From the DEPARTMENT OF WOMEN'S AND CHILDREN'S HEALTH
Division of Obstetrics and Gynecology
Karolinska Institutet, Stockholm, Sweden

HORMONAL AND METABOLIC EFFECTS OF DIET AND PHYSICAL EXERCISE IN OVERWEIGHT/OBESE WOMEN WITH POLYCYSTIC OVARY SYNDROME

Åsa Nybacka

Karolinska Institutet

Stockholm 2019

Published by Karolinska Institutet.
Printed by Exakta

ISBN 978-91-7831-017-3

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THESIS FOR LICENTIATE DEGREE

By

Åsa Nybacka

Principal Supervisor:
Professor Angelica Lindén Hirschberg
Karolinska Institutet
Department of Women's and Children's Health
Division of Obstetrics and Gynecology

Co-supervisor:
Professor Per M Hellström
Uppsala University
Department of Medical Sciences
Gastroenterology Unit

Examination Board:
Ass professor Anna-Clara Spetz Holm
Linköping University
Department of Clinical and Experimental Medicine
Division of Children's and Women's Health

Professor Tommy Cederholm
Uppsala University
Department of Public Health and Caring Sciences
Division of Clinical Nutrition and Metabolism

Ass professor Lars Hagenäs
Karolinska Institutet
Department of Women's and Children's Health
Division of Pediatric endocrinology

ABSTRACT

Background: Overweight/obese women with polycystic ovary syndrome (PCOS) often have hormonal problems characterized by irregular menses and decreased fertility. In addition, the overweight/obese women carry metabolic disturbances with a long-term risk of type 2 diabetes and cardiovascular disease.

Aims: The purpose of the study was to imply an individualized intervention employing either diet, exercise or the combination of both, to disentangle which of these treatment options that show the most favorable outcome in terms of hormone levels, menstrual regularity and ovarian function. Furthermore, effects on metabolic outcome variables were evaluated.

Methods: In a randomized comparative study, 57 overweight/obese PCOS women were assigned to an intervention program with either diet, exercise or the combination of both for a period of four months. The goal was to reduce the food intake by 600 kcal and increase the physical activity to 45–60 minutes of moderate to vigorous training 2–3 times per week. All patients were monthly monitored for adherence to the intervention program by either a dietician or physiotherapist, and subjected to a long-term evaluation after 12 months or more. Menstrual bleedings were recorded and ovarian parameters evaluated by ultrasound. The specific endocrine measurements consisted of changes in estradiol, progesterone, 17-OH-progesterone, luteinizing hormone, follicle stimulating hormone, sex hormone binding globulin, and dehydroepiandrosterone, as well as fasting glucose and oral glucose tolerance test, insulin, insulin-like growth factor-I, IGF-I binding protein 1 and serum levels of total cholesterol, high density lipoprotein, low density lipoprotein (LDL), triglycerides and C-reactive protein. Calculations were made for Homeostasis Model of Assessment (HOMA) index and free androgen index. Dual-energy X-ray absorptiometry was used to evaluate body composition. The values are presented as means ± standard deviation, or 95% confidence interval, or as medians with interquartile range. The data was evaluated using intention to treat or per protocol approach.

Results: There were no differences between the three groups at baseline. None of the participants were diabetic, but the majority displayed a HOMA index exceeding 3, indicating insulin resistance. The most important dietary change during the study was a reduced energy intake with more fiber, and less saturated fat and trans fatty acids based on the Swedish Nutritional Recommendations Objectified. Physically activity increased significantly in the exercise group as assessed by the number of steps per day. The summarized effects of the lifestyle interventions showed that dieting was the most effective way to reduce body weight excess, endocrinological and metabolic disturbances. Exercise alone was less effective but was superior to reduce upper body fat and maintain lean body mass. The combination of diet and exercise was too pressing for many women to pursue the intervention program in full. However, the three lifestyle interventions were equally effective in normalization of menstrual pattern and ovulation. Furthermore, there was an amelioration of the biomarkers of hyperandrogenism primarily in the diet group. Metabolic biomarkers including HOMA index, total cholesterol and LDL also improved in this group. The strongest factor to predict a reduction of BMI was increased fiber intake, while a decrease in trans fatty acid intake predicted reduced insulinogenic index.

Conclusion: Dietary management and exercise, alone or in combination, are equally effective in improving reproductive function in overweight/obese women with PCOS. However, diet alone was more effective than the other interventions to improve the general metabolic disturbances. Increased fiber and reduced trans fatty acids intake are primary predictors of metabolic improvement and weight control.

