Endocrine and metabolic characteristics in polycystic ovary syndrome

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List of papers

1. Glintborg D, Henriksen JE, Andersen M, Hagen C, Hangaard J, Rasmussen PE, Schousboe K, Hermann AP. The prevalence of endocrine diseases and abnormal glucose tolerance tests in 340 Caucasian, premenopausal women with hirsutism as primary diagnosis. Fertil Steril 2004 [1]

2. Glintborg D, Hermann AP, Brusgaard K, Hangaard J, Hagen C, Andersen M. Significantly higher ACTH-stimulated cortisol and 17- hydroxyprogesterone levels in 337 consecutive, premenopausal, Caucasian, hirsute patients compared to healthy controls. J Clin Endocrinol Metab. 2005 [2]

3. Glintborg D, Andersen M, Hagen C, Frystyk J, Hulstrøm V, Flyvbjerg A, Hermann AP. Evaluation of metabolic risk markers in PCOS. Adiponectin, ghrelin, leptin and body composition in hirsute PCOS patients and controls. Eur J Endocrinol. 2006 [3]

4. Glintborg D, Altinok ML, Mumm H, Buch K, Ravn P, Andersen M. Prolactin as a metabolic risk marker in 1007 women with polycystic ovary syndrome. Human Reproduction 2014 [4]

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7. Glintborg D, Frystyk J, Højlund K, Andersen KK, Henriksen JE, Hermann AP, Hagen C, Flyvbjerg A, Andersen M. Total and high-molecular-weight (HMW) adiponectin levels and measures of glucose and lipid metabolism following pioglitazone treatment in a randomized placebo controlled study in polycystic ovary syndrome. Clin Endocrinol 2007 [7]

8. Glintborg D, Højlund K, Andersen M, Henriksen JE, Beck-Nielsen H, Handberg A. Elevated risk markers of atherosclerosis in polycystic ovary syndrome (PCOS) were significantly reduced during pioglitazone treatment in a randomized placebo controlled study. Diabetes Care 2007 [8]

9. Glintborg D, Altinok ML, Mumm H, Hermann AP, Ravn P, Andersen M. Body composition is improved during 12 months treatment with metformin alone or combined with oral contraceptives compared to treatment with oral contraceptives in polycystic ovary syndrome. A randomized controlled clinical trial. J Clin Endocrinol Metab 2014 [9]

List of abbreviations

17OHP	17-hydroxyprogesterone
ACTH	Adrenocorticotroph hormone
BMI	Body mass index
DHEAS	Dehydroepiandrosterone
DXA	Dual-energy X-ray absorptiometry
FFA	Free fatty acid
FSH	Follicular stimulating hormone
GnRH	gonadotropin releasing hormone
HbA1c	Hemoglobin A1c
HDL	High density lipoprotein
HOMA	Homeostasis assessment model
HPA	Hypothalamic-pituitary-adrenal
LDL	Low density lipoprotein
LH	Luteinizing hormone
NC-ACS	Late onset adrenogenital syndrome
OCP	Oral contraceptive pills
OGTT	Oral glucose tolerance test
PCOS	Polycystic ovary syndrome
PPAR-γ	Peroxisome proliferator activated receptor γ
SD	Standard deviation
SF-36	Short Form-36
SHBG	Sex hormone binding globulin
T2D	Type 2 diabetes
TG	Triglyceride
WHR	Waist-to-hip ratio

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Background

PCOS was first described by Stein and Leventhal in 1935 [10], but until 2003 no agreement existed as to the diagnostic criteria [11]. In 2003, the Rotterdam criteria were proposed [12]. The Rotterdam criteria are the following:

1. Irregular/no ovulations

2. Clinical/paraclinical hyperandrogenaemia

3. Polycystic ovaries

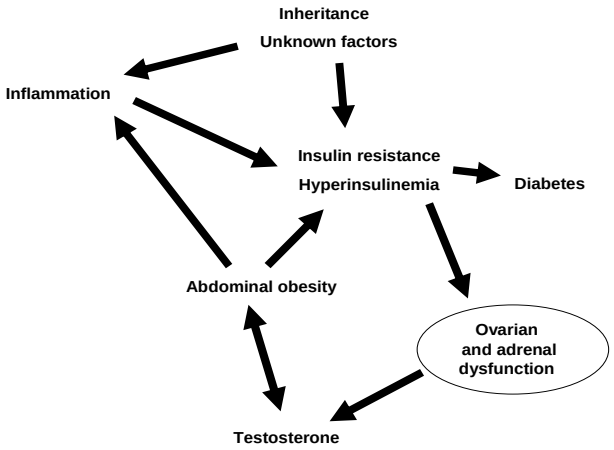
Two out of three criteria need to be fulfilled and other causes of the patient's symptoms should be excluded.

Most patients with PCOS are insulin resistant and about 50% of patients fulfil the criteria for the metabolic syndrome [13;14]. The exact mechanism for insulin resistance is undetermined, but the patients may have defects in the insulin-stimulated glucose metabolism [13;15]. Impaired beta-cell function is also present in PCOS and the risk for type 2 diabetes (T2D) is five- to eight fold increased in patients with PCOS compared to weight and age matched female controls [1]. Insulin stimulates p450c17 activity in ovaries and adrenals leading to increased androgen production [16]. The pathogenesis of PCOS involves hyperandrogenemia, central obesity, and insulin resistance/ hyperinsulinemia **(Figure 1)** [16]. High testosterone levels promote abdominal obesity, which may induce insulin resistance [17]. Insulin resistance induces hyperinsulinemia and subsequently stimulates the ovarian and adrenal hormonal production, inhibits sex hormone binding globulin (SHBG) production, and thereby testosterone activity increases.

Insulin resistance and central obesity in PCOS is associated with increased inflammatory activity and increased secretion of adipokines, interleukins, and chemokines [16], which may increase the long tem risk of diabetes and cardiovascular disease [18].

Which metabolic risk markers that associate best with the long term metabolic risk in PCOS remain to be established.

Figure 1: Pathogenic mechanisms of PCOS



Four different PCOS phenotypes may be defined when the Rotterdam criteria are applied [19]. One of these phenotypes includes patients with polycystic ovaries, irregular menses, and no signs of hyperandrogenemia [12]. The inclusion of patients without hyperandrogenemia in the definition of PCOS is currently debated. The metabolic disturbances of PCOS are more pronounced in hyperandrogen patients compared to patients with no hyperandrogenaemia [20]. The PCOS phenotype may therefore be a predictor of metabolic and cardiovascular risk. The task force of the Androgen Excess and PCOS Society suggested that the diagnosis of PCOS should not be established in patients without signs of hyperandrogenaemia [20]. How PCOS is best diagnosed remains to be determined. The estimated prevalence of PCOS varies from 5-20% depending of the applied criteria [21]. PCOS is therefore the most frequent endocrinopathy in reproductive-aged women [21;22]. In the present thesis, the used definition of PCOS has been described in each paper.

Hirsutism is defined as an increased growth of terminal hair in a male pattern in women. The prevalence of hirsutism is more than 25% in reproductive aged women [22]. Hirsutism is caused by increased androgenicity in the pilo-sebacceus gland resulting in increased growth of terminal hair [23;24]. Hirsute patients have increased dermal activity of the enzyme 5α-reductase, which is responsible for the conversion of testosterone to the more potent androgen dihydrotestosterone [25]. Individual variations in dermal 5α-reductase activity may explain that hirsute patient often have often near-normal testosterone levels and that circulating testosterone levels are not associated with clinical hirsute manifestations [25].

95% of hirsute patients are diagnosed with PCOS or idiopathic hirsutism. Idiopathic hirsutism is defined as hirsutism with regular ovulation and normal testosterone levels [25]. Depending on the methods applied, the prevalence of idiopathic hirsutism varies from 5-25% of hirsute patients [23;26]. In daily practice, PCOS and idiopathic hirsutism is difficult do distinguish and the two terms probably represent a continuum [16]. Several studies described metabolic and endocrine disturbances in idiopathic hirsutism similar to what is observed in PCOS [27;28].

Treatment modalities in PCOS aim at decreasing hyperandrogenism and improving insulin sensitivity, but the long term treatment strategy in patients with PCOS is still debated [29;30]. The most commonly used treatment modalities in PCOS patients without current pregnancy wish are oral contraceptives (OCP) and insulin sensitizers. OCP regulates menstrual cycles and SHBG levels are increased, leading to decreased levels of free testosterone and decreased hirsutism scores [31]. However, in short term studies OCP was associated with adverse effects on glucose metabolism [32]. OCP cannot be used in very obese patients and patients with other contraindications for OCP including previous or family history of thrombosis or breast cancer, coagulatory defects or heavily smokers.

Insulin sensitizing treatment most commonly includes lifestyle intervention and metformin treatment. The aim of insulin sensitizing treatment is to improve insulin resistance and, thereby, insulin stimulation of adrenal and ovarian androgen production decreases [33]. Weight loss improves clinical and biochemical manifestations of PCOS, but the patients' adherence to life style intervention is often limited [34]. Treatment with metformin increases insulin sensitivity and improves ovulatory function in PCOS [31], whereas androgen levels and hirsutism scores are only mildly improved or unchanged [31;35]. Metformin treatment is associated with gastrointestinal side effects including nausea, which often wear off during long-term treatment and could affect endocrine and metabolic outcomes.

The thiazolidinediones rosiglitazone and pioglitazone stimulate the peroxisome proliferator-activated (PPAR)-γ receptors in the cell nucleus and activate the transcription of genes that affect glucose and lipid metabolism mediating decreased peripheral adipocyte lipolysis, decreased free fatty acid (FFA) levels, and decreased visceral fat mass [36;37]. The treatment with PPAR-γ agonists may be associated with decreased bone mineral density and is contraindicated in patients with pregnancy wish [33]. In the present thesis, studies on PPAR-γ-agonist treatment were used to investigate pathogenic mechanisms of insulin resistance in PCOS. In the daily clinic, PPAR-γ-agonist treatment is not indicated in patients with PCOS.

Methods

Evaluation of hormone concentrations

In the present theses, blood samples were collected in the morning during follicular phase whenever possible. Medical treatment was paused three months before evaluation. Hormone concentrations are the result of oscillatory secretions, influenced both by time of day and by period of the menstrual cycle [38-40]. Therefore, single measurements of hormone concentrations are characterized by high biological variation [40]. In paper [6], we applied 20 minutes LH measurements to further characterize 24h changes in LH secretion during pioglitazone treatment as described previously by Veldhuis [41;42]. This method is time consuming and can not be applied in the daily clinic. The gold standard method for measuring total testosterone is mass spectrometry, whereas commercially available direct assays generally overestimate the steroid concentration [43;44]. Ether extraction followed by specific radio immunoassay can be applied to avoid over estimation of steroid hormones [45]. This method shows close correlation with the determination of testosterone levels by using mass spectrometry [46]. In this thesis, extraction methods as described by Lykkesfeldt were therefore applied when possible [46].

