Polycystic ovary syndrome in women with severe obesity

- effects of a 12-month weight loss intervention

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”*Utan tvivel är man inte klok”*

Tage Danielsson

Abstract

Background: Polycystic ovary syndrome (PCOS) affects one out of eight women and is associated with reproductive, metabolic, and psychiatric features. There is a strong association with obesity but studies on PCOS in women with severe and morbid obesity are lacking.

Aim: To estimate the prevalence of PCOS in women with severe obesity (body mass index [BMI] ≥ 35 kg/m^2), and to compare hormonal and metabolic features, anxiety and depression, health-related quality of life (HRQoL), energy intake, physical activity, and eating behavior by PCOS diagnosis and evaluate the effects of a 12-month weight loss intervention.

Methods: Participants with severe obesity were recruited from the obesity unit at Sahlgrenska University Hospital where they had been referred for weight loss treatment. Participants were divided into groups by PCOS-status, diagnosed with the National Institutes of Health-criteria, and assessed with clinical examination and questionnaires at baseline and after a 12-month weight-loss intervention.

Results: PCOS was present in 25.6% (n=63/246). Participants with PCOS had higher androgen levels, lower low-density lipo-protein cholesterol (LDL-C) and lower total cholesterol compared to women without PCOS. Groups did not differ in the prevalence of metabolic syndrome, symptoms of anxiety and depression, HRQoL, energy intake, or physical activity at baseline. Those with PCOS had higher cognitive restraint eating behavior at baseline. Anti-müllerian hormone (AMH) was higher in those with PCOS, but due to low sensitivity and specificity it was not possible to use AMH as a discriminator between women with and without PCOS. Over the course of the 12-month weight loss intervention, 70% (n=174) of participants dropped out leaving 72 women for follow up (PCOS n=16, non-PCOS n=56). Both groups lost weight (PCOS -12.5 ± 9.3 kg p <0.001; non-PCOS -14.0 ± 12.5 kg p <0.001), with no difference between groups. In women without PCOS, weight loss was associated with lower androgens, insulin and blood lipids, less symptoms of anxiety and depression and higher mental HRQoL. Further, those without PCOS reported reduced energy intake, and changed eating behavior. From baseline to follow-up, in comparison between the two groups, women without PCOS reported larger increase in cognitive restraint than those with PCOS and larger reduction in carbohydrates and sugars compared to women with PCOS, whereas women with PCOS reported larger reduction in fat intake.

Conclusion: In this unique cohort of women with severe obesity, PCOS was present in one out of four. AMH could not be used as a single surrogate marker

of the syndrome. Before obesity treatment, women with PCOS had more conscious control regarding eating. Importantly, using a structured weight loss intervention, those with PCOS lost weight to the same extent as women without PCOS. Comparing groups regarding change from baseline, there were no major discernible differences except that women without PCOS changed more in eating behavior with more cognitive restraint towards a behavior more favorable for further weight loss.
Keywords: polycystic ovary syndrome, severe obesity, weight loss, AMH, anxiety and depression, eating behavior

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List of papers

This thesis is based on the following studies, referred to in the text by their roman numerals.

I. Kataoka J, Larsson I, Björkman S, Elisasson B, Schmidt J, Stener-Victorin E. **Prevalence of polycystic ovary syndrome in women with severe obesity - effects of a structured weight loss programme.** Clinical Endocrinology (Oxf). 2019 Dec;91(6):750-758

II. Kataoka J, Larsson I, Lindgren E, Kindstrand LO, Schmidt J, Stener-Victorin E. **Circulating Anti-Müllerian hormone in a cohort study of women with severe obesity with and without polycystic ovary syndrome and the effect of a one-year weight loss intervention.** Reproductive Biology and Endocrinology 2022 Oct 29;20(1):143

III. Kataoka J, Olsson M, Lindgren E, Larsson I, Benrick A, Schmidt J, Stener-Victorin E. **Symptoms of anxiety and depression and health-related quality of life in women with severe obesity and polycystic ovary syndrome and the effect of a one-year weight loss intervention.** Submitted to journal.

IV. Kataoka J, Stener-Victorin E, Schmidt J, Larsson I**.** **A prospective 12-month structured weight loss intervention study in women with severe obesity and polycystic ovary syndrome: focusing on eating behavior, energy intake, physical activity and impact of weight loss on these behaviors.** Submitted to journal.

Additional publication performed during the PhD-studies, not included in the thesis:

Kataoka J, Tassone EC, Misso M, Joham AE, Stener-Victorin E, Teede H, Moran L.J. **Weight Management Interventions in Women with and without PCOS: A Systematic Review.** Nutrients. 2017 Sep 8;9(9):996.

Sammanfattning på svenska

Polycystiskt ovariesyndrom (PCOS) är vanligt och förekommer hos ca en av åtta kvinnor. Trots detta är det ett tillstånd som ofta är underdiagnostiserat och undermåligt behandlat. PCOS kännetecknas av glesa eller inga menstruationer, ökad hårväxt av manlig typ och en typisk ultraljudsbild av äggstockarna med många små omogna äggblåsor (antralfolliklar). Kvinnor med PCOS har oftast högre nivåer av både manligt könshormon (androgener) och insulin i blodet. PCOS är också associerat med ökad förekomst av utebliven ägglossning och infertilitet, typ 2-diabetes, övervikt/obesitas, höga blodfetter, högt blodtryck, psykisk ohälsa, försämrad livskvalitet och störningar i ätbeteende. Vad som orsakar PCOS är inte helt känt, men ärftlighet spelar stor roll och miljön i fosterlivet har troligen betydelse för dess uppkomst. Då man inte vet orsaken till PCOS är behandlingen fokuserad på symtom och på att förebygga och behandla följdsjukdomar. Livsstilsförändringar, med kost och fysisk aktivitet som leder till viktminskning eller förebyggande av viktuppgång, är en viktig behandling som har visat sig förbättra alla symtom. Över hälften med PCOS har övervikt (BMI ≥25 kg/m^2) eller obesitas (BMI ≥30 kg/m^2), vilket försämrar alla symtom förknippade med syndromet, dock är sambandet mellan PCOS och obesitas fortfarande oklart. Trots att förekomsten av obesitas har tredubblats de sista decennierna, med en förekomst av 13% i världen och 16% i Sverige, och trots kunskapen om att PCOS är en bidragande orsak till ohälsa hos kvinnor, finns det få studier av PCOS hos kvinnor med svår obesitas (BMI ≥35 kg/m^2) och hur viktminskning påverkar. I dessa studier har vi undersökt förekomsten av PCOS i en grupp av kvinnor med svår obesitas och delat in gruppen beroende på om de har PCOS eller inte. Dessa grupper jämfördes vid start av studien, och efter att de genomgått ett strukturerat 12 månaders viktminskningsprogram som inkluderade en period där all mat ersättes av lågenergipulverdrycker, följt av en period med energireducerad kost. Vi fann att förekomsten av PCOS hos kvinnor med svår obesitas var 25,6% vilket är dubblerat jämfört med förekomsten i den övriga befolkningen. Kvinnor med och utan PCOS skiljde sig inte åt vad avser kroppskonstitution, förekomst av metabola syndromet, symtom på ångest och depression, livskvalitet, energiintag och fysisk aktivitet. Dock hade kvinnor med PCOS ett mer restriktivt ätbeteende. Anti-müllerskt hormon (AMH) var högre hos kvinnor med PCOS men kunde inte användas för att särskilja kvinnor med och utan diagnosen PCOS. Både kvinnor med och utan PCOS svarade bra på en strukturerad viktminskningsbehandling med en lika stor viktminskning (ca. 12%). Efter viktminskning sågs förbättring av metabola och reproduktiva

parametrar, psykiskt mående och ett förändrat ätbeteende endast hos kvinnor utan PCOS. Det var dock få skillnader vad avser förändringar som skedde under viktminskningsbehandlingen när man jämförde grupperna. Restriktivt ätbeteende ökade och intag av kolhydrater och socker minskade mer hos kvinnor utan PCOS. Kvinnor utan PCOS minskade sitt fettintag mer.

Sammanfattningsvis förekom PCOS hos en av fyra kvinnor med svår obesitas. Före obesitasbehandling hade kvinnor med PCOS ett mer kontrollerat ätbeteende. Under obesitasbehandling minskade båda grupperna lika mycket i vikt, men det var få skillnader när man jämförde förändring mellan grupper förutom att kvinnor utan PCOS ökade kontrollerat ätbeteende mer, vilket är en faktor som kan främja bibehållande av en lägre vikt över tid.

Content

Abbreviations

AMH	Anti-müllerian hormone
BMI	Body Mass Index
BSA-S	Brief Scale for Anxiety self-rating
CPRS-SA	Comprehensive psychopathological rating scale for affective syndromes self-assessed
DEXA	Dual energy x-ray absorptiometry
FAI	Free androgen index
FFQ	Food frequency questionnaire
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
HOMA-B	Homeostatic model assessment of pancreatic beta cell function
HOMA-IR	Homeostatic model assessment of insulin resistance
HRQoL	Health-related quality of life
LH	Luteinizing hormone
MDRS-S	Montgomery Åsberg Depression Rating Scale self-rating
mFG-score	modified Ferriman-Gallwey score
MetS	Metabolic syndrome
NIH	National Institutes of Health
PCOM	Polycystic ovarian morphology
PCOS	Polycystic ovary syndrome
QWEPR	Questionnaire of eating and weight patterns revised
SF-36	Short-form 36
SHBG	Sexual hormone binding globulin
TFEQ-R21	Three factor eating questionnaire Revised 21
VLED	Very low energy diet
WHO	World health organization
WHR	Waist-hip ratio

Introduction

Polycystic ovary syndrome (PCOS) is a common disorder affecting approximately one out of eight women. (1, 2). Prevalence varies depending on the diagnostic criteria used (3-5). PCOS was originally described as a reproductive disorder and is indeed associated with menstrual irregularity, reduced fertility, and pregnancy complications (6). However, the syndrome is now more recognized as a multiorgan condition closely associated with obesity, with increased risk of metabolic features with increased prevalence of insulin resistance, hyperinsulinemia, type 2-diabetes, hypertension, and dyslipidemia (7, 8), as well as psychological features including anxiety, depression (9, 10) and disordered eating (11), all linked to lower health related quality of life (HRQoL) (12). Despite this recognition, PCOS remains poorly understood with challenges in diagnosis and treatment. For diagnosis of PCOS in adults, evidence-based guidelines for the assessment and management of PCOS, recommends two out of three clinical features of ovulatory dysfunction, clinical and/or biochemical hyperandrogenism and polycystic ovaries on ultrasound (which can be replaced by elevated levels of anti-müllerian hormone (AMH) (2). Whilst considering the increasing rates of obesity globally, studies on PCOS in women with severe and morbid obesity are lacking.

Obesity

Obesity is a major global health concern, leading to morbidities like type 2-diabetes, cardiovascular disease, and cancer and significantly decreases quality of life and life expectancy (13). Obesity has been defined as excessive fat accumulation that can impair health (14), and is classified as a disease by the World Health Organisation (WHO). The heritability rate of obesity is suggested to be 40-70%, which explains the variation of bodyweight in response to the obesogenic environment (15). Definitions of weight classes are seen in **Table 1**, where severe or morbid obesity are associated with higher mortality compared to groups with lower body mass index (BMI) (16). The prevalence of obesity has more than tripled over the last half decade and continues to rise, with a global prevalence of obesity of 13% in 2016 (17). In Sweden, the prevalence of obesity is 16%, with a higher prevalence of severe or morbid obesity among women than men (18).

Table 1.WHO classification of weight by BMI

Weight class	**BMI (kg/m^2)**
Underweight	<18.5
Normal weight	18.5 - 24.9
Overweight	25.0 - 29.9
Obesity class I/obesity	30.0 - 34.9
Obesity class II/severe obesity	35.0 - 39.9
Obesity class III/morbid obesity	≥40.0

WHO; World Health Organization; BMI, Body Mass Index

The cause of obesity is a long-term energy imbalance between intake and expenditure of calories (13). Multiple factors contribute to the development of obesity, including an environment with excess food, psychological and sociocultural factors leading to overeating, biological factors leading to low energy expenditure, and physical inactivity due to sociocultural factors among many others (13). Obesity is associated with a dysregulation of satiety and hunger signals in the hypothalamus affected by hormones from the gut and adipose tissue (13). Adipose tissue is considered a highly active endocrine organ (19). It consists mainly of adipocytes which secrete adipokines, like adiponectin and leptin, and inflammatory cytokines, which can induce insulin resistance, effect the metabolism of glucose and lipids and activate inflammatory pathways (14, 20). It is well known that obesity is closely associated with low-grade inflammation and the degree of inflammation seem to correlate with the severity of type 2-diabetes and insulin resistance (21).