LIST OF SCIENTIFIC PAPERS

I. Nybacka Å, Carlström K, Ståhle A, Nyrén S, Hellström PM, Hirschberg AL. Randomized comparison of the influence of dietary management and/or physical exercise on ovarian function and metabolic parameters in overweight women with polycystic ovary syndrome. *Fertil Steril 2011;96:1508-1513.*

II. Nybacka Å, Hellström PM, Hirschberg AL. Increased fibre and reduced trans fatty acids intake are primary predictors of metabolic improvement in overweight polycystic ovary syndrome – substudy of randomized trial between diet, exercise and diet plus exercise for weight control. *Clin Endocrinol (Oxf). 2017;87:680-688.*

LIST OF A RELATED PAPER BY THE AUTHOR

Nybacka Å, Carlström K, Fabri F, Hellström PM, Hirschberg AL. Serum anti-Müllerian hormone in response to randomized dietary management and/or physical exercise in overweight/obese women with polycystic ovary syndrome. *Fertil Steril 2013;100:1096-1102.*

CONTENTS

LIST OF ABBREVIATIONS

AM	Amenorrhea
AMH	Anti-Müllerian hormone
ANOVA	Analysis of variance
AUC	Area under the curve
CAH	Congenital adrenal hyperplasia
CEIA	Chemiluminescence enzyme immunoassay
CI	Confidence interval
CV	Coefficient of variation
DHEAS	Dehydroepiandrosterone sulphate
E%	Energy percent
E2	Estradiol-17ß
ELISA	Enzyme-linked immunosorbent assay
FSH	Follicle-stimulating hormone
fT	Free testosterone
GnRH	Gonadotropin-releasing hormone
HDL	High density lipoprotein cholesterol
HOMA	Homeostatic model assessment
HOMA-IR	Homeostatic model assessment of insulin resistance
hsCRP	High-sensitive C-reactive protein
IGF-I	Insulin like growth factor-I
IGFBP-1	IGF binding protein-1
IQR	Inter quartile range
IRMA	Immunoradiometric assay
IRP	International Reference Preparation
ISO	International organization for standardization
LDL	Low density lipoprotein cholesterol
LH	Luteinizing hormone
Lp(a)	Lipoprotein (a)
MUFA	Monounsaturated fatty acids
NNR	Nordic nutrition recommendations
OGTT	Oral glucose tolerance test
17OHP	17-hydroxy progesterone
OHSS	Ovarian hyperstimulation syndrome

OM	Oligomenorrhea
PCO	Polycystic ovaries
PCOS	Polycystic ovary syndrome
RCT	Randomized controlled trial
RIA	Radioimmunoassay
RM	Regular menstruation
SD	Standard deviation
SHBG	Sex hormone-binding globulin
SNO	Swedish nutrition recommendation objectified
T	Testosterone
TFA	Trans fatty acids
TG	Triglycerides
TSH	Thyroid-stimulating hormone

1 INTRODUCTION

Polycystic ovary syndrome (PCOS) affects about 10% of women of fertile age and is one of the most common causes of impaired fertility. It is characterized by oligo- or anovulation, hyperandrogenism (clinical and/or biochemical) and polycystic ovaries (PCO) (Azziz et al 2016, Rosenfield and Ehrmann 2016). The syndrome was first described in 1935 by Stein and Leventhal (Stein and Leventhal 1935) presenting seven women with amenorrhea, hirsutism and polycystic ovarian morphology (PCO) (Figure 1). Initially, the syndrome was named after these authors and was called the Stein-Leventhal syndrome. Besides the abovementioned clinical features, PCOS is associated with insulin resistance, accumulation of abdominal fat, and obesity (BMI > 30 kg/m^2) in 50% of the cases (Diamanti-Kandarakis and Dunaif 2012, Lim et al 2012). The metabolic long-term consequences are increased risk of developing type 2 diabetes mellitus, cardiovascular disease and dyslipidemia, i.e. the complete metabolic syndrome (Dumesic et al 2015, Gilbert et al 2018). The syndrome is recognized as having major impact on the reproductive and metabolic health of women throughout life.