Evaluation of insulin sensitivity

The euglycemic hyperinsulinemic clamp is considered the gold standard for the establishment of insulin sensitivity [47]. The euglycemic clamp method assesses whole-body insulin-mediated glucose disposal. The clamp method was used in paper [6], as we aimed for a precise characterization of degree and mechanism of insulin resistance. However, the clamp method is time-consuming and requires technical expertise not widely available.

Fasting insulin and homeostasis assessment model (HOMA) are easily applied in daily clinical practice as measures of insulin resistance. Fasting insulin levels correlate well with the measures of insulin sensitivity from clamp studies [48]. However, fasting insulin levels are the result of both insulin secretion and metabolism. Fasting insulin therefore do not provide accurate information on insulin sensitivity in individuals with beta-cell dysfunction. Especially when glucose levels are high as in T2D, insulin secretion is stimulated, thus resulting in higher insulin levels. HOMA, defined as fasting insulin*fasting glucose/22.5, has been shown to correlate better with insulin resistance than fasting insulin in some studies [49].

Evaluation of body composition

Weight and BMI are the simplest methods for the establishment of total body fat. These variables are inexpensive, easily applied in daily praxis, and offer a minimum of discomfort for the patient. However, due to the importance of fat distribution for metabolic and hormonal variables, measurements of body composition are needed as well as measures of total body fat. Waist circumference is a good estimate of abdominal fat [50], and can be applied in the daily clinic.

DXA scans offer the opportunity for establishing total body fat and make it possible to estimate abdominal and extremital fat mass [51;52]. However, the method is limited by the lack of ability to distinguish intra-abdominal fat mass from subcutaneous fat mass. Recent studies, however, suggested that subcutaneous abdominal and intra-abdominal fat have similar adverse effects on insulin resistance [53].

Aims of thesis

In the present thesis, the diagnosis, pathogenesis, risk factors, and medical intervention in hirsutism and PCOS are discussed. The thesis includes cross sectional data and results from two randomized controlled studies.

The aims of the present thesis can be grouped under these headings:

Diagnostic strategies in patients with hirsutism and PCOS

- To evaluate the prevalence of endocrine diseases in hirsutism [1]
- To establish the prevalence of T2D and to discuss the use of HbA1c and OGTT [1;5]
- To evaluate the use of ACTH tests [2]

Pathogenic mechanisms in PCOS

- To evaluate mechanisms of insulin resistance [6]
- To evaluate adrenal activity [2]
- To investigate the importance of the following inflammatory markers in PCOS
    - Adiponectin [3;7]
    - Ghrelin [3]
    - Leptin [3]
    - hsCRP and IL-6 [8]

- Cardiovascular risk markers and sCD-36 [8]
- Prolactin [4]

Medical intervention

To investigate the effect of different treatment principles in PCOS

- Oral contraceptives [9]
- Insulin sensitizing treatment [6-9]

Evaluation of endocrine diseases in hirsutism and PCOS

Hirsutism may be a manifestation of Cushing's syndrome, androgen producing tumors, acromegaly, or late onset adrenogenital syndrome (NC-AGS). Thyroid diseases, prolactinomas, and early menopause are associated with menstrual disturbances and should not be confused with PCOS. According to the Rotterdam criteria [12], PCOS is a diagnosis of exclusion. PCOS and idiopathic hirsutism are treated with OCP, lifestyle intervention, metformin, and cosmetic treatment, whereas other treatment modalities are needed in patients with endocrine diseases. Patients with androgen producing tumors and Cushing's Syndome need surgery. Hirsutism and PCOS has a prevalence of more than 10% in reproductive aged women and menstrual irregularities are often occurring, especially in women < 20 years [20;54]. General practitioners, gynecologists, and endocrinologists therefore need reliable screening tools to exclude endocrine diseases in the daily clinic.

The optimal screening programme for patients with hirsutism/ suspected PCOS has been debated [54-56]. In Paper [1], we applied a standardized evaluation program in all newly referred patients with hirsutism/ PCOS. The evaluation programme included clinical evaluation, fasting blood samples during follicular phase, transvaginal US, ACTH test, and OGTT [1]. In our population 5/340 = 1.5% patients were diagnosed with serious endocrine diseases [1]. This prevalence corresponded to results in previous studies [57-60]. The overall prevalence of endocrine diseases was 5.4% in a total of 7563 patients of mixed ethnical origin and diverse referral diagnoses of hyperandrogenaemia, anovulation, and/or PCOS [20].

CYP21 defects are autosomal recessive disorders, causing decreased cortisol secretion by the adrenals due to decreased activity of the 21-hydroxylase enzyme. The overall prevalence of NC-AGS in patients with hyperandrogenaemia is 1.3% [20], but varies according to the examined population. Symptoms in patients with NC-AGS and PCOS are similar and the two conditions can not be distinguished based on clinical symptoms alone. Patients with NC-AGS all had elevated basal 17-hydroxyprogesterone (17OHP) [1;57-60]. Some studies found CYP21 defects in patients with only modesty increased 17OHP levels and therefore the cut-off for 17OHP, above which 21-hydroxylase defects should be excluded, is most often set at 6-10 nmol/l [20;61]. This level corresponds to 17OHP levels in luteal phase and therefore 17OHP measurements should be repeated in patients with near-normal 17OHP levels before ordering a confirmatory ACTH test.

It was previously suggested that patients with PCOS had relative dopamine deficiency, which could lead to abnormal GnRH pulsatility, increased LH secretion, and a tendency to increased prolactin levels [62]. The majority of studies could not confirm this hypothesis [63], and it now generally recognized that patients with PCOS do not have increased prolactin levels. In contrast, we found that prolactin levels were significantly decreased in patients with PCOS vs. controls [4] and could be associated with metabolic risk in PCOS. We found 8/340 patients with prolactin above reference interval and 1/8 patients was diagnosed with a microprolactinoma [1]. Our finding of low prevalence of prolactinomas in a population with hyperandrogen symptoms was in accordance with other studies [58;60]. Therefore, prolactinomas seem to occur only rarely in hirsute patients. Hyperprolactinemia should be treated with dopamine agonists and it is therefore recommended that patients with increased prolactin levels should be referred to an endocrine department for further evaluation and treatment [20;64].

Table 2. Initial evaluation of hirsute patients

Clinical examination	Height, weight, waist, blood pressure, degree of hirsutism
	Transvaginal US (PCO and endometrial thickness)
Blood samples	Total testosterone, sex hormone binding globulin (free testosterone calculated)
	LH, FSH
	Prolactin
	17-OHP
	HbA1c, lipids
	TSH
Secondary evaluation	Suspected Cushing's syndrome: 24-h urinary cortisol, short dexamethasone test or midnight cortisol.
	MR/CT of the adrenals when testosterone is more than two times increased
	OGTT acromegaly in patients with clinical symptoms of acromegaly

Patients should be evaluated following >3 months OCP pause and in follicular phase

Gonadotropins are secreted in a pulsatile pattern, and therefore an increased LH/FSH ratio is no longer part of the PCOS diagnosis [12]. The measurement of LH and FSH is however relevant to exclude premature ovarian failure or central amenorrhea.

Androgen producing tumors are often rapidly progressing and surgery is the first line treatment. In the literature, women diagnosed with androgen producing tumors experienced rapid progression of hyperandrogen symptoms during a rather short time period and the medical history in combination with the clinical appearance raised the suspicion of androgen producing tumors [1;65]. The cut-off limit for total testosterone above which an androgen producing tumor should be excluded is usually set as more than two times above the upper reference limit [1;12;16]. It is, however, important that a reliable method of testosterone measurement is used. Commercially available direct assays generally overestimate the steroid concentration [43;44]. Over-estimation of androgen levels increase the risk of ordering unnecessary ultrasound and magnetic resonance imaging in healthy women and incidentalomas may be diagnosed [66].

Cushing's syndrome is caused by excess glucocorticoids produced by the adrenal cortex. ACTH levels are used to discriminate between adrenal or pituitary/ectopic tumours. One patient diagnosed with Cushing's syndrome could be diagnosed using medical history and clinical examination [1]. During the study inclusion

time, 25 patients at the department were diagnosed with Cushing's syndrome. These patients were all referred on account of hypertension and/ or suspicion of Cushing's syndrome. The majority of androgen producing adrenal tumors co-secretes cortisol, which may explain why many patients do not primarily present with PCOS-associated symptoms [67]. Only 6% of adrenal tumors secrete androgens alone [67].

In conclusion, patients with regular menses during a period of 6 – 12 months are unlikely to suffer from serious endocrine diseases. Most often, patients with irregular menses have PCOS. General practitioners can perform major part of the evaluation program and will be able to exclude serious endocrine diseases requiring treatment at a specialist center. **Table 2** includes a suggested evaluation program in premenopausal patients referred with hirsutism and/or PCOS. The use of HbA1c and metabolic screening will be discussed further below.

Ovarian and adrenal androgen production in PCOS

Patients with PCOS have supra-normal androgen responses to a short-time GnRH stimulation test suggesting overproduction of ovarian androgens [68;69]. Furthermore, adrenal suppression with dexamethasone is associated with persisting hyperandrogenemia in PCOS [70;71]. The LH pulse amplitude and pulse frequency is increased and irregular in PCOS [72]. When the concentration of LH increases relative to FSH, the ovarian androgen production by the theca cells increases and the development of the oocyte is disturbed [72]. Increased LH pulse frequency in PCOS could be due to increased pulse frequency of GnRH [73]. A primary hypothalamic defect could therefore be the primary cause of increased LH levels in PCOS [74]. Due to the pulsatile secretion pattern of LH, a random LH concentration measurement is of minor clinical value [41].
Increased ovarian androgen production is not only the result of increased LH stimulation. *In vitro* studies on theca cells from polycystic ovaries showed increased conversion of androgen precursors to testosterone both basally and after gonadotropin stimulation [75;76]. These findings support that theca cells from patients with PCOS have constitutional defects, which lead to increased ovarian androgen production [73].