Besides the co-morbidities mentioned, obesity affects fertility with impaired ovulation and implantation (22), and increases the risk for many pregnancy complications such as gestational diabetes, gestational hypertension and preeclampsia (23). It also increases the risk of depression, anxiety and other psychiatric disorders (24). The risk of depression is suggested to be directly proportionally to BMI (25), and increased in those with severe obesity (26). Obesity is also associated with disordered eating and eating disorders (27), with a higher prevalence of binge eating disorder in those with obesity,

compared to those without (28). Treatment for obesity is a great challenge in the modern world and should include both individual intervention and changes in the environment and society.

Etiology of PCOS

The origin of PCOS is unknown, but considered to be multifactorial, with genetic, environmental, and intrauterine factors contributing to its development (29). PCOS is a highly heritable condition, with a heredity around 70% (30), and daughters of mothers with PCOS have more than five times higher risk to develop PCOS (31). There are around 20 identified genetic risk loci for PCOS, however, these genes account for only 10% of the heritability (32). There is also emerging evidence that environmental factors could contribute to the pathogenesis of PCOS. Such factors could be maternal hyperandrogenism with or without obesity creating an adverse intrauterine environment resulting in epigenetic changes, either alone or in combination with genetics (33). In animal models of PCOS, elevated levels of intrauterine androgen and/or anti-müllerian hormone (AMH) affect the fetus and result in a PCOS-like offspring that is transmitted across generations (31, 34). However, to date, human studies have only focused on first generations (31). Several studies show that PCOS in mothers lead to metabolic dysfunction (35), neuropsychiatric disorders (36), and anxiety in the offspring (37). There is also evidence of shared genetic factors between PCOS and type 2-diabetes (38) and epigenetic changes in adipose tissue from women with PCOS (39). Recent years, mendelian randomization from genome wide association studies (GWAS) suggest that obesity can cause PCOS, but not the other way around (40-42).

Pathophysiology of PCOS

The pathophysiology of PCOS is considered complex and not yet fully understood. The main feature of the syndrome is elevated levels of circulating androgens (43). PCOS is also associated with defects in insulin secretion and action, which leads to insulin resistance in peripheral organs and tissues, and hyperinsulinemia (44, 45). Main principles of the suggested pathophysiology are shown in **Figure 1**.

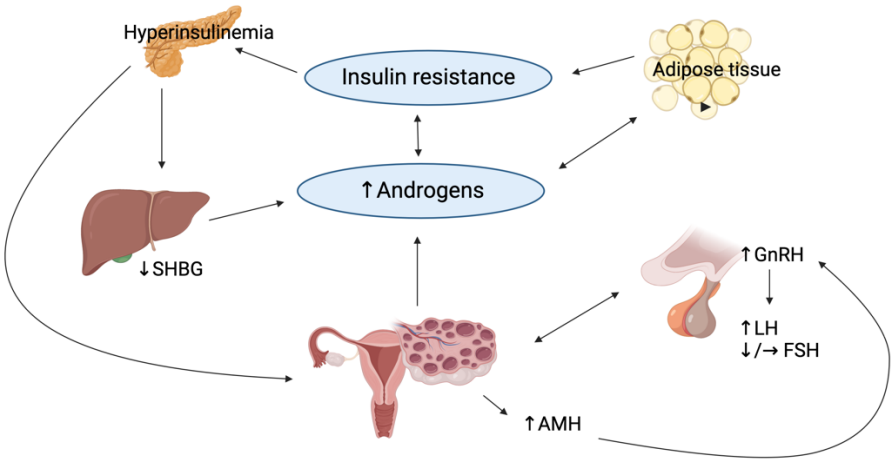

Figure 1. Suggested pathophysiology of PCOS. In PCOS, a disturbance in the hypothalamic-pituitary-gonadal axis with increased LH-pulses has been shown. This stimulates the ovarian theca-cell to produce and secrete excessive amounts of androgens leading to arrested follicular development and accumulation of small antral follicles. Insulin resistance, deriving from altered adipose tissue and elevated androgen levels leads to hyperinsulinemia which further stimulates the theca cell androgen production and lowers hepatic production of SHBG, which increases the amount of the non-bound androgens in the circulation. PCOS, polycystic ovary syndrome; AMH, anti-müllerian hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG sexual hormone binding globuline. Figure created in Biorender.

In PCOS, alterations in the hypothalamic-pituitary-gonadal axis, with a disturbed gonadotrophin releasing hormone (GnRH)-pulsatility, results in elevated luteinizing hormone (LH) pulse frequency. This drives the ovarian theca cells to increased androgen production, which together with relatively low levels of FSH arrests follicle development and prevents the development of a leading follicle (46, 47). Women with PCOS have higher levels of hypothalamic kisspeptin (48), which together with neurokinin B are key regulators of GnRH pulse frequency (49). This support the hyphothesis that an overactive hyptothalamic–pituary–gonadal (HPG)-axis can have a role in the pathogenesis of PCOS (50)

Defects in insulin secretion and action and insulin resistance could possibly derive from an altered adipose tissue morphology and function in PCOS, with enlarged adipocytes and decreased production of adiponectin associated with insulin resistance (51). In a PCOS-like mouse model, higher adiponectin has

been associated with lower insulin resistance and improved metabolic health (52). Insulin resistance causes elevated levels of insulin in the circulation, which enhances the effect on LH in stimulating ovarian theca cells to produce androgens (53). Insulin also decreases hepatic production of sex hormone binding globulin (SHBG) (54), which further increases the free circulating levels of androgens. Androgens can also contribute to insulin resistance in skeletal muscle and adipose tissue, and increase visceral obesity in women with PCOS (55), and antiandrogen therapy is shown to reverese insulin resistance in women with PCOS (56). There is also evidence of activation of androgens in adipose tissue (57) and higher androgen concentrations in adipose tissue in women with PCOS compared to controls (58).

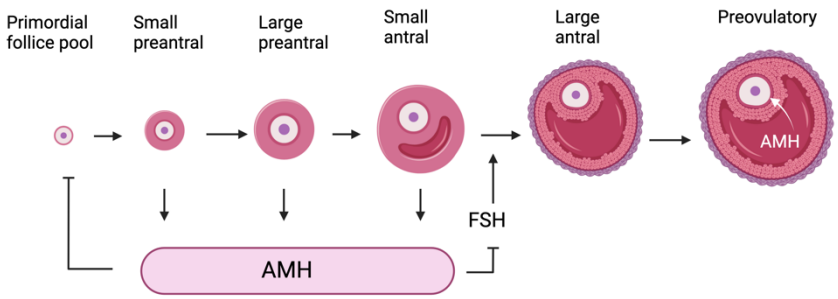

Figure 2. AMH and follicle development modified from Dewailly et al. (59). AMH is produced in the granulosa cells of small growing follicles, particularly in preantral and small antral follicles. It is suggested to inhibit initial follicle recruitment and FSH-dependent growth of small antral follicles and selection of a leading follicle, which lead to the polycystic ovarian morphology. AMH, anti- müllerian hormone; FSH, follicle-stimulating hormone. Figure created in Biorender.

Ovaries in women with PCOS are characterized with an increased ovarian reserve with a high number of arrested antral follicles (60), with the theca cell layer of these follicles producing androgens (61). Anti–müllerian hormone (AMH) is produced by granulosa cells in these antral follicles/small growing follicles (62), and circulating levels are elevated in women with PCOS (63, 64). AMH inhibits the initial follicle recruitment and the FSH-dependent growth and selection of a leading follicle. AMH also inhibits the gene expression of CYP19A1, which codes for aromatase, an enzyme transforming androgens to estrogens (65). This leads to a stop in the conversion from androgens to estrogens, contributing further to elevated androgen levels. *In vivo* and *in vitro* studies also show that GnRH-neurons express the AMH-

receptor and that AMH stimulates the secretion of LH (66), further stimulating ovarian hyperandrogenism. All these aberrations cause a vicious cycle in women with PCOS.

Hyperandrogenism

Mild and moderate hyperandrogenism is one of the main features of PCOS and prevalent in women with PCOS during fertile ages, affecting 60-100% with a significant impact on quality of life in women with PCOS (2). It can be identified either clinically or biochemically. Clinical manifestations of mild or moderate hyperandrogenism can be hirsutism -male pattern hair growth, female pattern hair loss and acne, Hirsutism is the most common clinical and best predicitive sign of hyperandrogenism and affects around 65-75% of those with PCOS (67, 68). It is most commonly assessed using the modified Ferriman-Gallwey (mFG) score. Regarding female pattern hair loss and acne, the predicitive values of these as markers for hyperandrogenism are unclear (1).

Biochemical hyperandrogenism is defined as elevated levels of androgens in the circulation, and is found in 80% of women with PCOS (69). Measurement of androgen levels is a challenge due to its variation with methods, ethnicity, life stage and confounding factors such as body weight (2). Testosterone in the circulation is mostly bound to albumin and SHBG, with a small fraction unbound. The unbound, or free testosterone reflects the clinical status of hyperandrogenism better than total testosterone. Free testosteone can be estimated either with the calculation with a specific formula, taking measurement of SHBG into account and assuming a fixed albumin concentration (70), or calculation of free androgen index (FAI), which is the ratio of total testosterone divided by SHBG x 100. Other weaker androgens such as androstenedione and dehydroepiandrosterone sulfate (DHEAS) secreted from the adrenals have a poor specificity for hyperandrogenism, and should not be used to diagnose biochemical hyperandrogenism, unless testosterone or free testosterone measures are not available (2). Up to late postmenopausal age, both clinical and biochemical hyperandrogenism remain more prevalent in women with PCOS compared to controls (71, 72).

AMH

AMH is a glycoprotein produced in the granulosa-cells of small growing follicles and is suggested to have an important role in both the development and pathogenesis of PCOS (73). It is well correlated with antral follicle count (74) and elevated in women with PCOS (63, 64, 75). AMH is also positively correlated to a more severe phenotype of PCOS, and negatively correlated to BMI (76, 77). Studies on weight loss in women with PCOS, show both reduced levels of AMH in those with overweight or obesity (78-80), and improved reproductive function with no changes in AMH (81, 82). Studies has shown that AMH can be used for diagnosing PCOS (83) especially in those with hyperandrogenic PCOS (77), and in adolescents (84), but to date there is not enough evidence to support the use of AHM as a single diagnostic test for PCOS (1). However, the recently updated guidelines for PCOS now support the use of AMH as a marker for polycystic ovarian morphology (PCOM) instead of ultrasound in the diagnostic algorithm for PCOS (2).

PCOS and obesity

PCOS is strongly associated with excess weight and abdominal obesity (85) and with increased weight gain through life, especially in early adulthood (86, 87). Excess weight is one of the greatest concerns in those with PCOS, and a highly prioritized research area for patients (2). It has been shown that women with PCOS report more weight loss attempts (88), and are more likely to follow weight loss diets compared to those without PCOS (89).

The prevalence of overweight and obesity in women with PCOS has increased from 51% to 74% since the 1990s according to American studies (85, 90). Studies in the Nordic countries are scares, but one Nordic study have found the prevalence of overweight and obesity in women with PCOS to be 34% (86). There are few studies of women with PCOS in peri- and postmenopausal ages, with only one prospective study following women with PCOS and controls up to mean age of 70 years, showing no increase in weight over 21 years in women with PCOS, (91).

The relationship between PCOS and obesity, and the exact mechanisms that lead to the excess body weight and central body fat distribution in those with PCOS is still not clear. If a woman with PCOS is predisposed to obesity or if obesity unmask latent PCOS is yet to find out. What is clear though, is that obesity and central adiposity increases insulin resistance and worsen all

symptoms of PCOS (92, 93). The mechanisms leading to the excess weight and increased BMI in PCOS could be both extrinsic, such as alterations in diet and physical activity, or intrinsic, with alterations in appetite regulation and metabolic rate and/or hormonal abnormalities such as insulin resistance and hyperandrogenism. Regarding appetite regulation and basal metabolic rate, the few studies that exist show no differences in these intrinsic factors between women with and without PCOS (1).

