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EFFECTS OF LIFESTYLE INTERVENTION IN OVERWEIGHT WOMEN WITH POLYCYSTIC OVARY SYNDROME –

ASPECTS ON REPRODUCTION, METABOLISM, PSYCHOLOGICAL WELL-BEING AND SLEEP

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To everyone who has helped me complete this thesis, in particular the women participating in the study.

POPULAR SCIENCE SUMMARY OF THE THESIS

Polycystic Ovary Syndrome, or PCOS, is the most common hormonal disturbance in women of a childbearing age, with up to one in ten women being affected. Some of the most common symptoms relate to the menstrual period with irregular or even absent periods and many women have difficulties falling pregnant. Women with PCOS often have raised levels of androgens in the blood. Androgens are sex-hormones, sometimes referred to as "male" hormones, although they are also normally present in women but in lower quantities. Testosterone is an example of an androgen. The raised levels of androgens can result in symptoms such as increased body hair often on the stomach, chest or back as well as acne. There are other problems associated with PCOS as well, such as type 2 diabetes, increased weight often located to the abdomen, increased cholesterol and levels of inflammation. In addition, women with PCOS are more likely to suffer from depression, anxiety and they often have a worse quality of life. We do not know exactly what causes PCOS but we think a combination of genetic and lifestyle factors are involved.

The recommended first-line treatment for PCOS is to improve the lifestyle by healthy eating, exercising or through some kind of behavioral change program that would lead to a healthier lifestyle. Previous studies have shown that weight loss, even as little as 5% - 10% can lead to normalized menstrual periods and therefore for the fertility to come back. In fact, the majority of PCOS symptoms improve when losing weight. Other treatment options for women with PCOS include the contraceptive pill, which can lower the levels of androgens and therefore improve the symptoms, as well as controlling the irregular periods. Sometimes diabetes medication, such as metformin, is used. Furthermore, women with PCOS might also need fertility treatment. We know that being over-weight increases the risk of miscarriage and also that pregnancies in over-weight mothers are more likely to involve complications for both the mother and the child. This is why in many countries, Sweden being one of them, there is an upper weight limit of body mass index (BMI) of 30-35 kg/m^2 to be able to access state funded and often even privately funded fertility treatment. This result in a large group of obese women with PCOS who do not qualify for fertility treatment.

This study set out to investigate if changing the lifestyle trough an intervention focusing on the study participants behavior would have an effect on the symptoms such as menstrual regularity, weight, levels of androgens such as testosterone, psychological well-being, sleep quality and inflammation. The women taking part in the study were 68 overweight or obese women with PCOS that were otherwise healthy and did not take any regular medication.

Study participants were randomly allocated to receive either the intervention or to control treatment. The intervention was a four-month long course that included weekly meetings in small groups with a course leader/ lifestyle coach as well as individual meetings with the coach every three weeks where the personal progress was reviewed. The course focused on topics such as personal leadership, mindfulness, stress management, healthy eating and physical activity. The women also received reading material and homework to prepare for each meeting and had to formulate their own goals. The women allocated to control treatment received the

normal healthy living information that is offered in ordinary gynecological clinics. At four months, the control treatment group received the intervention for an additional four months. All women taking part in the study were assessed at the start of the study, after four months and then again after 12 months.

The results of the study showed improvements in several PCOS related symptoms after having undergone the training course. We found that despite only managing to lose a small amount of weight, several other symptoms improved. The menstrual periods became more regular, the insulin resistance, which is related to the development of diabetes, improved, the waist measurement decreased, the blood lipids improved and the levels of several inflammatory markers in the blood decreased, of particular interest was the reduction in some related to inflammation in the brain.

We also found that the whole group of women with PCOS had a very low psychological well-being at the beginning of the study, much lower than what would be expected in a general population of women, however some aspects of the well-being improved after they had undergone the intervention. In terms of sleep at night, the whole group of women with PCOS had sleep amounts within the normal range of recommended sleep, however when comparing the sleep in the women with PCOS with a group of healthy women without PCOS but with a similar age and weight, we found that the sleep efficiency was lower in the women with PCOS.

The results of this study are important as we do not know the exact effect of lifestyle treatment on improving all symptoms of PCOS. We believe that our proposed intervention of addressing the behavior is an ideal treatment, in particular for the group of women with PCOS and fertility issues who are unable to access fertility treatment because of a high BMI.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Polycystiskt ovariesyndrom, eller PCOS, är den vanligaste hormonstörningen hos kvinnor i fertil ålder med runt 10% av alla kvinnor berörda. Mensstörningar i form av oregelbunden- eller helt utebliven menstruation är ett av de vanligaste symptomen och detta kan leda till svårigheter att bli gravid. Kvinnor med PCOS har också ofta förhöjda värden av androgener i blodet. Androgener är könshormoner, som ibland också kallas ”manliga” könshormoner, trots att de också normalt produceras av kvinnor, dock i mycket mindre mängd. Ett exempel på androgener är testosteron. Förhöjda androgennivåer hos kvinnor kan leda till bl.a. ökad behåring på mage, bröst, rygg och ansikte samt acne. Andra problem associerade med PCOS är ökad risk för att utveckla typ 2 diabetes, viktuppgång, framförallt bukfetma samt förhöjda blodfetter och inflammationsnivåer. Dessutom så har kvinnor med PCOS ökad risk för att utveckla depression och oro samt för att ha sämre livskvalité. Vi vet inte exakt vad som orsakar PCOS men vi tror att det är en kombination av genetiska- och livsstilsfaktorer.

Första linjens behandling som rekommenderas till kvinnor med PCOS är livsstilsförändringar. Dessa involverar förändringar i kosten, ökad fysisk aktivitet och/eller program för att ändra beteendet. Vi vet från tidigare studier att en så liten viktnedgång som 5% - 10% kan resultera i en normaliserad mensfunktion och därför också förbättrad fertilitet. De flesta symptom från PCOS förbättras efter viktnedgång. Andra behandlingsmöjligheter för kvinnor med PCOS är p-piller. De sänker androgennivåerna viket leder till minskad behåring och acne och de kontrollerar oregelbundna menstruationer. Ibland används även andra läkemedel såsom diabetesläkemedlet metformin. Kvinnor med PCOS kan också behöva fertilitetsbehandling om de har graviditetsönskan

Vi vet att övervikt ökar risken för missfall och att graviditeter hos överviktiga mödrar ofta innebär komplikationer för både mamman och barnet. Därför har många länder, inklusive Sverige, en övre BMI gräns på 30-35 kg/m^2 för att få tillgång till både offentligt finansierad och privata fertilitetsbehandlingar. Detta har till följd att många obesa kvinnor med PCOS inte uppfyller kraven för att få genomgå fertilitetsbehandling.

I den här studien ville vi undersöka om livsstilsförändringar efter en behandlingskurs som fokuserade på aspekter av studiedeltagarmas beteende, påverkade mensfunktion, vikt, androgennivåer, livskvalité, sömnkvalité och inflammation. Sextioåtta överviktiga kvinnor med PCOS deltog i studien. Kvinnorna var i övrigt friska och tog inga mediciner.

Studiedeltagarna delades slumpmässigt upp i två grupper där den ena gruppen (behandlingsgruppen) genomgick en 4-månader strukturerad kurs. Kursen leddes av en livsstilscoach och bestod av veckovisa möten i små grupper med andra studiedeltagare, samt individuella möten med kursledaren var 3:e vecka där den personliga progressen utvärderades. Kursen fokuserade på ämnen som personligt ledarskap, mindfulness, stresshantering, problemlösning och information kring kost och fysisk aktivitet. Deltagarna fick sätta upp personliga mål och inför varje kurstillfälle hade de hemläxa där de skulle läsa delar ur boken ”Hjärnkoll på vikten” av Martin Ingvar och Gunilla Eldh. Den andra hälften av kvinnorna

(kontrollgruppen) fick standardbehandling med enkla livsstilsråd, liknande de som ges på en vanlig gynekologimottagning. Efter 4 månader fick kontrollgruppen också genomgå den strukturerade kursen i 4 månader. Alla kvinnor undersöktes i början av studien, vid fyra månader och vid 12 månader.

Resultaten från vår studie visade förbättringar i flera PCOS-symptom, både vid 4 månader och vid 12 månader. Trots att kvinnorna bara gick ned ett par procent i vikt så förbättrades många andra symptom. Menstruationerna blev mer regelbundna, insulinresistensen, vilken är kopplad till diabetes, förbättrades, midjemåttet minskade och nivåerna av flera inflammationsmarkörer i blodet sjönk. Vi såg till exempel att markörer relaterade till inflammation i hjärnan förbättrades efter interventionen.

Vi undersökte också livskvalitén hos alla kvinnor med PCOS i början av studien och fann att den var mycket lägre än vad vi skulle ha förväntat oss hos en generell grupp med kvinnor utan PCOS. Vissa aspekter av livskvalitén förbättrades dock efter kvinnorna genomgått behandlingen. Vi jämförde också sömnen hos kvinnorna med PCOS med en grupp friska kvinnor utan PCOS men med liknande BMI och ålder och såg att kvinnorna med PCOS hade normala sömnmängder men sämre sömneffektivitet än de friska kvinnorna.

Hittills känner vi inte riktigt till alla effekter av livsstilsintervention på PCOS-symptom. Resultaten från den här studien är viktiga då de stödjer slutsatsen att livsstilsintervention kan ha en positiv effekt på många aspekter av PCOS. Baserat på resultaten från vår studie föreslår vi att livsstilsintervention med beteendeförändring är en behandling som passar bra framförallt för kvinnor med PCOS som försöker bli gravida utan att lyckas men som har ett för högt BMI för att kunna genomgå fertilitetsbehandling.

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ABSTRACT

Lifestyle intervention is the first line treatment for many symptoms in women with polycystic ovary syndrome (PCOS). However, the efficacy of this treatment in comparison to minimal intervention for improving aspects such menstrual function is still unclear.

The objective of this study was to assess the efficacy of a behavioral modification intervention in comparison to minimal intervention on a number of parameters, such as menstrual function, body weight and composition, endocrine- and metabolic-variables, psychological well-being, objectively measured sleep variables, as well as inflammatory proteins in over-weight/obese women with PCOS. Furthermore, we wanted to compare the sleep in our PCOS population with healthy non-PCOS controls but with a similar age and body weight.

We designed a randomized controlled trial where 68 over-weight/obese women, fulfilling all Rotterdam PCOS diagnostic criteria were randomized on a 1:1 ratio to 4 months of either behavioral modification intervention (intervention) or minimal intervention (control treatment), with a further assessment at 12 months. All participants were aged 18-40 years with a BMI $\geq$ 27 kg/m^2.

Following the 4-month intervention, we found that a higher proportion of women having received the intervention improved menstrual function compared to control treatment, mean difference 35% (95% CI:16-60), P = 0.003. In addition, the weight loss in the intervention group was small but significant (-2.1%, P = 0.002), however we found no difference between the groups. At the 12-month follow-up, we found within group improvements in menstrual function, ovulation, body weight, biochemical hyperandrogenism, insulin resistance and blood lipids. Furthermore, the over-weight/obese women with POCS had a severely impacted psychological well-being compared to that of a general population. At baseline 60% of the women had a global well-being score corresponding to severe distress and 40% to moderate distress. However, some aspects of well-being improved following intervention (reduced levels of anxiety, P = 0.035, increased general health P = 0.012 and less depressed mood P=0.033). In terms of sleep, we demonstrated that the sleep duration for the women with PCOS (7.2 hours) was within the normal range, but that the amount of sleep was shorter and the sleep efficiency lower than for the healthy controls (P = 0.049 and P <0.001, respectively). Furthermore, the intervention appeared to reduce the amount of daytime sleep. At the 12-month follow-up, we also found improvements in a number of inflammatory proteins including proteins associated with neurodegeneration, autophagy and atherogenesis.

In conclusion, we believe behavioral modification intervention is a useful tool to improve menstrual function as well as other POCS symptoms in over-weight/obese women with PCOS, in particular where fertility is the key concern.

LIST OF SCIENTIFIC PAPERS

I. Oberg E*, Gidlöf S*, Jakson I, Mitsell M, Tollet Egnell P, Hirschberg AL. **Improved menstrual function in obese women with PCOS after behavioral modification intervention – A randomized controlled trial.** *Clinical Endocrinology. 2019;00:1-11*

II. Oberg E*, Lundell C*, Blomberg L, Gidlöf SB, Egnell PT, Hirschberg AL. **Psychological well-being and personality in relation to weight loss following behavioral modification intervention in obese women with polycystic ovary syndrome: a randomized controlled trial.** *European Journal of Endocrinology.* 2020 Jul;183(1):1-11.

III. Oberg E, Blomberg L, Åkerstedt T, Hirschberg AL.
Different sleep pattern in over-weight/obese women with polycystic ovary syndrome.
Submitted manuscript

IV. Oberg E*, Gunn H*, Alvarsson M, Viner R, Hällqvist J, Heywood W, Mills K, Brismar K, Hirschberg AL.
Markers of inflammation in overweight women with PCOS following behavioral modification intervention.
Manuscript

*Shared first author.

CONTENTS

List of abbreviations

A	Amenorrhea
ACN	Acetonitrile
BL	Baseline
BMI	Body mass index
CEIA	Chemiluminescence enzyme immunoassay
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
DEXA	Dual energy X-ray absorptiometry
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DKK3	Dickkopf-related protein 3
DNA	Deoxyribonucleic acid
DTE	Dithioerythritol
ECLIA	Electrochemiluminescence immunoassay
FAI	Free androgen index
FFA	Free fatty acids
FSH	Follicle stimulating hormone
GCP	Good clinical practice
GWAS	Genome wide-association study
HDL	High density lipoprotein
HOMA	Homeostatic model of assessment
HRQoL	Health related quality of life
IL	Interleukin
ITT	Intention to treat
IQR	Interquartile range
IVF	In-vitro fertilization
KI	Karolinska Institutet
KPS	Karolinska university scales of personality
LC-MS/MS	Liquid chromatography-tandem mass spectrometry

LDL	Low density lipoprotein
LH	Luteinizing hormone
MIEN1	Migration and invasion enhancer 1
MF	Mindfulness
mFG	Modified Ferriman-Gallwey score
MRM	Multiple reaction monitoring
n	Number
NfL	Neurofilament light polypeptide
Nrp1	Neurophilin-1
NIH	National institutes of health
OM	Oligomenorrhea
PCOM	Polycystic ovarian morphology
PCOS	Polycystic ovary syndrome
PGWBI	Psychological general well being index
PGRN	Progranulin
RCT	Rrandomized controlled trial
RM	Regular menstruation
RNA	Ribonucleic acid
SAA	Serum amyloid A-1 protein
SD	Standard deviation
SHBG	Sex hormone-binding globulin
SPE	Solid phase extraction
SSP	Swedish universities scales of personality
SOAL	Stop, observe, accept, let go
T2DM	Type 2 diabetes mellitus
TCI	Temperament and character inventory
TFA	Trifluoroaceitic acid
TFEB	Transcription factor EB
TG	Triglycerides
TGWB	Global psychological well-being score
TIB	Time in bed

TNF- α	Tumor necrosis factor - α
ToR	Time of rising
TST	Total sleep time
WASO	Wakefulness after sleepp onset
WHR	Waist hip ratio

1 BACKGROUND

1.1 PCOS DIAGNOSIS IN A HISTORICAL CONTEXT

Polycystic Ovary Syndrome (PCOS) is a multifactorial syndrome characterized by hyperandrogenism, menstrual disturbances and polycystic ovarian morphology. Clinical manifestations of increased androgens include hirsutism and acne and many women with PCOS suffer from reduced fertility.[1]

What is now known as PCOS was in 1935 described by Stein and Leventhal as women with enlarged, polycystic ovaries associated with menstrual irregularity, infertility, hirsutism and obesity, then named the Stein-Leventhal syndrome.[2] Subsequently, over the years three slightly different definitions of PCOS have emerged as outlined in Table 1, reflecting different thoughts on the pathogenesis of the syndrome. All definitions are based on expert opinion and require the exclusion of other androgen excess conditions including androgen-producing tumors, non-classical adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia and thyroid diseases, before diagnosis is made. The definition recommended by the most recent international guidelines on PCOS is the Rotterdam Consensus from 2003: which requires at least two out of three of the following: oligomenorrhea or amenorrhea; clinical or biochemical hyperandrogenism; and displaying polycystic ovaries on a transvaginal ultrasound scan.[3,4] Polycystic ovarian morphology (PCOM) is defined as either having an ovarian volume of at least 10 ml, or an increased number of antral follicles present in the ovary. The exact number necessary for diagnosis depends on the frequency bandwidth of the ultrasound but should be at least 12.[3,4] The National Institutes of Health's (NIH) PCOS criteria from 1990 was the first attempt to define PCOS clinically and is still often used. It requires having both clinical and/or biochemical hyperandrogenism as well as menstrual dysfunction.[1,5] The androgen excess and PCOS (AE-PCOS) society guidelines from 2006 requires clinical or biochemical hyperandrogenism as well as either one or both of polycystic ovaries on ultrasound and oligo- or anovulation.[6]

PCOS was previously regarded as a gynecological and endocrine condition, however in recent years several other aspects have been recognized and it is now considered a multi-systems disorder with comorbidities not only affecting women of a fertile age but instead being a lifelong condition.[7,8]

With a lifetime perspective, PCOS often presents in adolescence with early menarche, menstrual disturbances, hirsutism, persistent acne, excess weight as well as psychological co-morbidities such as depression, anxiety, lower self-esteem and dissatisfied body perception.[9,10] For adult women, infertility and hirsutism are the most common presenting complaints, other aspects include gaining excess weight, abdominal obesity and insulin resistance. Furthermore pregnancy complications and psychological co-morbidities such as anxiety, depression and reduced health related well-being are common.[10-12] Women with PCOS often experience a later menopause.[10] At peri- and post-menopause, abdominal obesity, type 2 diabetes mellitus

(T2DM), addressing cardiovascular risk factors and an increased risk of endometrial carcinoma become key areas of concern.[10]

Table 1: Different diagnostic criteria for PCOS and the resulting phenotypes

Phenotype	Rotterdam Consensus (2003, 2018)				National Institute of Health (1990)	Androgen Excess Society (2006)		
	A	B	C	D				
Hyper-androgenism	√	√	√		√	√	√	√
Oligo- or amenorrhea	√	√		√	√	√		√
Polycystic ovaries	√		√	√			√	√

A tick in the box, √, represents the PCOS phenotypes. Development of diagram from *Day et al* (2018).[13]

1.2 PCOS PHENOTYPES

PCOS is a heterogeneous syndrome and attempts have been made to group the condition into subtypes. Four different phenotypes have been suggested based on the Rotterdam criteria of clinical presentation and risk profile (Table 1).[1,6,14,15] Phenotype A is the classic and most severe type, with all three diagnostic criteria fulfilled (hyperandrogenism, chronic anovulation and polycystic ovaries), it is also associated with insulin resistance and often lipid alterations.[1] Phenotype B is characterized by hyperandrogenism and chronic anovulation, but with normal ovaries, it is strongly associated with obesity and lipid alterations and often with insulin resistance.[1] Phenotype C is ovulatory and diagnosed by hyperandrogenism and polycystic ovaries, it is associated with lipid alterations and sometimes with insulin resistance.[1] Phenotype D is normoandrogenic and diagnosed by chronic anovulation and polycystic ovaries, it is not characterized by obesity, insulin resistance or lipid alterations.[1]

1.3 EPIDEMIOLOGY

PCOS is the most common endocrinopathy in women of a fertile age with a prevalence between 8% to 13% depending on diagnostic criteria used and population studied, furthermore, among over-weight women the prevalence is as high as one in three.[16-19] The prevalence has increased with the use of the Rotterdam consensus for diagnosis but community studies of the prevalence of PCOS highlight that many women with the condition remain undiagnosed.[16,20]