DHEAS is mainly produced in the adrenal glands and therefore increased DHEAS can be used as a marker of increased adrenal activity in PCOS [77]. DHEAS levels are increased in 20-30% patients with PCOS [78]. Levels of DHEAS decrease with age independent of PCOS status and race [78;79]. Brothers of PCOS patients have increased DHEAS levels suggesting that adrenal hyperactivity may be an inherited trait of PCOS [80]. Several studies described similarities between the hormonal secretion pattern in females with precocious pubarche and PCOS patients and precocious pubarche and adrenarche was a forerunner of PCOS in some patients [81;82]. Ibanez suggested that increased adrenal activity could be caused by intrauterine stress causing precocious pubarche and later on PCOS [83].
About 25-50% of PCOS patients have increased ACTH-stimulated 17OHP responses [2]. Initial studies reported that more than 20% of hyperandrogen patients were carriers of 21-hydroxylase defects compared to a prevalence of less than 5-10% in the background population [84;85]. It was therefore suggested that CYP21 heterozygosity could be an important pathogenic factor in PCOS despite the recessive mode of inheritance [86]. To confirm this hypothesis we included 337 hirsute patients, sequenced the whole CYP21 gene, and performed additional ACTH tests measuring cortisol and 17OHP levels [2]. In our study population, CYP21 carrier status in hirsute patients was similar compared to controls and cortisol levels were significantly increased [2]. These findings therefore supported that increased 17OHP levels in hirsutism were caused by increased adrenal activity and not by CYP21 defects [2]. This hypothesis is supported by studies measuring significantly increased ACTH-stimulated cortisol levels in PCOS patients vs. controls [2;87;88].

Different mechanisms may lead to increased hypothalamic-pituitary-adrenal (HPA) activity in PCOS. Previous studies reported increased urinary excretion of cortisol [89-92] and androgen [89-91] metabolites in PCOS patients compared with controls. Increased adrenal drive in PCOS could therefore be explained by increased cortisol turnover, whereas levels of cortisol in serum are usually normal in PCOS [93;94].
The enzyme 5α-reductase is present in the dermal *papillae* and mediates the conversion of testosterone to the more active androgen dihydrotestosterone. Dermal 5α-reductase activity is increased in PCOS [95;96]. High dihydrotestosterone levels increase terminal hair growth in the *dermal papilla* and therefore, 5α-reductase inhibitors can be used for the treatment of hirsutism [97]. Increased hepatic 5α-reductase activity in PCOS patients could be a mechanism for increased cortisol metabolism possibly leading to hyperactivity of the hypothalamic-pituitary-adrenal axis in PCOS [89;92;98]. Therefore, PCOS could be caused by increased 5α-reductase activity in skin and liver [89]. Supporting that a more general increased 5α-reductase activity may be important in PCOS, ovarian 5α-reductase activity was increased in polycystic ovaries [99]. We found that 5α-reductase activity decreased during pioglitazone treatment, which supported that 5α-reductase activity could be associated with insulin resistance in PCOS [100]. As an alternate mechanism for increased cortisol turnover, Stewart *et al.* furthermore demonstrated impaired reactivation of cortisol by 11β-hydroxysteroid dehydrogenase 1 in PCOS patients [89;93].

Insulin resistance

The physiologic effect of insulin is to stimulate glucose uptake in muscle and adipocytes and to suppress hepatic glucose production. Insulin suppresses lipolysis and therefore levels of FFA decrease. Insulin resistance is defined as a decreased ability of insulin to mediate the metabolic actions on glucose and lipid metabolism. Therefore, increased amounts of insulin are required to achieve a given metabolic action. Insulin resistance is therefore characterized by increased levels of insulin as long as the pancreatic beta-cell function is intact [101]. The measurement of insulin resistance is described in the Methods section.

The link between PCOS and insulin resistance was first described in 1980 by Burghen [102]. It is now well-established that more than 50% of PCOS patients are insulin resistant [103]. Insulin resistance is closely associated with BMI, but is also present in normal weight patients with PCOS [1]. The exact mechanism for insulin resistance in PCOS is still unknown. Patients with PCOS have similar insulin receptor amount and affinity compared with controls and therefore insulin resistance is probably mediated through changes in the insulin receptor-mediated signal transduction cascade [6;104]. Impaired insulin-mediated glucose disposal in PCOS compared to controls was described in previous studies [6;105-109]. Furthermore, oxidative and non-oxidative glucose

metabolism was impaired in PCOS in studies using indirect calorimetry and clamp techniques [6;107]. In these studies, insulin stimulated non-oxidative glucose metabolism was more severely impaired than oxidative glucose metabolism supporting decreased glycogen synthase activity in PCOS [6]. Impaired glycogen synthase activity was confirmed by studies on muscle biopsies from PCOS patients [110]. In muscle biopsies, patients with PCOS had significantly impaired insulin signalling through Akt and AS160 and impaired insulin-stimulated activity of glycogen synthase activity compared to controls [15;110]. Some PCOS patients had increased serine phosphorylation of insulin receptor β, but also distant parts of the insulin receptor cascade were affected [15;110-112].
Insulin resistance in PCOS may be inherited or could be due to adaptive mechanisms such as obesity and hyperandrogenism. These mechanisms were further evaluated in cultured myotubes obtained from insulin resistant patients with PCOS and controls [113;114]. Defects in insulin action that persist in cells removed from the *in vivo* environment for several passages suggest that these changes are the results of mutations in genes regulating these pathways. As recently reviewed [101], data on insulin action in myotubes from patients with PCOS vs. controls were conflicting. We found that glucose uptake and oxidation, glycogen synthesis and lipid uptake were comparable between patients with PCOS and controls along with comparable activity of the mitochondria [113;114]. These results suggested that insulin resistance in PCOS is the result of adaptive rather than inherited defects in the insulin signalling cascade.

Insulin secretion from the pancreatic beta-cell increases to compensate for insulin resistance. The hyperinsulinemia in PCOS may therefore be an adaptive mechanism of the pancreatic beta-cells to insulin resistance. The relationship between insulin secretion and insulin sensitivity is usually a constant hyperbolic function, which is described as the disposition index [101]. The disposition index predicts the risk of T2D. Pancreatic beta-cell dysfunction is required for the development of T2D. Dysglycemia develops when the pancreatic beta-cell is no longer able to secrete sufficient insulin to meet the increased requirements in insulin resistance. Increased risk of T2D in PCOS suggests impaired beta-cell function as well as insulin resistance. Several studies found decreased disposition index in patients with PCOS vs. controls [115;116]. However, other studies found increased [107;117] beta-cell responses to glucose stimulation in PCOS and the degree of beta-cell dysfunction in PCOS remains to be established.
In our study, the disposition index was unchanged during pioglitazone treatment suggesting unchanged beta-cell function [6].
Impaired hepatic insulin clearance also results in hyperinsulinemia [6;108;109]. Some studies measured increased insulin/C-peptide ratio in PCOS suggesting decreased hepatic extraction of insulin in PCOS [101].

Insulin resistance and hyperandrogenemia in PCOS

Since the first discovery of insulin resistance in PCOS patients, the possible link between hyperinsulinemia and high androgen levels has been investigated. Insulin may stimulate androgen production in ovaries and adrenals and/or hyperandrogenemia may promote insulin resistance.
Insulin receptors are present in normal and in polycystic ovaries [101]. Insulin in synergy with LH stimulated p450c17 activity in ovaries and adrenals and resulted in increased androgen production [118;119]. Studies supported that the theca cells from patients with PCOS are more responsive to the androgen stimulating actions of insulin than normal ovaries [101]. Therefore, insulin may act as a co-gonadotropin to increase androgen synthesis from theca cells. Furthermore, hyperinsulinemia decreases SHBG production by the liver and through this mechanism free testosterone levels increase [120]. Low SHBG levels predicted the diagnosis of PCOS [121] and correlated with low insulin sensitivity during euglycemic hyperinsulinemic clamps [122].
The relation between hyperinsulinemia and hyperandrogenemia in PCOS was further investigated in studies using diazoxide treatment to inhibit insulin secretion [123]. Diazoxide treatment was followed by decreased total and free testosterone levels and increased SHBG levels [123]. Furthermore, treatment with metformin and glitazone treatment was associated with higher ovulation rates [35;124;125]. These findings supported that hyperinsulinemia and insulin resistance are pathogenic factors in PCOS. Suggesting a more complex interaction between androgens and insulin sensitivity, glitazone treatment was associated with improved ovarian function and increased SHBG levels [6;126-128], but unchanged testosterone levels and FG score [6;126]. Furthermore, the exact mechanism of action of metformin is unknown which complicates interpretation of studies using metformin treatment. The effect of metformin on insulin sensitivity is probably indirectly mediated via impaired hepatic gluconeogenesis [129]. In a recent study, metformin decreased hepatic glucose output and improved glucose effectiveness without changing insulin sensitivity [129]. Metformin treatment is often followed by slight weight loss, which may improve insulin sensitivity [9;35].

The association between insulin resistance and adrenal activity in PCOS is not clear. DHEAS was not associated with fasting insulin in cross sectional studies [1;2;77;130]. The results of studies using insulin sensitizing treatment have been conflicting as some reported unchanged adrenal androgen levels [100;131-133], whereas other studies found decreased adrenal activity during metformin [134;135] or PPAR-γ agonist treatment [126;136;137]. We found decreased 5-α-reductase activity during pioglitazone treatment in PCOS, which could suggest decreased cortisol metabolism and therefore decreased HPA drive [100].

Testosterone may stimulate insulin resistance directly or indirectly. Testosterone applied in supra-physiological doses in women was directly followed by insulin resistance evaluated by euglycemic clamp studies [138]. Furthermore, high testosterone levels may promote abdominal obesity, which may indirectly induce insulin resistance [17]. PCOS phenotypes with hyperandrogenemia were more insulin resistant than phenotypes without hyperandrogenemia, which further supported the importance of hyperandrogenemia for insulin resistance in PCOS [138].
The effects of decreased androgen levels on insulin resistance were evaluated after androgen inhibition with GnRH agonists [138;139] or ovarian wedge resection [140;141]. In the majority of studies, insulin sensitivity however, did not increase despite decreased androgen levels.
Cyproterone acetate and OCP inhibit androgen levels, but were associated with increased insulin resistance rather than increased insulin sensitivity [142-144]. Adverse effects of OCP could however be due to the progesterone component [145] as anti-androgen treatment with flutamide was followed by increased insulin sensitivity and reduced inflammatory markers [146]. Increased inflammatory markers and unchanged central obesity were observed in PCOS during medical intervention with OCP, which may

explain that decreased androgen levels did not improve insulin sensitivity in PCOS [9].