Diagnostic criteria

How to define a disease is a common issue in the medical field. PCOS is considered a syndrome, and by the nature of a syndrome it is a heterogenous condition giving rise to a spectrum of subgroups/phenotypes. Throughout the years different diagnostic criteria for PCOS has developed and been under debate. PCOS was first introduced by Stein and Leventhal who were the first to describe the triad of polycystic ovaries, hirsutism and oligo-amenorrhea, and the condition was then known under the name Stein-Leventahl syndrome (94).

National Institutes of Health criteria

At the National Institutes of child health and human development conference on PCOS in 1989, there was an expert agreement for diagnosis of PCOS which resulted in the National Institutes of Health (NIH)-criteria, requiring both hyperandrogenism and oligo-/anovulation with the exclusion of other disorders that can mimic PCOS, such as hypothyreosis, Cushing's syndrome, hyperprolactinemia, and congenital adrenal hyperplasia (95).

The Rotterdam criteria

In 2003 there was a workshop held regarding the diagnostic criteria of PCOS with aim to gain consensus. The workshop resulted in the Rotterdam criteria (96), which required two out of three of the following clinical features: 1) oligo-/anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, 3) polycystic ovaries, defined as ≥12 follicles of 2-9 mm/ovary, or ovarian volume of >10 ml measured with ultrasound, with the exclusion of other endocrine disorders.

Androgen Excess and PCOS-society criteria

The Androgen excess and PCOS-society, including experts in PCOS, held a conference in 2006 and stated that PCOS should be a condition requiring hyperandrogenism. They constructed the Androgen excess and PCOS-society –(AE-PCOS)-criteria (43), which required hyperandrogenism in combination with either oligo-/anovulation or polycystic ovaries.

Evidence based criteria for diagnosis of PCOS

In 2018, the first international evidence-based guidelines for the assessment and management of polycystic ovary syndrome were published (97), and included a revised definition of the Rotterdam criteria for the diagnosis of PCOS. The guidelines endorsed the Rotterdam PCOS diagnostic criteria in adults and revised the ultrasound criterion for polycystic ovaries/polycystic ovarian morphology (PCOM). According to advancing technology with ultrasound bandwidth 8 MHz, ≥20 follicles of 2-9 mm or ovarian volume of ≥10 ml in one ovary was required to fulfil the ultrasound criterion of PCOM. Further, regarding diagnosis in adolescents, guidelines stated that within eight years after menarche, both hyperandrogenism and ovulatory dysfunction was required, and ultrasound not recommended (97).

In 2023, the evidence-based guidelines were revised and based on further evidence, the definition of PCOS was updated and the diagnostic tools were refined focusing on clinical features in diagnosis. Guidelines highlight the use of an algorithm to diagnose PCOS (**Figure 3**), and if irregular menstrual cycles and hyperandrogenism are present, the diagnostic procedure do not require ultrasound. Updated diagnostic criteria also include the possibility to use AMH instead of PCOM in adults (1). Further, guidelines recommend not to use ultrasound or AMH within 8 years from menarche, but instead to identify and re-evaluate those individuals at risk. A recent publication has summarized and adapted the guidelines for use in the Nordic countries (98).

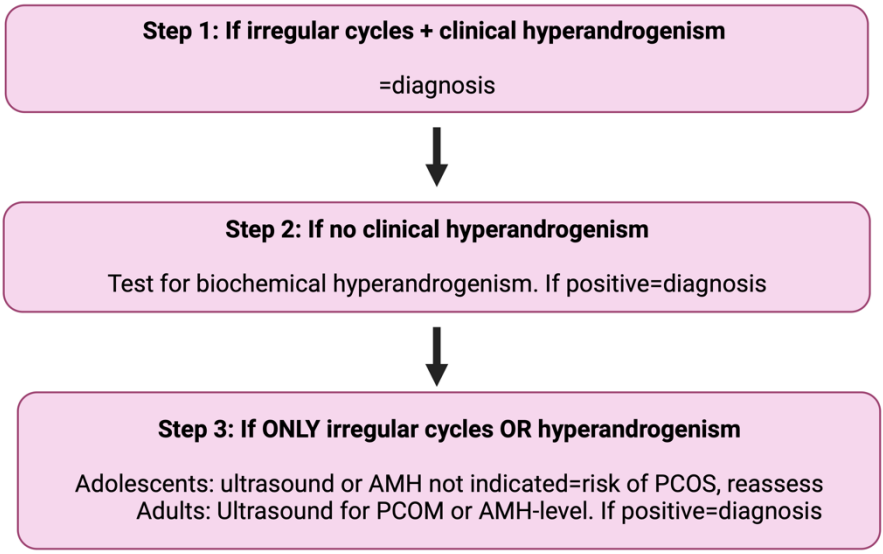

Figure 3. Algorithm for the diagnosis of PCOS according to evidence-based criteria. All steps include the exclusion of other possible causes for PCOS.

In adults, the diagnosis of PCOS require two out of three clinical features:

- Ovulatory dysfunction
- Clinical and/or biochemical hyperandrogenism
- Polycystic ovaries on ultrasound or elevated levels of AMH

With the exclusion of other causes following the algorithm in **figure 3.** The first two features are required in adolescsents, and if only one feature is present, a new evaluation should be performed eight years after menarche (2).

The following definitions are recommended according to the international guidelines for PCOS (2):

Clinical hyperandrogenism: History and physical examination regarding symptoms of terminal hair. The Ferriman- Gallwey (FG)-score is the principal instrument for assessing hirsutism. It is a visual scale, where participants grade/ evaluate terminal hair growth in eight parts of the body (upper lip, chin, chest, lower abdomen, arms, thighs, back and lower back), with a score range of 0-4 in each area, rendering a total score of 0-36, with a higher score indicating more hirustism (99). A total score of >4-6 is used for diagnosis of hirsutism.

Biochemical hyperandrogenism: To assess hyperandrogenism, total or free testosterone should be measured, and the recommended method is the liquid chromatography–tandem mass spectrometry (LC-MS/MS)(2). Free testosterone can be assessed either by free androgen index (FAI), which is calculated by testosterone divided by SHBG x 100, or by calculation using one of the formulas available (70), or by equilibrium dialysis or ammonium sulfate precipitation.

Ovulatory dysfunction: Irregular menstrual cycles, defined as <21 or >35 days or <8 cycles per year, more than three years after menarche. In adolescents, >1 to <3 years after menarche irregular cycles is defined as cycle length <21 days or >45 days.

Polycystic ovarian morphology: PCOM or polycystic ovaries should be defined as ≥20 follicles of 2-9 mm per ovary, or ≥10 follicles per cross-section, or ovarian volume <10 ml/cm^3, using transvaginal ultrasound with a bandwidth of 8 MHz. This criterion is fulfilled if one of the ovaries is showing this typical morphology.

Antimüllerian hormone: AMH is now considered an appropriate substitute for PCOM in the diagnosis of PCOS. Laboratories should use population- and assay specific cut-off levels, since AMH decrease with age, increasing BMI and with the use of combined oral contraceptive pills (1, 2).

Phenotypes of PCOS

When using the evidence-based criteria or modified Rotterdam criteria, four phenotypes are possible for PCOS. In the literature they are often referred to as hyperandrogenic-NIH or classic PCOS (A and B), ovulatory PCOS (C) and non-hyperandrogenic PCOS (D) (100). Phenotypes A and B account for approximately two thirds in a referral population and are the most metabolically affected phenotypes, associated with hyperinsulinemia, insulin resistance, dyslipidemia and obesity, while phenotype C is also associated with the above symptoms but to a less extent, and phenotype D have a low association with metabolic disturbances, but the reproductive dysfunction distinct this phenotype from women without PCOS (100-103). Genetic studies point to distinct genetic architecture underlying the different subtypes of PCOS (104).

Table 2. Possible phenotypes of PCOS**:** A-D, and the phenotypes categorized by different diagnostic criteria.

	A	B	C	D
Hyperandrogenism	√	√	√	
Ovulatory dysfunction	√	√		√
Polycystic ovarian morphology	√		√	√
NIH-criteria	√	√		
AE-PCOS-criteria	√	√	√	
Rotterdam-criteria	√	√	√	√

NIH, national institutes of health; AE-PCOS, androgen excess and PCOS society

Reproductive consequences of PCOS

PCOS is the most common reason for anovulatory infertility (105), and women with PCOS have a higher risk of gestational hypertension, gestational diabetes,, preeclampsia and preterm delivery, even after adjustment for BMI (1, 106). However, there is a good prognosis for live birth and women with PCOS are as likely to have their wanted number of children as women without PCOS (107, 108), but at an older age (107). There are studies indicating that women with PCOS have compromised endometrial function, which may predispose for implantation failure, miscarriage, and pregnancy complications, possibly due to altered trophoblastic invasion and defect placentation (2, 109-111).

Metabolic consequences of PCOS

Insulin resistance is a characteristic of PCOS, independent of BMI (92). Insulin resistance is the inability of cells to react on insulin, which impairs the uptake of glucose and leads to higher levels of glucose and insulin in the blood. Insulin resistance precedes type 2-diabetes and is closely associated with obesity, abdominal adiposity and is a risk factor for cardiovascular disease (112). Women with PCOS of fertile age have a higher incidence of type 2-diabetes,

and often receive the diagnose at a younger age compared to women without PCOS (113, 114). Type 2-diabetes in PCOS is independent of BMI but increases with obesity (92, 115) and hyperandrogenism (116). In peri- and postmenopausal ages, studies show a higher prevalence of type 2-diabetes, independent of BMI at perimenopause (117, 118), (119) but no difference in type 2-diabetes in women with normal weight with PCOS in post-menopause (91) and in late menopause (120). Compared to women without PCOS, those with PCOS have a higher risk of hypertension independent of overweight and obesity (121), an altered blood lipid profile with higher triglycerides and higher low -density lipoprotein (LDL)-cholesterol, and lower high-density lipoprotein (HDL)-cholesterol (122, 123). With increasing BMI, levels of HDL-C decrease, and LDL-C increase (124). In women with PCOS, the increased risk of hypertension remains in ages around the menopause (119, 125) and until late postmenopausal ages, where also hypertriglyceridemia remains higher in women with PCOS compared to controls (91, 126).

Metabolic syndrome (MetS) is a cluster of risk factors (hypertension, abdominal obesity, elevated glucose levels, low HDL-C and high LDL-C) predicting type 2-diabetes and cardiovascular events (127). Women with PCOS have a two-fold increased risk of MetS (128-130) even after adjusting for BMI *(*129*)*. A Swedish study showed a prevalence of MetS of 23.8% in women with PCOS and overweight, compared to controls where the prevalence was 8% (130). American studies have shown a prevalence of MetS in PCOS and BMI >30 to be 56-60% (131) (128).

The prevalence of risk factors for cardiovascular disease (insulin resistance, hypertension, obesity) is higher in those with PCOS compared to controls (73, 132), especially in those with PCOS with hyperandrogenism (116, 133). However, there has been a lack of evidence to evaluate the risk of cardiovascular events and mortality in women with PCOS (134). Previous studies have found no elevated risk of cardiovascular events or mortality from these events in women with PCOS in postmenopausal (135) or late postmenopausal age (91). However, recent studies show not only elevated risk factors for cardiovascular disease, but a higher incidence of major cardiovascular events in women with PCOS (136-138), independent of diagnostic criteria and BMI (137, 138). There is yet no evidence of increased mortality from cardiovascular disease in women with PCOS (138).

Psychological consequences of PCOS

Anxiety and depression

The prevalence of anxiety and depression is approximately 80% in women with PCOS, and a higher risk of both depression (OR 2.59 [2.11-3.16]) and anxiety (OR 2.68 [2.08-3.44]) is shown in women with PCOS compared with their non-PCOS counterparts (1, 2). This higher risk remains even after adjusting for BMI (139), and is consistent between phenotypes (140). Women with PCOS also have a higher risk of other psychiatric disorders and there have been suggestions of a shared genetic pathogenesis between PCOS and psychiatric disorders (10). However, recent genetic studies show no shared genetics (141). Symptoms of anxiety and depression in women with PCOS increase with BMI and insulin resistance (142, 143).