1.4 PATHOPHYSIOLOGY

Hyperandrogenism and insulin resistance often in combination with, and exacerbated by, obesity, are the key factors underpinning the clinical manifestations of PCOS (Figure 1). In terms of the ovary, women with PCOS have been found to have an intrinsic dysfunction of the theca cells, that leads to an excessive androgen production and overexpression of luteinizing hormone (LH) receptors independent of endocrine and paracrine regulation.[1,5] In addition, a dysfunction of the granulosa cells in the ovary can also contribute to the androgen excess

through the production of paracrine factors such as Inhibin-B.[1] There is also a continuous production of estradiol leading to a negative feedback on the pituitary gland, and a suppression of follicle stimulating hormone (FSH) secretion that together with the overexpression of LH receptors results in a raised LH/FSH ratio.[1] Increased levels of androgens enhance the follicle recruitment resulting in a large amount of small antral follicles, and initiates premature luteinization which prevents ovulation leading to oligo- or amenorrhea. Androgens also causes the typical macroscopic features of polycystic ovaries of increased stroma, cortical thickening and increased number of small antral follicles.[1] In addition, androgens facilitate the development of abdominal, particularly visceral, obesity which is common in women with PCOS and contributes to the development of insulin resistance by stimulating lipolysis which increases the free fatty acids in the blood.[21,22] The effects of androgen excess on relevant tissues are outlined in Table 2. Other symptoms resulting from hyperandrogenism are hirsutism, male pattern baldness and acne.[1]

Table 2: Effects of hyperinsulinemia and androgen excess on relevant tissues in PCOS

Tissue type	Effect of hyperinsulinemia	Effect of androgen excess
Ovary	- ↑ ovarian responsiveness to LH → increased testosterone - Premature luteinization→ hinders ovulation - Augmentation of LH action - Direct stimulation of androgen synthesis	- Enhances recruitment of primordial follicles → growth of small antral follicles - Initiates premature luteinization → hinders ovulation - ↑ stroma, cortical thickening, ↑ number of small antral follicles
Liver	- Inhibition of SHBG synthesis	- Inhibition of SHBG synthesis - Facilitates lipolysis → exposes liver to high flux of FFA → hepatic insulin resistance
Visceral fat	- Stimulation of adipogenesis and lipogenesis	- Visceral fat accumulation - Insulin resistance - Stimulates lipolysis
Adrenal gland	- Direct stimulation of androgen synthesis	

Abbreviations: FFA free fatty acids; LH luteinizing hormone; SHBG sex hormone-binding globulin

Furthermore, women with PCOS, independently of body weight, are thought to have a post receptor defect in insulin signaling, causing insulin resistance that disturbs metabolic but not mitogenic functions in tissues such as skeletal muscles, adipose tissue and the ovaries which results in hyperinsulinemia.[1,23,24] This excess of insulin causes an increased androgen production and overexpression of LH receptors in the ovarian theca cells as well as an increased androgen production from the adrenal gland.[1] Both insulin and androgens inhibit sex hormone-binding globulin (SHBG) synthesis and secretion in the liver, resulting in higher levels of bioavailable testosterone.[25] Insulin also stimulates adipogenesis and lipogenesis contributing to the development of predominantly abdominal obesity predisposing women with PCOS to T2DM and hyperlipidemia.[1,23] The effects of hyperinsulinemia on relevant tissues is PCOS are outlined in Table 2.

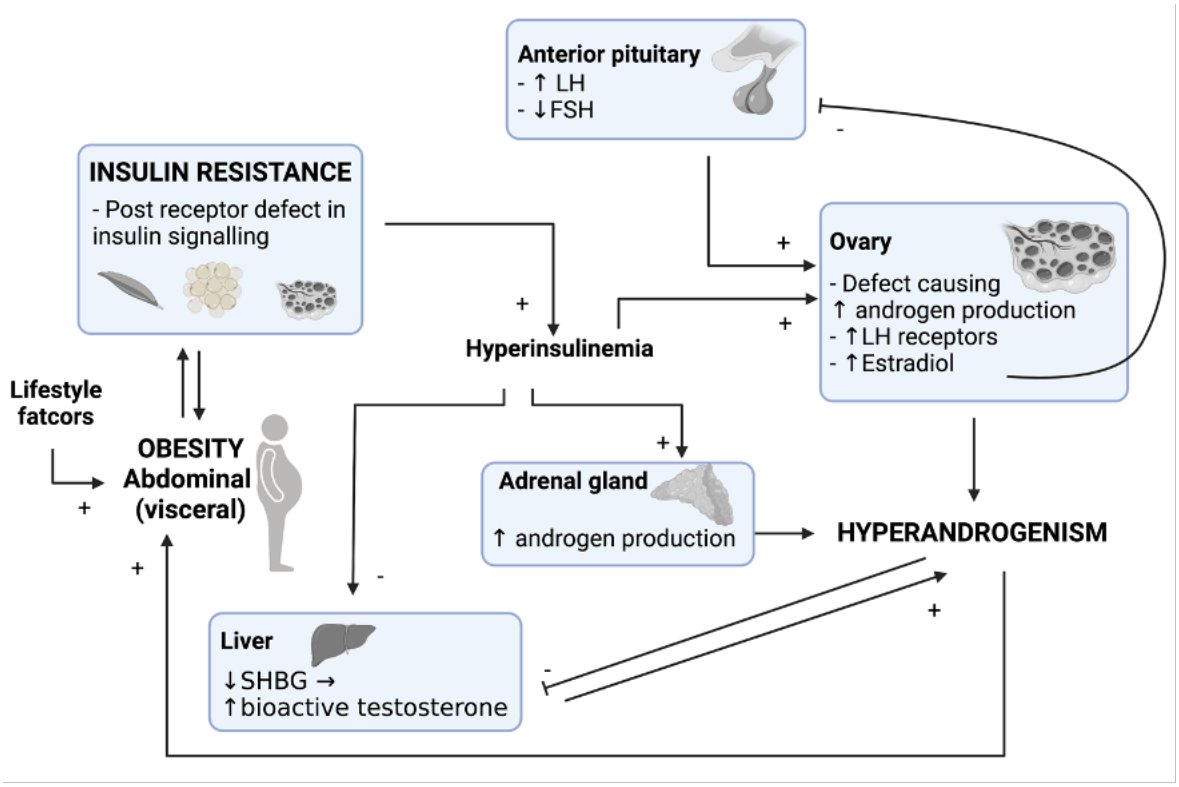

Figure 1: Schematic illustrating the relationship between hyperandrogenism, insulin resistance and obesity in the pathogenesis of PCOS. Figure created with BioRender.com

1.5 ETIOLOGY

1.5.1 Genetics of PCOS

There is a clear genetic component in the etiology of PCOS however no single gene has been identified. Familial studies looking at the inheritance of PCOS traits in first-degree relatives found a prevalence of 35-67%.[26] A large Dutch study found the heritability of PCOS to be as high as 70% in monozygotic twin sisters.[27]

In a recent meta-analysis of several Genome Wide Association Studies (GWAS), 14 independent loci significantly associated with PCOS were found.[13] In GWAS studies, regions on the chromosomes, loci, are identified, not specific genes. The majority of the suggested candidate genes at the loci identified by the GWAS studies were related to insulin resistance, hormones and organ growth.[26]

The inheritance of PCOS seems to be similar to other common complex disorders such as T2DM, with a number of genetic variations contributing together with environmental and life style factors.[28]

1.5.2 Prenatal exposure

Animal studies have shown that rodents, monkeys and sheep exposed to supraphysiological doses of androgens in utero through maternal exposure to androgens developed a PCOS phenotype.[29] Evidence from humans with congenital adrenal hyperplasia, who have increased adrenal production of androgens during fetal life, show an association with PCOM.[30] It has therefore been suggested that perinatal androgen exposure in humans from maternal hyperandrogenism could be a possible cause of PCOS. However, one study showed no increased androgen concentrations in umbilical cord blood from girls born to mothers with PCOS.[31] Another study found no correlation between maternal androgen levels at mid and late pregnancy or cord blood androgen concentration and the development of PCOS in adolescence.[32]

Low birth weight has also been associated with the development of PCOS where studies have shown that intrauterine growth restriction, with a weight catch-up during childhood as well as a high birth weight where the infant continue to increase in weight after birth, are both associated with premature pubarche, and subsequently with hyperinsulinemia and hyperandrogenism in adolescence.[33,34]

1.5.3 Obesity

It is well known that PCOS is associated with obesity in many but not all women with PCOS.[35] There is emerging thinking that in some women, PCOS arises secondary to obesity, as adipose tissue is considered to be an endocrine organ in itself that generates androgens. In addition, weight gain leads to insulin resistance which increases insulin production and causes reduced synthesis of SHBG in the liver, which increases the amounts of bioavailable testosterone as discussed above. Furthermore, in most but not all adult women, the PCOS phenotype disappears after substantial weight loss through either lifestyle changes or bariatric surgery.[1,36,37] This could potentially make obesity in itself a cause of anovulation and hyperandrogenemia in some women with PCOS.[1]

Obesity is often the symptom many women with PCOS suffer from the most and it drives the development of many of the other clinical features, making it a key aspect to address. It may be that obesity in itself is not the cause of PCOS but rather it can tip a genetically pre-disposed woman into the syndrome and worsen many of the symptoms in a vicious circle.[35]

In summary, the etiology of PCOS seems to be multifactorial including pre- and post-natal environmental components as well as and genetic and epigenetic factors.

1.6 CLINICAL MANIFESTATIONS OF PCOS

1.6.1 Gynecological and obstetric aspects of PCOS

1.6.1.1 Oligo- and anovulation

Oligo- or anovulation is one of the diagnostic criteria in PCOS and conversely, PCOS is the most common cause of anovulation in women of reproductive age.[3,17] In PCOS, local action of excess androgens within the ovary together with excessive LH secretion and insulin are thought to cause premature arrest of follicles. Furthermore, androgens contribute to aberrant development of the pre-antral follicles.[38] These effects result in absent or infrequent ovulation and therefore oligo- or amenorrhea. Excess androgens are also responsible for the typical macroscopic features of a polycystic ovary with increased cortical thickness, increased stroma and increased number of antral follicles.[1]

1.6.1.2 Fertility

Women with PCOS have reduced fertility compared to matched controls. A recent Swedish study found similar cumulative probabilities of a first childbirth in women without and with PCOS, however the PCOS women gave birth to fewer children and had a much lower probability of giving birth to a child conceived spontaneously than women without PCOS.[39] However, over time the cumulative probability of childbirth was similar between women with and without PCOS.[39] Anovulatory infertility is a main contributor to the lower fertility rates, however other factors are thought to contribute as well. The oocyte quality in women with PCOS has been found to be reduced because of differences in meiotic processes, increased oxidative stress in the follicles, higher levels of inflammation in the follicular fluid, aberrant expression of biomolecules with regular functions in oocyte development and altered oocyte glucose metabolism.[40] Furthermore, the endometrium of women with PCOS has been found to have primary abnormalities in glucose metabolism and in the expression of hormone receptors and proteins related to cell stress protection, as well as displaying an immune pattern typical of chronic low grade inflammation.[40]

1.6.1.3 Pregnancy complications

Women with PCOS have an increased risk of miscarriage, pre-term birth and stillbirth.[41-43] Furthermore, a recent systematic review and meta-analysis found that women with PCOS have an increased risk of the pregnancy complications of gestational diabetes mellitus, gestational hypertension and pre-eclampsia and that these increased risks were independent of obesity.[41] However, normo-androgenic women with PCOS did not have this increased risk profile (phenotype D).[41]

1.6.1.4 Endometrial pathologies

Women with PCOS have a four-fold increase of developing endometrial cancer compared to women without PCOS, even at a young age.[44] Reasons for this are thought to be the persistent progesterone deficiency associated with anovulation that maintains the endometrium in a

constant proliferative state.[44] Furthermore, obesity and insulin resistance, both common in women with PCOS, are also independent risk factors for developing endometrial hyperplasia and carcinoma.[44]

1.6.2 Dermatological symptoms

Clinical hyperandrogenism is one of the diagnostic criteria for PCOS and the most common sign of this is hirsutism, which is the excess growth of dark, coarse hair in a male-like pattern such as in the face, chest, abdomen and back.[17] Acne and alopecia are other clinical manifestations of hyperandrogenism.[17]

1.6.3 Metabolic consequences

PCOS is associated with metabolic complications such as accumulation of abdominal fat, insulin resistance, hyperinsulinemia and T2DM.[23,45] The increased insulin resistance is independent of body mass index (BMI) and caused by a post receptor defect in insulin signaling as outlined above.[1,23,24] The above are all risk factors for developing cardiovascular disease (CVD) at a later stage in life. In terms of the risk of developing CVD in PCOS, the evidence is limited and the results varied.[46] A recent meta-analysis looking at the difference between PCOS and non-PCOS groups found no significant difference in the risk of myocardial infarction, stroke and coronary artery/heart disease between the groups.[4] However, the studies included in the meta-analysis were small, and the women studied were relatively young, whilst CVD-related events usually occur later on in life.

1.6.4 Inflammation

In recent years PCOS has been recognized a condition associated with chronic, low-grade inflammation.[22,47-51] Low-grade inflammation is characterized by increased levels of inflammatory mediators such as tumor necrosis factor-α (TNF-α), Interleukin 6 and C-reactive protein (CRP), mediators that we know are raised in women with PCOS.[22] The mainly visceral accumulation of fat that is often present in PCOS is at least partly thought to cause this chronic inflammatory state as adipose tissue produces inflammatory molecules (cytokines, chemokines and adipokines) that act as mediators of systemic inflammation. However, the intrinsic insulin resistance present in PCOS as well as the influence androgens have on the development and distribution of fat tissue could also contribute to the development of a chronic low-grade inflammatory state in PCOS.[22]

1.6.5 Psychological implications

PCOS appears to be associated with psychological symptoms. A recent meta-analysis of GWAS studies based on 10 000 women with PCOS and 100 000 controls, showed shared genetic traits with depression.[13] Symptoms of moderate and severe depression are more common in women with PCOS compared with controls, independently of body weight.[11] Symptoms of anxiety are higher in women with PCOS compared to controls.[12] The Health related Quality of Life (HRQoL), which relates to the physical, social, and emotional effects of the disease and its associated treatments in women with PCOS also seems to be negatively

affected.[7] One meta-analysis found that the dimensions of Quality of Life mainly affected in PCOS were hirsutism and menstrual dysfunction whilst a more recent systematic review found that infertility and weight concerns affected the HRQoL the most.[7,52] In addition, a recent study showed that women with PCOS have increased odds of having a personality disorder, schizophrenia, bipolar disorder and a 40% increased risk of suicide attempts.[53]

Anxiety and emotional distress could at least partly be linked to the stigmatizing clinical features of PCOS such as hirsutism, obesity and infertility.[54] However, emotional distress has also been associated with increased levels of androgens, low-grade inflammation, diabetes and the metabolic syndrome.[54]

1.7 MANAGEMENT OF PCOS

Treatment of PCOS aims to address weight management, improve reproductive function, counteract clinical signs of hyperandrogenism such as hirsutism, and to manage or prevent metabolic complications.[4] A reduction in weight can improve all symptoms of PCOS and the latest international guidelines on PCOS from 2018 recommend lifestyle intervention through dietary modifications, exercise, behavioral modification or a combination of these as the first line treatment for PCOS symptoms.[4] A combination of lifestyle intervention modalities appears to be more effective than monotherapy.[55] A recent Cochrane review from 2019 confirmed that lifestyle intervention in comparison to minimal intervention may improve the free androgen index (FAI), weight and BMI, reduce abdominal obesity, and improve the blood lipid profile as well as insulin resistance and also improve quality of life.[56] There was however insufficient evidence for the review to assess the efficacy of lifestyle intervention on fertility outcomes.[56] Another recent systematic review and meta analysis from 2022 investigating the effects of lifestyle intevention on PCOS symptoms, found that lifestyle intevention improved reproductive function in terms of menstrual function and ovulation in incomparison to control treatment.[55]

Other standard treatment options for PCOS include combined oral contraceptives used to regularize the menses, reduce the risk for endometrial cancer and improve hyperandrogenism and therefore hirsutism and acne; insulin sensitizers e.g. metformin that can improve metabolic variables as well as fertility; and anti-androgens e.g. spironoloactone, finasteride and cyproterone acetate that can alleviate the symptoms of hyperandrogenism.[46] Where fertility is the main concern, assisted reproduction treatments could be needed.[46] Long term weight loss can also be achieved through bariatric surgery and there have been suggestions of including PCOS as an indication for surgery.[57,58] However, bariatric surgery results in an increased risk of pregnancy complications including small for gestational age, possibly neonatal mortality and it increases the risk substantially of having to undergo abdominal surgery during pregnancy.[58-60] Concerning psychiatric aspects of PCOS, the most recent international guidelines recommend that screening for emotional wellbeing should be carried out when diagnosing the condition, and if needed referral should be made to suitable qualified health professionals.[4] Inositol, a food supplement sold over the counter in many countries has been

shown in some studies to have a insulin-sensitising effect in PCOS.[61] However, the latest international guidelines on the treatment of PCOS from 2018 considers Inositol an experimental treatment in PCOS and suggests further research intro the use of the supplement.[4] Other experimental pharmacological treatments that are being investigated in POCS are glucagon-like peptide-1 (GLP-1) receptor agonists, which are antidiabetic drugs mimicing the actions of the gut hormone incretin and reduce hyperglycemia and body fat.[62]

Only a small number of studies have looked specifically at behavioral modification as a component of lifestyle management in PCOS with variable results.[63-68] Evidence from other areas such as from programs promoting dietary and lifestyle changes to prevent T2DM shows that behavioral modification techniques (e.g. goal-setting and self-monitoring) are beneficial.[69]

1.8 PERSONALITY TRAITS PREDICTING WEIGHT LOSS

Despite lifestyle intervention being the first line treatment in PCOS, achieving substantial weight loss in this population is challenging. Identifying the patients benefiting the most from lifestyle intervention would be useful. Some attempts at identifying patients susceptible to lifestyle intervention for weight loss purposes in other patient populations have been made through assessing the patients' personality prior to intervention with varying results.[70-73] Personality traits are features which influence mood, thoughts and behavior and are thought to be stable over time.[70]

Various tools have been used to assess personality in the literature. The Swedish Universities Scales of Personality (SSP), the tool used in this study, is a modernized and simplified version of the Karolinska University Scales of Personality (KPS). Some of the other commonly used scales for assessing personality are the Minnesota Multiphasic Inventory and the Temperament and Character Inventory (TCI).[71]

1.9 OMICS AS A TOOL TO UNDERSTAND THE PATHOPHYSIOLOGY IN PCOS

As discussed in previous sections there are still many unanswered questions around the processes underlying the heterogenous multifactorial syndrome of PCOS although we know that factors such as genetics, environmental influences and lifestyle choices all are involved.[74] In the last decade, the application of the high throughput technologies of omics, including genomics, transcriptomics, proteomics and metabolomics, have increasingly been used to better understand these processes.[75] Genomics, studying the gene expression, involving GWAS have identified a number of candidate genes associated with PCOS susceptibility, in particular genes related to ovarian and adrenal steroid hormone production, and to the response to gonadotrophins, androgens and insulin.[76] The genome directs the production of proteins using enzymes and messenger molecules and transcriptomics involves studying the presence and amounts of messenger RNA and RNA molecules.[75] Proteomics and metabolomics involve

looking at the last steps of the process where information flows from DNA to proteins and metabolites as outlined in Figure 2.[74]