Diabetes risk in PCOS

Nearly 50% of PCOS patients fulfill the criteria of the metabolic syndrome and PCOS is associated with increased risk of diabetes [1;147]. The prevalence of diabetes in cross sectional studies was 1.5-10% and the prevalence of impaired glucose tolerance (IGT) was 10-36% [1;147;148]. Using OGTT, we found that the prevalence of previously undiagnosed diabetes was 4.7% resulting in a total diabetes prevalence of 6.6%. The median BMI of our study cohort was 27 kg/m^2 [1]. 13/14 patients diagnosed with diabetes in our study population was overweight supporting that obesity is an important predictor of diabetes in PCOS [1]. Based on available studies it is currently estimated that PCOS is associated with 5-8 times increased diabetes risk compared to age and weight-matched controls [103].

Until recently it was recommended that OGTT should be performed as a baseline screening in all PCOS patients, but fasting plasma glucose could be applied where OGTTs were not available [12;14]. In daily practice the performance of OGTT is inconvenient and time consuming because the patient has to be seen in a fasting state and the test duration is two hours. There are considerable intra-individual variations in fasting and 2 hour glucose levels, which may lead to misclassification of abnormal glucose tolerance [149;150]. HbA1c is a widely used marker of chronic glycemia and reflects the average blood glucose levels over a two- to three-month period [151]. HbA1c has higher repeatability than fasting glucose and can be assessed in the non-fasting state [151]. HbA1c may however be affected by genetic, hematologic, and illness-related factors [151;152]. Recently, HbA1c ≥ 6.5% was applied as the cut-off point for diagnosing T2D in asymptomatic patients [151]. The cut-off point for HbA1c was based on the established association between HbA1c and microvascular disease [151].

In our population of patients with PCOS, HbA1c had a low sensitivity of 35% for the diagnosis of T2D when OGTT was used as the gold standard for the diagnosis of diabetes [5]. However, the specificity of HbA1c for the diagnosis of diabetes was high and the more severe cases of T2D established by the OGTT were also identified by the HbA1c method. These results were in agreement with recent studies in Turkish [153] and Austrian [154] populations with PCOS and supported that HbA1c can not be used to diagnose diabetes when OGTT is considered the gold standard test. Celic and Lerchbaum therefore concluded that OGTT should be performed in all patients with PCOS [153;154]. This conclusion was based on the importance of diagnosing diabetes in an early stage to make sure that treatment with metformin and lifestyle intervention was initiated [153;154]. Our findings supported that increased HbA1c levels could be used as a marker of cardiovascular risk in PCOS [5]. Increasing HbA1c was associated with higher waist, BMI, and a more adverse lipid profile [5]. These results were supported by studies in non-PCOS populations where HbA1c and fasting glucose levels were similarly associated with risk of diabetes, but HbA1c was more strongly associated with risk of cardiovascular disease and death from any cause than fasting glucose [155]. Population based studies found that increasing HbA1c levels within the reference range were associated with cardiovascular disease [155-157]. Different study populations may therefore be identified when HbA1c and glucose levels are applied for the diagnosis of diabetes.

At present, the indication for performance of OGTT in patients with PCOS is unclear. Previous guidelines recommended that OGTT should be performed in all patients [14], whereas recent guidelines suggested that OGTT should be performed in high risk patients with BMI > 30 kg/m^2, age > 40 years, a history of GDM, or a family history of T2D [1;153;154]. Up to 30% patients with PCOS and T2D according to OGTT would however not be diagnosed if these criteria were applied [154]. In our study, 1/13 patients diagnosed with diabetes had BMI < 25 kg/m^2. In the studies by Lerchbaum [154] and Vribkova [158], 0/298 and 1/104 PCOS patients with BMI < 25 kg/m^2 were diagnosed with diabetes during OGTT, respectively. Therefore, if the aim is to diagnose T2D, the value of performing OGTT in normal weight patients with PCOS seems to be limited. The importance of diagnosing pre-diabetes is currently discussed. Pre-diabetes is defined as blood glucose levels higher than normal but below diabetes thresholds. Pre-diabetes can be diagnosed in patients with impaired fasting glucose (fasting glucose ≥ 6.1 mmol/l and < 7.0 mmol/l), impaired glucose tolerance (2 hour glucose levels ≥ 7.8 mmol/l and < 11.0 mmol/l), and/or HbA1c 5.7 – 6.4% [159]. It is estimated that 5-10% individuals with pre-diabetes convert to diabetes/ year and up to 70% individuals with pre-diabetes will develop diabetes [159]. Pre-diabetes should be treated with life style intervention, but treatment with metformin also postpones the development of diabetes [159]. It remains to be determined whether the performance of OGTT is relevant with the aim of diagnosing pre-diabetes or as a tool for estimating prognosis and/or choice of treatment modality in patients with PCOS. Whether HbA1c is superior to OGTT regarding long term metabolic and cardiovascular risk in PCOS remains to be established in future studies.

Central obesity and fat metabolism in PCOS

Approximately 75% PCOS patients are overweight and central obesity is seen in both normal and overweight PCOS patients [160-162]. Increased fat ingestion in PCOS patients vs. controls was found in some studies [163;164], but could not be reproduced by others [165]. Ghrelin secretion following meals were less suppressed in PCOS compared to controls, which suggesting impaired appetite regulation [166]. The prevalence of eating disorders was nearly 40% in women presenting with hirsutism [167], and conversely, PCOS was overrepresented in bulimic women [168]. The metabolic rate is not decreased in PCOS patients [6;169;170] and randomized studies showed no differences in the ability to loose weight between PCOS patients and weight-matched controls on the same diet [171;172]. In previous studies, decreased quality of life in PCOS was associated to increased body weight [173].

Visceral adiposity is associated with insulin resistance and increased morbidity [174], suggested to be at least partly mediated via a state of low-grade inflammation [175;176]. In PCOS, increased fat mass is associated with hyperandrogenism, irregular ovulations, and reduced fertility [16]. The adipose tissue produces and releases a number of bioactive proteins, collectively referred to as adipokines [177]. Except for leptin and adiponectin, adipokines are not exclusively produced by adipocytes, but are primarily secreted by adipose tissue-resident macrophages [178]. In obesity, the number of adipose tissue-resident macrophages is increased in both subcutaneous abdominal and visceral adipose tissue [179;180] and the circulating mononuclear cells are more inflammatory active [175]. Increased adipokine secretion predicts the metabolic syndrome and increases the risk of diabetes. We and others investigated the importance of several adipokines in

PCOS and the effects of medical intervention. Some of these adipokines are mentioned below. High inflammatory activity in PCOS could be due to central obesity and/or increased testosterone levels. A close relation between inflammation and testosterone levels is supported by intervention studies using simvastatin [181] and flutamide in PCOS [146]. In these studies simvastatin decreased androgen levels [181] and flutamide decreased markers of inflammation [146].

Levels of free fatty acids (FFA) are increased in PCOS both in the fasting state and during the hyperinsulinemic clamp period [6;117;182]. FFAs influence glucose metabolism as they compete with glucose uptake in peripheral tissues [183]. Chronically elevated FFA levels increase gluconeogenesis and insulin resistance while insulin secretion is impaired [184;185]. The positive effect of PPAR-γ agonists on insulin sensitivity may be mediated through improved lipid metabolism with decreased FFA levels and decreased visceral fat volume [36;37]. Thiazolidinedione treatment in PCOS was associated with unchanged [6;186;187] or increased [128] BMI. Body composition evaluated by DXA-scan [188] and WHR [128;187;188] was unchanged during thiazolidinedione treatment in PCOS. The effects of glitazone treatment on lipid metabolism in PCOS were evaluated in randomized studies using insulin infusions of 40 [6], 80 [126], and 300 mU/m^2 min [128]. In these studies, measures of insulin sensitivity and glucose metabolism significantly improved, whereas improved insulin stimulated suppression of FFA levels [6] and decreased lipid metabolism [6;126] was reported in two studies. Pioglitazone treatment improved lipid profiles in patients with insulin resistance and/or T2D [189;190], but not in PCOS [8;187;191]. Improved lipid profiles during glitazone treatment in patients with T2D suggest that the effect on lipid profile may be related to reduced blood glucose levels [189].

Limited data are available on the effects of metformin and OCP on regional fat mass in PCOS. In our study, a 3rd generation OCP was associated with a median weight gain of 1.2 kg, which was evenly distributed on the upper and lower body regions [9]. Changes in testosterone levels were not associated with changes in fat distribution during bivariate regression analyses. Therefore, our data did not support that the android fat distribution was improved during treatment with OCP in PCOS. In previous six-months studies, weight was unchanged in PCOS [192;193] and non-PCOS populations [194] during treatment with different generation OCPs. Previous studies supported that OCP may improve LDL, total cholesterol, and HDL, whereas TG levels increased [195].

In a recent review, the effect of metformin on weight loss was limited in PCOS [35], but the study duration of included studies was six months or less, which could be too short to document effects on body composition measures. We found a median weight loss 1.6 kg after 6 months and 3.0 kg after 12 months treatment with metformin mono-therapy, which was not affected by BMI at study inclusion [9]. Therefore normal weight patients with PCOS could also benefit from metformin treatment. Our data support that weight loss during metformin treatment in PCOS is not due to initial side effects alone, but the exact mechanism for improved body composition could not be concluded. Metformin treatment inhibited gastric ghrelin secretion *in vitro,* which could suggest improved appetite regulation [196]. We found no significant changes in adiponectin levels during metformin treatment [197] and the effect of metformin treatment on ghrelin secretion needs further testing in PCOS. The importance of weight loss for treatment of PCOS is further discussed in the Treatment section.

Several studies supported that metformin treatment is associated with improved lipid profile with decreased LDL and TG levels, and increased HDL without changes in total cholesterol levels [35].

The metabolic syndrome in PCOS

The elements of the metabolic syndrome in PCOS are given below [12]:

- Waist ≥ 88 cm
- Impaired glucose tolerance
- Blood pressure >130/85 mmHg
- HDL <1.3 mmol/l
- TG >1.7 mmol/l

A high percentage of PCOS patients have abnormal lipid profiles including increased total cholesterol, TG, and LDL, whereas HDL levels are decreased [8;198]. It was estimated that 70% women with PCOS had borderline or high lipid levels [199]. As recently reported, dyslipidemia may depend on ethnicity [200] and age [79] in PCOS.

It is generally recommended that patients with PCOS are screened for the elements of the metabolic syndrome. Whether this screening should include other metabolic risk markers remains to be determined.

Some of the most important metabolic risk markers in PCOS are presented below. Some of these markers may add information on different metabolic aspects in PCOS such as inflammatory activity and cardiovascular risk.