The underlying mechanisms of anxiety and depression in PCOS are not clear and cannot be explained only by the clinical features of PCOS (9). In the general population, hyperinsulinemia/insulin resistance has been suggested to have a role in pathogenesis of depression (144), and there are associations between the metabolic syndrome and symptoms of anxiety and depression (145). In women with PCOS, both insulin resistance and hyperandrogenism have been associated with symptoms of anxiety and depression (146, 147), also in adolescents (148). According to long-term follow up studies, symptoms of anxiety and depression seem to remain into peri- and postmenopausal ages in women with PCOS (139, 149).

These afore mentioned psychiatric comorbidities have a great impact on quality of life, in women with PCOS, and can also affect the engagement in lifestyle interventions. Evidence-based guidelines recommend screening women with PCOS for depression and anxiety with validated screening tools (2).

Health related quality of life

Health -Related Quality of Life (HRQoL) is a way of examining the impact of a disease or disorder on quality of life. Women with PCOS have lower HRQoL and decreased life satisfaction compared with women without PCOS (1, 12, 150). Weight concerns and hirsutism as typical symptoms of PCOS, are associated with lower HRQoL (151, 152). In one study assessing HRQoL in women with severe obesity, there were no differences between those with and

without PCOS (Wang 2021). The increased body image distress that has been found in women with PCOS, contributes further to symptoms of anxiety and depression (153), which in turn decrease quality of life and may negatively influence self-management and engagement in lifestyle-interventions.

Dietary intake and physical activity in PCOS

There is no current evidence that lifestyle behavior, such as energy intake, macronutrient intake and physical activity, differs between women with, and without PCOS (1). Studies conducted include women from premenopausal to perimenopausal ages in a BMI-range from normal weight to obesity class I, (154-158), with only one study on women with PCOS with severe obesity (159).

Eating behavior and eating disorders in PCOS

Eating behavior is an important factor in weight management. Women with PCOS have several risk factors for eating disorders, such as excess weight, anxiety and depression (9), poor self-esteem and negative body image (160). They are also more likely to have disordered eating with an increased risk of any eating disorder (OR 1.53[1.29-1.83]) (1). A recent review highlights the importance of assessing eating disorders and behavior in treatment of PCOS (161).

Disordered eating can be of behavioral, cognitive, and emotional character. Three domains of eating behavior that has been studied for long time are uncontrolled eating, cognitive restraint- and emotional eating (162, 163). Emotional eating measure eating as a response to emotions, cognitive restraint eating measures the ability to consciously restrict food intake in attempt to lose weight or maintain weight, and uncontrolled eating measure the loss of control in eating (163). Regarding eating behavior, studies have shown that in women with and withoutPCOS, eating behavior do not differ between groups in women with normal weight (164) or in those with severe obesity (159). However, women with PCOS show greater concerns about weight and diet (165), and have an increased risk of abnormal eating behavior scores compared to controls, which is independent of obesity, and increasing with symptoms of anxiety (166).

Binge eating disorder (BED) is a psychiatric disorder characterized with frequent intake of unusually large amount of food and the inability to stop eating. It encompasses individuals with severe stress and dysfunction due to binge eating and is more common in those with obesity (167), and associated with anxiety and depression (168). A recent systematic review informing guidelines for PCOS show elevated risk of BED in women with PCOS (OR 2.0 [1.18-3.72]) (1). Studies investigating eating behavior in women of peri-/postmenopausal age are lacking.

Management of PCOS

There is a limited understanding of the causes of PCOS, and therefore available treatment is tailored to manage specific symptoms. Since obesity is prevalent and worsens all symptoms, weight management and treatment of obesity should be prioritized in women with PCOS to improve metabolic health, including central adiposity and dyslipidemia (2).

Lifestyle intervention

A healthy lifestyle and weight management, i.e. the prevention of weight gain, weight loss or maintaining weight after weight loss is considered important throughout the lifespan for women with PCOS, and have been shown to improve metabolic, reproductive and psychological symptoms in women with PCOS (1, 169). Lifestyle intervention can include improvement in dietary quality and quantity, diet energy restriction when needed, increased physical activity, and can also include behavioral intervention. There is no evidence that any specific diet composition or physical exercise is superior in women with PCOS (1), and therefore, recommendations of diet and physical activity for those with PCOS should follow recommendations for the general population. A healthy diet för the general population include a high intake of vegetables, fruits and whole grains, seafood, low-fat dairy, legumes and nuts and seeds and vegetable oils and margarines and low in red and processed meats, sugar-sweetened beverages, sugary foods and refined grains (170). The recommendations for regular physical activity for the general population in Sweden include 150-300 min/week of medium active activity which heighten the pulse, muscle strengthening activity two times per week, and reduction of sedentary activity (171).

Regarding the effect of weight loss in women with PCOS, there are a few studies, all hampered with a small number of patients, indicating that a weight

loss of 5-10% leads to more regular cycles and restores ovulation (172-175), improves insulin sensitivity with lower insulin and lower HOMA-IR (176-179) and improves dyslipidemia (177). Weight loss in women with PCOS and obesity has also shown better HRQoL and less symptoms of depression in women with PCOS (180-182). However, studies on weight loss in women with PCOS include women with overweight and obesity class 1, thus BMI 25 to <35 kg/m^2, with only a few studies including a very low number of patients reporting of women with severe obesity (175, 176, 179). Further, studies are focused on premenopausal women with PCOS, and studies on weight loss in women with severe obesity (BMI ≥35 kg/m^2) and in the peri- and postmenopausal years are lacking. A recent weight loss intervention study examining the effect of recommendations on healthy diet and exercise on women with severe obesity with and without PCOS planned for in-vitro fertilization (IVF) (183), showed statistically significant weight loss, but no improvement in cardiometabolic variables . However, clinical weight loss was modest.

Women with PCOS report barriers to lose or maintain weight (184). However, the origin of these barriers are not clear. Women with PCOS have a similar resting energy expenditure (165), but there is limited knowledge about gut hormones and other intrinsic factors that can affect the ability to lose weight (1). The capability to lose weight but can also be affected by psychiatric symptoms and weight stigma, which are important features of PCOS (150). Compared to controls, women with PCOS report a negative body image (160), body image distress (153), more weight loss attempts, and a perception of having overweight (88). Despite the reported barriers of weight loss in women with PCOS, studies comparing weight loss in women with and without PCOS are limited, and have shown no differences in the ability to lose weight between women with and without PCOS (183, 185).

Pharmacological treatment for weight loss and metabolic symptoms

Metformin is the recommended insulin sensitising treatment in women with PCOS and is suggested to be used as an addition to lifestyle changes in women with PCOS (186). It has been shown to improve anthropometric and metabolic features such as excessive weight, insulin resistance, glucose and lipid profile in women with PCOS, where the best evidence is found in women with overweight or obesity (187).

Several pharmacological agents can be used for weight management. There is Orlistat, an agent that blocks the pancreatic lipase, an enzyme digesting fat, and prevents the uptake of fat in the gut (188). Other agents that can be used for treatment of obesity are the glucagon-like-peptide (GLP)-1 receptor agonists (RA), also known as GLP-1 analogues (189). GLP-1 analogues increase insulin secretion after a meal and control glucose levels in the blood, but also have important central effects such as reduced hunger and increased satiety (190). GLP-1 analogues such as semaglutide and liraglutide are increasing in use for treatment of type 2-diabetes and obesity (189, 191, 192). A recent study on women with PCOS who were resistant to lifestyle treatment, showed that six months treatment with semaglutide reduced mean weight with 11.5 kg, and reduced insulin and HOMA-IR in 80% of participants, with better results for those with obesity class I (BMI 30-34.9 kg/m^2) compared to those with severe obesity (BMI >35 kg/m^2) (193). However, existing evidence is not sufficient to form recommendations of the use of these agents in women with PCOS, and therefore, recommendations for women with PCOS should follow those for the general population with obesity (194).

Surgical treatment for weight loss

Bariatric surgery is an effective treatment for those with obesity leading to large weight loss and remission of many obesity-related co-morbidities (195). Weigh loss after bariatric surgery has also been shown to be sustainable over time (196). However, weight regain after bariatric surgery is seen in one out of six patients and has been associated with preoperative risk factors like depressive symptoms, lower self-esteem, an eating pattern with uncontrolled and emotional eating (197). In PCOS, weight loss is important to improve metabolic features, but also for fertility reasons (198), and for reducing pregnancy complications due to excess BMI (199). Bariatric surgery is however associated with nutritional defects that can affect the fetus, preterm birth, children being born small for gestational age and possibly higher perinatal mortality (200, 201). This is important to bear in mind when advising women of fertile age with PCOS regarding obesity management. Studies on bariatric surgery in those with PCOS show large weight loss and improvement in metabolic and hormonal variables (202-204) as well as in eating behavior (205). Bhandari et. al. showed improvements in menstrual cycles and hirsutism and sustainable weight loss in women with PCOS even five years after surgery (Bhandari et al., 2022). There are currently no randomized controlled trial investigating the efficacy of bariatric surgery on women with PCOS and

therefore there are no specific recommendations for bariatric surgery in women with PCOS besides those for the general population.

Treatment for symptoms of hyperandrogenemia

Treatment for symptoms of hyperandrogenism (hirsutism, acne, alopecia) is prioritized by those with PCOS, and combined oral contraceptive pills (COCP) or cosmetic treatment are the first choices. COCP increases SHBG and reduces androgen levels and are recommended for treating irregular menstrual bleedings and/or hirsutism) (1). No specific type of COCP has proven to be superior for use in women with PCOS (206). However, COCP containing the antiandrogenic cyproterone-acetate in combination with ethinyl estradiol have been shown to have marginal benefits, but a higher risk of venous thrombosis as a side effect (206). Therefore, recommendations of type of COCP in treating women with PCOS, should be the same as for women in the general population. Mechanical laser or light treatment for hirsutism can be used for removal of facial hair. Laser is better for treating hirsutism, however women with PCOS might need more treatments than those with idiopathic hirsutism (1). If the above treatments are not sufficient for symptom relief, treatment that reduces androgens can be used. Least side-effects are seen with spironolactone, an aldosterone receptor antagonist. Antiandrogens like flutamide and finasteride can also be used, but are not recommended specifically for treating PCOS, since the evidence for these substances is limited, and treatment with these agents are shown to have more side-effects compared to other treatments, with liver toxicity being one (207).

Fertility treatment

There is a good prognosis for live birth in women with PCOS, but many women with PCOS will need treatment for anovulation (208). The first recommendations to improve fertility is to optimize reproductive health with education of a healthy lifestyle and to prevent excess weight. If women with PCOS have oligo-or anovulation and need infertility treatment, Letrozole is considered the first-line treatment after excluding other causes of infertility . If the response is poor, gonadotrophin therapy can be used as a second line treatment for anovulation. Assisted reproductive technology including in vitro fertilization (IVF) is a third line treatment when there is letrozole resistance, or if gonadotrophin stimulation produces many follicles, or if not pregnancy occurs within 6-9 cycles of letrozole stimulation with ovulation (1, 209).

Knowledge gap

PCOS is strongly associated with obesity, an issue with increasing prevalence across the world. Despite this, there are limited studies on PCOS in women with severe obesity. The few studies that exist include small sample sizes and are contradictory. One study reports the same prevalence of PCOS across BMI-categories (90) whilst another reports higher prevalence in women with severe or morbid obesity (210). When initiating this study, there were no studies on the effect of weight loss through a structured weight loss intervention comparing women with and without PCOS with severe obesity.

Aims

The overall aim of the thesis was to study the prevalence of PCOS in women with severe obesity and to study the effects of a structured weight loss intervention in women with and without PCOS.

The specific aims were:

Paper I

To estimate the prevalence of PCOS in a cohort of women with severe obesity, and to examine if metabolic syndrome was more common in women with PCOS in this cohort. Further to investigate the effects of a 12-month weight loss intervention on hormonal and metabolic variables.

Paper II

To study circulating levels of AMH in women with severe obesity with and without PCOS and determine if AMH can be used as a single surrogate marker for PCOS. Further, to study the effect of a 12-month weight loss intervention on AMH.