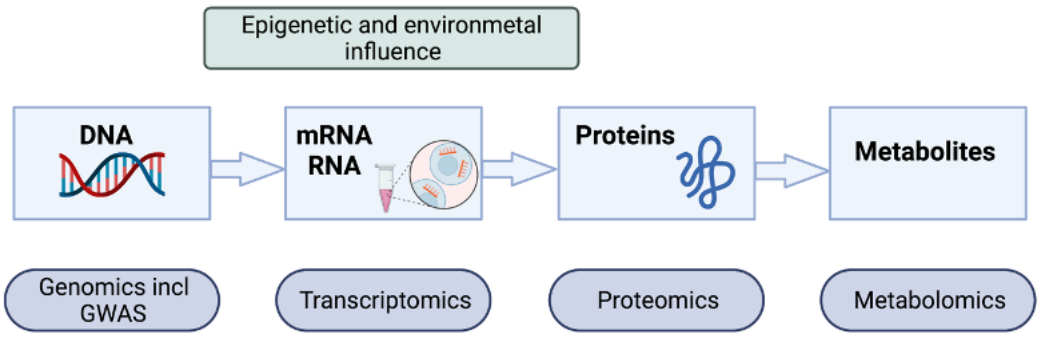

Figure 2: Diagram outlining the process of information flow from DNA, via transcription of genes into mRNA through to the synthesis of proteins and metabolites, including the technique used to study each step. Adapted from Insenser *et al* (2013).[74]
Abbreviations: GWAS genome wide association studies; m messenger; RNA ribonucleic acid.
Figure created with BioRender.com

1.9.1 Proteomics

The proteome includes all proteins expressed by the genome, it is dynamic and reflects environmental factors and as well as epigenetics.[77] Proteomics involve the analysis of the proteome and can be used to help us understand disease processes, identify drug targets to treat disease and to find diagnostic-markers associated with a disease.[77]

Non-targeted proteomics, which enables the identification of all proteins present in a certain cell or tissue, allows for the identification of differently expressed proteins in a PCOS population compared to controls without PCOS.[74] Non-targeted proteomic analysis on ovarian and adipose tissues, follicular fluid as well as different blood components has revealed a number of proteins differently express in women with PCOS in areas that affect the metabolism of lipids and glucose, the immune response including inflammation, the thrombotic and fibrinolytic systems as well as proteins related to oxidative stress.[74,78-85]

Based on the findings from non-targeted proteomic analysis as well as pre-existing information from inflammatory pathways, a targeted proteomics panel of proteins to be analyzed can be developed. A benefit of targeted proteomics is that a large number of relevant proteins can be identified without the need for using expensive antibody assays, making the analysis ideal for investigating less well-known pathways.[86]

1.10 KNOWLEDGE GAPS

At the time of initiating this randomized controlled trial (RCT) in 2012, evidence from assessing the efficacy of lifestyle intervention compared to minimal intervention on improving a number of clinical symptoms in overweight/obese women with PCOS, was limited. Only three previous RCTs comparing lifestyle intervention with minimal intervention in women with PCOS had reported on weight.[63,64,87] In terms of menstrual regularity following lifestyle intervention compared to minimal intervention four studies had reported on this. [63,64,87,88] In a Cochrane review on lifestyle changes in women with PCOS from 2011 it was concluded that there existed no literature assessing the effect of lifestyle intervention on quality of life in this patient group, furthermore the review called for more quality research on the effects, including the long-term ones, of lifestyle intervention, specifically in the areas of menstrual function, fertility outcomes as well as blood lipids and glucose levels.[89] The knowledge, particularly around fertility in women with PCOS, has improved to a great extent recently however, the issue around managing the weight still constitutes a challenge.

The clinical impression is that there is a large group of overweight women with PCOS, predominantly phenotype A (Table 1), that have struggled to achieve sustainable, long term weight loss, despite having tried several lifestyle management programs focusing on diet and exercise. These women have therefore missed out on the improvements in other PCOS symptoms that weight loss can result in. In addition, the current consensus in Sweden and elsewhere, is that no fertility treatment should be offered to women with a BMI > 35 or in some parts of the country BMI >30, leaving many obese women with PCOS unable to access assisted reproduction.[90,91] The reason for this recommendation around BMI is the negative effects obesity has on fertility with increased miscarriage rates and also increased risks of obstetric complications for both the mother and the child.[92]

1.11 HYPOTHESIS

This RCT was designed, as one of the first in its field, to assess the efficacy of lifestyle intervention, specifically our proposed intervention "behavioral modification intervention", on improving key clinical manifestations of PCOS compared to standard treatment. Evidence from other fields has shown that introducing a behavioral modification component to lifestyle intervention could yield good, long lasting results for maintaining weight loss in other patient populations.[93] We hypothesized that behavioral modification intervention would improve the clinical manifestations of PCOS.

2 RESEARCH AIMS

2.1 GENERAL AIMS

The overall aim of this thesis was to study the effects of behavioral modification intervention on reproductive function, metabolic parameters, inflammation, psychological well-being and sleep quality in overweight/obese women with PCOS in a RCT.

2.2 STUDY SPECIFIC AIMS

Study I: Evaluate effects of a behavioral modification program on reproductive outcomes through weight loss in over-weight/obese women with PCOS.

Study II: Assess psychological wellbeing in over-weight/obese women with PCOS and investigate the effects on wellbeing following a behavioral modification program.

Study III: Compare sleep variables in over-weight/obese women with PCOS with healthy controls and assess the effect of behavioral modification on sleep in women with PCOS.

Study IV: To assess the effect of behavioral modification intervention on a large panel of inflammatory proteins in over-weight/obese women with PCOS.

3 MATERIALS AND METHODS

3.1 OVERVIEW OF STUDIES

This thesis includes four studies. Table 3 shows a summary of subjects included, study design, interventions and methods used for each study.

Table 3: Tabulated overview of materials and methods of all four studies

Study	Participants	Design	Intervention	Methods	Analyses
I	68 women with PCOS and BMI ≥ 27 kg/m^2	RCT	4-months: - behavioral modification intervention - control treatment	Menstrual diaries/app LC-MS/MS ECLIA CEIA DEXA	Menstrual function Steroid hormones Progesterone Other hormones & binding proteins Body composition
II	68 women with PCOS and BMI ≥ 27 kg/m^2	RCT	4-months: - behavioral modification intervention - control treatment	PGWBI SSP LC-MS/MS CEIA	Well-being Personality Steroid hormones Other hormones & binding proteins
III	- 39 women with PCOS and BMI ≥ 27 kg/m^2 - 21 age- and weight-matched healthy controls	- RCT - separate healthy control group	4-months: - behavioral modification intervention - control treatment	Actigraphy LC-MS/MS CEIA	Sleep variables Steroid hormones Other hormones & binding proteins
IV	42 women with PCOS and BMI ≥ 27 kg/m^2	RCT	4-months: - behavioral modification intervention - control treatment	Targeted Proteomics LC-MS/MS CEIA	96 pro- and anti-inflammatory proteins Steroid hormones Other hormones & binding proteins

Abbreviations: BMI body mass index; CEIA chemiluminescence enzyme immunoassay; DEXA dual energy X-ray absorptiometry; ECLIA electrochemiluminescence immunoassay; LC-MS/MS liquid chromatography-tandem mass spectrometry; RCT randomized controlled trial; PCOS Polycystic Ovary Syndrome; PGWBI psychological general well-being index; SSP Swedish universities scales of personality.

3.2 STUDY DESIGN

The study design was a RCT where 68 overweight/obese women with PCOS were randomized on a 1:1 ratio to intervention (behavioral modification intervention) or control treatment (minimal intervention). Neither participants nor investigators were blinded to the allocation due to the obvious differences in the two interventions.

The intervention lasted for 4 months following which the control group received 4 months of intervention. The patients were assessed at baseline, 4 months, as well as at 12 months when all study participants had received the intervention, albeit at different times. Figure 3 shows an outline of the RCT design including drop-outs.

In Study III, a group of 21 healthy controls without PCOS was introduced. This group received no intervention and was only assessed at baseline.

3.3 STUDY POPULATION AND ENROLMENT

The same cohort of 68 over-weight/obese women with PCOS, or a subset thereof, was used for all four studies included in the thesis. For Study III, a healthy control group (n=21) of a similar age and BMI as the women with PCOS was introduced. Written informed consent was obtained from all study participants. The study protocol was approved by the Regional Review Board of Ethics in Research in Stockholm (2012/146-31/3, 2012/1762-32, 2014/1406-32). The clinical trial registry number was ISRCTN48947168.

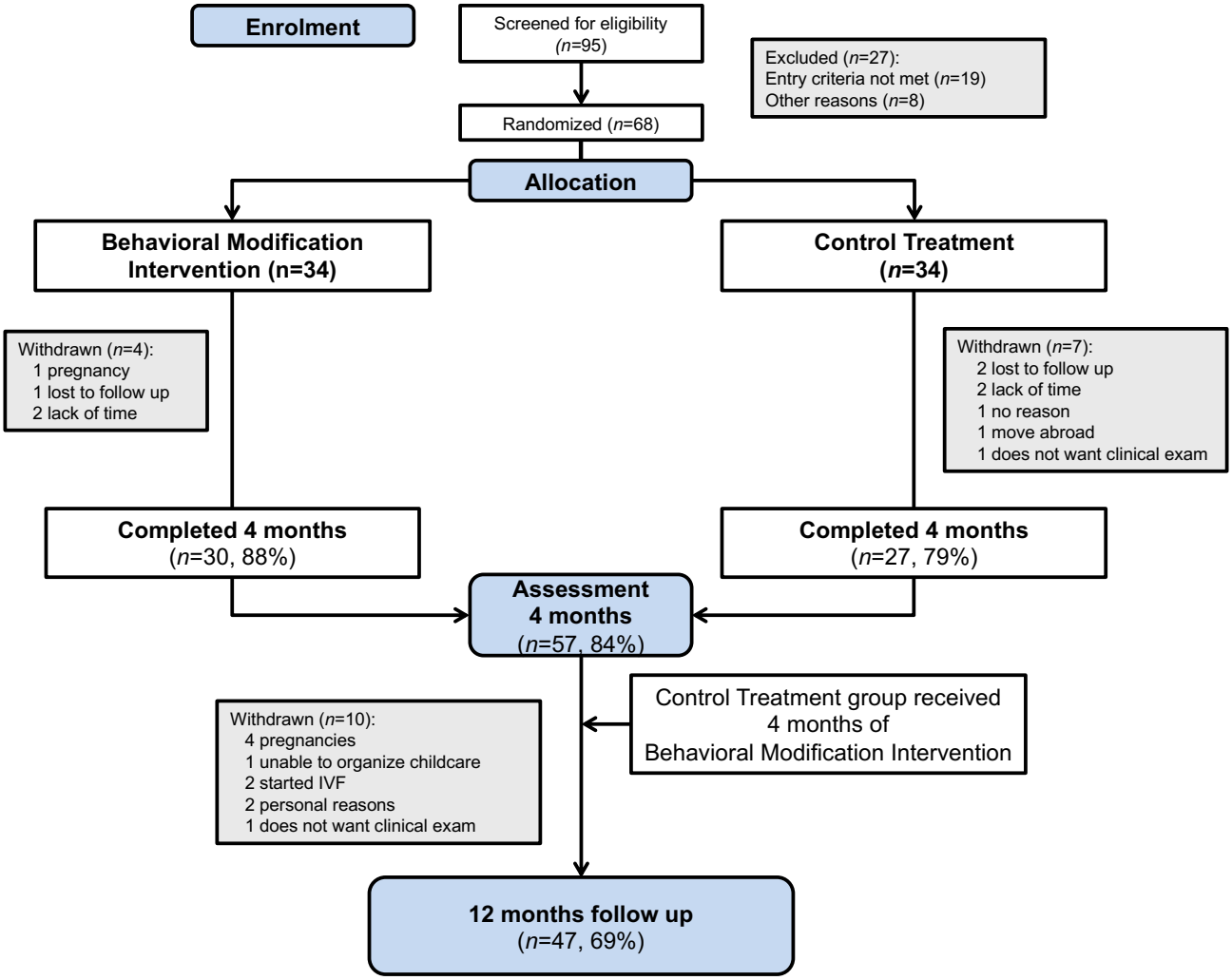

Figure 3: Flow diagram outlining the study design of the randomized controlled trial (Study I-IV)

3.3.1 PCOS population (Study I-IV)

Ninety-five women were assessed for eligibility to participate, and 68 women were entered into the trial. Table 4 outlines the entry and exclusion criteria for participating in the study. Recruitment was made through adverts in a local newspaper. The enrolment of study participants took place from 4 April 2012 to 23 January 2015.

In Study I, data from the whole cohort of 68 women was used. In Study II, psychological general well-being index (PGWBI) questionnaires from 12 women were missing resulting in 56 women being included in the well-being analysis. For the personality assessment using the SSP, parts of the questionnaire were incompletely filled in or missing for some participants resulting in 57 women being included in the analysis. In Study III, the sleep variable measurement using actigraphy was introduced part of the way through the study, resulting in 39 women with PCOS taking part in this analysis. For Study IV the technical constraints of using a 96-well-plate only allowing for the analysis of 96 samples, limited the targeted proteomics analysis to a subset of 42 women from the initial cohort, to be analyzed at baseline and at the 12-month follow-up, only 9 samples were analyzed.

Table 4: Entry- and exclusion criteria for study participants (Study I-IV)

Women with PCOS (Study I-IV)		**Healthy controls (Study III)**	
Entry criteria	**Exclusion criteria**	**Entry criteria**	**Exclusion criteria**
- Age 18-40 years - BMI ≥ 27 kg/m^2 - Fulfilling all 3 Rotterdam Consensus diagnostic criteria of PCOS[3]	- Having another chronic illness - Taking regular medication* - Current pregnancy - Breast-feeding - Smoking - Substantial weight change in the past year	- Age 18-40 years - BMI ≥ 27 kg/m^2 - Regular periods - Normal androgen levels - No previous PCOS diagnosis	- Having another chronic illness - Taking regular medication* - Current pregnancy - Breast-feeding - Smoking - Substantial weight change in the past year

*A three-month washout period was applied if using a hormonal contraceptive

3.3.2 Healthy controls (Study III)

In Study III, sleep variables were studied in the PCOS women before and after intervention. In this study, we also recruited healthy controls to enable comparison of sleep variables with the PCOS cohort at baseline. Twenty-six women were screened to take part as healthy controls in Study III, and 21 women entered the study. The entry and exclusion criteria are outlined in Table 4. Recruitment was made through adverts on a website for clinical studies and the enrolment took place from June 2020 to September 2021.

3.4 INTERVENTION

3.4.1 Behavioral modification intervention (intervention)

The behavioral modification intervention (intervention) involved a 4-month long intensive course designed to offer the participants a structured approach to achieve behavioral change resulting in long-term weight control with subsequent improvements in other parameters such as menstrual function, metabolic parameters and sleep behavior. The course was led by a lifestyle coach with a PhD in endocrinology and metabolism and was based on up-to-date knowledge on personal leadership, mindfulness, weight control as well as diet and physical activity with an aim help the participants to find motivation, learn to think in new ways and to start practicing new habits. It included weekly meetings in small groups with fellow study participants. The women had to prepare for each meeting through personal reflection, completing tasks and by reading chapters from the book "Hjärnkoll på vikten" (Ingvar *et al*, 2010).[94] Topics addressed in the meetings were personal leadership, goal setting, techniques to avoid instant gratification and the development of exercise and dietary plans. The study participants were also offered monthly one-to one sessions with the course leader to review progress, receive personalized support and discuss appropriate diet changes and training regimens. Table 5 shows an outline course plan used for the intervention.

3.4.2 Minimal intervention (control treatment)

The minimal intervention (control treatment) was designed to reflect standard care and consisted of basic oral and written information about healthy lifestyle delivered at one occasion by a research midwife.

To reduce hunger feelings, all women from both the intervention and control treatment groups received a protein dink free of charge, Natural Balance Shake, Formulation Pre Mix™ (Indevex Biotech (TM)®) containing 65 kcal, which they were encouraged to drink 30 minutes prior to each major meal.

Table 5: Outline course plan used during the behavioral modification intervention.

Week	Lesson	Home work
1	Introduction of participants, course plan, introduction of book and crib sheet Personal leadership; identify the problem MF; breathing exercises	Reading: chapter 1-3 (Don't feed the sugar monster) To do: start using the crib sheet To do: take the first step towards controlling the weight
2	MF; breathing exercises Don't feed the sugar monster Personal leadership; focus on dreams, goals and target vision MF; breathing exercises /target vision	Reading: chapter 4 - 5 (Don't feed the sugar monster) To do: find your sugar traps To do: describe your target vision
3	MF; breathing exercises /mindful eating Don't feed the sugar monster Personal leadership: progress? MF; breathing exercises /target vision	Reading: chapter 6-7 (Stop dieting) To do: describe your "ideal food day" To do: Divide your final goal into intermediate goals and design a plan of action
4	Individual coaching session	
5	MF; hourglass meditation Stop dieting Personal leadership: focus on intermediate goals / plan of action MF; breathing exercises /target vision	Reading: chapter 8-9 (stop dieting) To do: write a food diary To do: Evaluate, revise and refine your plan of action
6	MF; hourglass meditation /focused attention Stop dieting Personal leadership - "just do it" MF; breathing exercises / target vision	Reading: chapter 10-11 (unwind) To do: write a food diary To do: take the next step towards controlling the weight
7	Individual coaching session	
8	MF; hourglass meditation /focused attention Unwind Personal leadership, focus on follow up (how is it going?) MF; hourglass meditation /Stop, Observe, Accept, Let go- (SOAL)	Reading: chapter 10-11 (unwind) To do: find your "stress traps" To do: find a way of giving yourself feedback
9	MF; hourglass meditation /focused attention Unwind Personal leadership: focus on symbols and affirmations MF; hourglass meditation /SOAL	Reading: chapter 12-13 (empty the depots) To do: practice using SOAL To do: find your symbols and write down your affirmations
10	MF; hourglass meditation/ who stands on your internal stage? Empty the depots Personal leadership, focus on symbols and affirmations MF; change the time frame	Reading: article on the effects of exercise To do: exercise plan for the following 6 weeks To do: Evaluate, revise and refine your plan of action
11	Individual coaching session	
12	MF; hourglass meditation/ who stands on your internal stage? Empty the depots (practical advice) Personal leadership – "just do it" MF; change the time frame	Reading chapter 14-16 (the world and us) To do: write in your exercise diary To do: Evaluate, revise and refine your plan of action
13	MF; silent meditation The world and us Personal leadership: focus on rewards MF; breathing exercises / target vision	Reading: all yellow boxes in book To do: write in your exercise diary To do: take the next steps towards controlling your weight
14	Individual coaching session	
15	MF; silent meditation Summary of the book and other knowledge Personal leadership: focus on rewards MF; breathing exercises /target vision	To do: write your own summary of the most important knowledge you have gained to date and how to maintain your good habits for the rest of the year
16	Last day of the course	

The course text book "Hjärnkoll på vikten" formed the basis of the parts of the course focusing on metabolic control.[94] The principles of personal leadership were introduced during the lessons. All lessons started and ended with a mindfulness (MF) exercise. Individual coaching took place approximately every three weeks.

3.5 PROCEDURES AND ANALYTICAL METHODS

Figure 4 outlines the investigations, assessments and procedures carried out at each review-point (baseline, 4 months and 12 months) for the women taking part in the study. The sections below further describe the procedures and analytical methods used.