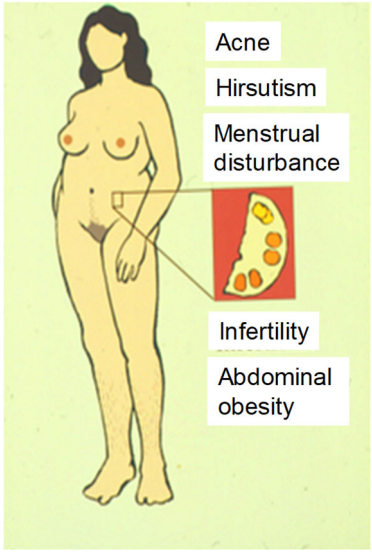

Figure 1. Clinical features of PCOS.

1.1 DIAGNOSTIC CRITERIA OF PCOS

PCOS is a heterogeneous disorder, presenting a spectrum of symptoms and manifestations that vary over time but also between individuals. This creates problems to diagnose and evaluate the syndrome. The diagnostic criteria of PCOS were first introduced in 1990 by experts from the National Institutes of Health (NIH) in USA (Table 1). The syndrome was defined as clinical and/or biochemical hyperandrogenism in combination with oligo/anovulation. In 2003, the diagnostic criteria for PCOS were revised at an International Expert Conference in Rotterdam (The Rotterdam consensus 2004) including also the ultrasound picture of PCO as an important feature. According to Rotterdam criteria, the diagnosis of PCOS includes at least two of the three following features; oligo/anovulation, hyperandrogenism and PCO after exclusion of secondary causes e.g. congenital adrenal hyperplasia (CAH), Cushing's syndrome, acromegaly, and virilizing tumors. Consequently, this definition expands the population of women fulfilling the criteria of PCOS since various combinations of the three criteria are possible. In 2006, the Androgen Excess and PCOS (AE-PCOS) Society recommended that PCOS should be considered a disorder of hyperandrogenism and that the original NIH criteria should be accepted with some

modifications (Azziz et al 2006). In 2012, an Evidence-Based Methodology PCOS Workshop by NIH recommended the use of the broader Rotterdam criteria but with inclusion of a description of the specific PCOS phenotype. However, in recent international guidelines, the criteria of the Rotterdam consensus of 2003 were considered the best evidence-based diagnostic criteria (Teede et al 2018).

Table 1. Different diagnostic criteria of PCOS over time.

	NIH 1990	**Rotterdam 2003**	**AE-PCOS 2006**	**NIH 2012 extension of Rotterdam**
Criteria	1. HA	1. HA	1. HA	1. HA
	2. OA	2. OA	2. OA and/or PCO	2. OA
		3. PCO		3. PCO
Requirements	Two of two criteria required	Two of three criteria required	Two of two criteria required	Two of three criteria required and identification of specific phenotype
Possible phenotypes	A: HA + OA	A: HA + OA + PCO	A: HA + OA	A: HA + OA + PCO
		B: HA + OA	B: HA + PCO	B: HA + OA
		C: HA + PCO	C: HA + OA + PCO	C: HA + PCO
		D: OA + PCO		D: OA + PCO

HA, hyperandrogenism; OA, oligo-/anovulation; PCO, polycystic ovary morphology on ultrasound

1.2 EPIDEMIOLOGY

PCOS is probably the most common endocrine disorder in women of fertile age. However, the prevalence is uncertain and varies depending on the diagnostic criteria that have been used. With the initial NIH criteria, the prevalence was reported to be 6-10 percent, while the Rotterdam criteria, which have extended the definition, give a prevalence of 15-20 percent in the same population (Yildiz et al 2012). Furthermore, the incidence of PCOS appears to vary in different populations, and high prevalence has been reported in ethnic groups of Mexican, Indian and Aboriginal origins, while the prevalence in China is relatively low (Dumesic et al 2015).

1.3 ETIOLOGY

The etiology of PCOS is largely unknown. However, several etiological factors have been proposed including genetic causes, androgen programming *in utero*, as well as environmental factors (Norman et al 2007). Twin studies and increased familial occurrence of PCOS speak for a hereditary component of the syndrome, but no single genetic defect has been detected and therefore it is more likely that it is a polygenetic disorder (Vink et al 2005). Experimental animal models also indicate that the hormonal environment during fetal life may predispose for later development of PCOS (Abbott et al 2004). In humans, this can be supported by the fact that diseases such as congenital adrenal hyperplasia (CAH), which involve increased androgen production from the adrenals during fetal life,

are associated with PCOS (Hague et al 1990). Furthermore, weight gain and obesity seem to be of great importance for the development of PCOS (Hirschberg 2009).