Adiponectin

Adiponectin is the most abundant secreted protein and is secreted exclusively by the adipose tissue [201]. Adiponectin secretion is down regulated in obesity [202]. Low circulating levels of adiponectin was associated with increased risk for insulin resistance and T2D [203]. The mechanisms, by which adiponectin affect insulin sensitivity, are not fully clarified. Animal and *in vitro* studies found that recombinant adiponectin stimulated muscular and hepatic glucose uptake, decreased hepatic gluconeogenesis, and promoted FFA oxidation in skeletal muscle [201;204;205]. Therefore, adiponectin reduced TG levels and increased insulin sensitivity. Low adiponectin levels were associated with impaired insulin-stimulated oxidative and non-oxidative glucose metabolism in healthy individuals and in T2D, suggesting that low plasma adiponectin levels contribute to impaired glucose transport and glycogen synthesis [206-209].

Adiponectin levels are decreased in PCOS patients compared to weight matched controls [3;210] and inversely correlate with fasting insulin [3;211], central fat mass [3;7], and whole body- and non-oxidative glucose metabolism [7;212]. Adiponectin correlated inversely with lipid oxidation and FFA levels during insulin stimulation in PCOS [7]. This indicates that adiponectin may improve the capacity to switch from lipid to glucose metabolism and to store glucose as glycogen in response to insulin [7]. Adiponectin was positively associated with ghrelin and inversely associated with leptin [3].

Total testosterone was both positively and negatively associated with adiponectin in previous studies. We found that total testosterone was positively associated with adiponectin levels in PCOS, which remained significant after correcting for WHR and total fat mass [3]. In another study population, total testosterone was inversely associated with adiponectin [7]. In these studies, no significant associations were found between free testosterone levels

and adiponectin [3;7]. The results of these studies were included in a meta-analysis, concluding that total testosterone levels were not associated with adiponectin [210]. In agreement with this, increased insulin sensitivity was associated with increased adiponectin levels despite unchanged testosterone [7] and changes in testosterone levels were not associated with changes in adiponectin [7]. Therefore, factors other than testosterone may be important for adiponectin secretion in PCOS.

Adiponectin may have direct effects on ovarian function. Adiponectin receptors are found in the ovaries and in the endometrium [201]. Theca cells in patients with PCOS had decreased expression of adiponectin receptors compared to healthy ovaries [213]. Adiponectin stimulation was associated with decreased ovarian androgen production [213]. These findings support important relations between obesity, adiponectin, and hyperandrogenism in PCOS. Increased testosterone levels in obese patients with PCOS could be mediated indirectly by decreased adiponectin levels.

The beneficial effects of PPAR-γ agonist treatment on insulin sensitivity may be caused in part by the ability to increase adiponectin levels [214]. In PCOS, adiponectin levels increased during PPAR-γ agonist treatment [7;212;215]. Following pioglitazone treatment, increased adiponectin levels were associated with increased insulin-stimulated glucose metabolism and decreased lipid oxidation [7]. Weight and fat mass measures were unchanged during pioglitazone treatment, which supported that changes in insulin sensitivity predicted adiponectin levels [7]. In contrast to findings with glitazones, adiponectin levels were unchanged during metformin treatment [35;197;216;217]. We found that body composition improved during 12 months randomized treatment with metformin and/or OCP treatment, but changes in adiponectin levels were comparable in the three treatment arms [197]. It was reported that a weight loss more than 10% was needed to induce significant increases in adiponectin [218]. In agreement with this, a moderate weight loss of 3.8% during lifestyle intervention did not change adiponectin levels in PCOS [219]. We found unchanged HOMA-levels during metformin treatment [9]. These findings could suggest that increased insulin sensitivity is a more important mechanism for increased adiponectin secretion than smaller improvements in body composition. The effects of physical activity on adiponectin secretion in PCOS should be further evaluated.

Adiponectin circulates in different polymer-complexes classified as high-molecular weight multimers, medium-molecular weight hexamers, and low-molecular weight trimers [220]. It was suggested that the effect of adiponectin on insulin-stimulated glucose metabolism was mediated primarily by the high-molecular weight form of adiponectin [221-224]. In these studies, only high-molecular weight adiponectin showed a similar close association with progression to T2D [224], level of insulin resistance [221;222], and the presence of the metabolic syndrome [222] as total adiponectin. Data in patients with PCOS were conflicting. We found weaker correlations between measures of insulin-stimulated glucose metabolism and absolute or relative levels of high-molecular weight adiponectin than with total adiponectin [7]. These data suggested that no further information is gained by measurement of high-molecular weight adiponectin compared to total adiponectin in PCOS [7] and were supported by other studies [210]. In contrast, testosterone treatment was associated with decreased adipocyte production of high-molecular weight adiponectin and high-molecular weight adiponectin was negatively associated with free testosterone levels [225]. These findings support that the regulation of high-molecular weight adiponectin and adiponectin secretion may differ and that both markers could give valuable information of inflammatory status in PCOS.

Ghrelin

Ghrelin is mainly secreted from the entero-endocrine cells in the stomach [226;227]. Ghrelin levels increase during hunger and are suppressed during eating. Ghrelin secretion is down regulated during conditions of positive energy balance such as obesity [227]. Ghrelin is expressed in pancreatic beta-cells and may inhibit insulin secretion. Low ghrelin levels were associated with insulin resistance and diabetes [228]. Ghrelin was positively associated with adiponectin and inversely associated with leptin [3]. Previous studies reported lower ghrelin levels in PCOS patients than in weight matched controls [3;166;229-231] and ghrelin was inversely associated with fasting insulin [3]. We and others found evidence that central fat mass is the most important predictor of ghrelin secretion in PCOS [3;166;232;233]. In our cross sectional study, ghrelin showed significant negative correlations with total, central, and extremital fat mass in PCOS patients with the highest correlation coefficient for central fat mass [3]. No significant associations between insulin or HOMA and ghrelin remained after adjusting for fat mass, which supported that fat mass is more important for ghrelin secretion than insulin [3]. In agreement with this hypothesis, fasting insulin levels were higher in PCOS vs. weight matched controls but ghrelin levels were comparable [232]. A hypocaloric diet combined with metformin treatment did not change ghrelin levels despite significantly improved insulin sensitivity [231].
The ghrelin receptor is distributed not only in the central nervous system but also in ovarian tissue, thus suggesting a possible reproductive function for ghrelin [234]. Testosterone levels were inversely correlated with ghrelin [3;231;233;235;236] and this association remained significant after adjusting for WHR and total fat mass [3]. An association between ghrelin and sex hormones was further supported by studies reporting significantly increased ghrelin levels as testosterone levels decreased during flutamide [237] or OCP [236] therapy in PCOS. Changes in ghrelin levels were inversely correlated with changes in testosterone and these differences remained significant after adjusting for HOMA [237].

Changed ghrelin secretion in PCOS may have important implications for the energy intake in PCOS. An inverse relationship between ghrelin and leptin has previously been established in both healthy individuals [238] and in PCOS [3;239] and supports that the effects of ghrelin on energy homeostasis are opposite the effects of leptin [238]. Ghrelin levels decrease following food intake and the postprandial suppression of ghrelin may be important for the feeling of satiety and meal termination [240]. Previous studies documented that patients with PCOS had blunted ghrelin suppression following meals [166] and during OGTT [241]. Based on these limited data, PCOS patients may have decreased feeling of satiety and tend to over-eat during meals.
In conclusion, ghrelin levels are decreased in PCOS and are inversely associated with measures of insulin resistance, fat mass, and testosterone. It remains to be established whether changes in ghrelin secretion is responsible for possible abnormal appetite regulation and obesity in patients with PCOS.

Leptin

Leptin was the first described adipokine and has important effects on the regulation of food intake and energy expenditure [242]. Leptin is secreted from adipocytes and suppresses food intake and promotes energy expenditure [201]. Ob/Ob mice lacking leptin are massively obese, whereas leptin levels are increased in obesity and overfeeding, suggesting that obesity may be characterized as a leptin-resistant state [243]. Leptin is expressed in the hypothalamus and pituitary and may affect not only the hypothalamic regulation of appetite but also the sympathetic nervous system. In mice, leptin injections improved ovarian follicle development and leptin receptors were found in ovaries, suggesting that leptin may be important for gonadal function [244;245].
In PCOS, previous studies showed close positive associations between leptin and BMI, waist circumference, and measures of insulin resistance [3;246-252]. No significant differences were measured in leptin levels between patients with PCOS and weight matched controls despite significant differences in insulin sensitivity [3;247;248;250;253] and leptin levels were unchanged during glitazone treatment [201]. Data on the effect of metformin treatment on leptin levels were conflicting [201;254], but could be due to decreased fat mass during metformin treatment. Most studies showed no significant association between leptin and androgen levels [3;247;249] and leptin levels were unchanged during anti-androgen treatment in PCOS [253]. These results suggest that fat mass is the most important predictor of leptin secretion in PCOS. Available data do not support a hypothesis of a potential contribution of changed leptin secretion for the pathogenesis of PCOS.

oxLDL and sCD36

LDL must be oxidized to be taken up by macrophages, therefore making oxLDL the atherogenic form of LDL [255;256]. OxLDL levels were increased in PCOS patients compared to weight matched controls [8;257]. OxLDL levels were comparable in normal weight and overweight PCOS patients, therefore suggesting a minor association between body weight and oxLDL [257]. These findings were in agreement with our study reporting a BMI independent correlation between oxLDL and glucose and lipid metabolism [8]. We and others found a positive association between oxLDL and free testosterone, but this correlation became insignificant after correcting for BMI [8;257;258]. Increased levels of oxLDL in PCOS therefore seem more closely related to insulin resistance than with hyperandrogenaemia in PCOS.