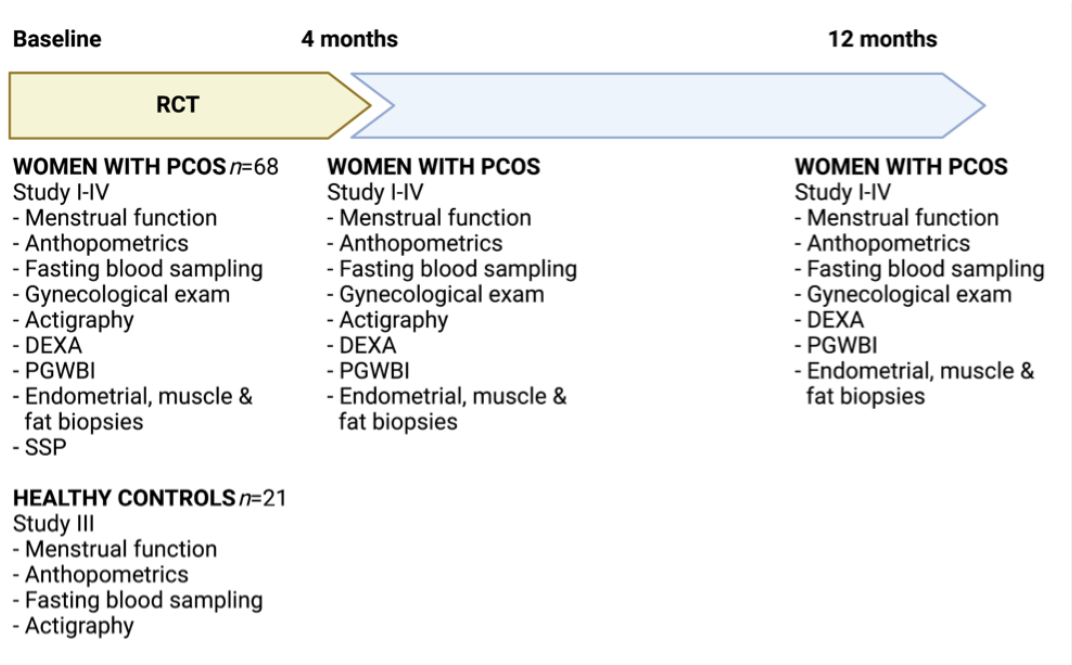

Figure 4: Study review points and procedures carried out. Created with BioRender.com
Abbreviations: DEXA dual energy x-ray absorptiometry; PCOS polycystic ovarian syndrome; PGWBI psychological general well-being index; SSP Swedish universities scales of personality. Figure created with BioRender.com

3.5.1 Procedures

At all review points physical examination to collect data as well as blood sampling, was carried out on menstrual cycle day 6-8. For women with no spontaneous menstruation, a bleeding was induced through administration of 10 mg medroxyprogesterone for seven days.

Anthropometric measurements (height, weight, hip and waist) were made in a standardized manner. Fasting peripheral venous blood sampling was carried out for analysis of hormones, binding proteins, metabolic variables and proteomics analysis.

Hirsutism was used as a measure of clinical hyperandrogenism, and the modified Ferriman-Gallwey (FG) score was used to assess this where a score of ≥ 8 denoted hirsutism.

The gynecological exam included a transvaginal ultrasound (Sonoline SI-250, Siemens Health Care Diagnostics) where the endometrial thickness, antral follicle count and measurements for ovarian volume were obtained.[95]

As part of this RCT, biopsies of the endometrium, muscle and subcutaneous fat tissue were taken under local anesthesia, at baseline, 4 and 12 months, however the results of these procedures are not included in this thesis.

3.5.2 Menstrual function, ovulation assessment and pregnancies

The women with PCOS recorded their menstrual periods using a menstrual diary or an app throughout the 12-month study period. Regular menstrual periods were defined as having a cycle length of 21-35 days, oligomenorrhea as having a cycle length >35 days but less than 3 months and amenorrhea as having no spontaneous menstrual bleeding in the last 3 months.

Women were encouraged to report spontaneous menstrual bleedings and if so, blood samples were collected on cycle day 21-23 so that ovulation could be confirmed by progesterone analysis.

Pregnancy data for the women with PCOS for 12 months after study completion was collected from medical records and patients' own reports.

3.5.3 Body composition

Dual-energy X-ray absorptiometry (DEXA) scanner Hologic Discovery A, was used to assess the body composition variables of total fat percentage, trunk fat mass and lean body mass.

3.5.4 Psychological assessments

3.5.4.1 Psychological general well-being index (PGWBI)

The validated non-disease specific psychological general well-being index (PGWBI) was used to determine the well-being in the women with PCOS. The PGWBI includes 22 questions divided into six dimensions as outlined in Table 6. A global score was obtained by adding all dimensions together. The global PGWBI score has cut-off levels for categories of wellbeing: severe distress (0-60), moderate stress (61-72) and positive well-being (73-110).[96] Both the global score and that of the individual dimensions can be normalized to a scale of 0-100 to facilitate comparisons between studies.[96] A higher score always reflects a greater well-being.

Table 6: The separate dimensions of the psychological general well-being index (PGWBI) their meaning and possible range of scores.[96]

Dimension	Meaning low score	Meaning high score	Range of scores
Anxiety (ANX)	Extremely bothered by nervousness, tense most of the time, feeling highly strung and under stress and pressure.	Never bothered, nervous anxious, worried or upset nor feeling tense. Feeling relaxed and at ease, and never under pressure.	0-25
Depressed mood (DEP)	Suicidal intent, feeling down heart, sad and blue all the time. Feeling discouraged and hopeless, at the point of giving up.	Never feeling depressed, down heart, sad or hopeless.	0-15
Positive well-being (PWB)	Feeling very low in spirits, dissatisfied or unhappy. Never cheerful or light-hearted.	Feeling in excellent spirits, happy, satisfied and cheerful.	0-20
Self-control (SC)	Feeling emotionally unstable and unsure of oneself. Disturbed not to be in firm control of behaviour, thoughts emotions and feelings.	Feeling emotionally stable and in firm control of behaviour, thoughts, emotions and feelings.	0-15
General health (GH)	Bothered by illness, aches or pains and extremely concerned about health. Needing help with most thing.	Not bothered by illness, can carry out all things one likes doing. No health concerns or worries.	0-15
Vitality (VT)	Lack of energy, drained, never feel fresh and rested. Feeling dull, sluggish, tired and worn out.	Full of energy. Feeling fresh and rested, active and vigorous. Never feeling tired, worn out or exhausted.	0-20
Global score (TGWB)	Sum of the above	Sum of the above	0-110

3.5.4.2 Swedish universities scales of personality (SSP)

The Swedish universities scales of personality (SSP) was used to assess personality traits at baseline in the population of overweight women with PCOS. The SSP tool, or parts of it, can be used both in healthy subjects and in psychiatric disease and can be used for both research purposes and in clinical practice.[97] The tool contains 91 questions that can be grouped into 13 personality scales as outlined in Table 7 together with descriptions of each scale.[97] Based on the scores for each personality scale, a T-score can be calculated. A T-score of 50 with a standard deviation of 10, represents the mean score in a gender stratified healthy Swedish population.[97] A higher score indicates that the person expresses more of a particular personality scale and a lower score that the person expresses less of that personality scale.

Table 7: The 13 personality scales used in SSP, including descriptions of a person with high scores.[97]

Personality scale	Meaning of a high score
Mistrust	Distrusting other people's motives, suspicious
Trait Irritability	Impatient and irritable
Physical Trait Aggression	Starts and gets into fights, hits back
Verbal Trait Aggression	Gets into arguments and criticizes others when annoyed
Social Desirability	Helpful and friendly, socially conforming
Embitterment	Blames and envies others, gets into arguments
Detachment	Withdrawn and avoids involvement with other people
Adventure Seeking	Wants action and change, avoids routine
Impulsiveness	Impulsive, lack of planning
Lack of Assertiveness	Inability to speak up and be self-assertive
Stress Susceptibility	Gets tired easily, uneasy when asked to speed up
Psychic Trait Anxiety	Lacks self-confidence, worries a lot
Somatic Trait Anxiety	Restless, tense, having autonomic disturbances

3.5.5 Actigraphy for sleep assessment

An actigraph, ActiSleep+ (ActiGraph) was used to record sleep variables, step count and energy expenditure in the overweight women with PCOS as well as in the healthy controls. The study participants were encouraged to wear the device for 7 consecutive days around their non-dominant wrist.

The actigraph measured movements in three dimensions using an accelerometer. The acceleration data was then converted to sleep parameters. ActiSleep+ uses the validated Sadeh Sleep algorithm to do this.[98] To extract data from the actigraph, the ActiLife 6 (ActiGraph)

software was used. The sleep reports were then reviewed manually and the following sleep variables were recorded: total sleep time over 24h (TST 24h, min), time in bed at night (TIB, min), total sleep time at night (TST night, min) and total sleep time during the day (TST day, min) (defined as a new episode of sleep initiated 30 minutes or later after time of rising, when occurring after 08:00). Furthermore, the time of rising (ToR) and the bed time were obtained. In addition, the number of wakeups (*n*), wakefulness after sleep onset (WASO, min), together with expenditure (kcal/day) and steps taken (*n*) were recorded and Sleep efficiency (TST night /TIB x100) was calculated.

3.5.6 Proteomics analysis

The plasma proteomics analysis was carried out by the Translational mass spectrometry research group at University College London, Institute of Child Health, United Kingdom, using an in-house developed panel of 96 pro- and anti-inflammatory biomarkers multiplexed into one assay (see https://www.protocols.io/view/mrm-lc-ms-ms-assay-for-inflammatrory-associated-pr-ewov148zovr2/v1, Table 1).[99] The biomarkers included were specifically associated with cardiovascular, low grade acute and chronic inflammation as well as neuroinflammation.[86,100] Only proteins with fold changes in normalized abundance ratios of ≥1.5 were included in the presented results.

3.5.6.1 Plasma Sample Preparation for Targeted Proteomics

In brief, plasma samples were thawed, ammonium sulphate added and the samples vortexed to enable detection of low-and medium abundant proteins. Whole yeast enolase protein (Sigma, United Kingdom) was added to the protein pellets together with a digestion buffer and shaken until the protein pellet had been re-suspended.

Next, dithioerythritol (DTE) was added, the samples were shaken and diluted with water following which the proteins were precipitated and digested to peptides using Trypsin.

The peptide samples underwent solid phase extraction (SPE) clean-up using Bone Elut C18 96-well-plates (Agilent, Santa Clara, CA, United States) and were then diluted with trifluoroacetic acid (TFA). The C18 solid phase extraction plates were equilibrated with (acetonitrile) ACN and TFA. The samples were then loaded onto the plates and washed with TFA. Peptides were eluted into Waters 700 µl 96-well plates with ACN and TFA and solvents evaporated using a SpeedVac (Eppendorf, Hamburg, Germany). The plates with the dried pellets were covered and stored at -20°C.

3.5.6.2 Targeted Proteomics MRM LC-MS/MS

The dried pellets with peptides were thawed and reconstituted in ACN and TFA. The peptides were separated by reverse phase chromatography over a 16 minutes ACN gradient using Waters Acquity Ultra Performance Liquid Chromatography system (Waters, Manchester, United Kingdom). The Liquid Chromatography system was coupled to a Waters Xevo-TQ-S

triple quadrupole mass spectrometer for multiple reaction monitoring (MRM) detection in positive electrospray ionization mode. There were three injections performed for each sample.

The raw data was imported to and processed using Skyline open-source software v19 (MacCoss Lab Software, University of Washington, United States). Five custom synthetic standard peptides (Genscript, United States) were used to evaluate the stability and to make sure the correct peaks were identified. We normalized the peak intensity data to the internal standard using yeast enolase to get the normalized abundance ratios of the peptides. The data was then exported to Microsoft Excel and SPSS v26 (IBM, Stockholm, Sweden) for further analysis.

3.5.6.3 Categorization of proteins based on biological function

Information on the biological function of the proteins was obtained using the Uniprot proteomic database and the proteins could be categorized into functional groups.[101]

3.5.7 Biochemical measurements

As outlined in Table 8, the analysis of steroid hormones were made using liquid chromatography tandem-mass spectrometry (LC-MS/MS).[102]

To obtain the FAI, testosterone (nmol/L) was divided by SHBG (nmol/L) times by 100. A serum testosterone of >310 pg/mL was used to define biochemical hyperandrogenism.[103] To assess the insulin resistance, the Homeostatic Model of Assessment (HOMA) index was calculated (insulin (mIU/L) x glucose (mg/dL) /405).[104]

The analysis of glucose, serum cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triglycerides (TG) were made using enzymatic clinical routine methods (DXC 800 Access, Beckman Coulter Inc, Fullerton, CA until June 2015, thereafter Cobas 8000, Roche Diagnostics). Serum levels of insulin were analyzed using Cobas e, Roche Diagnostics. These are all standard methods used in clinical practice and accredited by the Karolinska University Hospital. Table 8 shows the methods used for analysis of the remainder of the hormones.

Table 8: Methods, detection limits, within and between assay coefficient of variation (CV) for hormones and binding proteins

Analyte	Method	Manufacturer	Detection limit	Within assay CV	Between assay CV	Overall precision CV
FSH	CEIA	Immulite (Siemens/DPC)	0.1 U/L	8%	8%	
LH	CEIA	Immulite (Siemens/DPC)	0.7 U/L	6%	9%	
SHBG	CEIA	Immulite (Siemens/DPC)	0.2 nmol/L	6.5%	8.7%	
Progesterone	Access immunoassay[A]	Beckman Coulter Inc	0.3 nmol/L	6.1%	7.5%	
	ECLIA[B]	Roche Diagnostics AG	0.2 nmol/L	2.5%	6.5%	
Testosterone	LC-MS/MS	Endoceutics laboratory[C]	50 pg/mL			2.8%
		Xevo TQ-S Waters Sweden[D]	0.06 nmol/L			6.2%
DHEA	LC-MS/MS	Endoceutics laboratory[C]	500 pg/ml			2.4%
DHT	LC-MS/MS	Endoceutics laboratory[C]	10 pg/mL			4.9%
Androstenedione	LC-MS/MS	Endoceutics laboratory[C]	100 pg/mL			1.2%
		Xevo TQ-S Waters Sweden[D]	0.1 nmol/L			5.5%
Estradiol	LC-MS/MS	Edoceutics laboratory[C]	1pg/mL			1.5%
		Xevo TQ-S Waters Sweden	4 pmol/L			8%

[A] From start of study until May 2015, cut off value to detect ovulation > 17 nmol/L.
[B] From May 2015 until end of study, cut off value to detect ovulation of > 5.3 nmol/L.
[C] Sex steroid hormones analyzed at the Endoceutics Laboratory, Qubec, Canada, using liquid LC-MS/MS as previously described[105,106].
[D] Sex steroid hormones analyzed at the Department of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden using UHP LC-MS/MS, Xevo TQ-S, Waters Sweden AB.
Abbreviations: CEIA chemiluminescence enzyme immunoassay; DHEA dehydroepiandrosterone; DHT dihydrotestosterone; ECLIA electrochemiluminescence immunoassay; FSH follicle stimulating hormone; LC-MS/MS liquid chromatography tandem-mass spectrometry; LH luteinizing hormone; SHBG sex hormone-binding globulin.
Conversion to SI units: Testosterone nmol/L 0.003467; Estradiol pmol/L 3.671

3.6 STATISTICAL ANALYSIS

The statistical analyses were carried out using the statistical software SPSS version 24 (Study I&II) and version 26 (Study III &IV). The baseline continuous data is presented as means ± standard deviations (SD) for normally distributed variables, and if not normally distributed as a median and interquartile range (IQR). Categorical data is given as percentages and proportions. The baseline difference between groups were calculated using the Student's t-test for continuous data and Fisher's exact test for categorical data. For continuous variables, the within and between group treatment effects at 4 months and between baseline and 12 months were calculated using a mixed model.

In Study II, the women participating in the study were divided into those who managed to lose ≥5% weight and those who did not at 12 months based on previously published data suggesting that a weight loss of 5%-10% is needed to improve menstrual function.[63,107-112] A mixed model was used to calculate the difference between these weight loss groups. The analyses were carried out using an intention to treat (ITT) approach. For categorical variables, the chi-square test was used to assess within and between group changes over time. In Study III analysis of covariance was used to compare the sleep variables in the group of women with PCOS with healthy controls adjusting for confounders. In Study II, the mean personality T-scores of the women participating in the study were compared with the expected constant T-score of 50 for a normative population and the score was considered significantly different from the normative score if the confidence interval (CI) of the T-score of the study population did not include 50. The Mann-Whitney U-test was used to look for an association between the personality scores and successful weight loss. The last value carried forward method was used for these analyses to ensure an ITT approach. To investigate associations between variables or changes to variables following intervention, logistic regression analysis was used in Study I and in Study II, III and IV the Spearman's rank correlation was used.

We considered P values < 0.05 statistically significant. In terms of changes to normalized protein abundances we only included proteins with a fold change ≥ 1.5 (Study IV).

The initial power calculation based on an assumption that the participants would achieve at least a 5% weight loss following intervention, resulted in that approximately 20 patients in each intervention group would be needed to provide 80% power to detect a difference in reproductive function at the 5%-level. As the expected weight loss of 5% was not achieved, the number of study participants was increased.

A randomization list was created electronically by a statistician, where study participants were randomized to intervention (n=34) or control treatment (n=34) using blocks of eight patients, using SAS Systems 9.1 (SAS Institute Inc, Cary, NC, USA).

3.7 ETHICAL CONSIDERATIONS

Many of the women with PCOS in our study had experienced several, sometimes stigmatizing, symptoms such as being overweight, suffering from hirsutism and having fertility problems. This introduced several important ethical aspects to consider when interacting with the participants. In addition, a number of the women expressed a feeling of previously having been judged by healthcare professionals for example for not managing to lose weight. This made the whole research-team having to ensure that the patients were treated with upmost respect and not in a judgmental way. Furthermore, strategies for addressing the disappointment experienced by the women not succeeding in losing weight following the intervention had to be developed.

As several of the women participating in the study previously had tried a number of weight loss strategies without success many of the participants regarded taking part in this study as an exciting opportunity and were very hopeful and positive towards taking part. To avoid the disappointment of being randomized to control treatment, the study was designed so that the control treatment group received the intervention after the 4-month RCT part of the study was completed. This also allowed for a larger number of women having received the intervention at the 12-month follow-up.

The results from the psychological questionnaires used in the study showed that this group of women had a very low level of general well-being. All patients exhibiting signs and symptoms of depression or other psychiatric disease, were referred for psychological help.

Participation in the study required a substantial commitment of the participants' time over many months, and many participants had to take time off work to attend the clinical examinations, investigations and group meetings. We therefore tried to accommodate the appointment times to suit the study participants.

Participants undertaking the DEXA-scans were exposed to radiation, however the amount of radiation used in DEXA scans is very low and comparable to standard background radiation.[113]

The collection of biopsies that were made as part of the study, although the results not included in this thesis, could be perceived as invasive and potentially traumatic for the patients. However, the women received a thorough oral and written explanation of the procedures carried out and we were very clear to point out that the patients could take part in the study even without giving the biopsies. We used local anesthesia before the biopsy-taking and the participants received financial compensation for the more invasive procedures.

4 RESULTS

4.1 RANDOMIZATION, EXCLUSIONS AND WITHDRAWALS

Ninety-five women were assessed for eligibility to enter the study, 27 women were excluded, the majority because of not meeting the inclusion criteria, resulting in 68 overweight/obese women with PCOS being randomized to either behavioral modification intervention (*n*=34) or minimal intervention (*n*=34). At 4 months, 30 women remained in the behavioral modification intervention group and 27 in the minimal intervention (control treatment) group (84% in total). At 12 months, 47 women (69%) completed the study. The main reason for withdrawal was fertility related (pregnancy or start of IVF-treatment) as outlined in Figure 3.

4.2 BASELINE CHARACTERISTICS OF THE STUDY POPULATION AND HEALTHY CONTROLS

The baseline characteristics of the 68 overweight/obese women with PCOS are shown in Table 9. There were no differences between the behavioral modification intervention and the control treatment groups.