1.4 PATHOPHYSIOLOGY

Hyperandrogenism and insulin resistance are the two most important factors that can explain the various symptoms of PCOS. There is evidence of increased androgen production and release by the theca cells of the ovaries (Rosenfield and Ehrmann 2016). The ovarian androgen excess is augmented by disordered feedback control of pulsatile gonadotropin-releasing hormone (GnRH) secretion in the hypothalamus, resulting in stimulated luteinizing hormone (LH) secretion from the anterior pituitary and a relative follicle-stimulating hormone (FSH) deficiency, which will favor androgen synthesis (Rosenfield and Ehrmann 2016). Enhanced production of androgens will impair follicular development and increase the degree of follicular atresia leading to an elevated number of small follicles and enlarged stroma of the ovary. The clinical consequences of hyperandrogenism are the typical polycystic ovarian morphology (Figure 2), anovulation causing menstrual disorders, reduced fertility, and hirsutism and acne vulgaris (Dumesic et al 2015, Azziz et al 2016, Rosenfield and Ehrmann 2016).

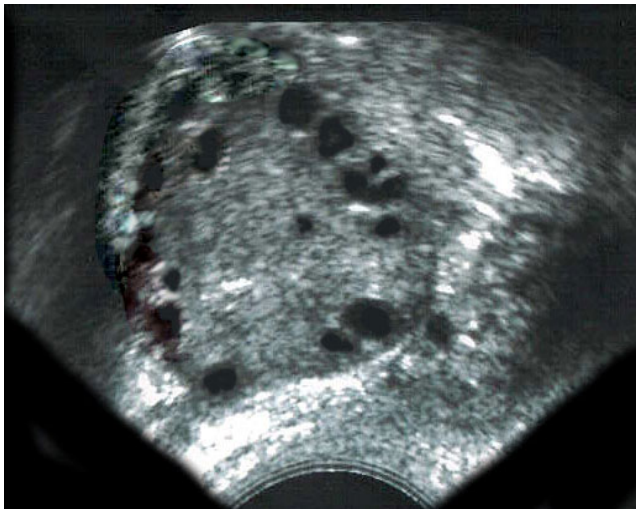

Figure 2. Ultrasound picture of a typical enlarged polycystic ovary with an increased number of small follicles.

Women with PCOS also have an increased occurrence of insulin resistance, independent of obesity, leading to secondary hyperinsulinemia. The molecular mechanism seems to be a post-receptor defect in insulin signaling due to increased insulin receptor substrate-1 serine phosphorylation that selectively affects metabolic but not mitogenic pathways in muscle, fat tissue and in the ovary (Diamanti-Kandarakis and Duanif 2012). Hypersecretion of insulin directly or synergistically with LH stimulates androgen production from the ovarian theca cells. Furthermore, insulin inhibits the hepatic synthesis of sex hormone-binding globulin (SHBG) and insulin-like growth factor binding protein 1 (IGFBP-1), and thereby increases free and bioavailable testosterone and insulin-like growth factor (IGF) concentration in the ovary. Thus, hyperinsulinemia contributes to hyperandrogenism and ovarian dysfunction in women with PCOS (Table 2). Insulin resistance may cause metabolic symptoms including abdominal obesity that predisposes to type 2 diabetes, hypertension, hyperlipidemia and cardiovascular disease (Diamanti-Kandarakis and Duanif 2012, Dumesic et al 2015, Azziz et al 2016).

Testosterone may in turn induce insulin resistance by facilitating catecholamine-stimulated lipolysis in visceral fat tissue, thus exposing the liver to a high flux of free fatty acids, which could result in hepatic insulin resistance (Ek et al 2002). Furthermore, increased

testosterone could cause insulin resistance by inducing decreased capillary density in peripheral muscle tissue, as well as visceral fat accumulation as demonstrated by testosterone treatment in women (Hirschberg 2009). It is still unclear, whether hyperandrogenism or insulin resistance is the primary defect in PCOS or if combination of both factors is necessary for the development of the syndrome.

Table 2. Effects of insulin that contribute to hyperandrogenism in women.

