only one anatomical location (abdominal superficial subcutaneous adipose tissue) was another limitation, as different adipose tissue depots have different profiles regarding inflammatory marker expression [170, 179, 180].

In conclusion, fat mass rather than PCOS diagnosis per se appears the main determinant of increased inflammatory markers in adipose tissue. Further studies investigating the different clinical phenotypes of PCOS in terms of gene expression pattern of inflammatory markers in abdominal superficial subcutaneous tissue are warranted.

Weight reduction treatment in women with PCOS

The use of sibutramine together with lifestyle modification resulted in a greater weight loss in women with PCOS than for placebo and lifestyle modification alone (Paper IV). Women treated with sibutramine lost on average 7.8 kg (-9.0%) during treatment, compared to a weight loss of 2.8 kg (-1.7%) in the placebo group. Successful weight loss after sibutramine treatment was associated with lower age and low SHBG levels at baseline. Although testosterone levels at baseline were not associated with weight reduction, this finding could indicate young women with pronounced hyperandrogenism would benefit from sibutramine treatment. The weight reduction obtained with sibutramine and lifestyle modification in women with PCOS was similar to previously reported weight reductions in other obese patient groups [137-140, 181, 182].

The importance of lifestyle modification in addition to anti-obesity treatments has been stressed. Wadden et al [138] present data indicating organized and intense lifestyle modification together with sibutramine treatment is more efficacious than sibutramine alone and that organized lifestyle modifi-
cation alone is as powerful as sibutramine alone in reducing weight in obese subjects.

Both sibutramine and placebo treatment resulted in increased menstrual frequency but there was no difference between treatments: this finding was in agreement with other studies [71, 124, 183] on weight loss in PCOS patients. The frequency of menstruations can be an indirect measurement of ovulation, although urinary luteinizing hormone kits or progesterone serum concentrations should preferably have been used to estimate the number of ovulatory cycles. With longer-term treatment and sustained weight reduction, further improvements in menstrual pattern could possibly be detected.

The European Medicines Agency (EMA) recently recommended the suspension of marketing authorization of sibutramine. This statement was initiated by a safety review of preliminary data from the SCOUT-study where 9800 obese or overweight patients with known cardiovascular disease were treated with sibutramine or placebo for a six-year period [144]. The SCOUT-study concluded that patients treated with sibutramine have an increased risk of serious, non-fatal cardiovascular events, such as stroke and myocardial infarction and weight reduction was modest with long-time treatment. An EMA press release stated the 21 of January 2010; sibutramine should no longer be prescribed by doctors nor dispensed by pharmacists. At the time this thesis was written, the SCOUT data, upon which the recommendations are based, had not been published.

In the young and presumably otherwise healthy women with PCOS, improvements in lipid profile were obtained with sibutramine treatment. Sibutramine treatment resulted in lower levels of triglycerides, lower levels of ApoB and a lower ApoB/ApoA-1 ratio, which concurred with other findings for sibutramine treatment, where favorable effects on HDL-cholesterol and triglycerides and total:HDL cholesterol ratio are reported [181]. In addition, SHBG levels increased in the sibutramine group: increased SHBG levels result in decreased availability of biologically active free testosterone [184] and may be a marker of metabolic benefit, as low SHBG is associated with insulin resistance and high plasma insulin levels [185]. However, sibutramine did not have any beneficial effects on blood pressure, fat distribution or insulin sensitivity index in young women with PCOS. Finally, compliance to the contraceptive advice given at study inclusion was not always followed by the participants, as four women became pregnant during the course of the study.

The EMA recommendations have been heavily criticized by patient organizations and health care professionals. The patients who were included in the SCOUT study had known contra-indications for sibutramine use, and it can be argued these subjects would not have received treatment in clinical practice. However, according to the European authority, overweight and obese patients have an increased risk of cardiovascular events, and this is why the SCOUT study results are relevant in the clinic, even for patients
without evident cardiovascular disease. These recommendations are a major drawback for women with PCOS (and other obese patients) who are in need of rapid weight reduction for the initiation of fertility treatment. These women are left with life-style intervention [122], orlistat [186] or, bariatric surgery [187]. However, as the full report of the SCOUT study is not yet available, a comprehensive assessment of the result is not possible.

In conclusion, sibutramine in combination with lifestyle intervention can reduce weight in overweight and obese women with PCOS. However, the use of sibutramine has been precluded because of recommendations from the European Medicines Agency. Further recommendations on possible usability in younger patients need to be awaited.

Concluding remarks
The new Rotterdam criteria appeared to increase the heterogeneity of an already heterogeneous PCOS population. With the implementation of the Rotterdam criteria, physicians and women are further confused about the long-term consequences of PCOS. Such confusion might have only been an academic problem, if young women with PCOS, had not found numerous indications the Internet that their future health is at great risk.