Furthermore, there were no differences at baseline in the global psychological well-being score (TGWB) or for the scores in the individual well-being dimensions between the groups (Study II). In addition, there were no differences between the personality T-scores at baseline between the two groups. In terms of actigraphy measured sleep variables (Study III), the control treatment group had a significantly shorter total daytime sleep time (TST daytime, min) than the behavioral modification intervention group, but there were no other differences in sleep variables between the groups at baseline.

Table 9: Baseline characteristics of the women with PCOS in the behavioral modification intervention group and the control treatment group

	Behavioral Modification Intervention (*n*=34)	Control Treatment (*n*=34)
Demographics		
Age (year)	31.0 ± 5.1	29.9 ± 5.7
Anthropometry & body composition		
Body weight (kg)	92.9 ± 18.0	93.8 ± 14.4
BMI (kg/m^2)	33.5 ± 5.13	34.3 ± 4.93
BMI>30	27/34 (79.4%)	27/34 (79.4%)
Waist circumference (cm)	103.3 ± 12.4	103.4 ± 10.2
WHR		
DEXA total fat (%)	42.4 ± 3.6	43.5 ± 4.2
Menstrual function		
Regular periods	0/34 (0)	0/34 (0)
Oligomenorrhea	23/34 (67.6%)	26/34 (76.5%)
Amenorrhea	11/34 (32.4%)	8/34 (23.5%)
Endocrine variables		
FSH (IU/L)	6.7 ± 3.8	5.5 ± 1.5
LH (IU/L)	6.4 ± 3.4	7.7 ± 4.5
Testosterone (pg/mL)	358 ± 110	414 ± 158
SHBG (nmol/l)	27.6 ± 12.8	26.1 ± 15.7
Free Androgen Index	5.5 ± 3.1	5.7 ± 5.1
Androstenedione (pg/mL)	1570 ± 474	1668 ± 592
Estradiol (pg/mL)	38.0 (32.4 -55.4)	47.3 (37.9 - 58.3)
Hyperandrogenism		
Biochemical hyperandrogenism[a]	71.9%	70.0%
mFG score	10.5 ± 6.9	11.5 ± 7.9
mFG ≥ 8 (%)	61.3%	59.4%
Metabolic variables		
Fasting Insulin (mIU/L)	15.1 ± 10.0	15.5± 10.3
Fasting Glucose (mmol/L)	4.7 (4.4 - 5.0)	4.4 (4.2 - 5.1)
HOMA	3.3 ± 2.4	3.5 ± 2.8
Blood lipids		
HDL (mmol/L)	0.99 ± 0.2	1.1 ± 0.3
LDL (mmol/L)	3.3 ±0.8	3.1 ± 0.6
Triglycerides (mmol/L)	1.2 ± 0.5	1.4 ± 0.7

[a]Biochemical hyperandrogenism defined as Testosterone >310 pg/mL[20]
Baseline data given as mean ± SD or median (IQR, 25th-75th percentile) unless otherwise stated.
Calculated by Student T-test.
Abbreviations**:** BMI body mass index; DEXA dual-energy X-ray absorptiometry; FSH follicle stimulating hormone; HDL high density lipoproteins; LDL low density lipoproteins; mFG modified Ferriman-Gallwey; HOMA; Homeostatic Model Assessment; LH luteinizing hormone; SHBG sex hormone-binding globulin; WHR waist hip ratio
Free androgen index calculated as testosterone nmol/L divided by SHBG nmol/L x 100.
Homeostatic Model Assessment (HOMA) index calculated using the formula (insulin mIU/L) x (glucose mg/dL) / 405
Conversion to SI units: Testosterone nmol/L 0.003467; Estradiol pmol/L 3.671

In Study III, a control group of 21 women without PCOS, but with similar age and BMI to the women with PCOS was introduced and compared with a subset of overweight/obese woman with PCOS (*n*=39). The baseline characteristics of both groups are outlined in Table 10. As expected, there were differences in some of the women with PCOS having higher testosterone ($P = 0.001$) and FAI ($P < 0.001$), as well as lower SHBG ($P < 0.001$) than the healthy controls. The women with PCOS also had a larger waist/hip ratio ($P < 0.001$) than the controls. In addition, there were differences in some of the key demographic variables between the groups, where the women with PCOS were more likely to be in a stable relationship ($P = 0.028$) and to have children ($P = 0.044$) than the healthy control group.

Table 10: Baseline characteristics of the PCOS population and the healthy control group (Study III)

	PCOS population (*n*=39)	**Healthy controls (*n*=21)**	***P*-value**
Demographics			
Age (year)	30.1 ± 5.4	29.9 ± 5.2	0.849
Current student	8/39 (20.5%)	8/21 (38.1%)	0.220
Unemployed	0/39	0/21	
In a stable relationship	26/39 (66.7%)	8/21 (38.1%)	**0.028**
Have children	16/39 (41.0%)	3/21 (14.3%)	**0.044**
Anthropometric			
Body weight (kg)	93.2 ± 16.2	86.6 ± 10.7	0.083
BMI (kg/m^2)	34.1 ± 5.0	31.6 ± 3.4	0.064
WHR	0.89 ± 0.06	0.81 ± 0.05	**<0.001**
FSH (IU/L)	6.6 ± 3.4	7.1 ± 2.1	0.584
LH (IU/L)	6.7 ± 3.2	6.5 ± 2.9	0.887
Testosterone (pg/ml)	392.5 ± 137.9	270.0 ± 95.2	**0.001**
SHBG (nmol/l)	27.3 ± 15.1	54.8 ± 40.1	**<0.001**
Free Androgen Index	6.5 ± 3.9	2.3 ± 1.0	**<0.001**
Androstenedione (pg/ml)	1627.8 ± 525.2	1055.3 ± 250.7	**<0.001**
Estradiol (pg/ml)	49.9 ± 26.7	48.9 ± 32.3	0.906

Baseline continuous data is presented as means ± SD, categorical data is presented as a proportion/percentage.
The independent sample t-test was used to determine the difference between groups for continuous data and Fisher's exact test for categorical data.
Abbreviations: BMI body mass index; FSH follicle stimulating hormone; LH luteinizing hormone; SHBG sex hormone-binding globulin; WHR waist/hip ratio.
Free androgen index calculated as testosterone nmol/L divided by SHBG nmol/L x 100
Conversion to SI units: Testosterone nmol/L 0.003467; Estradiol pmol/L 3.671

4.3 MENSTRUAL FUNCTION, OVULATION & PREGNANCIES (STUDY I)

In Study I, we assessed improvements in menstrual regularity, defined as moving from either oligomenorrhea to regular cycles or from amenorrhea to oligomenorrhea or regular cycles. We found that a higher proportion of the women in the behavioral modification intervention group improved their menstrual cycle at 4 months compared to control treatment, 59% (*n*=20/34) vs 24% (*n*=8/34), with a mean difference between the groups of 35% (95% CI: 16 to 60), *P* = 0.003 as shown in Figure 5. At 4 months 21% of the women (*n* = 7/34) in each intervention group had ovulated, compared to zero percent at baseline, resulting in no difference in improved ovulation rate between the intervention groups at 4 months (Figure 5). The only significant predictor of improved menstrual function at 4 months was having received the intervention (OR 3.9, 95% CI 1.3 to 11.9).

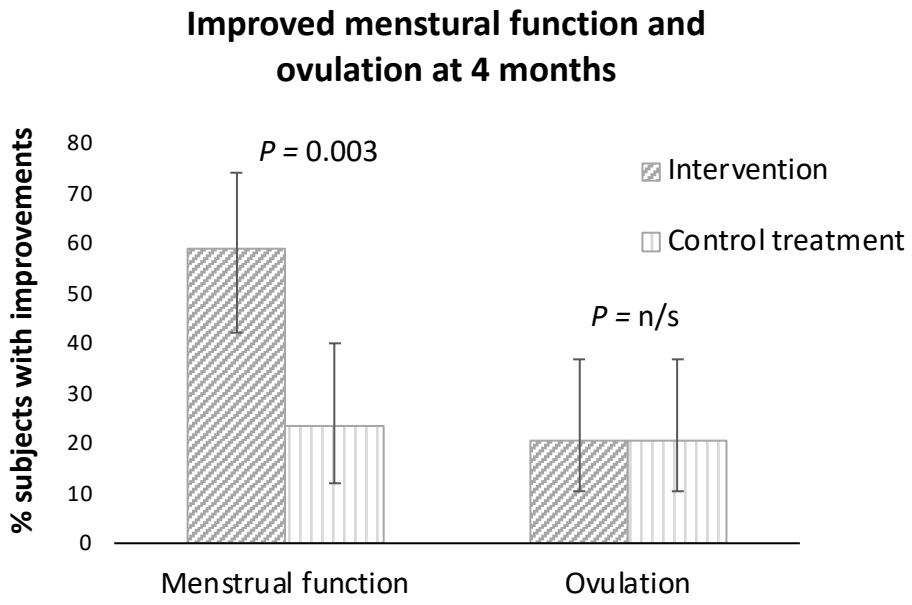

Figure 5: Improved menstrual function and ovulation (% of subjects) after 4 months in both treatment groups. Data given as percentages and confidence intervals. *P*-values refer to between intervention group differences.

Figure 6 shows the distribution of menstrual cycle types for all women at baseline and 12 months when all women with PCOS had received 4 months of intervention.

When comparing the menstrual function at baseline with that at 12 months, 54% of the women (*n*=37/68) (95% CI 42-66), *P* < 0.001) had improved their menstrual function based on an ITT analysis.

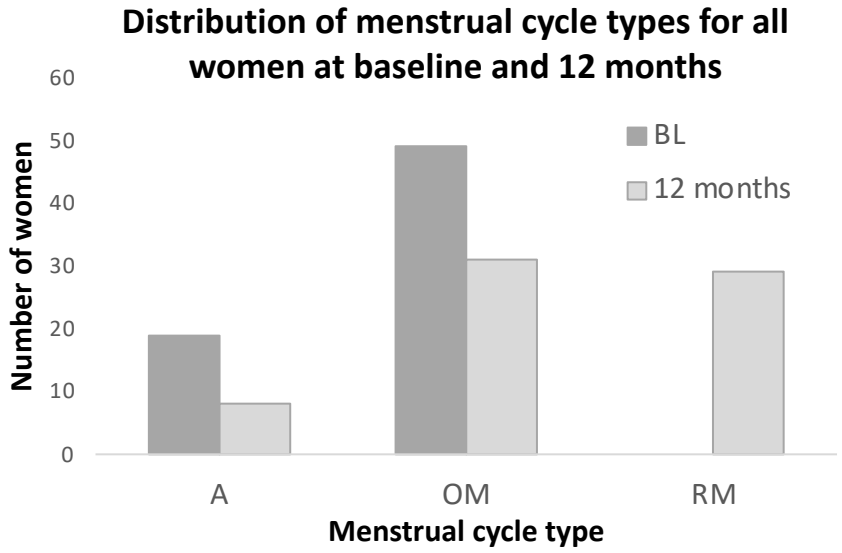

Figure 6: Number of women with each menstrual cycle type amenorrhea (A), oligomenorrhea (OM) and regular menstruation (RM) at baseline and 12 months. Data shown as per ITT.

Logistic regression analysis showed no correlation between improved menstrual function and weight loss.

Furthermore, we found improvements in other fertility related parameters at 12 months. The ovulation rate at 12 months was 43% (n=29/68) (95% CI:31-54) compared with zero at baseline ($P < 0.001$) and the number of ovarian follicles (right ovary) decreased from 13.3 (95% CI: 11.8-14.8) at baseline to 10.6 (8.7-2.4) at 12 months, ($P = 0.012$).

In terms of pregnancies, 29 of the 68 women entering the study wished to become pregnant. Eleven of the 29 women (38%) (95% CI: 23-56) achieved pregnancy within one year of completing the trial, 2 through ovarian stimulation, and 9 spontaneously.

4.4 WEIGHT AND BODY COMPOSITION (STUDY I)

In study I, we found that following 4 months of intervention, the body weight decreased slightly in the behavioral modification intervention group ($P = 0.002$), whereas there was a small non-significant decrease in weight in the control treatment group, but there was no difference in the weight change between intervention groups (

Figure 7 A). There were also reductions in the DEXA variables total fat percentage ($P = 0.021$) and in trunk mass ($P = 0.042$) in the behavioral modification intervention group at 4 months, however we found no significant differences in change from baseline to 4 months between the groups (

Figure 7 A and B).

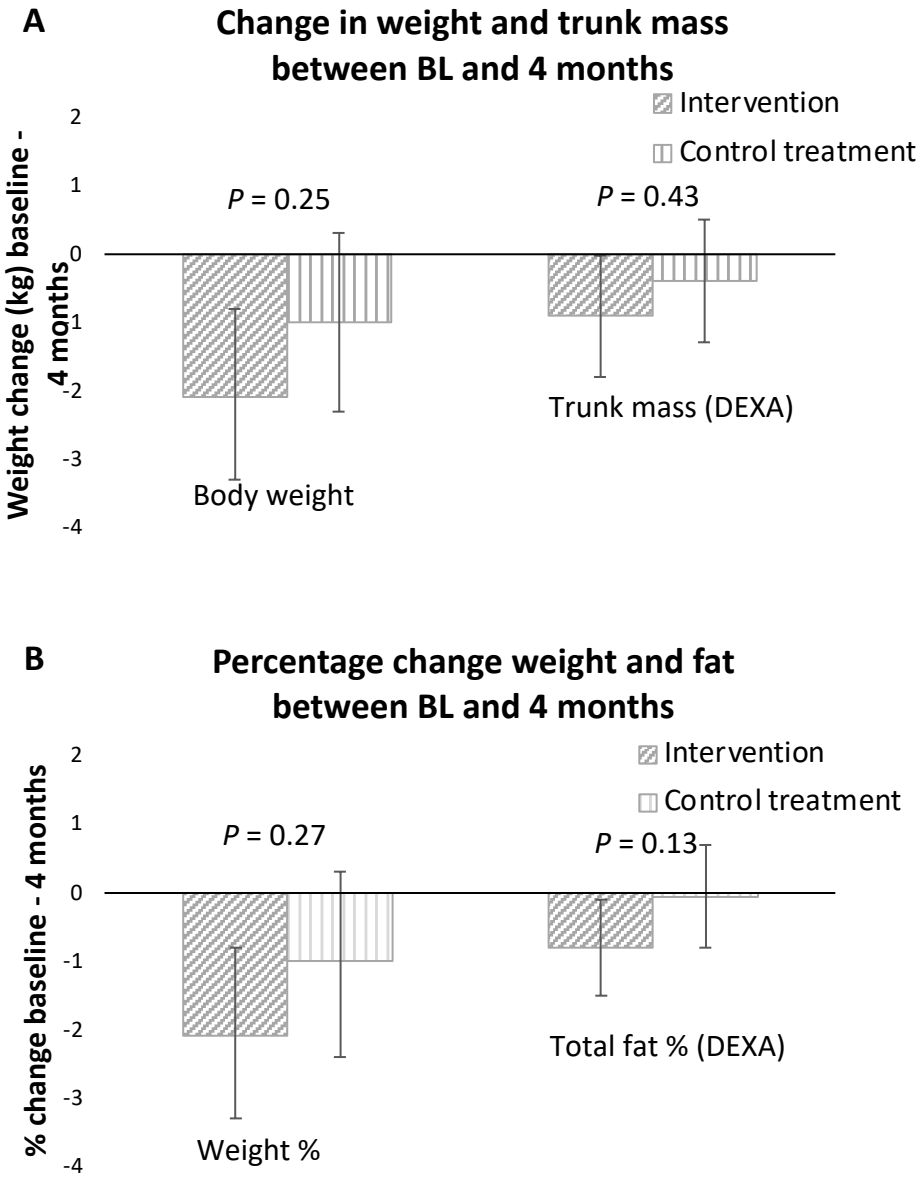

Figure 7: A: Change in body weight and trunk mass (DEXA) both after 4 months in both intervention groups. B: Change in weight (%) and total fat % (DEXA). Data given as mean percentage change from baseline and confidence intervals and obtained using a mixed model on an ITT basis.
Abbreviations: BL baseline; DEXA dual energy X-ray absorptiometry.

Furthermore, at 4 months we found reductions in the waist circumference in both the intervention and control treatment group (-2.4 cm 95% CI: -4.1 to -0.8, *P*=0.005 and -1.9 cm, 95% CI: -3.7 to -0.1, *P*=0.04), but no difference in the change between the groups.

At the 12-month follow-up, when both groups had received the intervention, we found small but significant reductions in weight, trunk mass and total fat % as show in Figure 8.

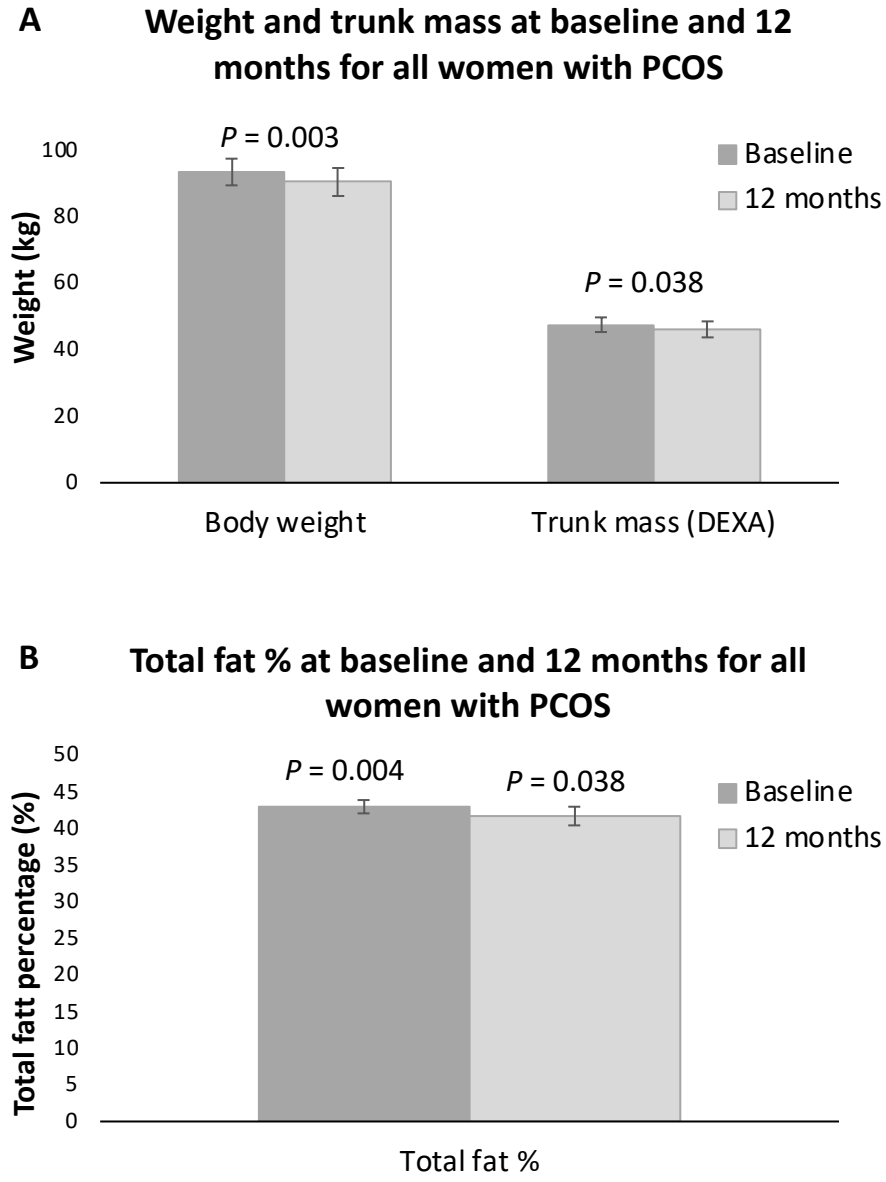

Figure 8: A: Body weight and trunk mass (DEXA). B: Total fat % from DEXA scan. All values are given as means including CI at baseline and 12 months. Data includes all women with PCOS, calculated using a mixed model on an ITT basis.
Abbreviations: DEXA dual energy X-ray absorptiometry.