CD36 is expressed on the surface of monoytes and macrophages [259]. The foam cell formation process is initiated and enhanced by the binding of oxLDL to CD36 receptors, making CD36 activity a risk factor of cardiovascular disease [259]. Soluble CD36 (sCD36) can be measured in plasma and correlated inversely with insulin stimulated glucose disposal, whereas a positive association was found between sCD36 and insulin and BMI [260]. Patients with PCOS had higher sCD36 levels than weight matched controls [8]. sCD36 showed a BMI independent significant inverse associations with insulin sensitivity and insulin stimulated oxidative glucose metabolism, whereas positive correlations were found with FFA and lipid oxidation during insulin stimulation [8].
During pioglitazone-induced increased insulin sensitivity, sCD36 levels significantly decreased, whereas no significant changes were observed in central fat mass. Multiple regression analyses further supported the fat mass independent correlation between sCD36 and insulin sensitivity and suggested that sCD36 is an independent marker of insulin resistance in PCOS [8]. oxLDL and sCD36 showed similar significant associations to measures of fat mass and glucose and lipid metabolism and therefore supported the hypothesis of a pathogenic relation between CD36 and oxLDL [8]. oxLDL decreased during metformin treatment in patients with T2D [261], but at present no studies evaluated the effect of metformin and/or OCP treatment on sCD36 or oxLDL levels in patients with PCOS.
In conclusion, sCD36 could be an important marker of cardiovascular risk in PCOS, but long term studies are needed to test this hypothesis and the effects of medical intervention on sCD36 should be further tested.

hsCRP and IL-6

hsCRP is secreted in response to cytokines including IL-6. Increased hsCRP was the strongest univariate predictor for the risk of cardiovascular events [262]. hsCRP may not only be a marker of inflammatory disease but may also amplify the inflammation process by further activation of monocytes and endothelial cells [262;263]. Patients with PCOS had significantly higher levels of hsCRP compared to weight matched controls in several previous studies [8;264-269], whereas other studies found no significant differences in hsCRP [270;271]. In recent meta-analyses, CPR levels were on average 96% increased in PCOS vs. controls and remained increased after correcting for BMI [272]. We found that hsCRP correlated positively with DEXA-scan established fat mass measures, whereas no significant correlation was found with testosterone or measures of glucose metabolism [8]. Pioglitazone-mediated improved insulin sensitivity was accompanied by decreased hsCRP levels, whereas no significant changes were measured in body composition or testosterone levels, therefore supporting parallel improvements of insulin sensitivity and hsCRP [8]. Previous studies found no significant differences in IL-6 between PCOS patients and controls and no effect of metformin and glitazone treatment [8;270;271]. In these studies, hsCRP and IL-6 were closely associated and similar positive associations were found between IL-6 and hsCRP and body composition [8;265].
In conclusion, levels of hsCRP were associated with fat mass and insulin resistance in PCOS, whereas IL-6 did not seem to contribute to the pathogenesis of PCOS.

Prolactin

Prolactin is secreted not only from the pituitary gland, but also from macrophages in the adipose tissue in response to inflammation and high glucose concentrations [273]. In cross-sectional studies, high prolactin was associated with increased white blood cell count [274] and autoimmune diseases [275]. The hypothesis that prolactin can act as an adipokine was supported by studies in patients with prolactinomas. Patients with prolactinomas were insulin resistant and insulin sensitivity increased during treatment with dopamine agonist [276-279]. Recently, bromocriptine was approved for the therapy of T2D in the United States [280].
We recently reported significantly lower prolactin levels in patients with PCOS vs. controls [4]. Prolactin levels were inversely associated with age, smoking status, waist, total cholesterol, TG, and LDL and positively associated with HDL in our PCOS population. In multiple regression analyses, prolactin was inversely associated with LDL after correcting for age, BMI, and smoking status. The use of prolactin as a cardiovascular risk marker in PCOS should be confirmed in other studies, but were in accordance

with recent cross-sectional findings in healthy individuals. In these studies, low prolactin levels predicted adverse metabolic outcomes [281;282] and prolactin significantly increased following lifestyle intervention in obese children [283].
The findings from previous studies therefore suggested that the associations between prolactin and metabolic risk factors could be different within and outside the physiological range. In agreement with this hypothesis, *in vitro* studies found that both low and high prolactin levels had adverse effects on beta-cell function. Prolactin knockout and prolactin receptor deficiency was associated with reduced beta-cell activity and glucose intolerance [284]. In animal studies, prolactin injection was associated with weight loss and improved insulin sensitivity [285]. Hypogonadism is associated with insulin resistance and obesity [286]. Hyperprolactinemia induces hypogonadism, which is reversed during treatment with dopamine agonists and could increase insulin sensitivity along with decreased BMI.

We found that prolactin levels were positively associated with estradiol, total testosterone, DHEAS, 17-hydroxyprogesterone, and cortisol levels in patients with PCOS [4]. In multiple regression analyses, prolactin was positively associated with estradiol, 17-OHP, and cortisol after correcting for age, BMI, and smoking status. In cell and animal studies, prolactin had direct stimulatory effects on adrenocortical cell proliferation and prolactin stimulation promoted increased weight of the adrenal glands [287]. More studies are needed to determine the role of prolactin for adrenal activity in PCOS.

Conclusion
Levels of inflammatory markers in patients with PCOS vs. controls are summarized in **Table 3** along with possible associations between inflammatory markers and measures of fat mass, insulin, and testosterone levels. Future studies are needed to determine which of these markers and the cut-offs that should be applied in the daily clinic as predictors of for metabolic and cardiovascular risk in patients with PCOS.

Table 3: inflammatory markers in PCOS.

Inflammatory marker	Levels in PCOS	Associations with BMI/ fat mass	Insulin sensitivity	Testosterone
Adiponectin	**Decreased**	**(÷)**	**÷ ÷**	**?**
Ghrelin	**Decreased**	**÷ ÷**	**(÷)**	**÷**
Prolactin	**Decreased**	**(÷)**	**(÷)**	**+**
sCD36, ox-LDL	**Increased**	**+**	**+ +**	**none**
CRP	**Increased**	**+**	**+**	**none**
Leptin	**Unchanged**	**+ +**	**(+)**	**none**
IL-6	**Unchanged**	**+**	**none**	**none**

÷ ÷ strong inverse association, ÷ inverse association, (÷) weak inverse association
+ + strong positive association, + positive association, (+) weak positive association
None: no association

Other inflammatory and metabolic markers in PCOS

Recently, a wide range of inflammatory and metabolic risk markers was measured in PCOS. Some of these markers include the chemokines migration inhibitor factor (MIF), monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1α, the adipokines visfatin and resistin and several others. Data on these risk markers have been conflicting and the importance in PCOS remains to be established.

Chemokines
Chemoattractant proteins, or chemokines, are small proteins that activate (chemoattract) leucocytes during the process of inflammation. The mechanism for increased flux of monocytes to the adipose tissue with increasing adiposity and the concomitant differentiation to macrophages in the adipose tissue may involve adipose tissue-released chemokines [179;288]. Obesity is positively associated with increased levels of the chemokines MIF [289], MCP-1 [290], and MIP-1α [291]. Increased MCP-1 levels were reported in PCOS vs. controls matched for weight [292-294] and fat mass [292], therefore supporting a hypothesis of increased inflammatory activity of the fat tissue in hyperandrogen patients. Chemokine secretion was higher in visceral than in subcutaneous fat [180], which could explain increased chemokine secretion in PCOS [292].
MCP-1 secretion may be involved in follicular development, ovulation, steroidogenesis, and corpus luteum function, supporting a relationship between chemokine secretion and sex hormones [295]. In agreement with this hypothesis, we reported a BMI and SHBG independent correlation between chemokine levels and testosterone in PCOS patients [292]. Testosterone treatment in men were associated with increased levels of MIF [296], supporting an adverse effect of testosterone on chemokine secretion.
In obese patients, PPAR-γ agonist treatment reduced chemokine levels *in vivo* [297] and *in vitro* [180], whereas glitazone, metformin, and OCP treatment failed to improve plasma chemokine levels in PCOS [197;292] despite increased insulin sensitivity [292] and decreased testosterone levels [197]. These findings support that increased chemokine secretion in PCOS is primarily associated with fat mass.

Osteoprotegerin
High levels of osteoprotegerin predicted cardiovascular events [298;299] and heart disease [298;300] in non-PCOS populations. These findings suggested that osteoprotegerin could a marker of cardiovascular disease. Osteoprotegerin is a soluble decoy receptor for RANKL and was initially discovered as a key regulator in bone metabolism [301]. Osteoprotegein is produced in diverse tissues including bone, heart, and vascular smooth muscle cells [302;303]. Preserved BMD and increased inflammatory state in PCOS could be associated with increased osteoprotegerin levels [304]. Osteoprotegerin levels were however decreased [305;306] or unchanged [304] in PCOS. We found that osteoprotegerin levels were unassociated with measures of insulin resistance and pioglitazone treatment significantly decreased inflammatory markers, insulin sensitivity, and bone mineral density without affecting osteoprotegerin levels [304]. Based on these results, osteoprotegerin is not a good imflammatory and bone metabolic marker in PCOS. Osteoprotegerin could however be a marker of manifest cardiovascular disease in PCOS, but this hypothesis remains to be confirmed.

Resistin

Resistin is an adipokine secreted by macrophages and stromal cells in the adipose tissue [201;307]. Resistin may inhibit adipocyte differentiation and is associated with obesity and diabetes. Data in patients with PCOS were conflicting as some studies reported no differences in resistin levels between patients with PCOS vs. controls, whereas other studies reported increased resistin levels in PCOS [201]. Resistin was positively associated with testosterone and increased ovarian androgen production [201]. PPAR-γ agonist treatment was associated with decreased resistin levels suggesting that resistin may be a marker of insulin resistance in PCOS.

Visfatin

Visfatin is an adipokine secreted by adipocytes, lymfocytes, and several other cell types [201;307]. Visfatin may stimulate the cellular expression of cytokines including TNF-α and IL-6. Visfatin is significantly increased in obese individuals and is positively associated with BMI. Data on visfatin levels after bariatric surgery were conflicting as some studies reported decreased levels, but other studies reported increased levels of visfatin [201].
In PCOS, visfatin levels were unchanged or increased and a positive association between visfatin and measures of insulin resistance was not a consistent finding in previous studies [201]. Therefore the importance for visfatin in PCOS remains to be established.

Other investigated markers in PCOS were omentin-1 (low levels in PCOS), retinol binding protein-4 (increased, normal, or low levels in PCOS), and chemerin (increased levels in PCOS). Whether these or other markers may add more knowledge on inflammatory status in PCOS is undetermined.
The possible use of HbA1c as a cardiovascular and metabolic risk marker is discussed above.

Treatment principles in PCOS

Currently the most used treatment modalities in PCOS are OCP and/or metformin in combination with life style intervention. Some of the effects of OCP and insulin sensitizing treatment on hormonal and metabolic outcomes in patients with PCOS have been mentioned already in the previous sections.