Tissue system	Effects of insulin
Liver	Inhibits SHBG synthesis
Liver, ovary	Inhibits IGFBP-1 synthesis
Ovary	Directly stimulates androgen synthesis
Ovary	Stimulates androgen synthesis by acting synergistically with LH
Ovary	Stimulates ovarian growth and cyst formation by acting synergistically with LH
Ovary	Up-regulates LH receptors
Peripheral circulation	Increases biologically active testosterone by stimulating ovarian testosterone and inhibiting hepatic SHBG synthesis

1.5 REPRODUCTIVE DYSFUNCTION

1.5.1 Impaired fertility

PCOS is the most common cause of anovulatory infertility, being responsible for about 70% of the cases (Broekmans et al 2006). Increased ovarian androgen production stimulates the initiation of primordial follicles and small antral follicles to growth. LH hypersecretion amplifies androgen production by the theca cells, resulting in increased follicular activation. However, then further follicular growth is arrested (Azziz et al 2016, Rosenfield and Ehrmann 2016). This could be explained by decreased levels of FSH, which are not high enough to stimulate full follicular maturation and selection of the dominant follicle. Androgen excess also initiates premature luteinization of granulosa cells, which inhibits a dominant follicle to develop. Increased insulin could contribute to inhibition of follicular development by potentiating LH-induced androgen synthesis (Azziz et al 2016, Rosenfield and Ehrmann 2016). Together these abnormalities will eventually result in anovulation and retrieval of follicles.

There is also increasing evidence that the endocrine and metabolic abnormalities that are present in PCOS may have complex effects on the endometrium. Thus, higher frequency of implantation failure and miscarriage has been reported in PCOS (Bahri Khomami et al 2019), indicating the importance of endometrial factors for reproductive dysfunction in PCOS. It is still debated whether PCOS is an independent risk factor for miscarriage or if it is explained by obesity (Bahri Khomami et al 2019). The endocrine conditions in PCOS including unopposed estrogen, hyperandrogenism and hyperinsulinemia, may negatively affect the endometrium and constitute a part of the infertility. Moreover, reduced oocyte and embryo quality have been related to PCOS (Lai et al 2018).

All sequelae of PCOS, including infertility, are aggravated by obesity, especially of the abdominal type (Hirschberg 2009). Thus, in PCOS women, obesity is associated with more frequent anovulation and increased frequency of infertility and moreover with a lower responsiveness to ovulation induction and a reduced pregnancy rate following treatment (Joham et al 2016). There are also reports on a lower embryo quality and a reduced implantation rate following in vitro fertilization (IVF) treatment in obese PCOS patients compared with those who are lean. Obesity in PCOS has furthermore been associated with a greater risk of miscarriage than normal weight women with PCOS (Hirschberg 2009). This has been ascribed to disturbances in fibrinolytic factors, related to both obesity and PCOS, which may in turn cause placental thrombosis and subsequent fetal loss.

1.5.2 Pregnancy outcome

Besides difficulties to become pregnant for women with PCOS, the syndrome is associated with significant implications for pregnancy and neonatal outcomes. Systematic reviews and meta-analyses have demonstrated a 3-fold increased risk of gestational diabetes in PCOS, and a 3 to 4-fold increased risk of pregnancy-induced hypertension and preeclampsia. These risks in pregnant women with PCOS have been attributed to hyperandrogenism but could be exacerbated by insulin resistance and obesity (Hirschberg 2009, Roos et al 2011). However, the specific mechanisms involved remain to be elucidated. Furthermore, a 2-fold higher risk of preterm delivery has been reported and increased risk of neonatal intensive care of the children (Palomba et al 2015).

1.6 OBESITY AND DISTURBED APPETITE REGULATION

The estimated prevalence of obesity in women with PCOS is 50% according to a systematic review (Lim et al 2012), and when adding overweight (BMI >25) the prevalence increases to 61%. Abdominal fat is particularly characteristic of PCOS and this clinical feature, which is dependent on hyperandrogenism, has a prevalence of 54% (Lim et al 2012).
Weight gain often precedes the development of PCOS. However, there is no evidence that obesity causes the syndrome. Increased weight will result in insulin resistance and compensatory hyperinsulinemia which may induce a vicious circle of endocrine and metabolic abnormalities and aggravating clinical symptoms including hirsutism, sub-fertility, and metabolic complications (Figure 3).