We found reductions in BMI and waist circumference between baseline and 12 months as shown in Table 11.

Table 11: Baseline and 12 months anthropometric variables for the whole study population of women with PCOS

	Mean value at BL (95% CI)	**Mean value at 12 months (95% CI)**	***P*-value**
Anthropometric variables			
BMI (kg/m^2)	33.9 (32.7 to 35.2)	32.8 (31.5 to 34.1)	**0.003**
Waist circumference (cm)	103.4 (100.6 to 106.2)	99.8 (96.2 to 103.4)	**0.003**

Data given as mean and 95% Confidence Interval (CI) calculated using a mixed model on an ITT basis. Abbreviations**:** BL baseline; BMI body mass index

4.5 ENDOCRINE VARIABLES (STUDY I)

We found a tendency of a significant decrease in DHEA in the intervention group between baseline and 4 months ($P = 0.07$), and a significant increase in the control treatment group ($P = 0.05$) resulting in a significant difference in the change between groups ($P = 0.008$). We found no other differences in the change from baseline to 4 months between the groups.

As shown in Figure 9 we found a significant increase in SHBG at 12 months compared to baseline when looking at the whole population of women with PCOS ($P = 0.021$), in addition the FAI decreased at 12 months compared to baseline ($P = 0.001$). We found no other significant changes to endocrine variables between baseline and 12 months.

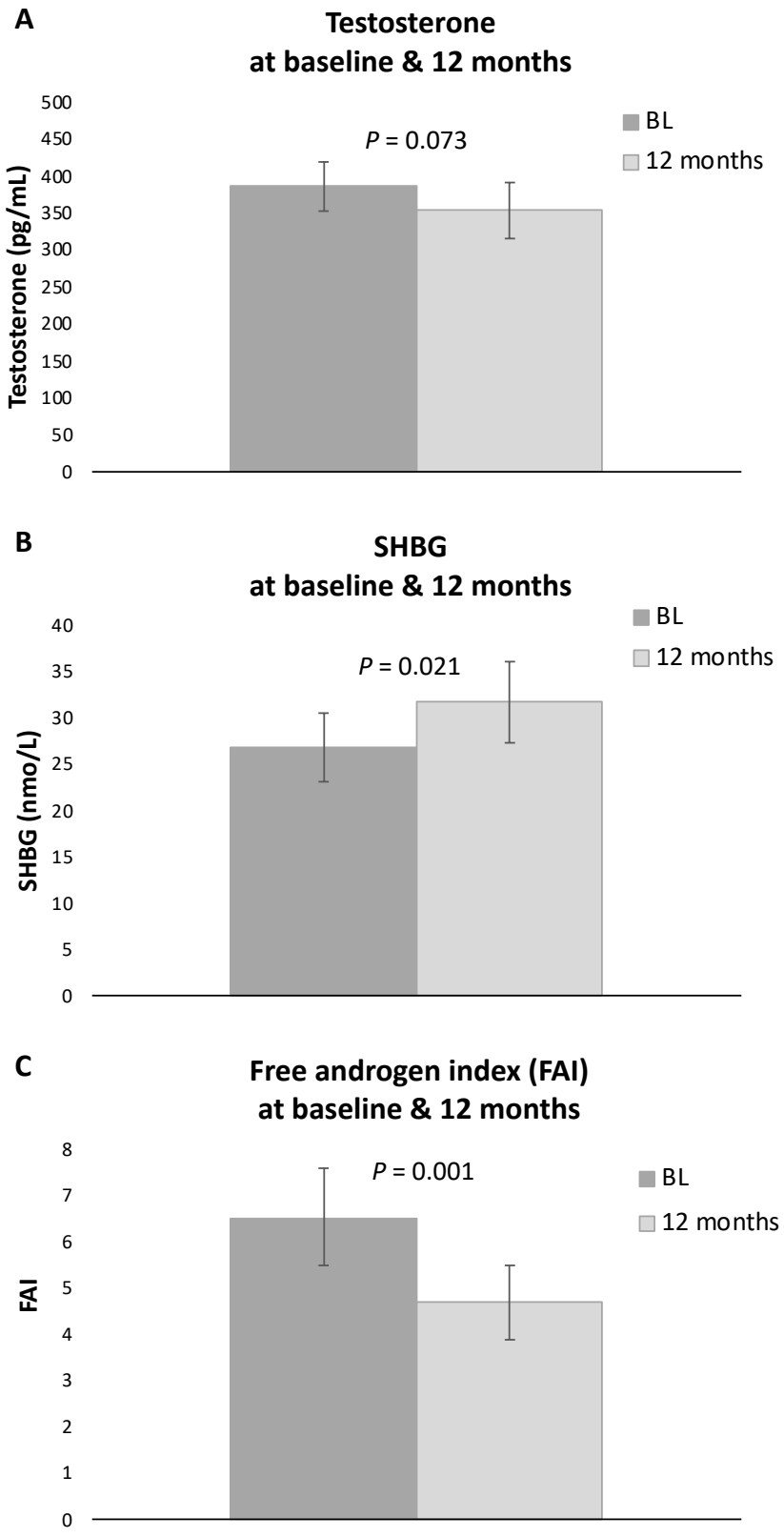

Figure 9: Baseline and 12 months endocrine parameters for the whole population of women with PCOS

Data given as mean and 95% Confidence Interval (CI) calculated using a mixed model on an ITT basis.Free androgen index calculated as testosterone nmol/L divided by SHBG nmol/L x 100. Abbreviations: BL baseline; FAI free androgen index; SHBG sex hormone-binding globulin. Conversion to SI units: Testosterone nmol/L 0.003467.

4.6 METABOLIC AND BLOOD LIPID VARIABLES

In terms of blood lipids, there was a significant decrease in LDL in the behavioral modification intervention group at 4 months compared to baseline, 0.3 mmol/L (95% CI: -0.5 to -0.1) (*P* = 0.003). We found no other within or between group differences for the metabolic variables of fasting insulin, fasting glucose, HOMA, LDL, HDL and triglycerides following 4 months of intervention.

However, when comparing baseline variables with those at the 12 months follow-up for the whole study population as shown in Table 12, we found significant reductions in fasting insulin and HOMA as well as an increase in HDL.

Table 12: Baseline and 12 months metabolic parameters for the whole study population of women with PCOS

	Mean value at BL (95% CI)	**Mean value at 12 months (95% CI)**	***P*-value**
Metabolic variables			
Fasting Insulin (mIU/L)	15.4 (12.8 to 17.9)	12.3 (9.5 to 15.1)	**0.003**
Fasting Glucose (mmol/L)[a]	4.8 (4.6 to 5.0)	4.5 (4.5 to 4.9)	0.209
HOMA	3.4 (2.8 to 4.1)	2.8 (2.1 to 3.5)	**0.007**
Blood lipids			
HDL (mmol/L)	1.0 (1.0 to 1.1)	1.2 (1.1 to 1.3)	**<0.001**
LDL (mmol/L)	3.2 (3.1 to 3.4)	3.1 (2.9 to 3.3)	0.154
Triglycerides (mmol/L)	1.3 (1.2 to 1.5)	1.2 (1.1 to 1.4)	0.272

Data given as mean and 95% Confidence Interval (CI) calculated using a mixed model on an ITT basis.
[a] log transformed values.
Abbreviations**:** BL baseline; HDL high density lipoproteins; LDL low density lipoproteins; HOMA; Homeostatic Model Assessment.
HOMA index calculated using the formula (insulin mIU/L) x (glucose mg/dL) / 405

4.7 PSYCHOLOGICAL ASSESSMENTS

4.7.1 Psychological well-being (Study II)

In Study II, we found that none of the overweight/obese women with PCOS came into the category of *positive well-being* based on the global PGWBI score as shown in Figure 10. The majority of the women were categorized into *severe distress* (Figure 10).

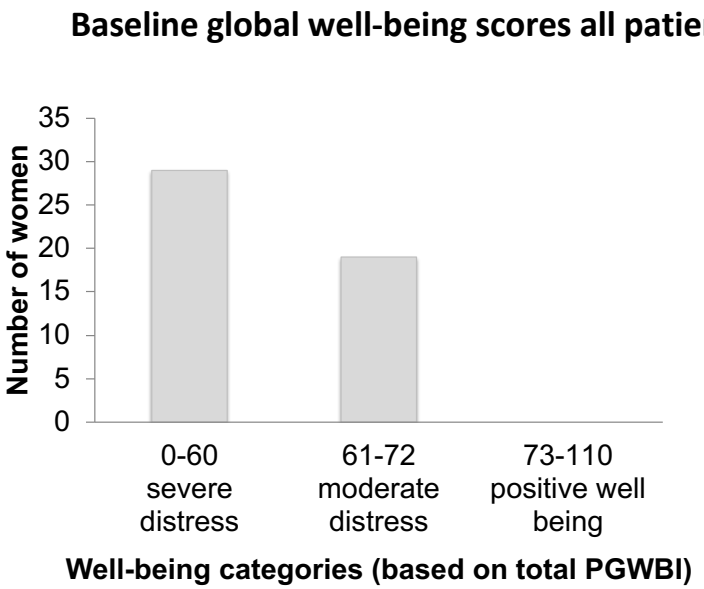

Figure 10: Number of overweight/obese women with PCOS in each category of well-being based on the global PGWBI score at baseline.

We found no change within or between treatment groups to the global PGWBI score following the 4 months behavioral modification intervention. However, there were improvements in the behavioral modification group for the normalized mean score for three of the individual dimensions of PGWBI, *anxiety* (P=0.04), *general health* (P=0.01) and *depressed mood (*P=0.03) following intervention as shown in Figure 11. There was a tendency to significance between the change in anxiety and general health between the two groups (P=0.06, respectively).

When comparing the PGWBI score between baseline and 12 months, we found no difference in the proportion of women falling into each category of well-being (severe-distress, moderate distress and positive well-being), nor in any of the individual well-being dimensions or global mean PGWBI score.

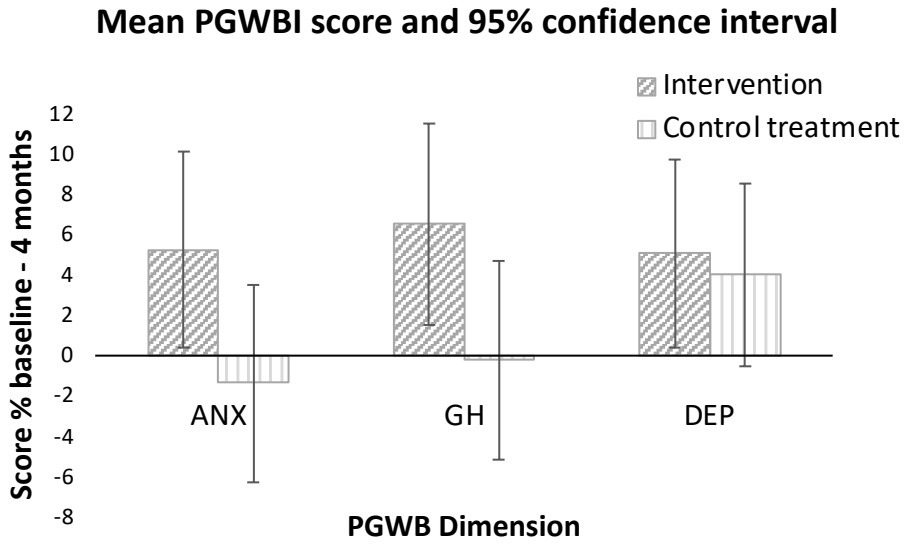

Figure 11: Normalized PGWBI change at 4 months % from baseline for the dimension anxiety (ANX), general health (GH) and depressed mood (DEP), per treatment group, intervention (*n*=23) and control treatment (*n*=26). Values given are mean including the 95% confidence interval calculated using a mixed model on an ITT basis. For all dimensions, a higher score indicates a higher level of well-being.

However, when relating the PGWBI scores at baseline to successful weight loss at 12 months (≥ 5% weight loss) and non-weight loss (< 5% weight loss), we found a significant difference between the baseline score for anxiety for the two groups, with the weight loss group expressing significantly less anxiety (expressed by a higher anxiety score), (*P*=0.004) as shown in Figure 12.

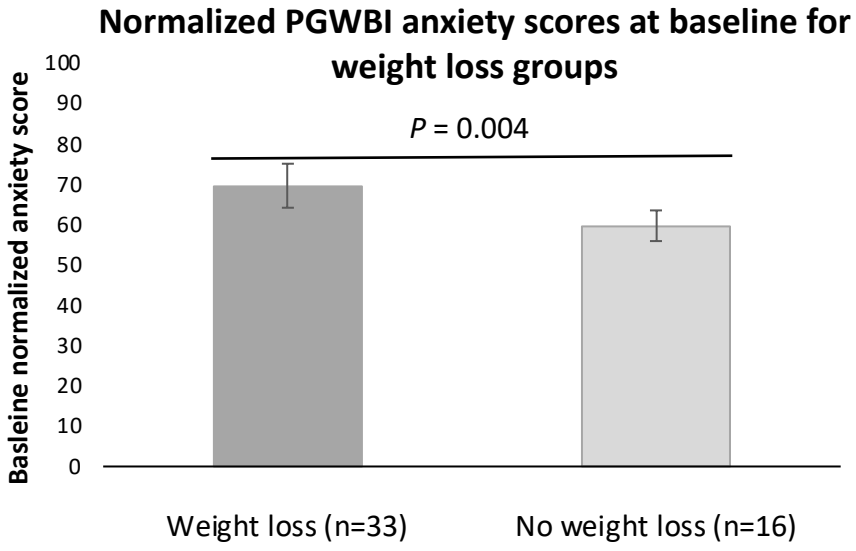

Figure 12: Normalized anxiety scores at baseline for the 12-month weight-loss (≥ 5% weight loss) and non-weight loss (< 5% weight loss) groups. Significance calculated using a mixed model. Analysis done on an ITT basis. A higher anxiety PGWBI score indicates less anxiety.

4.7.2 Personality assessment (Study II)

In Study II, we assessed the personality score at baseline and related that to successful weight loss at 12 months (≥ 5% weight loss) and non-weight loss (< 5% weight loss). As shown in Figure 13, we found the *Social Desirability* score to be higher in the weight loss group than in the non-weight loss group ($P = 0.03$) and the *Embitterment* score to be lower in the weight loss group than in the non-weight loss group ($P = 0.02$).

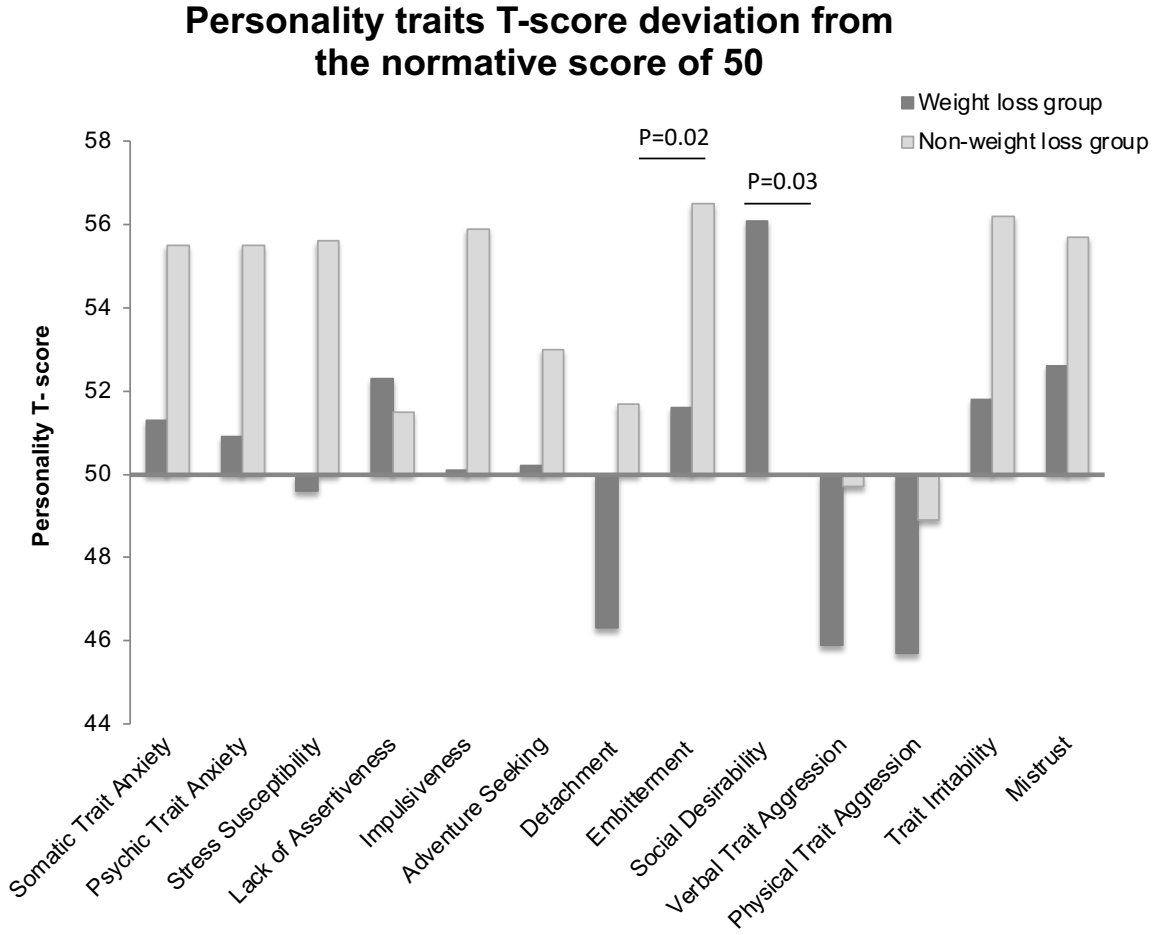

Figure 13: The deviation from the normative value of 50 for personality scores in the two subgroups of successful weight loss *(n*=32) in non-weight loss (*n*=17). P-values given where there is a significant difference in personality score between weight loss groups and calculated using the Mann-Whitney *U*-test**.** Analysis done on an ITT basis.

Furthermore, as shown in Table 13, at baseline, the weight loss group expressed significantly more *Social Desirability* and less *Physical Trait Aggression* than a normative female population that would be expected to score 50. In addition, the non-weight loss group scored significantly higher in the scales of *Mistrust*, *Trait Irritability*, *Embitterment*, *Impulsiveness*, *Stress Susceptibility*, *Psychic Trait Anxiety*, *Somatic Trait Anxiety* than what would be expected from a normative female population (Table 13).

Table 13: Scores for the personality traits assessed at baseline categorized into the weight loss group (≥ 5% weight loss) and non-weight loss group (< 5% weight loss) at 12 months.