OCP

OCP regulates menstrual cycles and SHBG levels are increased, leading to decreased levels of free testosterone and improved hirsutism [21;31]. The estrogen component of OCP furthermore suppresses LH secretion and thereby the stimulation of ovarian androgen production [195]. OCP are superior to insulin sensitizers regarding menstrual regulation and treatment of hirsutism [31]. Therefore OCP is often the first-choice in patients with PCOS. Possible side effects however need to be considered. OCP is associated with an increased risk of thrombo-embolic episodes [308]. Increased risk of thrombo-embolic episodes was associated with increased SHBG levels during OCP treatment [308]. Multiple types of OCP are available on the market and which types of OCP should be preferred in PCOS is debated [30;309]. The estrogen fraction of OCP is most often ethinyl estradiol, whereas the progestin component varies. 2nd generation OCPs have the lowest thrombo-embolic risk and is therefore recommended as first treatment choice in PCOS. Norgestrel (2nd generation) and levonorgestrel (2nd generation) may have more androgenic activity than desogestrel (3rd generation), norgestimat (3rd generation), and gestodene (3rd generation) [310]. In patients with severe hirsutism, OCPs containing a progesterin with antiandrogen activity such as cyproterone acetate (Diane Mite) or drospirenone (4th generation) therefore could be considered, but limited data are available comparing differences in metabolic and clinical outcomes between different generations of OCP [30;309;310].

The possible effects of OCP on metabolic risk factors and glucose metabolism are currently debated [311]. High testosterone levels could redistribute extremital fat to abdominal fat and therefore increase the inflammatory activity in PCOS [17]. Body composition and insulin sensitivity were however unchanged following suppression of androgen levels with OCP or ovarian wedge resection [139;312]. We found that 1 year's OCP was associated with a median weight gain of 1.2 kg, which was evenly distributed on the upper and lower body regions [9]. As recently discussed [9], combined treatment with OCP and metformin could be considered in normal weight patients with PCOS to avoid weight gain.
In the general population, OCP was associated with increased insulin levels, but the long term risk of diabetes was not increased [308]. Limited data are available in PCOS, but the majority of studies reported unchanged insulin sensitivity [311] and inflammatory markers [197] during OCP treatment.
Previous studies supported that OCP may improve LDL, total cholesterol, and HDL, whereas TG levels increased [195]. Previous meta-analyses included different types of OCP, which could have affected study outcomes. Different generation OCPs could have divergent effects on the metabolic risk in PCOS [313]. Six months anti-androgen treatment with Diane vs. metformin was associated with significantly increased adiponectin levels during OCP [314]. Unchanged BMI and unchanged insulin sensitivity supported that increased adiponectin levels could be caused by the anti-androgen effect of Diane, but bivariate associations between changes in testosterone and changes in adiponectin levels were non significant [314]. Thus the improvement of adiponectin could be mediated at the receptor level. More studies are needed to determine if anti-androgen OCPs are superior to2nd and 3rd generation OCPs regarding anti-inflammatory effects.

Patient with contraindications such as obesity, previous or family history of thrombosis, breast cancer, coagulatory defects, hypertension, or heavily smokers should not be treated with OCP. Alternative treatment modalities are listed in table 4. Hirsutism can be treated cosmetically or with local anti-androgen treatment. Patients with contraindications for OCP can be treated with spironolactone [54]. The effect of spironolactone on hirsutism is comparable to OCP. Spironolactone is contraindicated in pregnancy and must be combined with safe birth control such as an intrauterine-device.

Life style intervention and metformin

Life style intervention and weight loss in PCOS: It is generally accepted that a more than 5-10% weight loss improves fertility and menstrual cycles in women with PCOS [34]. Available data suggest that the diet should be restricted to 1500 kcal/day [34]. Improved menstrual cycles and decreased testosterone levels were observed when the weight loss was obtained over 2-3 months [34], whereas hirsutism was not improved during life style intervention [34].
The optimal diet in PCOS remains to be established. A low glycemic index diet may improve insulin sensitivity by reducing the postprandial glucose level and reactive hyperinsulinemia and could be of benefit in patients with PCOS [315]. Diets with low glycemic index diet often have higher protein content. It has been speculated that a high protein diet may cause less hunger and

craving for carbohydrates ("carbo-craving") and compliance may therefore increase on a high protein compared to a low protein diet [315;316]. In previous studies, the diet composition did not affect weight loss [34]. Furthermore, the percentage of weight loss seemed to determine the diet effect on hormonal and metabolic outcomes, whereas the type of diet was of minor importance [317;318]. In a recent review, patients on a low glycemic index diet tended to have a higher weight loss than patients on other diet types [315]. The drop out rate was however higher in patients randomized to low glycemic index diets compared to other diet types [315]. Diet-intervention studies of longer duration showed an almost linear weight loss during the first 3 months followed by a regression towards baseline values [34]. The high drop out rate in previous studies support that patient's individual preferences are important to maintain life style intervention and weight loss [34]. In the daily clinic, a comprehensible, healthy diet composition seems important for long-term weight loss attainment.
Gastric bypass and gastric banding are associated with significantly improved metabolic risk profile, decreased hirsutism, and more regular menstrual cycles [319], but can only be recommended in few selected patients. Recent studies suggested that GLP-1 agonists could be applied in PCOS to induce weight loss [320], but at present PCOS is not registered as an indication for GLP-1 treatment. Especially, pregnancy should be avoided during GLP-1 treatment.

Metformin and insulin sensitizers in PCOS: Metformin treatment and lifestyle intervention improve insulin resistance and therefore insulin stimulation of the adrenals and ovaries decreases. Furthermore, metformin may have beneficial effects on liver, muscle, fat tissue, ovary, and inflammatory markers as already described in this thesis.
Weight loss during metformin treatment alone is limited [9;35;321]. Metformin combined with life style intervention was superior to either treatment alone regarding weight loss [171;322;323]. Therefore in the daily clinic metformin could be added to life style intervention when life style intervention alone does not induce sufficient weight loss. No long term follow up studies however evaluated the ability of PCOS patients to achieve permanent weight loss on diverse treatment regimes.
Metformin treatment may slow the progression of diabetes in PCOS, but no long term prospective studies exist in PCOS populations [125]. The indication for medical preventive treatment in high risk groups of diabetes has been debated [324]. Insulin sensitizing treatment could be prescribed to postpone diabetes similar to preventive treatment for dyslipidemia or hypertension. However, several studies showed that life style modification in PCOS patients had the same positive effects on hormonal and metabolic status than metformin treatment [317;318;322;325;326]. In the Diabetes Prevention Study, intensive life style modification was more efficient than metformin treatment in decreasing the risk of progression to T2D in high risk individuals [327]. Future studies need to evaluate the effect of long term treatment with insulin sensitizers to postpone diabetes in insulin resistant PCOS individuals [324;328]. At present, diet, exercise, and weight loss remain the most important treatment modalities in PCOS.
The use of metformin treatment in normal weight patients with PCOS is debated. As already discussed, also normal weight patients with PCOS may develop T2D [329]. In our study, the effect of metformin on body composition was independent of BMI [9]. One retrospective, observational study found similar persistence rates with metformin and OCP in PCOS, but at 12 months, less than 30% patients were still on medical treatment [330]. These findings emphasize the difficulty of long term studies in PCOS. In our study, only 65/90 patients finished the study [9] and especially the drop out rate was high in normal weight patients. Treatment in patients with PCOS therefore needs to be individualized to ensure adherence to medical treatment. Longer prospective studies should determine the indication for treatment in normal weight PCOS patients and in patients with only slightly deteriorated insulin sensitivity.

Insulin sensitizing treatment is associated with only modest decreases in testosterone activity and OCP is more efficient than metformin and life style intervention in improving hirsutism and acne [35;331-333]. In studies using PPAR-γ agonists as an insulin sensitizer, the ovulation rate increased without significant changes in testosterone levels [6;136;186;334;335]. The FSH-stimulated estradiol response increased after pioglitazone treatment in PCOS patients [335]. Regulation of menses during pioglitazone treatment may be caused by increased insulin sensitivity in the ovarian granulosa cells and consecutive normalized estradiol secretion. The inability to measure decreased androgen levels despite increased ovulation rate could be due to the applied methods. Measurements of peripheral serum androgen metabolites may be too imprecise to detect minor changes in ovarian hormonal secretion. Measurements of pulsatile testosterone secretion and other ovarian secreted hormones may be needed in order to characterize discrete improvements of ovarian hormonal secretion. Ovarian catherization was performed in few studies, but this procedure can not be carried out in larger clinical studies. PPAR-γ agonists are weight neutral. It is possible that the small but significant decreases in weight during metformin treatment could explain the discrepant effects on sex hormones during glitazone and metformin treatment.
A potential limitation of previous studies is the relatively short study duration. When PCOS is considered a vicious cycle as described in the introduction, hormonal disturbances should improve when the cycle is broken. However, once established during puberty, the androgen production by the ovaries may become more or less autonomous. Therefore, even if insulin stimulation of the ovary is diminished, abnormal hormone production may continue. Given the limited effects of metformin and life style intervention on hyperandrogenism, OCP is generally first treatment choice in patients with hirsutism.
Some of the treatment principles in PCOS are shown in table 4.

Table 4 treatment principles in PCOS

Hyperandrogenism

- OCP
- Spironolactone
- (Lifestyle intervention)

Cosmetic treatment

Menstrual irregularities

- Lifestyle intervention
- OCP
- Gestagen IUD
- Metformin

The metabolic syndrome

- Lifestyle intervention
- Metformin

Future perspectives

Hirsutism and PCOS are very common conditions in the daily clinic. It is therefore important that general practitioners are familiar with the diagnosis and treatment of patients with PCOS. Endocrine diseases are rare in hirsute patients, but should always be ruled out. The general practitioner has access to the blood samples, which are needed to rule out the majority of endocrine diseases in patients with hirsutism and/or irregular menses. Several issues, however, remain unclear in PCOS and await further studies. Close collaboration between general practitioners, endocrinologists, and gynaecologists is therefore necessary to ensure optimal evaluation and treatment in patients with PCOS.

One main concern in daily praxis is the increased risk of metabolic disturbances in patients with PCOS. Insulin resistance is prevalent in PCOS and therefore, measurement of fasting metabolic parameters should be part of the routine diagnostic evaluation. It is generally recommended that patients with PCOS are screened for the metabolic syndrome, but the optimal use of clinical and biochemical risk markers remain to be determined. In the present thesis several inflammatory markers have been presented in the context of PCOS, but the use of these markers in the clinic awaits clarification. Furthermore, the presence of obesity and hyperandrogenemia is associated with insulin resistance [20;336]. When the Rotterdam criteria are applied, four different phenotypes of PCOS are identified depending on which of the criteria are fulfilled [337]. The metabolic and long term risk profile in the four different phenotypes may differ. Several studies supported that patients with anovulation and hyperandrogenism had more severe metabolic manifestations than patients with regular menses and patients without hirsutism [337]. It is therefore possible that screening and follow up should be adjusted to the patients individual risk profile. The manifestations of PCOS may change according to life style intervention, age, and use of OCP. Weight gain could determine ovulatory function in patients with hyperandrogenism and PCO [101]. It remains to be established how often individual patients may move from one phenotype to another and how this should affect evaluation and follow up.