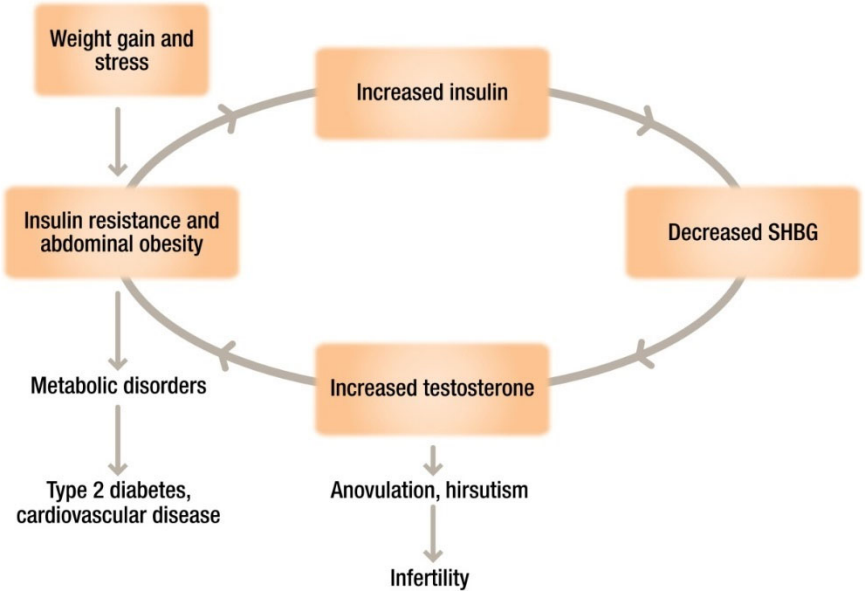

Figure 3. Vicious circle of endocrine and metabolic abnormalities and aggravating clinical symptoms of PCOS.

On the other hand, decreased weight is associated with resolution of PCOS symptoms. However, despite struggling to control their body weight many women with PCOS fail to maintain normal weight and gradually develop obesity. This could partly be explained by disturbed appetite regulation in these women.
Many women with PCOS suffer from cravings of carbohydrates and reduced feelings of satiety (Hirschberg et al 2004). Furthermore, there is an association between PCOS and binge-eating/bulimic behavior (Cesta et al 2016). It has been demonstrated that women with PCOS have altered secretion of appetite regulating hormones, including the satiety hormone cholecystokinin and the appetite-stimulating hormone ghrelin (Hirschberg et al 2004, Moran et al 2004). Thus, there is evidence of disturbed appetite regulation in women with PCOS. Increased appetite may be part of an anabolic constitution that predisposes an individual to obesity. However, whether this is a primary defect in PCOS or not is not known.

1.7 LONG-TERM MEDICAL COMPLICATIONS

1.7.1 Type 2 diabetes

It is well-known that PCOS is associated with insulin resistance independent of obesity. The prevalence of insulin resistance has been reported to range from 44% to 70% depending on the criteria used (Diamanti-Kandarakis and Dunaif 2012). The insulin resistance in PCOS has been demonstrated in classic insulin target tissues such as muscle, adipose tissue and the liver, but also in the ovary (Diamanti-Kandarakis and Dunaif 2012). It can be selective and affect only metabolic but not mitogenic action of insulin (Bajaj and DeFronzo 2003, Diamanti-Kandarakis and Dunaif 2012). A relative insulin resistance leads to a compensatory increase in insulin secretion from the pancreatic ß-cells, and thus to hyperinsulinemia.
As a consequence of insulin resistance and hyperinsulinemia, women with PCOS have an increased risk of type 2 diabetes. In U.S. populations of women with PCOS, it has been demonstrated that up to 40% have impaired glucose tolerance and 10% develop type 2 diabetes by their fourth decade (Ehrmann et al 1999). The risk of these complications is higher in obese women with PCOS but also lean women have increased rates of impaired glucose tolerance and type 2 diabetes (Legro et al 1999). However, in European populations the prevalence rates appear to be lower (Gambineri et al 2004). A meta-analysis of 30 BMI-matched studies reported increased odds ratios (OR) for both impaired glucose tolerance (2.54, 95% CI 1.44-4.47) and type 2 diabetes (4.00, 90% CI 1.97-8.10) (Moran et al 2010). Today it is well accepted that PCOS should be regarded as a prediabetic condition.