	T-scores	
Personality scales	**Weight loss group** (*n*=17)	**Non-weight loss group** (*n*=32)
Mistrust	52.6 (46.9 to 58.4)	**55.7 (50.8 to 60.6)**
Trait Irritability	51.8 (47.4 to 56.1)	**56.2 (51.4 to 61.0)**
Physical Trait Aggression	**45.7 (41.7 to 49.7)**	48.9 (45.6 to 52.2)
Verbal Trait Aggression	45.9 (40.7 to 51.0)	49.7 (45.4 to 54.0)
Social Desirability	**56.1 (52.3 to 59.9)**	50.0 (45.6 to 54.4)
Embitterment	51.6 (45.1 to 58.0)	**56.5 (53.0 to 60.0)**
Detachment	46.3 (41.0 to 51.7)	51.7 (48.0 to 55.5)
Adventure Seeking	50.2 (46.9 to 53.5)	53.0 (48.9 to 57.0)
Impulsiveness	50.1 (44.6 to 55.7)	**55.9 (52.2 to 59.6)**
Lack of Assertiveness	52.3 (47.0 to 57.6)	51.5 (47.6 to 55.3)
Stress Susceptibility	49.6 (44.7 to 54.5)	**55.6 (50.7 to 60.5)**
Psychic Trait Anxiety	50.9 (46.1 to 55.8)	**55.5 (51.3 to 59.6)**
Somatic Trait Anxiety	51.3 (46.8 to 55.9)	**55.5 (51.6 to 59.4)**

Data given as means and 95% Confidence Interval (CI). T-scores in bold indicate those that differ significantly from the normative female score of 50.
T-score for all personality traits in a female normative population expected to be 50
P-values for difference between weight loss and non-weight loss groups calculated using the Mann-Whitney *U*-test, analysis done on an ITT basis.

4.7.3 Sleep (Study III)

When comparing the sleep of over-weight/obese women with PCOS and healthy controls of a similar age and weight, we found that the women with PCOS had a lower sleep efficiency, and shorter TST 24 h, TIB night and TST night, as well as longer WASO (adjusted *P* -values), as shown in Figure 14. There were no differences in bed time or time of rising between the groups when controlling for being in a relationship, having children, BMI and waist circumference as covariates.

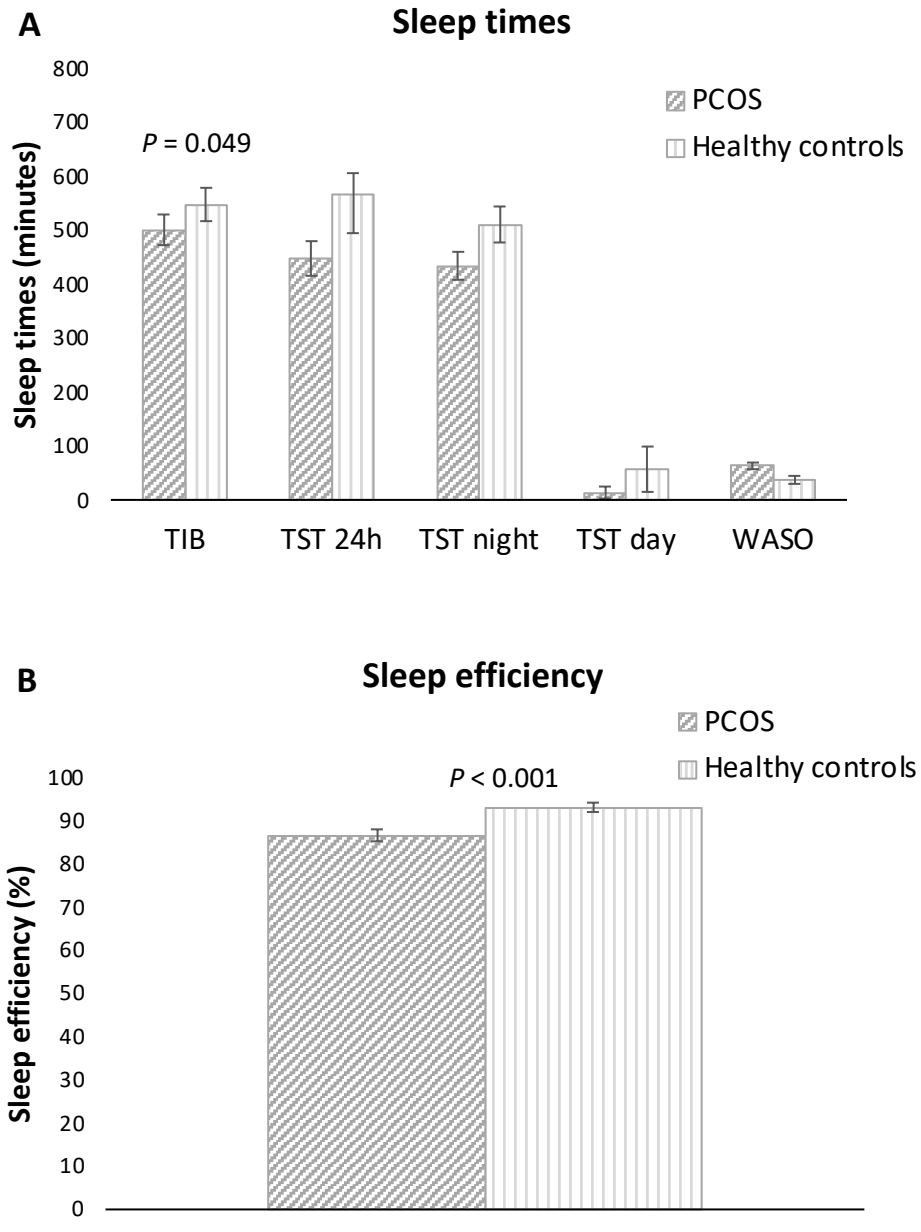

Figure 14: Actigraphy measured sleep variables for overweight/obese women with PCOS (*n*=39) and healthy controls (*n*=21) of a similar age and weight presented as means including the 95% CI. *P*-values are adjusted: controlling for being in a relationship, having children, BMI and waist circumference as covariates. Abbreviations: h hours; min minutes; TIB time in bed; TST total sleep time; WASO wakefulness after sleep onset.

All between group differences remained when splitting the data into weekdays only (Monday to Thursday), apart from TIB where we no longer found a difference between groups. For the weekend (Friday and Saturday) sleep parameters, the women with PCOS had a significantly shorter TST 24h (P=0.041, TST night (P=0.015) and a worse sleep efficiency (P<0.001) as well as a longer WASO (P<0.001).

Sleep assessment following the 4 months behavioral modification intervention in the group of overweight women with PCOS, revealed non-significant reductions in the TST 24h and TST daytime in the intervention group. The same variables increased significantly in the control treatment group resulting in significant between group differences in the change from baseline for TST 24h and TST daytime as outlined in Figure 15. We found no other within or between group differences to the sleep parameters following the intervention. When analyzing the data separately for weekdays (Monday to Thursday) and weekend (Friday and Saturday) we found no within or between group differences following intervention.

In terms of correlations for the baseline sleep variables for the woman with PCOS, we found a positive correlation between sleep efficiency and the psychological well-being index (PGWBI) dimension of self-control (r=0.41, P=0.023) and a negative correlation between WASO and self-control (r=-0.38, P=0.035).

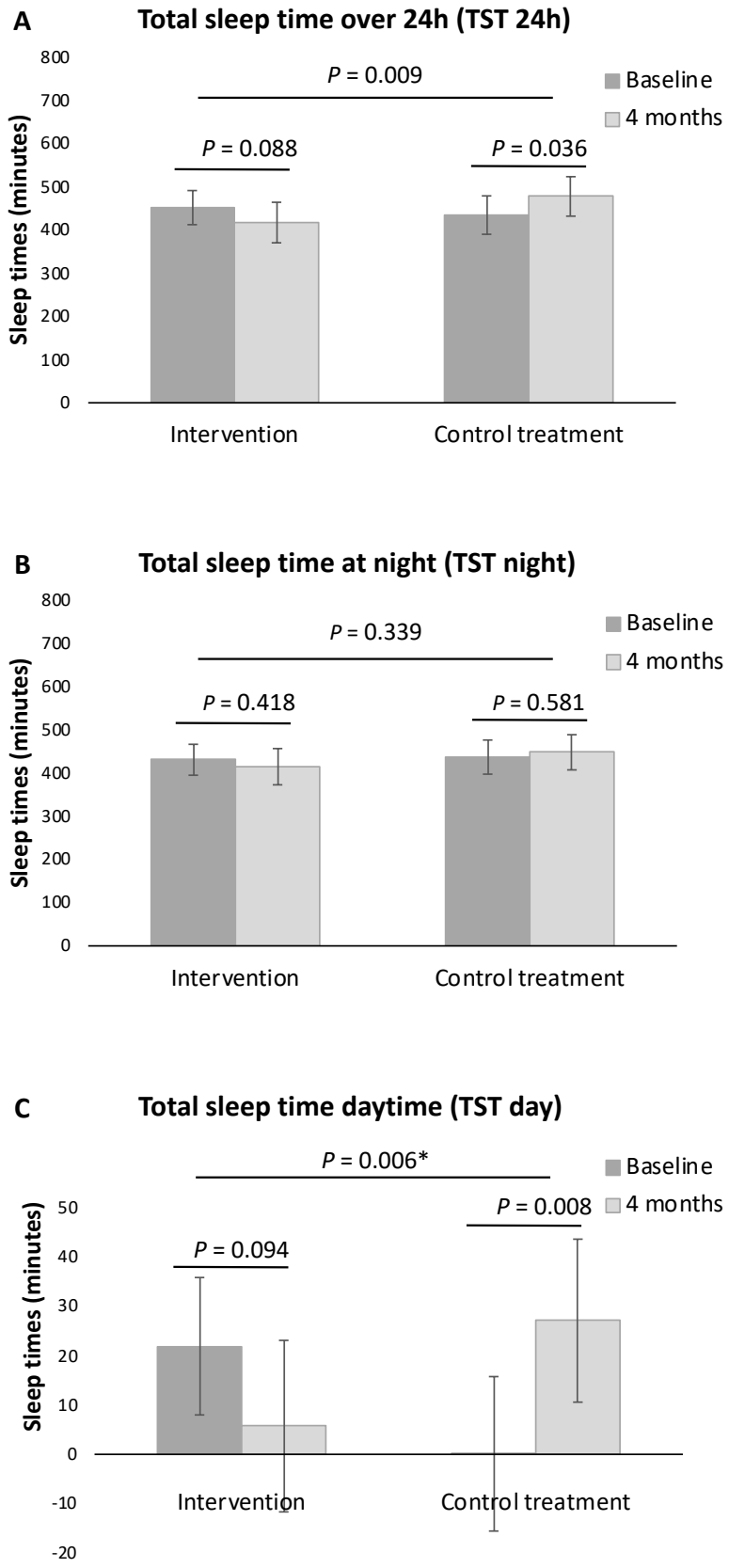

Figure 15: Actigraphy registered sleep variables at baseline and following 4 months intervention for the overweight women with PCOS.
Data given mean ± standard error and mean differences with its 95% Confidence Interval calculated using a mixed model on an ITT basis. **P*-value corrected for the cofounder TST day using analysis of covariance. Abbreviations: TST total sleep time.

4.7.4 Inflammation (Study IV)

In study IV, proteomics analysis using a multiplex panel of 96 pro-and anti-inflammatory proteins of the plasma from 9 overweight women with PCOS showed a between group difference in the change in normalized abundance in the protein E-selectin ($P = 0.003$). When looking at the change in normalized abundance between baseline and 12 months, we found changes in 10 proteins. All but one protein decreased in amount as outlined in Table 14.

Table 14: Proteins with significant fold change between baseline and 12 months for 9 overweight women with PCOS

Protein	Function	Fold change normalized abundance, *P*-value
Interleukin-4 (Il-4)	Inflammation Lipoprotein metabolism	↓ 4.5 *
Serum amyloid A-1 protein (SAA)	Inflammation	↓ 2.3 *
Alpha-1-antitrypsin	Inflammation Coagulation	↓ 2.0 **
Transcription factor EB (TFEB)	Inflammation Lipoprotein metabolism	↓ 4.0 ****
Neuropilin-1 (Nrp1)	Lipoprotein metabolism Cell stress protection	↑ 2.0 **
Neurofilament light polypeptide (NfL)	Cell stress protection	↓ 1.6 *
Migration and invasion enhancer 1 (MIEN1)	Cell stress protection	↓ 3.1 **
Dickkopf-related protein 3 (DKK3)	Lipoprotein metabolism Cell stress protection	↓ 1.7 *
NAD-dependent protein deacetylase sirtuin-2	Lipoprotein metabolism Cell stress protection	↓ 1.7 *
Progranulin (PGRN)	Inflammation Cell stress protection	↓ 2.6 *

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$
Arrow indicates direction of change in relative abundance of protein.

In terms of correlations at baseline, we found that the normalized abundance of the protein Interleukin-4 (IL-4) was positively associated with the baseline variables of weight (r=0.420, *P*=0.006), DEXA trunk mass (r=0.408, *P*=0.007), fasting insulin (r=0.379, *P*=0.015) and HOMA (r=0.397, *P*=0.010). In addition, the normalized abundance of the proteins Alpha-1-antitrypsin and Migration and invasion enhancer 1 (MIEN1) were positively associated with baseline triglycerides (r=0.342, *P*=0.027 and r=0.404, *P*=0.008 respectively).

We found that changes between baseline and 12 months to the proteins Transcription factor EB (TFEB), Dickkopf-related protein 3 (DKK3) and progranulin (PGRN) were associated with changes in fasting insulin and HOMA as outlined in Table 15.

Table 15: Spearman's correlations between changes in proteins and metabolic variables from baseline to 12 months for 9 over-weight/obese women with PCOS

Change in proteins (12m-BL)	**Change in metabolic variables (12m-BL)**	
	Δ fasting insulin	Δ HOMA
Δ Transcription factor EB	**r=-0.75** ***P*=0.020**	**r=-0.70** ***P*=0.035**
Δ Dickkopf-related protein 3	**r=0.77** ***P*=0.016**	**r=0.70** ***P*=0.035**
Δ Progranulin	**r=0.85** ***P*=0.004**	**r=0.78** ***P*=0.014**

5 DISCUSSION

In this thesis, we investigated the efficacy of behavioral modification intervention in 68 over-weight/obese women with PCOS, on improving menstrual function, body weight, metabolic and inflammatory variables, psychological well-being and sleep. In **Study I,** we showed that a significantly higher proportion of women having received behavioral modification intervention improved menstrual function compared to women receiving control treatment, this is despite only a small, but significant, weight loss was achieved by the intervention group. At 12-months, we found improvements in both menstrual regularity and ovulation compared to baseline. **Study II** demonstrated that the psychological well-being in over-weight/obese women with PCOS is low, but that some aspects of well-being (anxiety, general health and depressed mood) improved following intervention with a tendency to significance when compared to control treatment. We also demonstrated an association between successful weight loss and a lower personality score for embitterment and a higher personality score for social desirability and we showed the importance of well-being for achieving weight loss. In **Study III,** we found that sleep amount at night for over-weight/obese women with PCOS is within the normal range, however the sleep efficiency is lower and the wakefulness after sleep onset is higher in women with PCOS than for healthy controls with a similar BMI and age. Furthermore, the women with PCOS receiving the intervention reduced their amount of daytime napping significantly compared to control treatment. In **Study IV,** we performed proteomic analysis of a large panel av pro-and anti-inflammatory markers on a subset of the over-weight/obese women with PCOS and found reductions in the abundance of 9 proteins and an increase in one at the 12-month follow-up compared to baseline. We also found that improvements in insulin resistance was associated with changes to a number of the proteins.

5.1 CHARACTERIZATION OF OUR STUDY POPULATION AT BASELINE

The 68 women participating in this study all fell into the PCOS phenotype A category at baseline, which means displaying all three diagnostic criteria of clinical or biochemical hyperandrogenism, oligo- or anovulation and PCOM. At baseline, 60% of the women had significant hirsutism, the measure we used to assess clinical hyperandrogenism, defined as a mFG score of ≥ 8. In addition, 71% had biochemical hyperandrogenism, defined as a serum Testosterone > 310 pg/mL.[20] Furthermore 21% were overweight and 79% obese.

None of the participants had been diagnosed with insulin resistance prior to joining, however, we found in Study I, that at baseline the mean HOMA score, used as a measure for insulin resistance was just below the suggested value for diagnosing T2DM of 3.6 for a female European population (HOMA 3.3 for the intervention group and 3.5 for the control treatment group), and above the cut-off of 1.8 for pre-diabetes.[114] This corresponds with data from other authors who have found insulin resistance assessed by euglycemic-hyperinsulinemic clamp, to be present in 95% of over-weight (BMI ≥ 27 kg/m^2) women with PCOS and in 75% of lean women with PCOS (BMI < 27 kg/m^2).[115]

In addition, we found, that this group of women we studied had a very low psychological well-being. We showed in Study II that none of the women scored high enough on the PGWBI score to fall into the category of positive well-being, where we would expect to find 70% of a normal population.[96] In addition, comparing the psychological well-being scores from our study population with studies looking at patient groups with other medical conditions, our population scored markedly lower. [96,116,117] Our results are in agreement with other authors who have found worse health related quality of life in women with PCOS compared not only with healthy controls but also with patients suffering from other chronic conditions such as diabetes, heart disease and arthritis. [118,119]

In terms of sleep, in Study III, we showed that the mean total sleep time at night of 7.2h for the overweight/obese women with PCOS in our study was within the normal span of recommended sleep times of 6-8h per night for healthy adults, where longer sleep times than 9h can indicate co-morbidity.[120] When comparing the sleep in the women with PCOS with that of a healthy population with similar age and BMI, we found that the women with PCOS had a shorter mean sleep time at night, during the day and over 24-hours even after adjusting for covariates (being in a relationship, having children, BMI and waist circumference). We know that sleep efficiency generally decreases with longer sleep times and improves with shorter sleep times. However, despite the longer sleep times seen in the healthy control group, they had better sleep efficiency and shorter wakefulness after sleep onset than the women with PCOS. Both these variables are indicators of sleep quality, implying the healthy controls had a better sleep quality despite a longer total sleep time.[121] A number of other studies investigating adults and adolescents with PCOS have also found lower sleep efficiency in women with PCOS than controls. [122-124] In addition, three small studies found reduced sleep efficiency in women with PCOS compared to healthy controls, although not reaching statistical significance.[125-127]

In terms of blood lipids (Triglycerides, LDL and HDL), the mean values at baseline fell within the normal ranges, however the mean value for HDL (1.0 mmol/L), was just at the lower limit of normal range for adult women (1.0 mmol/L-2.7 mmol/L).

5.2 EFFICACY OF BEHAVIORAL MODIFICATION INTERVENTION ON CLINICAL FEATURE OF PCOS

Lifestyle intervention, using dietary, exercise, behavioral modification techniques or a combination of these, is the recommended first line treatment for many symptoms relating to PCOS.[4] When designing our study, the majority of the little evidence already existing in this area, showed efficacy of lifestyle intervention in the short term.[63,64,87,88] We hypothesized that by using a behavioral modification intervention we would see results sustained over a longer time. The intervention showed efficacy in improving menstrual function and body weight both in the short- and long-term despite a very minor weight reduction. For other parameters such as endocrine-, metabolic- and inflammatory-variables we mainly found the intervention efficacious in the longer term.

5.2.1 Reproduction and weight loss

Subfertility is often a main concern for women with PCOS and improving the chances of conceiving is often important for this patient group. Traditionally there has been a general belief that a 5%-10% weight reduction can lead to resumed ovulation and improvements in menstrual regularity. [63,107-112] In Study I, we found that the women with PCOS having received 4 months of behavioral modification intervention, only had a significant mean weight loss of 2% and that only 15% of the women having succeeded in losing weight, had lost more than 5% of the body weight. Our intervention, using tools to attempt behavioral change, differs from pure diet or exercise interventions, and we were hoping to be able to see sustainable improvements to weight in the longer term, and not only a substantial short term weight loss. However, we had expected to see a larger weight loss than what we did. Despite this very modest weight reduction, the proportion of women with improved menstrual function was significantly higher among those having received 4 months intervention compared to control treatment (59% vs 24%).