Paper III

To study symptoms of anxiety and depression and HRQoL in women with severe obesity with and without PCOS, and to compare these symptoms across BMI-groups. Further to study the effect of a 12-month weight loss intervention on HRQoL and symptoms of anxiety and depression in women with severe obesity.

Paper IV

To study eating behavior, energy intake and physical activity in women with severe obesity with and without PCOS, and the effect of a 12-month weight loss intervention on these variables.

Patients and Methods

Setting, study population and enrollment

Paper I-IV

In all papers the same cohort was studied. Participants were recruited between 2011-2016 from the Regional Obesity Center at Sahlgrenska University Hospital, where they had been referred to for weight loss treatment from primary care or specialist clinics. The criteria for assessment at the Regional obesity center were ≥18 years of age and BMI ≥35 kg/m^2. All patients referred to the Obesity Center were invited to group meetings where they received information about treatment for obesity and were invited to participate in the present study. Inclusion criteria was 18-50 years of age. Exclusion criteria was presence of other endocrine disorders, pregnancy, or breastfeeding during the last six months, climacteric symptoms, and inability to understand oral and written information. Those who accepted participation and met the inclusion criteria were screened for PCOS diagnosis and assessed with baseline measurements.

Data on women who were eligible for the study but were not included was not available. Therefore, we estimated this number based on the number of women referred to the obesity center during the recruitment period of five years. There were seven information meetings per year at the obesity center, with approximately 70 persons per meeting. Proportion of female were 60%, and of these 80% were between 18-50 years. These estimations resulted in 1175 women eligible for inclusion. In total 298 women consented to the study. Complete data was not available for 52 women, with the most common reason being lack of data to diagnose PCOS. These women were excluded from analyses. At 12-month follow-up, 72 women had adhered to the treatment. Drop-out occurred mostly in the period after the 12-week of very low energy diet (VLED). Major reasons for drop-out were that participants did not have time to continue treatment, family reasons, not being allowed to be away from work, disappointment of limited weight loss and that study staff missed to re-schedule participants when an appointment was missed. **Figure 4** provides an overview of the study timeline from recruitment to follow-up.

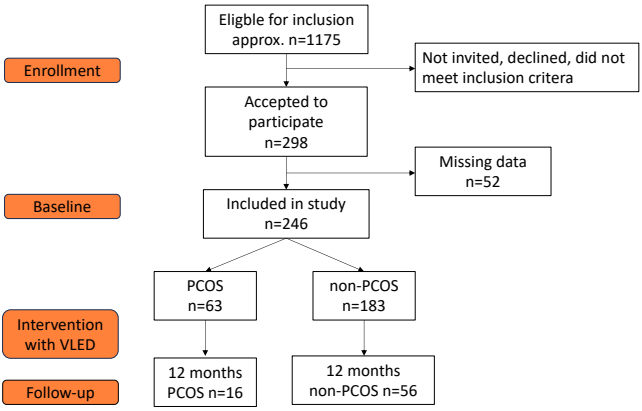

Figure 4. Flow chart of participants from inclusion to follow-up. VLED, very low energy diet; PCOS, polycystic ovary syndrome

Paper III

To compare symptoms of anxiety and depression and HRQoL in women with and without PCOS across weight categories, cohorts from earlier studies were included in paper III (39, 211, 212). Characteristics of the included studies are shown in **Table 2**. All studies recruited participants from the community and used the Rotterdam criteria for diagnosing PCOS. Age-span were 18–38 years.

Table 2. Characteristics of included studies in paper III

	Jedel et al. (211)	Johansson et al. (212)	Kokosar et al. (39)
Design	Case-control	RCT	Case-control
Year	2005 - 2008	2009 - 2010	2011 - 2013
N (PCOS/non-PCOS)	34 (20/14)	24 (24/0)	103 (72/31)
BMI PCOS, kg/m^2	31.3 ± 4.2	24.2 ± 3.5	28.2 ± 7.4
BMI non-PCOS, kg/m^2	30.6 ± 4.1	-	24.7 ± 5.0

RCT, randomized controlled study; PCOS, polycystic ovary syndrome.

Diagnosis of PCOS

PCOS was diagnosed using the NIH-criteria. The initial set-up included evaluation of PCOM with transvaginal ultrasound, however, this ultrasound criteria was later excluded due to difficulties in visualizing the ovaries and achieving adequate images due to obesity. When starting the present study, there were few prevalence studies on PCOS in women with severe obesity. To compare our findings with existing studies, we used cut-off values for hyperandrogenism from a Spanish study which showed a higher prevalence of PCOS in women with severe obesity compared to other weight categories, using the same criteria for diagnosing PCOS (210).

The following definitions and cut-off values were used:

- Clinical hyperandrogenism was defined as Ferriman-Gallwey (FG)-score ≥6.
- Biochemical hyperandrogenism was defined as free testosterone (fT) ≥0.035 nmol/L, or free androgen index (FAI) >5, or serum-testosterone >1.2 nmol/L.
- Oligo-/anovulation was considered if presence of amenorrhea or cycles <21 days or >35 days or <8 cycles/year according to self-reported data.

Diagnosis of Metabolic syndrome

A widely used assessment tool for metabolic syndrome is the National Cholesterol Education Program: Adult Treatment Panel III (NCEP:ATPIII) criteria (213), which require at least three out of five of the following criterion for women:

- Waist circumference >88 cm
- Triglycerides ≥150 mg/dL (>1.7 mmol/L)
- High density lipoprotein cholesterol (HDL-C) <50 mg/dL (<1.29 mmol/L)
- Blood pressure ≥130/≥85 mmHg
- Fasting and 2-h glucose from oral glucose tolerance test >110 mg/dL (>6.1 mmol/L)

Data collection

Data from the main study on women with severe obesity used in paper I-IV were collected at baseline and at 12-month follow up (**Figure 5**). Data from the three additional studies used in paper III was collected at baseline (height, weight, BMI, SF-36, CPRS-SA). All questionnaires used were validated, which means that they were developed to be used in the intended population, and measures what they intend to measure (214).

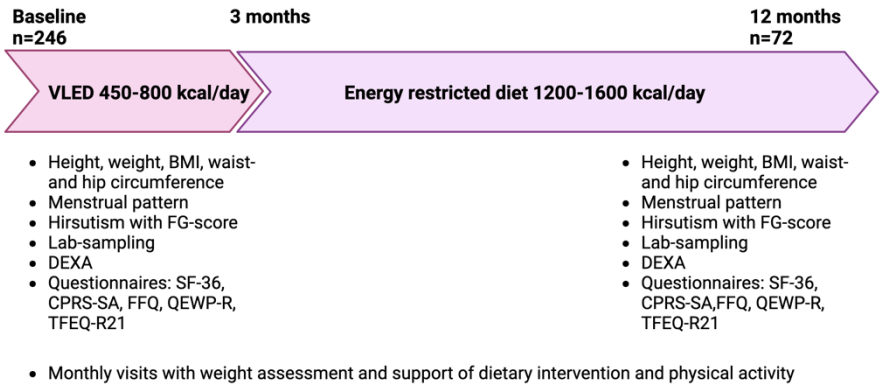

Figure 5. Data from the main study on women with severe obesity were collected at baseline and at 12-month follow-up. VLED, very low energy diet; BMI, body mass index; FG-score, Ferriman-Gallwey score; DEXA, dual-energy x-ray; SF-36, short form-36; CPRS-SA, comprehensive psychopathological rating scale for affective syndromes self-assessed; FFQ, food frequency questionnaire; QEWP-R, questionnaire of eating and weight patterns revised; TFEQ-R21, three factor eating questionnaire.

Anthropometry and blood pressure

Body weight was measured to the nearest 0.1 kg on calibrated scales with the participant dressed in indoor clothing with footwear removed. Height was measured in to the nearest 0.5 cm using a wall mounted stadiometer. Waist circumference was measured in a standing position midway between the most caudal part of the lateral costal arch and iliac crest after an exhalation. Hip circumference was measured at the symphysis-trochanter femoris level (215). BMI was calculated by dividing weight by squared height (kg/m^2), and waist-to-hip ratio (WHR) was calculated with waist circumference divided by hip circumference. Blood pressure was measured in the right arm, in the supine position after 15 minutes of rest with an electronic blood pressure device. The means from two separate measurements were used.

Body composition

Whole-body composition was measured by Dual Energy X-ray Absorptiometry (DEXA) which measured total fat, trunk fat mass, lean body mass and bone mineral content.

Biochemistry

All blood samples were analyzed at an ISO-accredited laboratory (15189:2012, ISO 22870:2016) at Sahlgrenska University Hospital, Gothenburg, Sweden. When starting this study, LC-MS/MS was difficult to access in clinical practice, and therefore the more common, electrochemiluminiscent immunoassay was used for analysis of steroids. The immunoassay tend to render higher values than mass spectrometry because of cross-reactions with steroid precursors (216), but is still comparable with the latter to distinguish women with PCOS from those without (217).

Table 3. Methods, detection limits, inter assay coefficient of variation (CV) for laboratory measurements.

Analyte	Method	Manufacturer	Detection limit	Inter assay CV
Testosterone	ECLIA	ACESS2, COBAS[A]	<0.4 nmol/L	[C]
SHBG	ECLIA, sandwich	ACESS2, COBAS[B]	<1 nmol/L	7%
Insulin	ECLIA, sandwich	Cobas Roche	1.0 mU/L	10%
Glucose	Fotometri, enzymatisk metod (Hexokinas/G-6-PDH)	Cobas Roche	0.2 mmol/L.	3%
s-Cholesterol	Fotometri, enzymatisk direktanalys	Cobas Roche	0.10 mmol/L	3%
s-Triglycerides	Fotometri, enzymatisk direktanalys	Cobas Roche	0.10 mmol/L	4%
s-HDL-C	Fotometri, enzymatisk direktanalys	Cobas Roche	0.08 mmol/L	5%
s-LDL-C	Fotometri, enzymatisk direktanalys	Cobas Roche	0.10 mmol/L	4%
s-TSH	ECLIA sandwich	Cobas Roche	0.014 mIU/L	7%
s-fT4	ECLIA kompetitiv	Cobas Roche	<5.5 nmol/L	10%

[A]ACCESS2 until May 2013, COBAS from June 2013

[B]ACCESS2 until March 2015, COBAS from April 2015

[C] Until 2014: at concentration = 5 nmol/L, 20 nmol/L and 40 nmol/L inter assay CV = 10%
From 2015: At concentration = 2 nmol/L inter assay CV=6%. At concentration = 3.5 nmol/L inter assay CV=5%

ECLIA, electrochemiluminiscent immunoassay with competitive analysis; CV, coefficient of variation; SHBG, sexual hormone bindning globuline; s, serum; s-HDL-C, serum high density lipoprotein cholesterol; s-LDL-C, serum low density lipoprotein cholesterol; s-TSH, serum thyroidea stimulating hormone; s-fT4, serum free thyroxin

Free testosterone was calculated using total testosterone and SHBG, assuming a fixed albumin concentration of 43 g/L using a matrix as described by Vermeulen et al. (70).

Anti-müllerian hormone (AMH) was analyzed at the institute of Physiology and Pharmacology at Karolinska Institute, Stockholm, Sweden. AMH was measured in serum using the Ultra-Sensitive AMH/MIS ELISA (AL-105, Ansh Labs, Texas, USA). This is a three-step sandwich-type immunoassay, where collected samples are added to an AMH antibody coated plate and uses stabilized recombinant human AMH as calibrators, with an analytical measure range of 0.08 - 14.2 ng/ml.

Insulin resistance: Homeostatic model assessment of insulin resistance *(HOMA-IR)* is a method used to quantify insulin resistance and was calculated according to (f-insulin mU/L × f-glucose mmol/L) / 22.5 and homeostatic model assessment of pancreatic beta cell function *(HOMA-B)* was calculated according as (20 × f-insulin mU/L) / (f-glucose mmol/L – 3.5) (218).