1.7.2 Cardiovascular disease

It is acknowledged that women with PCOS have increased prevalence of risk factors for cardiovascular disease such as hypertension, hyperlipidemia, abdominal obesity and impaired glucose tolerance or type 2 diabetes (Lim et al 2012, Gilbert el 2018). Furthermore, it has been demonstrated that they have increased left ventricular mass, endothelial dysfunction and arterial stiffness in higher frequency (Calderon-Margalit et al 2014, Usselman et al 2019). Several of these risk factors are not fully explained by increased body weight in women with PCOS compared to non-PCOS women. However, the overall risk of cardiovascular disease in PCOS is controversial. Meta-analysis showed no statistical difference between PCOS and control groups in terms of myocardial infarction, stroke and cardiovascular mortality (deGroot et al 2011). Thus, it is yet to be demonstrated that elevated cardiovascular risk factors in PCOS convert to cardiovascular disease in the longer term.

1.7.3 Endometrial cancer

Three meta-analyses have reported increased risk of endometrial cancer in PCOS (Chittenden et al 2009, Haoula et al 2012, Barry et al 2014). The risk of endometrial cancer has been shown to be 2- to 6-fold higher in women with PCOS than the general population. However, the association is complex with many potential confounders including obesity, infertility and metabolic disorders, as well as treatment in PCOS. Where BMI was considered, associations with PCOS and endometrial cancer are less clear (Harris and Terry 2016). The increased risk of endometrial cancer could be explained by unopposed estrogen stimulation of the endometrium during anovulation and lack of protection by progesterone.

1.8 TREATMENT

1.8.1 Surgery

Historically, PCOS was surgically treated with wedge resection of the ovaries by laparotomy (Stein 1964). This method was shown to be effective in terms of resumption of menses and fertility. A follow-up study in 149 patients 15-25 years after ovarian wedge resection demonstrated that regular menstruation pattern lasted in 88% of the patients and the cumulative/live birth rate was 78% (Lunde et al 2001). However, wedge resection is an invasive method, it is costly and could be complicated by adhesions.

Later, laparoscopic surgery (e.g. ovarian drilling) was introduced for ovulation induction in PCOS (Gjønnæss 1984). This method has been compared with metformin, clomiphene citrate, aromatase inhibitors, gonadotrophins and combinations of these treatments in different studies (Palomba et al 2004, Hamed et al 2010). All together it can be concluded that there is no evidence of inferiority for laparoscopic surgery over other ovulation induction agents. However, it is an invasive surgical intervention with a small risk of reduced ovarian reserve and adhesion formation. Laparoscopic ovarian surgery is therefore recommended as second line therapy for women with PCOS and infertility and who are resistant to clomiphene citrate or aromatase inhibitors.

In women with PCOS and severe obesity, bariatric surgery has been demonstrated to be highly effective, resulting in sustained weight loss, resolution of PCOS symptoms and improved fertility (Butterworth et al 2016). However, there is still controversy around efficacy for fertility and pregnancy outcome and bariatric surgery may adversely affect maternal and neonatal health (Johansson et al 2015). Perinatal risks include small for gestational age, premature delivery, and possibly increased infant mortality. It is recommended to avoid pregnancy for at least 12 months after bariatric surgery.

1.8.2 Combined oral contraceptives

Oral contraceptives containing both estrogen and synthetic progesterone (progestin) are used by many women for birth control, as well as for medical treatment of dysmenorrhea, heavy bleeding, endometriosis and menstrual disorders. The treatment is generally well tolerated with few side-effects. However, there is a small risk for venous thromboembolic events, which must be considered particularly in obese women (Sitruk-Ware 2016).

Today, combined oral contraceptives (COCs) are recommended as first line pharmacological treatment for management of clinical symptoms in women with PCOS without current pregnancy wish (Teede et al 2018). COCs will regulate the menstrual cycle and counteract the risk of endometrial pathology due to anovulation. Furthermore, COCs could be used to address symptoms of hyperandrogenism like hirsutism and acne. COCs reduce hyperandrogenism by several mechanisms including increased binding of circulating testosterone to SHBG, decrease ovarian production and secretion of testosterone and furthermore, the progestin component can have antiandrogenic properties (De Leo et al 2016).

Different combinations of COCs are available with varying pharmacological and clinical properties. In general, levonorgestrel-containing COCs are recommended as first choice due to the lowest relative risk of venous thrombosis (Sitruk-Ware 2016). On the other hand, third and fourth generations COCs with other progestins are more estrogen dominant and would therefore be preferable for women with PCOS. However, there is no scientific evidence that a specific formulation of COCs is more beneficial in PCOS than other alternatives. Various COCs have similar efficacy in treating hirsutism (Amiri et al 2018). It is therefore recommended to follow general guidelines when considering COC in women with PCOS (Teede et al 2018).