There was no difference in ovulation rate between the intervention groups at 4 months, but when comparing baseline with 12 moths for all women, 43% had confirmed ovulation and 54% experienced improved menstrual function. One reason for not finding a between group difference in ovulation rate, despite improvements in menstrual function, could be that our method of detecting ovulation through analyzing serum progesterone on cycle day 21-23, did not consider varying cycle lengths. Ideally weekly serum progesterone analysis, or urinary hormone determination, ought to have been made for all participant throughout the study period to ensure the capture of all ovulatory events.

It is remarkable that despite the minimal weight loss following intervention, we found such improvements in the menstrual function. Evolutionary it appears as if resuming fertility is of a high biological priority.

We have only found one previous secondary analysis reporting on improvements in menstrual function following lifestyle intervention in comparison to minimal intervention.[88,128] A number of other RCTs have not found a difference in menstrual function following lifestyle intervention compared to minimal intervention.[63,64,68] However, a recent systematic review and meta-analysis from 2022 (including our Study I) found improvements in menstrual function following lifestyle intervention compared to control treatment, although the control group included in one of the studies had received more extensive intervention than just minimal intevention.[55,56] A number of other studies have found within group improvements in ovulation and menstrual regularity following lifestyle intervention.[68,108,109,129-131]

We found no association between weight loss and improved menstrual function. This is in line with previous data published by our research group where no association between menstrual function and weight loss was found, but instead between improved menstrual function and enhanced insulin sensitivity.[129] In contrast, another study found that a reduction in body weight

had significant effects on improving the prevalence of ovulatory dysfunction (oligomenorrhea or amenorrhea).[68]

In Study I, we also found that 38% of the women with a desire to become pregnant did so within one year of study completion, the majority spontaneously. Little previous data exists on pregnancy rates following lifestyle intervention but two uncontrolled studies using dietary modifications as the intervention, describe pregnancy rates of 30%-56% in women with PCOS.[111,132] Another RCT, investigating infertile overweight women independently of PCOS status found that following an intensive weight reduction program, resulting in 9 kg weight loss prior to in-vitro fertilization (IVF), there was no difference in live birth rate between the intervention and control group, however the women in the intervention group achieved more spontaneous pregnancies.[92]

5.2.2 Body composition

In Study I, we found small reductions to the body composition measurements of total fat percentage and trunk mass both at 4- and 12-months following intervention but we found no difference to the change in body composition between the groups. Our results are in line with one study that found improvements in total fat percentage following dietary intervention however another study found no changes in body composition following aerobic exercise.[133,134]

5.2.1 Endocrine parameters, metabolism and blood lipids

In terms of endocrine variables, we only found improvements in DHEA comparing the intervention group with control treatment at four months (Study I). However, in the longer-term follow-up when comparing 12 months with baseline, we found an increase in SHBG and the FAI decreased. These results are in line with previously published work including the latest Cochrane review suggesting lifestyle intervention may improve biochemical hyperandrogenism.[56]

Furthermore, following the 4 months intervention, we found a reduction in LDL in the intervention group. It appears that the improvements in the metabolic parameters are more prominent in the longer term and at the 12-month follow-up we found a decrease in insulin resistance as well as an increase in HDL. Other surrogate markers of insulin resistance, abdominal circumference, and trunk mass also reduced following intervention. As insulin resistance plays a key role in the pathogenesis in PCOS this is an important finding and our results also corresponds with the finding in the most recent Cochrane review.[56]

5.2.2 Psychological aspects and sleep

5.2.2.1 Psychological well-being

In Study II, we found a tendency of improvement in the psychological well-being categories of anxiety and general health when comparing behavioral modification to minimal

intervention. The most recent Cochrane review concluded that quality of life may improve following lifestyle intervention in comparison to minimal intervention, at least in the dimensions of emotions and infertility (using the disease specific PCOS quality of life questionnaire, PCOSQ, tool).[56] The studies summarized in the review were based on different types of lifestyle interventions such as physical exercise, resistance training and a mindfulness stress management program and had used a combination of assessment tools (PCOSQ and Short form (SF)-36).[135-137] Following the Cochrane review, Jiskoot *et al* (2020) published the long-term effects of cognitive behavioral therapy lifestyle intervention on women with PCOS compared to control treatment and found significant improvements in the well-being dimensions of depression and self-esteem.[66]

In our Study II, published after the Cochrane review, we also found within group improvements in the anxiety, general health and depressed mood score following behavioral modification intervention. A number of other studies have found similar results following lifestyle intervention using other assessment tools.[66,135,136,138,139] As described in Study IV, we found improvements in a number of inflammatory proteins following intervention and this could perhaps in part contribute to some of the improvements in certain dimensions of well-being. A previous study of a general female population found that inflammation was related to several measures of well-being with higher levels of cytokines associated with lower well-being and quality of life.[140]

5.2.2.2 Objectively assessed sleep variables

To our knowledge, this study was the first one to investigate the effects of objectively measured sleep variables following behavioral modification intervention in a PCOS population. In Study III, we found a non-significant reduction in the total sleep time over 24 hours and in daytime sleep for the intervention group and increases to both total sleep time over 24 hours and daytime sleep in the control treatment group resulting in a significant between group difference. We found no difference between groups for the total sleep time at night, therefore the difference in total sleep time over 24 hours can be explained by less daytime napping in the behavioral modification intervention group. We know from other non-PCOS populations that daytime napping is associated with an increased risk of T2DM and increased risk of death of all causes.[141,142] As discussed above, we found improvements in insulin resistance as measured by the HOMA score following intervention this could at least partly contribute to the reductions to daytime napping durations. Furthermore, induced inflammation in an otherwise healthy population has been shown to be connected to the development of fatigue and sleepiness.[143] Our results in Study IV of improvements in several inflammatory proteins following intervention could in part contribute to the reduced daytime napping following intervention in the women with PCOS.

5.2.3 Chronic inflammation

PCOS is increasingly being recognized as a low grade, chronic inflammatory condition.[22,47-51] In Study IV, we found changes to the amounts of 10 proteins associated with inflammation following intervention when comparing baseline with the longer-term follow-up at12 months.

5.2.3.1 Neurodegeneration and neuronal inflammation

We found reductions to a number of proteins involved in brain injury and neurodegeneration at 12 months. Neurofilament light polypeptide (NfL), which is a brain biomarker that is increased in traumatic brain injury and chronic neurodegeneration, and DKK3 also involved in neurodegeneration, both decreased following intervention.[101,144-147] We also found a decrease in the amounts of both NAD-dependent protein deacetylase sirtuin-2 which, among other functions, is a regulator of autophagy in neuronal cells as a response to oxidative stress and to PGRN, which is a marker of neuronal inflammation and is known to be raised in women with PCOS.[101,148] Furthermore, MIEN1, a pro-apoptotic protein that is known to be raised in PCOS decreased following intervention.[101,149]

Women with PCOS often have untreated or undiagnosed insulin resistance, this is also true for the PCOS women studied here as discussed above. In other, non-PCOS populations it has been suggested that untreated insulin resistance can result in neurodegeneration.[150] We found improvements in fasting insulin and HOMA at 12 months following intervention. This together with the positive correlations between changes to fasting insulin and HOMA with changes in the proteins DKK3 and PGRN suggest that the reductions seen in proteins associated with neuroinflammation and neurodegeneration could be driven by improvements in insulin resistance following intervention. This is in line with the conclusions from another recent study that found a positive correlation between the inflammatory marker TNF-α and fasting insulin and HOMA.[151] Furthermore we (in Study II), and others, have found low levels of psychological well-being in women with PCOS and the prevalence of psychiatric co-morbidities is raised in this patient group.[11,52,53,152] This is relevant as a link between depression and chronic inflammation has been suggested.[153]

5.2.3.2 Inflammation and lipoprotein metabolism

Other proteins involved in the inflammatory response, IL-4, an anti-inflammatory cytokine and regulator of lipid metabolism, Serum amyloid A-1 protein (SAA) a major acute phase protein, Alpha-1-antitrypsin, another acute phase protein that is known to be raised in PCOS, and Transcription factor EB (TFEB) with anti-atherogenic functions and a key regulator of autophagy, lipid catabolism and energy metabolism were all lower at 12 months compared to baseline, suggesting lower levels of inflammation following intervention and the results correspond well with the improved metabolic profile seen in this study following intervention.[101,154-157]

There have been suggestions that the pro-inflammatory state observed in women with PCOS is in fact a result of obesity, which in itself causes chronic low-grade inflammation.[48,49]

However, the extremely small weight loss we found at 12 months coupled together with the positive correlations between changes in several inflammatory proteins with changes in blood lipids and insulin resistance rather than with weight loss, suggest that the long term changes in inflammation could be related to improvements in insulin resistance (HOMA).

5.2.4 Identifying responders to intervention

Our proposed intervention, behavioral modification intervention, requires substantial time commitment as well as determination from the participants. We showed in Study I, that weight loss for this population is difficult to achieve and this corresponds well with findings from other authors.[64,110,158] This poses the question of who the intervention is best suited for. It would be of great benefit to find ways of identifying specific characteristics that improve the chances of successful outcomes following intervention.

As previously described in the literature, a reduction in weight of 5%-10% can lead to improved menstrual function and following on from that hopefully to improved chances of conceiving, which is often a key concern for the population group studied here.[63,107-112] Therefore, we looked at weight loss at the 12-month follow-up of all women with PCOS. We defined successful weight loss as those having achieved ≥ 5% (n = 18/68) reduction in weight and the non-weight loss group as those having achieved < 5% (n = 50/68) weight loss.

In Study II, we demonstrated that the women achieving successful weight loss had a better psychological well-being in the domain of anxiety at baseline, than the non-weight loss group. Other authors have found similar results.[159,160] This highlights the importance of assessing the psychological well-being prior to embarking upon a lifestyle intervention program and if indicated offer management options to the woman before initiating the intervention.

In addition, in Study II, we found an association between weight loss and personality traits assessed by SSP. We found that the women achieving weight-loss scored significantly higher for the personality traits of social desirability and lower for embitterment than the non-weight-loss group. When comparing the scores with those of a normative female population, the scores from the weight loss group only differed for two personality traits (higher social desirability and lower physical trait aggression). However, the scores for the non-weigh loss group were higher than those of a normative female population for several personality traits (somatic trait anxiety, psychic trait anxiety, stress susceptibility, impulsiveness, embitterment, trait irritability and mistrust). We have found one other study assessing personality using the SSP tool in relation to weight loss, although for a mixed male and female population.[72] Here BMI was negatively related to a higher score for adventure seeking and uncontrolled eating related to higher embitterment, trait irritability, mistrust, detachment and lack of assertiveness.[72] A couple of other studies using the Character Inventory (TCI) tool for assessing personality in non-PCOS populations following medical and lifestyle intervention also found some aspects of personality associated with weight loss.[71,73]

In addition to identifying responders to treatment, identifying the young women with PCOS at risk of gaining weight during the adolescent period and trying to prevent this weight gain in the first place, perhaps by using behavioral modification intervention, would be the preferred strategy. A recent large population-based cohort study found that adolescent girls with obesity, independently of the presence of PCOS, had an increased risk of childlessness and infertility when becoming adults, not to mention all other metabolic, psychological and general health benefits of having a normal weight.[161] This further highlights the need to try to prevent the weight gain early on.

5.3 APPLICATIONS IN CLINICAL PRACTICE

Our findings of improved ovulation and menstrual regularity as well as pregnancy rates of 38% following behavioral modification intervention offer both women and health care providers important information and encouragement that the chances of conceiving may improve following lifestyle intervention. This is particularly relevant as the upper weight limit to qualify for state funded fertility treatment in some regions in Sweden is BMI 30, and in others, including Stockholm, where this study was conducted, BMI 35.[90,91] For the group of women with PCOS and a BMI over 30 or 35, who do not qualify for fertility treatment, behavioral modification intervention could offer a possibility to improve menstrual function and thereby most likely also the chances of conceiving. These BMI-cut offs for accessing fertility treatments have however been challenged. A recent RCT investigating the effects of weight reduction through dietary intervention prior to IVF in obese infertile women found no difference in the live birth rate between the groups despite a mean weight reduction of 9 kg in the intervention group, although a significantly larger number of spontaneous pregnancies occurred in the intervention group.[92] Although a caveat is that any potential differences in the long term outcomes for the children born to women in the two intervention groups were not investigated.[92] Furthermore, another study has found maternal BMI to be an independent risk factor for aspects of their children's neurocognitive development.[162] We suggest that behavioral modification intervention could be used as a first line treatment in overweight/obese women with PCOS where the main concern is improving fertility, in particular in women with a higher BMI than the cut-off for accessing fertility treatment. No analysis of the cost of implementing such an intervention has been made here. However, the cost of weekly meetings with a lifestyle coach in small groups during a 4-month period ought to compare favorably with the cost of undergoing bariatric surgery, or even with fertility treatment. Furthermore, publicly funded fertility treatment is not available in all countries, making our intervention suitable in places where only expensive privately provided fertility treatments are on offer. One practical challenge in implementing this intervention as a service on offer in commercial fertility clinics is that the potential financial incentive for offering a lifestyle intervention course is likely to be much lower than for offering fertility treatments.

Furthermore, an important finding in our study was the low levels of psychological well-being experienced by this patient group, where none of the study participants scored in the category

of positive well-being and women with higher well-being, in at least some dimensions, were more likely to lose-weight following the intervention. This highlights the importance of assessing and addressing the psychological status prior to initiating a quite demanding lifestyle modification intervention. This is also recommended in the latest international PCOS assessment and management guidelines.[4]

5.4 METHODOLOGICAL CONSIDERATIONS

In this thesis, we present novel findings regarding the effect of lifestyle intervention on objectively measured sleep variables, and markers of inflammation, in particular in the areas of neurodegeneration, autophagy and atherogenesis. We have also found associations between successful weight-loss and personality traits in this population that, to our knowledge, have not previously been investigated. In addition, we have added to the existing, sometimes limited, evidence around the effects of lifestyle intervention on menstrual function, changes to endocrine and metabolic parameters, and psychological well-being. Furthermore, the thesis makes an attempt to define in what settings and to who this intervention should be offered.

In terms of the study design, the randomized controlled design of the study (Figure 3) is a strength. In addition, the design, whereby the control treatment group received the intervention following the initial 4-months RCT period of the study, allowed for the assessment of a larger number of women having undergone the intervention at the 12-month assessment point as well as being done for ethical reasons to ensure all study participants received the intervention. Another strength was that a standardized course content (Table 5) delivered by the same lifestyle coach was used for all study participants.

A weakness of the study is the control group used for the sleep assessment in Study III. The control group was included and investigated during the extraordinary times of the Covid-19 pandemic, where many people's work-, study- and spare-time conditions radically changed, allowing for working from home and less social activities which could have resulted in larger available sleep windows. Furthermore, it was a challenge finding BMI-matched controls without PCOS, oligo- or amenorrhea or other endocrine disturbances. There were also some demographic differences between the control group and the women with PCOS such as the proportion of women with children or having a partner, however these differences were adjusted for in the statistical analysis.

Furthermore, there is a risk of type II errors occurring in our study. The initial power calculation, based on previous studies suggesting a 5% weight reduction was needed to improve menstrual function, revealed that approximately 20 women were needed in each intervention group to provide 80% power to detect a difference in reproductive function at the 5%-level. However, as the study proceeded, we realized that the study participants did not manage to achieve a mean weight loss of 5% following the intervention. This together with a larger drop-out rate than expected (16% at 4 months and 31% at 12 months) necessitated an increase of the study population to 34 women in each intervention group. Although larger than expected, the drop-out rate was close to or even lower than similar studies.[67,128,137-139]

The tool used for assessing the psychological well-being, PGWBI is a validated non disease specific tool that allowed for comparison of the well-being in our study population with other non-PCOS populations. However, we were not able to relate the well-being to disease specific symptoms, which can be done by when using the PCOSQ-tool.

The use of actigraphy for assessing sleep variables allowed for non-invasive, objective sleep assessments over a long period of time in the normal setting of a patient's home. This is in contrast to the majority of sleep studies on women with POCS that have been carried out using subjective reports of sleep. However, actigraphy does not detect sleep apnea, which is associated with PCOS.[127,163-165]

Furthermore, the proteomic analysis carried out in Study IV had a high instrument sensitivity and allowed for the analysis of a much larger number of proteins than if antibody assays had been employed. The multiplex panel of proteins studied also included proteins not previously investigated in PCOS, such as some relating to neurodegeneration, autophagy and atherogenesis. However, due to laboratory constraints related to the number of samples that could be analyzed using this experimental method, 22 women from the intervention group and 20 from the control group were selected for the analysis based on sample availability at all review points. At four months, 20 samples were analyzed from the intervention group and 17 from the control group. At 12 months only 9 samples in total were analyzed.

Despite showing promising results in terms of improvements in several symptoms of PCOS following our proposed behavioral modification intervention, the population studied here was a select group of PCOS patients having both a high BMI and fulfilling all three Rotterdam consensus criteria for diagnosing POCS. This calls for some caution when applying the results to women with different PCOS phenotypes.

6 CONCLUSION

In conclusion, we found that in a controlled trial over-weight/obese women with PCOS, menstrual function improved following behavioral modification intervention. We also found small amounts of weight loss following intervention, however improved menstrual function was not related to weight loss.

Furthermore, we found that the over-weight/obese women with POCS in our study had a severely impacted psychological well-being compared to a general population and that some aspects of well-being improved following intervention.

In terms of objectively assessed sleep variables, we demonstrated that over-weight/obese women with PCOS had sleep durations at night within the normal range but shorter that of a healthy control population of a similar age and weight and the sleep efficiency was lower in the women with PCOS. In addition, behavioral modification intervention could reduce the amount of daytime sleep.

Furthermore, we found improvements in a number of inflammatory proteins including proteins associated with neurodegeneration, autophagy and atherogenesis following intervention. The improvements in many of these variables, particularly the metabolic and inflammatory ones, were more marked at the longer-term, 12 months follow-up.

We believe behavioral modification intervention is a useful tool to improve menstrual function as well as other POCS symptoms for over-weight/obese women with PCOS in particular where fertility is the key concern.

6.1 FUTURE DIRECTIONS

Anecdotal evidence from speaking to the women with PCOS taking part in this study indicated a high patient satisfaction with the intervention. Comments such as “finally someone is taking me seriously” or “I wanted to join the study because I had heard so many positive comments about it from the PCOS community” were common. This is somewhat contradicted by 21% drop out rate in the intervention group following the 4-month intervention period. However, the patient satisfaction with the intervention was not formally assessed in our study. This lack of evidence regarding patient satisfaction in lifestyle intervention trials for women with PCOS has also been identified in the recent Cochrane review by Lim *et al* (2019) and is an important aspect to include in further work, not at least to ensure a better patient compliance to the lifestyle interventions offered.[56]

As outlined in Figure 3 some of the reasons for withdrawing from the study were lack of time and inability to organize childcare. This highlights the importance of finding more flexible interventions requiring less time commitment from the patients. Evidence from other patient groups, for example in T2DM, have shown efficacy in delivering a lifestyle intervention resulting in behavioral change, through a digital tool, focusing on self-affirmation and motivational interviewing leading to improved metabolic control and quality of life.[166] It would be of benefit to explore the efficacy of similar cheap and scalable digital tools in women with PCOS.

To get a better understanding of the role of obesity and insulin resistance as a cause of the changes to the inflammatory proteins, important next steps would be to run our multiplex-panel of inflammatory markers on samples from women with PCOS and a normal BMI and perhaps even on a group of women with PCOS and type 1 diabetes mellitus, without insulin resistance. It would also be of use to run the multiplex-panel on our age- and BMI-matched healthy control group of women without PCOS as well as to extend the analysis to other tissues such as adipose tissue, muscle and the endometrium.

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