Symptoms of anxiety and depression

Numerous questionnaires are available to assess symptoms of anxiety and depression. A well established, validated instrument used in both research and clinical practice to define symptoms of anxiety and depression is the Comprehensive Psychopathological Rating Scale for Affective Syndromes self -assessed (CPRS-S-A). From the CPRS-S-A, two subscales are extracted: the Brief Scale for Anxiety self-rating (BSA-S), which measures symptoms of anxiety and the Montgomery Åsberg Depression Rating Scale self - rating (MADRS-S), which measures symptoms of depression. The questionnaire consists of 19 domains which are designed to measure symptoms of anxiety, depression, and obsessive- compulsive symptoms. The latter is less sensitive and not used in this study. All domains are rated on a scale from 0 to 6. A rating of 0 means absence of symptoms, a rating of 2 represents a potentially pathologic deviation, a rating of 4 represents a pathological condition and a rating of 6 represents an extremely pathological condition. The domain ratings are summed, rendering a maximum value of 54 for each scale. Since symptoms of anxiety and depression can be present without any clinical relevance, a cut-off for clinically relevant symptoms of anxiety and depression was set at a score of $\geq$11, a value shown to discriminate health from disease in a general population (219, 220).

Health-related Quality of Life

HRQoL is a way of describing how a disease or disorder affects the physical and mental health status of a person. There are generic tools, which can be used regardless of the disease or disorder, or disease-specific tools which are adapted to certain diseases or disorders. The short form-36 (SF-36) is a generic tool designed to capture a person's perception of how their health status has influenced their physical, psychological and social functioning over the past four weeks (221). Used widely in both research and clinical practice, the SF-36 is a validated questionnaire with high reliability (222). It is divided into eight domains, each scored on a scale of 0-100, ranging from low to high health quality. Two summary scores reflecting physical and mental HRQoL are computed: physical component summary score (PCS) and mental component summary score (MCS). In studies on women with PCOS, the most common used generic instrument is the SF-36 and the most commonly used disease specific instrument is the PCOS questionnaire (PCOSQ). The PCOSQ also takes symptoms of hirsutism, menstrual disturbance and infertility into consideration (151). Since we aimed to compare women with PCOS with those without, the SF-36 was considered a more adequate tool for the present study.

Energy intake

Energy intake was assessed with a semi-quantitative Food Frequency Questionnaire (FFQ). This is a self-reported questionnaire covering habitual dietary intake during the last three months (223). Calculations of energy intake, macro- and micronutrients were done using the food database from the Swedish food Agency (224).

Physical activity

Physical activity was assessed with the short version of the International Physical Activity Questionnaire (IPAQ*)* (225). This is a validated questionnaire consisting of nine items which measures recalled physical activity over the last seven days. It covers information on time spent walking, time spent in activity of moderate or vigorous activity and time spent in sedentary activity. Measuring physical activity only during the last week can be a source of misreporting. It can also be difficult to code activity into intensity categories, and to estimate exact hours and minutes of each activity.

Eating behavior

Eating behavior was assessed with the self-reported version of the 21-item Three-Factor Eating Questionnaire (TFEQ-R21), originally constructed by Stunkard and Messick with 51 questions (162), which have been shortened into two different versions; TFEQ-R18 and TFEQ-R21. The latter have been revised and validated in the translated version by Karlsson et al. and is widely used in populations with obesity (163). TFEQ-R21 consists of three subscales each covering three different eating behavior domains: emotional eating, cognitive restraint eating and uncontrolled eating. The output of the questionnaires is in raw scales which have to be transformed into scaled scores of 1-100. Higher scores indicate greater emotional eating, greater cognitive restraint, and greater emotional eating.

Binge eating disorder

Binge eating disorder (BED) was identified with criteria from the Diagnostic and Statistical Manual of Mental Disorders, Fifth edition (DSM-V) (226), using questions from the Questionnaire of Eating and Weight Patterns-Revised (QEWP-R) (227, 228). BED is diagnosed when fulfilling the following five criteria: 1) Recurrent episodes of binge eating, 2) Episodes of binge eating associated with three or more of: eating more rapidly, eating until uncomfortably full, eating large amounts of food without feeling hungry, eating alone because of being embarrassed or feeling guilty or depressed, 3) Marked distress regarding binge eating, 4) Binge eating at least one day a week for the last three months, 5) Binge eating not associated with compensatory behavior like purging, fasting or excessive exercise, and do not occur during bulimia nervosa or anorexia nervosa.

QEWPR is a widely used questionnaire, validated in the Swedish translation, and focuses primarily on assessing diagnostic criteria for binge eating disorder according to DSM-5. It also includes questions about earlier weight loss attempts and night eating. To assess BED in clinical practice, QEWPR together with an interview is used. Participants are asked about meal order and current weight loss attempts to exclude that overeating is not due to limited food intake during the day leading to uncontrolled eating during the evening. Since this questionnaire is widely used, there are many studies to compare to. However, most studies on women with PCOS use other questionnaires (1).

Intervention

All participants started a 12-month dietary intervention, starting with a very low energy diet (VLED) for 12 weeks. During this period, participants only consumed a liquid diet of 450-800 kcal/day consisting of four pre-packed powder sachets per day. The powder was dissolved into cold or hot water. In addition to the VLED-portions, the participant could drink energy-free beverages such as water, coffee, tea (without sugar), bullion and zero-soda/ lemonade. During the strict VLED-period there were scheduled visits to a study nurse 4 - 5 times where well-being and weight was monitored, and participants were given support and counselling to be able to adhere to the VLED. After 12 weeks, meals with solid foods were re-introduced gradually for another 12 weeks. The prescribed energy intake was calculated for participants to achieve further weight reduction. Calculation was based on the Harris Benedict sex-specific equations (229), multiplied with a physical activity level of 1.3 - 1.4 and subtracting 30% for weight reduction. During this second period until the end of the intervention, participants had scheduled monthly individual meetings with a dietician. Participants were given dietary advice based on three main meals with specified portion sizes and intake of 1400 - 1600 kcal/day. The macronutrient composition of the energy reduced diet was based on Nordic Nutrition Recommendations (NNR) (230) and was consisting of 15 - 20 energy percent (E%) protein, 30 E% fat and 50 - 55 E% carbohydrates. Throughout the intervention, participants were given advice about daily physical activity and weight was monitored monthly.

Sample size

To estimate the prevalence of PCOS among women with severe obesity, there was no comparison and no test to be performed. Thus the sample size determination was not a question about power, but of precision reflected by a confidence interval. With a known PCOS prevalence of 4.8% in a general population of Northern Sweden (231), and with the hypothesizing of a PCOS prevalence of 10% in women with severe obesity in Sweden, the true probability (10 %) and expected confidence interval in a sample size of n=200 would be 0.058 - 0.142 (5.8 - 14.2%).

Statistical analyses

Statistical analyses were performed using IBM SPSS statistics version 22, 25, 27 and 29.

Kolmogorov-Smirnovs test and the normal plot were utilized to test normality of continuous variables. Normally distributed data is presented as mean and standard deviation SD, and skewed data as median and interquartile range (IQR). Categorical variables are presented as percentages.

To test the differences between groups, a two-sided t-test was applied if data was considered normally distributed, and Mann Whitney-U test was used for skewed data. The chi-square test was employed when comparing proportions. Adjusting for co-variates was done with analysis of covariance (ANCOVA).

Changes within groups from baseline to 12-month follow up was assessed using paired samples t-test if data was considered normally distributed and using Wilcoxon-signed rank test if data was skewed. Wilcoxon signed rank test was used comparing groups regarding change from baseline to follow up.

Receiver operating characteristics (ROC)-analyses and area under the curve (AUC) were used to assess the diagnostic utility of serum concentration of AMH in diagnosing PCOS.

Correlations were determined using Spearman´s rank correlations test,and presented as correlation coefficient rho.

$P < 0.05$ was considered significant.

Ethical permissions and considerations

The studies included were conducted following the declaration of Helsinki. Ethical approval was obtained for the four papers in the thesis from the regional ethical review board of Gothenburg, Gothenburg, Sweden with dnr. 106 - 11, approved 110331 for paper I – IV, and dnr. 679-08, approved 090121, dnr. 520 - 11, approved 110801 and dnr. 307 - 05, approved 051006 for paper III.

Women in the study gave their written informed consent and could withdraw at any time of the study. All data was collected and handled by authorized personnel. The main study was a clinical study where participants followed

clinical routines, except for filling out additional questionnaires, and one additional measure of body composition with DEXA. DEXA is performed with a low radiation dose of 0.01-0.03 mSv, comparable to one week of background radiation, which is considered a low amount of radiation. Further, diagnosing participants with PCOS can lead to worry over future issues such as infertility and the increased risk for metabolic and psychiatric consequences. However, awareness of a PCOS- diagnosis can be of benefit to the individual, who can understand experienced symptoms and will have the possibility for prevention and treatment. Most women also have a positive experience with caregivers taking their medical issues seriously and receiving information that the planned intervention is expected to reduce symptoms of PCOS. For women who have a known diagnose of PCOS, it enhances the possibilities to influence the development of the syndrome knowing that obesity worsen all symptoms.

Results

Baseline

In total, 246 women with severe obesity were included in the analyses. In the present cohort, the prevalence of PCOS was 25.6% (CI 95% 0.205 – 0.313). Women with PCOS were younger than women without PCOS and therefore all variables were adjusted for age. Variables that differed between women with and without PCOS are shown in **Table 5**.

Women with PCOS had higher androgen levels and more hirsutism than those without PCOS. They also had lower cholesterol and LDL-C, whereas insulin and measures of insulin resistance did not differ between groups, neither did the proportion of metabolic syndrome (PCOS 43.5%, non-PCOS 43.4%, p=n.s).

Circulating levels of AMH was higher in women with PCOS compared to women without PCOS. There was a positive correlation between AMH and circulating androgens in both groups. ROC-area under the curve (ROC_{AUC}) for AMH was 0.701 (95% CI, 0.622-0.780) and ROC_{AUC} for free testosterone was 0.770 (95% CI 0.700-0.890).

Energy intake and physical activity did not differ between groups. Eating behavior differed as women with PCOS had a higher cognitive restraint eating behavior, whereas the proportion of binge eating disorder did not differ between groups (PCOS 11.5%, non-PCOS 17.5%, p=0.27). In women with severe obesity, both in those with and without PCOS, cognitive restraint eating was negatively correlated to energy intake, and uncontrolled eating was positively correlated to energy intake. Emotional eating was positively correlated to energy intake in women without PCOS.

Table 4. Variables that differed at baseline between women with severe obesity with and without PCOS. An arrow indicates a higher or lower value in women with PCOS compared with women without PCOS.

Age, years	↓
FG-score	↑
Testosterone, nmol/L	↑
SHBG, nmol/L	↓
Free testosterone, nmol/L	↑
FAI	↑
Cholesterol, mmol/L	↓
LDL-C, mmol/L	↓
AMH, pmol/L	↑
Cognitive restraint eating	↑

PCOS, polycystic ovary syndrome; FG-score, Ferriman-Gallwey score, SHGB, sexual hormone binding globuline; FAI, free androgen index; LDL-C, low density lipoprotein cholesterol; AMH, anti-müllerian hormone

Symptoms of anxiety and depression and HRQoL in women in different BMI-categories

In women with severe obesity, there were no differences in scores of symptoms of anxiety and depression between women with and without PCOS. Independent of PCOS-diagnosis, clinically relevant symptoms of anxiety (PCOS 71.3%, non-PCOS 65.5%) and depression (PCOS 56.4%, non-PCOS 52.2%) were present in a majority of women with severe obesity, with no difference between groups. HRQoL did not differ between women with severe obesity with and without PCOS. To define the impact of BMI on symptoms of anxiety and depression and HRQoL, data from women with severe obesity was combined with data from three earlier studies on women with and without PCOS in different BMI-categories with a BMI-range of 18 - 59 kg/m^2, with results seen in **Figure 6**.