The symptoms of PCOS most often start in puberty and last throughout the reproductive period. The manifestations of PCOS are age dependent and age should be considered when the patient is evaluated. 80% healthy young women had polycystic ovaries suggesting that PCO is a normal variant in young women [338]. We recently reported that young patients with PCOS were characterized by PCO and biochemical hyperandrogenism, whereas older patients had more severe hirsutism and more cardiovascular and metabolic risk factors [79]. At present many patients request evaluation for PCOS. The consequence of pausing OCP for clinical and biochemical evaluation in the individual patient should however be considered. Given the high prevalence of PCO in young individuals, the consequence of performing ultrasound should be considered before referral to gynaecological evaluation. The majority of young patients with PCOS will benefit from treatment with OCP and general advice on life style intervention.

Ethnicity is associated with clinical presentation and metabolic risk in PCOS [200]. Inherited variations in 5α-reductase activity may explain differences in the prevalence of hirsutism among diverse ethnic populations [339]. Several studies documented that immigrants in western countries may be at increased risk for life style diseases such as obesity and diabetes [340]. Increased risk for diseases may be attributable to genetic predisposition or to environmental influences such as different exercise or eating habits. We recently reported that North European women with PCOS were significantly older and had higher BMI by the time of referral compared to women originating from the Middle East [200]. North European women with PCOS had increased blood pressure, smoked at a higher frequency, and tended to have a more adverse lipid profile than patients originating from the Middle East [200]. After correcting for differences in age and BMI, Middle Eastern women were characterized by increased androgenicity, which could be explained by decreased SHBG, and increased diabetes risk [200]. More studies are needed to determine whether different ethnic groups with PCOS should be monitored differently.

The long term risk of PCOS is debated. Recent guidelines recommended yearly follow up of all patients with PCOS with measurements of weight, waist circumference, blood pressure, fasting lipids, and screening for T2D [16;341]. In prospective studies, the most important predictive factor for the development of T2D was obesity [147;342]. Several authors described a high risk for deteriorated glucose tolerance during prospective follow-up studies in PCOS patients [147;342]. Especially obese PCOS patients have a high rate of conversion from normal glucose tolerance to IGT or T2D. American PCOS patients had an 8-year incidence of 13.4% for T2D in [343] and 16.4% Australian PCOS patients developed T2D during 6 years of follow-up [342]. No long term studies exist in normal weight PCOS populations. We evaluated clinical and metabolic outcomes in 69 hirsute patients with average BMI 25 kg/m^2 after a median observation period of 4 years [329]. During follow up, glucose levels had increased and 8.5% patients were diagnosed with T2D [329]. The increased glucose levels were seen despite unchanged BMI, and the development of IGT and T2D was seen in both normal weight and obese individuals. These findings suggest that measurement of HbA1c with intervals is reasonable also in normal weight patients with PCOS.

Patients with PCOS are considered to be at high risk of developing a cardiovascular disease due to the high prevalence of risk factors including coronary artery calcification and echocardiographic abnormalities [344-346]. At present, however, no long term studies exist on well-defined PCOS populations [347]. Retrospective studies found significantly increased risk of hypertension and CVD in women with irregular cycles and the current estimated risk for CVD is 4-11 fold increased in PCOS [348-350].The main problem in retrospective studies was difficulties in defining PCOS patients and finding appropriate control groups. Therefore prospective studies on well-defined patient populations are needed to confirm this hypothesis. Treatment with statins is rarely indicated in reproductive aged women with PCOS. In recent studies, simvastatin decreased androgen levels [181], but was associated with increased insulin resistance [351]. There are insufficient safety data on treatment with statins during pregnancy. Therefore, the consequence of measuring yearly lipid status in young patients with PCOS is questionable.

PCOS patients most often have irregular menses or amenorrhea, implying unopposed estradiol exposure of the endometrium. It is therefore speculated that PCOS patients carry an increased risk of endometrial cancer, but only few epidemiological studies have been conducted to confirm this hypothesis [352]. In our study population, PCOS was not associated with an increased risk of endometrial cancer [353]. Studies have not been able to document higher breast cancer risk in PCOS patients [354].

Several studies showed normal or higher bone mineral density in both PCOS and hirsute patients than in control subjects [355-357]. The positive combination of hyperandrogenemia and possibly high androgen sensitivity may be an explanation for higher BMD levels [358]. Furthermore, adiposity is positively associated with bone mineral density [359] and recent studies suggested that hyperinsulinemia independent of BMI may be protective for the development of osteoporosis [360;361]. Increased bone mineral density in some previous studies could be associated with decreased fracture risk, but limited fracture data exist in patient populations with PCOS [362]. Vitamin D levels were lower in patients with PCOS and hirsutism vs. controls [363-365] and the prevalence of vitamin D levels <50 nmol/l in PCOS was 31-85% [366]. Vitamin D increases calcium absorption, osteoblast activity, and bone formation, whereas vitamin D insufficiency causes defects in bone mineralisation, increases bone turnover, and fracture risk. Vitamin D may be important not only for bone metabolism, but also for the risk of cancer, cardiovascular, and autoimmune diseases. Vitamin D levels increased following vitamin D substitution in patients with PCOS, but insulin sensitivity and metabolic risk markers were unchanged [367]. The importance of low vitamin D levels in PCOS and the indication for vitamin D treatment therefore needs further evaluation.

Recent studies supported that quality of life is impaired in patients with PCOS and the risk of depression is approximately eight times increased compared to controls [173;368]. The short Form-36 (SF-36) may be used to reach a health state description and can describe the burden of a specific condition [369]. SF-36 scores were similar in patients with PCOS and patients with chronic diseases such as diabetes and asthma [173;370]. Cross sectional studies reported eight times increased risk for depression in patients with PCOS [371]. In meta-analyses, especially obesity and hirsutism were associated with impaired quality of life in PCOS [173;371], whereas menstrual irregularities were less important [370]. These results emphasize the possible negative effects of PCOS on the patients' quality of life. OCP did not change depressive symptoms in 36 patients with PCOS despite more regular menses and decreased hirsutism [372], but no long term randomized studies evaluated the effects of OCP or metformin on quality of life. Physical activity and lifestyle intervention may improve quality of life, but more studies are needed to determine which treatment modalities should be advised in patients with PCOS and decreased quality of life.
We found that 20% reproductive aged patients with PCOS had prescriptions of antidepressants during follow up compared to 15% healthy controls [373]. These data confirmed that a high percentage of Danish women use antidepressants. Increased urinary excretion of cortisol metabolites in PCOS patients and compensatory hyperactivity of the HPA-axis may be associated with depression and reduced quality of life [89]. Antidepressants may modulate the HPA-axis in PCOS and improve metabolic risk factors [374]. Future studies are needed to test this hypothesis. A recent Cochrane review found lack of data on the use of antidepressants in patients with PCOS and studies on their effectiveness in PCOS were requested [375].

Family studies showed a PCOS prevalence of 25-50% in first-degree relatives of PCOS patients suggesting a strong genetic component of PCOS [376]. Recent studies used the candidate gene approach and investigated genes involved in androgen synthesis, secretion, metabolism, and regulation along with genes affecting insulin resistance, insulin secretion, and inflammation [376-380]. The use of the candidate gene approach in PCOS is limited by the fact that the exact pathogenesis for PCOS is unknown. Like other common diseases such as T2D, PCOS is most likely a multi-genetic disease with several genes having small and additive effects, whereas other genes may protect against the syndrome [380]. Genetic studies in PCOS are challenged by the diverse definitions of PCOS used over time. PCOS is a heterogeneous disease and the genetic profile of different phenotypes may differ. Furthermore, PCOS can only be diagnosed in reproductive aged women and the male phenotype of the syndrome is unknown [376]. Lifestyle and environmental factors are of high importance in PCOS, the clinical expression may vary considerably over time, and the wide use of OCP may minimize the symptoms of PCOS. These challenges of genetic studies may explain why initial positive results could not be reconfirmed in other study populations. One genome wide association study found that three loci were associated with PCOS and future large scale and possibly multi-center studies are needed to determine the genetic background for PCOS.

Summary

Hirsutism affects 5-25% women and the condition is most often caused by PCOS. The initial evaluation of hirsute patients should include a thorough medical history, clinical evaluation, and standardized blood samples to diagnose the 5% hirsute patients with rare endocrine disorders. The majority of these examinations can be performed by the patient's general practitioner.
PCOS is a diagnosis of exclusion and is a multiorgan disease affecting most endocrine organs including ovaries, adrenals, pituitary, fat cells, and endocrine pancreas. The manifestations of PCOS are diverse and up to 50% patients are normal weight. In most cases, however, the severity of symptoms can be related to abdominal obesity. Increased inflammation in PCOS can be measured as decreased adiponectin levels and increased levels of adipokines, chemokines, and interleukins. In the present thesis the use of these inflammatory markers is reviewed, but more data including hard end points are needed to determine which of these markers that should be introduced to the daily clinic.
Abdominal obesity and insulin resistance stimulates ovarian and adrenal androgen production, whereas SHBG levels are decreased. Increased testosterone levels may further increase abdominal obesity and inflammation, therefore describing PCOS as a vicious cycle. Abdominal obesity and increased activation of the inflammatory system is seen in both normal weight and obese PCOS patients leading to an increased risk of dyslipidemia, diabetes, and possibly cardiovascular disease. Patients diagnosed with PCOS therefore should be screened for elements in the metabolic syndrome including weight, waist, blood pressure, HbA1c, and lipid status. Our data supported that prolactin and HbA1c levels could be markers of cardiovascular risk and should be confirmed by prospective studies.
PCOS is a life-long condition and treatment modalities involve lifestyle modification, insulin sensitizers such as metformin, or inhibition of testosterone levels with OCP. Treatment with pioglitazone

supported that increased insulin sensitivity in PCOS is associated with improved inflammatory and cardiovascular risk markers. Our data supported that one year's metformin treatment was associated with a minor but significant weight loss in patients with PCOS irrespective of BMI at study inclusion. Treatment with OCP improved sex-hormone levels, but was associated with minor weight gain. Based on the study results, clinicians should consider the combined treatment with metformin and OCP also in normal weight patients with PCOS.

The challenge in the future is to ensure sufficient evaluation and treatment of patients with hirsutism and PCOS and to determine which subgroups of patients should be treated by their general practitioner and which patients should be referred for hospital and/or gynecological evaluation and treatment. Furthermore more data are needed to determine the optimal follow up program regarding metabolic risk in different subgroups of patients with PCOS.

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