However, do young lean women with oligomenorrhea and PCO carry the same risk for diabetes and cardiovascular disease as the premenopausal women with persisting hyperandrogenism in addition to their obesity? From the findings presented here, there was no deterioration in fibrinolytic activity and no increase in adipose tissue inflammatory markers in lean PCOS patients; thus, these women appeared to carry fewer risk factors for metabolic and cardiovascular disturbances. Whether this translates to decreased risk of type 2 diabetes and cardiovascular disease among lean women with PCOS could not be answered in this thesis, and remains to be proven in the future. It can be hypothesized that lean women with PCOS and without marked hyperandrogenism are more likely to recover from the PCOS with increasing age and their future health risks are different from other women with other PCOS phenotypes. However, to determine this, long-term studies of different PCOS phenotypes and the respective long-term risks are warranted.
General conclusions

The prevalence of PCOS in Northern Sweden is similar to other Western countries, and is subsequently the most common endocrine disturbance among women of childbearing age.

Hyperandrogenism is more strongly associated with components of the metabolic syndrome than oligomenorrhea/amenorrhea.

With a few questions and limited biochemical analyses, it is possible to identify women with PCOS and concurrent metabolic disturbances.

Overweight women with PCOS have impaired fibrinolysis and among women with PCOS, decreased fibrinolytic activity is associated with increased free androgen levels.

Lean women with PCOS have normal fibrinolytic activity.

The gene expression pattern of inflammatory markers in abdominal superficial subcutaneous tissue is similar in overweight PCOS patients and overweight control subjects; whereas, lean PCOS patients display lower expression of adipose inflammatory markers.

Sibutramine combined with brief lifestyle interventions results in weight reduction in overweight and obese women with PCOS, but the usefulness of treatment is questioned by recent safety studies.
Polycystiskt ovarialsyndrom (PCOS) är den vanligaste endokrina rubbningen hos kvinnor i barnafödande åldrar. Enligt de diagnoskriterier som kom 2003 och som används av de flesta gynekologer ska kvinnan uppfvisa minst två av följande tre kriterier: tecken till ägglossningsstörning, tecken till hyperandrogenism eller typisk ultraljudsbild. Det finns studier som visar ökad förekomst av insulinresistens, typ 2 diabetes och försämrad blodfett hos kvinnor med PCOS jämfört med ålders- och viktmatchade kontroller men det är oklart om dessa risker även gäller för kvinnor som diagnostiserats enligt de nya kriterierna och om det även gäller för smala kvinnor med PCOS.

I mitt avhandlingssarbete har vi försökt bestämma förekomst av PCOS i norra Sverige. Vi finner att 4,8 % av kvinnor under 40 år uppfyller PCOS kriterierna enligt äldre, ej ultraljudsbaserade, kriterier. Kvinnor med enbart tecken på ägglossningsstörning skilde sig inte ur metabol synvinkel från kvinnor utan något symtom alls. Den grupp som upprivasade PCOS diagnos hade högre BMI, högre blodtryck och rapporterade i högre utsträckning tidigare högt blodtryck under graviditet och graviditetsdiabetes jämfört med de andra grupperna.

Störningar i det fibrinolytiska systemet är en viktig del i uppkomsten av hjärtkärlssjukdomar och kan mätas med PAI-1 (plasminogen aktivator hämmare) och tPA (vävnads plasminogen aktivator). Vi har undersökt det fibrinolytiska systemet hos kvinnor med PCOS med ålders och viktmatchade kontroller. De smala PCOS kvinnorna skilde sig inte från smala kontroller med avseende på fibrinolytisk aktivitet oavsett hur man ställt PCOS diagnos. Bland överviktiga kvinnor med PCOS fann vi ökade nivåer av tPA massa oavsett om hyperandrogenism inkluderas i diagnosen eller ej. PAI-1 aktivitet var endast ökad bland kvinnor med PCOS som uppvisade hyperandrogenism.

Fettväven är kroppens allra största endokrina organ som producerar och frisläpper hormoner, proinflammatoriska och antiinflammatoriska cytokiner. Inflammation i fettväv är kopplat till insulinresistens och ökad risk för hjärtkärlsjukdom. Särskilt bukfetma är en känd riskfaktor för metabola störningar. Man har tidigare visat att markörer för inflammation är ökad i blod hos överviktiga PCOS kvinnor jämfört med viktmatchade kontroller men studier direkt på fettväv finns inte sedan tidigare. Vi har jämfört överviktiga och
smala PCOS kvinnor med överviktiga friska kontroller och någon skillnad i genuttryck för inflammatoriska markörer mellan de två överviktiga grupperna sågs ej men de smala kvinnorna med PCOS hade lägre nivåer både jämfört med den överviktiga PCOS gruppen och den överviktiga kontrollgruppen.

Viktnedgång är förstahandsbehandling för överviktiga kvinnor med PCOS. Vi har provat om behandling med sibutramin, som är en serotonin/noradrenalin återupptagshämmare som verkar direkt på aptitecentrum i hjärnan, i kombination med livsstilsintervention kan leda till viktnedgång hos kvinnor med PCOS. Vi fann en signifikant bättre viktminskning med sibutramin och livsstilsintervention jämfört med placebo och livsstilsintervention. Vissa metabola parametrar förbättrades också med behandling men vi såg ingen förbättring i blodtryck trots viktminskningen. Sibutramin har dock nyligen blivit indraget då säkerhetsstudier visat på en ökad risk för kardiovaskulära komplikationer hos redan hjärtsjuka individer.
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