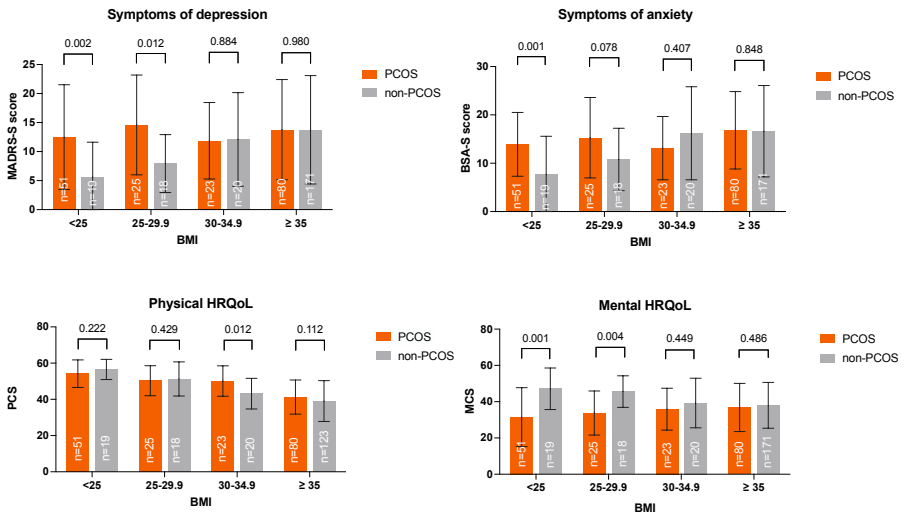

Figure 6. Symptoms of anxiety and depression and HRQoL in women with and without PCOS in different BMI categories. Data is presented as mean ± SD. SD, standard deviation; PCOS polycystic ovary syndrome; BSA-S, Brief Scale for Anxiety self-rating; MADRS-S Montgomery Åsberg Depression Rating Scale self-rating;, HRQoL, health-related quality of life; PCS, physical component score; MCS, mental component score, BMI, body mass index.

Women with PCOS with normal weight or overweight had more symptoms of depression than controls, whereas there were no differences between women with or without PCOS with obesity and severe obesity. Symptoms of anxiety were higher in women with PCOS in those with normal weight, but not in the

higher BMI-categories. Mental HRQoL was lower in women with PCOS with normal weight and overweight, but there were no differences between groups in women with obesity and severe obesity. Physical HRQoL, was higher in women with PCOS in those with obesity class I, but there were no differences in other BMI-categories.

To investigate if circulating androgens , hirsutism or insulin affected symptoms of anxiety and depression in different BMI-categories, we performed correlation analyses. To increase power we combined women with and without PCOS in the two lower BMI-categories (BMI<30 kg/ m^2), and the two higher BMI-categories (BMI >35 kg/ m^2) respectively. In women with BMI <30 kg/m^2, FG-score was positively associated with symptoms of anxiety and depression, and negatively associated with physical and mental HRQoL, and insulin was positively correlated to symptoms of anxiety and depression and negatively correlated to physical HRQoL. In women with BMI ≥30 kg/m^2 insulin was positively correlated to anxiety and depression.

12-month follow-up

During the intervention there was a drop-out, leaving 72 women (PCOS n=16, non-PCOS n=56) with complete data at 12-month follow up. Women who dropped out from the study did not differ from the ones who adhered regarding age, PCOS-status, anthropometric or metabolic characteristics. Measured variables that changed, and the direction of change are presented in **Table 5**.

Anthropometry and body composition: Weight loss in women with PCOS was -12.3 ± 10.7 kg ($p < 0.001$) and in women without PCOS -13.9 ± 13.4 kg ($p < 0.001$) with no difference in change between groups (p=0.796). Weight loss in percent was approximately 12% in both groups. Both groups decreased in BMI, weight circumference, hip circumference, WHR, as well as in total fat mass and total lean mass, with no difference between groups. Those without PCOS decreased in cholesterol, TG, LDL-C, insulin, and increased in HDL-C, with no change in women with PCOS. There were no differences between groups comparing change from baseline to follow-up.

Reproductive variables and hirsutism: Women without PCOS decreased in circulating free testosterone, total testosterone and FAI and increased in SHBG, with no change in women with PCOS. FG-score and AMH did not change in any group. There were no differences between groups comparing change from baseline to follow-up.

Anxiety and depression and HRQoL: Women without PCOS reduced in scores of symptoms of anxiety and depression, whereas women with PCOS did not change. Regarding HRQoL, both women with and without PCOS increased in summary score for physical quality of life, but summary score for mental quality of life did not change in either group. There were no differences between groups comparing change from baseline to follow up.

Physical activity, energy intake and eating behavior: Women without PCOS reported reduced energy intake, reduced energy percent (E%) carbohydrates and E% sugars, increased E% protein and iron intake, and decreased time spent in sedentary activity and walking. Women with PCOS reported reduced E% fat and increased time spent in medium to vigorous activity. Women without PCOS reported changes in eating behavior in all domains; uncontrolled eating and emotional eating decreased, whereas cognitive restraint eating increased. In women with PCOS there were no statistically significant changes in eating behavior from baseline to follow-up. Difference in change between groups were seen in cognitive restraint score where women without PCOS increased more than women without PCOS, E% fat intake where women with PCOS decreased more than women without PCOS, and E% carbohydrates and E% sugar where women without PCOS decreased more compared to women with PCOS.

Table 5. Changes from baseline to follow-up at 12 months in women with severe obesity with and without PCOS. The arrow in the first two columns indicate if there is a significant difference within group from baseline to follow-up. The last column shows difference between groups comparing change from baseline to follow-up.

	Follow up at 12 months		
	Within group		Between group
	PCOS	non-PCOS	
Weight	↓	↓	↔
BMI	↓	↓	↔
Waist hip ratio	↓	↓	↔
Testosterone	↔	↓	↔
SHBG	↔	↑	↔
Free testosterone	↔	↓	↔
FAI	↔	↓	↔
FG-score	↔	↓	↔
Insulin	↔	↓	↔
HOMA-IR	↔	↓	↔
HOMA-B	↔	↓	↔
Cholesterol	↔	↓	↔
TG	↔	↓	↔
HDL-C	↔	↑	↔
LDL-C	↓	↓	↔

f-T4	↔	↑	p=0.014
DEXA total bone	↔	↓	p=0.017
DEXA total fat	↓	↓	↔
DEXA total lean	↓	↓	↔
BP diastole	↔	↓	↔
BSA-S-score	↔	↓	↔
MADRS-S-score	↔	↓	↔
Physical HRQoL	↑	↑	↔
Energy intake	↔	↓	↔
Protein (E%)	↔	↑	↔
Fat intake (E%)	↓	↔	p=0.024
Carbohydrates (E%)	↔	↓	p=0.017
Sugar (E%)	↔	↓	p=0.017
Cognitive restraint	↔	↑	p=0.014
Uncontrolled eating	↔	↓	↔
Emotional eating	↔	↓	↔

PCOS, polycystic ovary syndrome; BMI, body mass index; SHBG, sexual hormone binding globuline; FAI, free androgen index; FG-score, Ferriman-Gallwey-score; HOMA-B, homeostatic model assessment of beta-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL,C, low density lipoprotein cholesterol; DEXA, dual-energy x-ray absorptiometry; BP, blood pressure; BSA-S, Brief Scale for Anxiety self-rating; MADRS-S Montgomery Åsberg Depression Rating Scale self-rating;, HRQoL, health-related quality of life; E%, energy percent

Discussion

PCOS is a common condition causing significant impacts on the individual and health care system. It is strongly associated with obesity and first line treatment is weight loss through lifestyle modifications. However, to date, studies on PCOS in women with severe obesity and the effect of weight loss intervention compared to women without PCOS have been lacking. This thesis set out to study the prevalence of PCOS in women with severe obesity and to compare women with and without PCOS regarding metabolic, hormonal, and psychiatric features as well as health-related quality of life, at baseline and through a 12-month structured weight loss intervention in the framework of an energy restricted diet.

Prevalence of PCOS

Previous studies on the prevalence of PCOS among women with severe obesity are few and have shown contradictory results. One American study of 675 women found the prevalence of PCOS diagnosed using the NIH-criteria, to be consistent through BMI-classes (90). In this American cohort, 57 women had severe obesity (BMI ≥35) of which 12.4% had PCOS and 53 women had morbid obesity (BMI ≥40) of which 11.5 %, had PCOS. In a Spanish study of 113 women with overweight and obesity, 28.3% were diagnosed with PCOS according to the NIH-criteria, with a similar prevalence in all categories of obesity (232). In the latter study, 30 women had severe obesity, of which 27% had PCOS, and 23 had morbid obesity, of which 26% had PCOS. Using a larger cohort, the current thesis found a prevalence of PCOS of 25.6%, a result in line with the Spanish study and much higher compared to the prevalence in a general population (1, 2), or the Swedish population (231). These results strengthen data from genetic studies, showing a causal connection on obesity on PCOS (40-42). This imply that a a predisposition of PCOS, triggered by obesity can lead to the syndrome.

Anti-müllerian hormone

In this cohort of women with severe obesity, AMH was higher in those with PCOS compared to those without PCOS, as shown in previous studies in lower BMI categories (63, 64, 75). AMH was positively correlated to testosterone, which is in line with previous studies, where AMH have been positively correlated to hyperandrogenism and to a more severe PCOS–phenotype (76).

In contrast to earlier studies (76, 77), AMH was not correlated to BMI, probably due to the limited variation in BMI with all women having severe obesity. AMH has been suggested to be used as a diagnostic test for PCOS, since it has been shown to be a good discriminator of PCOS (77, 83), especially the hyperandrogenic type (77). However, a recent meta-analysis informing the 2023 guidelines for PCOS concluded that there is no evidence to support this yet (1). In this cohort of women with severe obesity, AMH was not considered a good discriminator of PCOS due to low sensitivy and specificity. However, AMH is now part of PCOS diagnosis, and elevated levels of AMH can be used instead of polycystic ovarian morphology in adults.

Metabolic variables and metabolic syndrome

Women with obesity and hyperandrogenism, represent the most metabolically affected subgroup of PCOS (100-103). However, in this cohort of women with severe obesity, those with PCOS had lower total cholesterol and lower LDL-C compared to those without PCOS. This is a surprising finding, since both groups reported similar energy intake, fat intake and physical activity. Dyslipidemia increases with age (233), and women with PCOS were younger than those without PCOS, however, age was adjusted for in all analyses. Regarding socio-economic factors, smoking has been shown to decrease HDL-cholesterol (234), and alcohol intake has been shown to increase HDL-cholesterol (235), however there is no evidence of any effect on other blood lipids. Data on smoking was not collected in this study, and reported intake of alcohol did not differ between groups. There was an equally high prevalence of MetS of 43% in both groups, but no differences between groups as seen in previous studies where women with PCOS have a higher prevalence of MetS across weight categories (131, 236). The similar prevalence could be due to the severe obesity in the cohort which is a serious medical condition, and thus, the addition of PCOS or the hyperandrogenism may not have worsen the metabolic profile.

Anxiety and depression

When comparing symptoms of anxiety and depression in women with and without PCOS in different BMI-categories, there were differences between groups in women with normal weight and overweight, that disappeared in women with obesity class I and severe obesity, where no differences between women with and without PCOS were detected. The higher risk of anxiety and depression in women with PCOS, is well known (1), and this risk increases

with BMI, at least in women up to obesity class I (143). However, an important finding in this study is that most of the women with severe obesity had clinically significant symptoms of both anxiety and depression, independent of PCOS diagnosis. This stresses the fact that obesity increases the risk of anxiety and depression (237, 238), with studies showing that around 50% in a population with BMI >40 report a history of depression (26). The results from the different BMI-categories imply that symptoms of anxiety and depression are consistent in women with PCOS across BMI categories, but in women without PCOS, the influence of BMI on symptoms of anxiety and depression is more pronounced. Furthermore, in women with severe obesity there is no additional effect of PCOS on symptoms of anxiety and depression, possibly due to that these women already are heavily affected by the obesity *per se*. The finding that symptoms of anxiety and depression in women with a lower BMI are positively correlated to both insulin and hirsutism, but in women with a higher BMI only correlated to insulin but not to hirsutism further strengthens this observation.

Health-related Quality of Life

HRQoL did not differ between women with and without PCOS with severe obesity, which contradicts previous studies on women with PCOS (1) but resonates with a recent study comparing women with and without PCOS with a mean BMI 36 kg/m^2 that showed no differences in HRQoL between groups (159). However, the latter study was a post-hoc analysis of a randomized controlled study including women scheduled for fertility treatment, and HRQoL was not the primary outcome. These results should therefore be interpreted with this in mind. Psychiatric symptoms have a large impact on HRQoL. Other factors leading to a lower HRQoL could be hirsutism, obesity, weight stigma and infertility (9, 153, 181). In the cohort of this thesis, groups did not differ regarding symptoms of anxiety and depression or weight, and data on infertility was not collected. However, scores for hirsutism were higher in women with PCOS, but this PCOS-specific factor is not accounted for in the generic questionnaire used in this study. Of note, differences in HRQoL between groups were detected in the lower BMI categories, meaning that the questionnaire *per se* does not have an impact on the lack of difference in women with severe obesity.

Dietary intake and physical activity

There were similar self-reported energy- and macronutrient intake in women with severe obesity with and without PCOS, results in line with existing data (1). This imply that dietary intake in this group is influenced mostly by the obesity *per se,* and not by PCOS-status. There is a potential of general misreporting bias with both under- and overreporting dietary intake. In addition, there may be under-reporting of specific foods that are socially undesirable to eat, such as sweets, chocolate and pastries or over-reporting foods that are desirable to such as vegetables and fruits which lead to an under-reporting of energy intake. This type of misreporting tends to be more prevalent among groups with obesity (239, 240). However, the reported energy intake at baseline was equivalent to the mean estimated energy requirements for weight stability for the participants weight at that time according to Mifflin st jeor (241), implying a reasonably accurate energy report at baseline. The physical activity of both groups is below the recommended physical activity according to both international and Swedish recommendations (171, 242), but did not differ between groups. An higher level of physical activity according to the current national and international recommendations would be beneficial to women with severe obesity independent of PCOS, since it has been shown to improve psychiatric (243), metabolic and reproductive function, and can improve the effect of weight loss.

Eating behavior and Binge eating disorder

The higher cognitive restraint eating behavior at baseline which was seen in women with PCOS, a higher control of what and how much you eat, in order to influence body weight or body shape, is a behavior in favor for weight loss or weight maintenance (244). These results are in line with previous studies on women with overweight and obesity class I, where women with PCOS had greater concern about weight and dieting (165) and higher cognitive restraint compared to controls (245). The reason for greater concerns about weight in women with PCOS, could be due to a history of weight gain (87), weight gain in early adulthood (86), more weight loss attempts (88), and/or the negative body image that is well known in women with PCOS (153). In women with severe obesity, the prevalence of binge eating disorder did not differ between groups, in contrast to what is seen in women with lower BMI (1). However, the prevalence of binge eating disorder was similar to that found in another cohort of women with severe obesity (28).

Effect of weight loss intervention

The significant and similar weight loss of approximately 12% in both women with and without PCOS in this thesis, indicate that in a structured intervention, women with PCOS can achieve the same weight loss as women without PCOS. This is a positive finding that contradicts the spread opinion about barriers for weight loss in women with PCOS. However, a structured clinical setting, with strict schedules for eating and regular check-ups, is far from an environment without support, where women should lose weight by themselves, where other factors can contribute to failure in weight loss.

Women without PCOS decreased in androgen levels and hirsutism score whereas SHBG levels increased, while no changes were seen in women with PCOS over the 12-month follow-up. In previous studies on women with PCOS, a modest weight loss of 5%, has been shown to lead to lower androgens and higher SHBG (178, 179). Regarding metabolic profile, only women without PCOS improved in lipid-status and decreased in insulin levels from baseline to follow up, whilst there were no changes in insulin or lipids, except LDL-C, in women with PCOS. This contradicts previous studies in women with PCOS, showing lower insulin levels after weight loss (175, 178, 179, 246). Symptoms of anxiety and depression decreased in women without PCOS, with no difference in women with PCOS. Both groups increased in scores of physical quality of life, which is probably due to the weight loss of >10% of their baseline weight. These results are in line with a recent six month weight loss study in women with BMI 30-31 kg/m^2, with a weight loss of 5 kg (6%) that showed similar improvement in HRQoL in women with and without PCOS (180).

Only women without PCOS reported reduced energy intake, despite the similar weight loss in both groups. To achieve a weight loss of 12 kilos over 12 months, energy intake must decrease with approximately 230 kcal/day based on an energy content of 7000 kcal per kg body weight. Both groups lost weight and thus both groups must have decreased their energy intake to allow this. Mean reported energy intake in women with PCOS did reduce but did not reach statistical significance. One explanation of this could be that women with PCOS misreported energy intake to a higher degree compared to their non-PCOS counterparts, or that energy intake at 12 months had started to increase again in that group. Another likely explanation could be the low statistical power at follow up due to few participants at 12 months follow-up compared with baseline, and large variability between individuals in self-reported energy

intake leading to overlapping standard deviations. Both women with and without PCOS increased in mean values of physical activity, thus in the right direction in relation to the recommended levels of physical activity. However, standard deviations were large and overlapping, probably due to the large variability in physical activity between individuals in the group, and the lack of statistical power.

Women with severe obesity without PCOS decreased in both emotional eating and uncontrolled eating and increased in cognitive restraint. These are changes in eating behavior that favor further weight loss or weight maintenance. During the first part of the intervention, the VLED with intake of portions of liquid diet four times per day allows participants to take control over their eating behavior, at the same time as a large weight loss can be achieved (247). This control of eating behavior together with the achieved weight loss, are factors that promote further weight loss (248) and can reduce emotional eating. The introduction of meals with solid foods with a regular meal pattern makes it easier to eat proper portions and avoid hunger and over-eating. In women with PCOS, mean values for cognitive restraint eating increased while uncontrolled eating and emotional eating decreased but did not reach statistical significance. There is evidence of increased cognitive restraint in women with PCOS after a diet induced weight loss of 5% both after a 12 month intervention in women with overweight (249), and after a 12 week intervention in women with overweight and obesity class I (250). However, the first study was a post hoc analysis of a RCT, and both studies used other methods measuring eating behavior than in the present study.

Most of the weight loss studies mentioned above, included women with overweight or obesity class I, which stand in contrast to the cohort of this thesis with women with severe obesity. Despite a statistically and clinically significant weight loss, participants had a mean BMI around 35 kg/m^2 at follow-up, many still having severe obesity, which can be one explanation to the lack of changes seen in outcomes in women with PCOS. There is a possibility that further weight loss is warranted in women with severe obesity and PCOS for symptom alleviation. Support to this theory is that studies after bariatric surgery on women with PCOS, show a much larger weight loss of around 25% (203, 204), with improvement in both metabolic and reproductive outcomes, still remaining after five years follow-up (204). Another possible explanation of the lack of change could be the low statistical power in the PCOS-group at follow-up, due to the high drop-out during the intervention.

Strengths and limitations

One strength of the main study is the relatively large sample size at baseline, which is large enough to detect differences between groups. Another strength is that data was collected prospectively specifically for the research question. All patients, both women with PCOS and those without, were recruited at the same unit with the same staff, and patients received the same information about the treatment. Using validated questionnaires is a method of decreasing bias, knowing that we measure what we intend to measure. In this study, all questionnaires were validated. Data from queastionnaires were self-reported, which can introduce bias with patients over- or underreporting. Selection bias arise when a sample is not chosen from a representative population. In this study there was a selection of women in a clinical setting, from a university clinic, with a sample of women referred for obesity treatment, and not a sample from an unselected population. Participants recruited from a hospital setting, could be suffering from more co-morbidites compared to participants selected from the general population. Persons who accept to participate in a research study could also be the ones that are more motivated for treatment and may potentially have greater success in achieving set goals. There is also a possibility that participants have symptoms of, or a known diagnosis of PCOS, and are more motivated to enter the study and thus have more success in the intervention part. This was not a fertility study, but it cannot be excluded that women entering the study could have had a wish to conceive, and therefore would be more motivated for weight loss. Drop-out was high, and could also possibly introduce bias, since participants who adhere to these kinds of studies are the ones that succeed in treatment. However, analyses of those dropping out did not differ in age, baseline anthropometry, hormones, or metabolic variables. The large drop-out left a small sample size at follow-up, and therefore the risk of type-II errors must be taken in considerations when interpreting the results on follow-up regarding all variables. Due to the above reasons, the external validity of the study is low, and it is with caution one can apply these findings on a general population with PCOS. Furthermore, PCOS was diagnosed with the NIH-criteria, and therefore results will not be directly comparable if using other diagnostic criteria.

Conclusions

The results of this thesis demonstrate that the prevalence of PCOS in women with severe obesity is high, affecting one in four. Further, the results provide evidence that women with PCOS can lose weight to the same extent as women without the syndrome. In women with severe obesity, a condition associated with many co-morbidites, a diagnosis of PCOS did not further affect metabolic variables, psychiatric symptoms, or HRQoL. This suggests that the obesity *per se* has more impact than PCOS in women in this BMI-category. Whilst AMH was higher in women with severe obesity and PCOS compared with women without PCOS, due to low sensitivity and low specificity, it cannot be used as a single surrogate marker for PCOS diagnosis in this group. Symptoms of anxiety and depression were predominantly consistent across BMI-categories in women with PCOS, however in women without PCOS, the influence of BMI was more pronounced, with more symptoms of anxiety and depression in higher BMI-categories. Moreover, all women with severe obesity were heavily affected by symptoms of anxiety and depression, irrespective of PCOS. With regards to eating behavior, women with severe obesity and PCOS were more conscious about eating than women with severe obesity without PCOS. A weight loss of 12% improved hyperandrogenism, insulin, lipids, symptoms of anxiety and depression and mental HRQoL and changed eating behavior only in women without PCOS. Comparing groups with regards to change from baseline to follow-up, there were no major discernible differences except that women without PCOS changed more in eating behavior with more cognitive restraint towards a behavior more favorable for further weight loss.

Future perspectives

Weight loss through lifestyle intervention including diet and increased physical activity is considered first line treatment for women with PCOS and obesity. However, existing studies are low powered, show limited weight loss, high drop-out rates, and lack of long term follow-up. In the current study, both women with and without PCOS experienced similar and large weight loss, however with no other discernible improvements in women with PCOS. One conclusion from this is that women with PCOS may need more intense treatment than lifestyle interventions to achieve improvements in metabolic and psychological features after weight loss. A more intense treatment may include the GLP-1 analogues, which is gaining popularity in the treatment of obesity, and is an option understudied in women with PCOS (194). Another option that has been shown to lead to large and sustainable weight loss in women with PCOS is bariatric surgery. This treatment, however, is associated with various complications, both short-term and long-term. Bariatric surgery can also lead to pregnancy-related complications since it is associated with a high prevalence of nutritional deficiencies that can affect the fetus, and lead to consequences such as preterm birth and having an infant born small for their gestational age. Further, higher rates of perinatal mortality have been reported following bariatric surgery (200, 201). This is an important consideration when advising women of fertile age with PCOS regarding obesity management. It is well known however, that both obesity and PCOS are associated with many pregnancy complications, including the ones mentioned above, which can have a detrimental effect on both the woman and the child. The effects of obesity and PCOS *per se* on the fetus and on pregnancy complications needs to be weighed against the risks associated with the obesity management treatments available. To be able to inform treatment recommendations for women with PCOS and obesity, well designed randomized controlled studies comparing GLP-1 analogues or bariatric surgery with lifestyle intervention, or comparing bariatric surgery with GLP-1 analogues are needed. Study designs should include large sample sizes, have a multi-center approach, and include longer duration to be able to confirm the possible risk-reducing effects. Results from such studies will support clinicians in offering the most effective treatment for weight loss in women with PCOS. Further, the heterogeneity of PCOS calls for a more tailored approach to treatment. There is evidence of biological distinct subtypes in PCOS, i.e. the hyperandrogenic, reproductive or metabolic subtype (104), where the metabolic subtype with obesity, could benefit more from intensified weight loss treatment. Subtyping with clinical variables such

as antropometric, metabolic and hormonal variables, rather than with diagnostic criteria for PCOS decided from consensus, can lead the way for personalized treatment options depending on PCOS-subtype. Lastly, and of great clinical significance, is the importance of early diagnosis. An early diagnosis of PCOS, together with an awareness of the consequences of the condition, can facilitate prevention of weight gain in early adulthood and reduce the effect that obesity has on PCOS. The awareness of a PCOS-diagnosis also gives the opportunity for health-professionals to guide women with PCOS (with or without obesity) preconceptionally, and inform them about the consequences of weight gain and the the importance of maintaining a healthy weight during pregnancy. A known early diagnosis of PCOS also enables the opportunity to monitor women more closely throughout their pregnancies with the aim to prevent excessive weight gain and pregnancy related complications, and to possibly reduce transmission of PCOS to subsequent generations.

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