1.8.3 Metformin

Metformin is a biguanide that inhibits the production of hepatic glucose, and thereby decreases insulin secretion and increases glucose uptake in peripheral tissues (Wang YW et al 2017). It is a safe and low-cost medication, which has been extensively used for treatment of type 2 diabetes for several decades and is also used for management of hormonal and clinical PCOS features. However, dose dependent gastrointestinal side effects are common and affect compliance of the treatment. These side effects could be reduced by starting at a low dose and by increasing the dose gradually. Furthermore, it should be considered that long term use of metformin could result in low vitamin B12 levels.

There is evidence that metformin is beneficial to prevent weight gain, lower testosterone and improve metabolic features in PCOS (Teede et al 2018). However, metformin has no significant effect on hirsutism or protective associations with endometrial cancer (van Zuuren et al 2015). The beneficial effects are in general greater in overweight subgroups of PCOS than in lean women. Metformin could be recommended in addition to lifestyle changes for the treatment of weight, hormonal, and metabolic outcomes in PCOS (Hoeger et al 2008). The treatment may also offer greater benefit in high metabolic risk groups including those with diabetes risk factors, impaired glucose tolerance or high-risk ethnic groups, such as South East Asians, indigenous Australians and Africans. Metformin could as well be used to improve fertility but often in combination with other more effective ovulation induction agents, see below.

1.8.4 Fertility treatments

In women with anovulatory infertility, oral ovulation induction with letrozole is first-line pharmacological treatment (Wang R et al 2017). Letrozole is an aromatase inhibitor, which inhibits the conversion of androgens to estrogens. A daily dose of letrozole for five days in a row, will lead to a secondary release of LH and FSH, stimulating ovarian follicle development and maturation (Franik et al 2018). Clomiphene and metformin also have a role in ovulation induction, alone or in combination, but are less effective than letrozole to improve ovulation, pregnancy and live birth rate.

Second line of pharmacological treatment is subcutaneous injection of gonadotrophins to stimulate ovulation (Teede et al 2018). There are several considerations to make by this treatment including requirement of intensive ultrasound monitoring and risk of multiple pregnancy. Low dose gonadotrophin protocols are recommended to optimize monofollicular development. If the patient does not conceive by these strategies, in vitro fertilization (IVF) can be offered to women with PCOS and anovulatory infertility.

1.8.1 Lifestyle modification therapy

Lifestyle intervention including any combination of exercise, diet and behavioral modification intervention is the recommended first line treatment for overweight and obese women with PCOS (Teede et al 2018). Several studies have demonstrated that even a 5-10% loss in body weight loss can restore menstrual cyclicity and ovulation (Kiddy et al

1992, Huber-Buchholz et al 1999, Clark et al 1995, Crosignani et al 2003, Moran et al 2003, Hoeger et al 2004, Tolino et al 2005, Palomba et al 2008, Thomson et al 2008). The effects of different types of diets on the endocrine and metabolic parameters in PCOS have been compared in small randomized studies (Moran et al 2003, Stamets et al 2004). No diet seems to be clearly favorable over other diets with regard to the improvement of insulin sensitivity, weight reduction and maintenance of weight loss in PCOS patients (Moran et al 2003, Stamets et al 2004). Exercise as single intervention has also been shown to improve fertility, insulin sensitivity and cardiopulmonary functional capacity in PCOS women (Palomba et al 2008; Vigorito et al 2007). However, whether diet or exercise is the most effective therapy is not known.

Even though weight loss therapy may be successful initially, these programs do not take into account the problem of continued weight maintenance over forthcoming years, probably due to the low retention of changes in lifestyle (Anderson et al 2001). Because of poor compliance there is a need for different approaches to lifestyle intervention in women with PCOS. Structured lifestyle management with long-term support may improve patients' compliance. It is also important with realistic lifestyle goals. Healthy lifestyle may be beneficial for health and quality of life also in the absence of weight loss.

Our hypothesis was that weight reduction as brought about with diet, physical exercise or combination of both should normalize menstrual function and increase the number of ovulatory cycles in order to optimize fertility. We also hypothesized that dietary management and/or physical exercise should improve metabolic homeostasis and body composition in relation to dietary intake and composition.

2 AIMS OF THE THESIS

The overall aim of the present thesis was to study effects of a 4-month intervention of dietary management, physical exercise or the combination of both, in a randomized design, on hormonal, reproductive and metabolic outcomes in overweigh/obese women with PCOS.

Specific aims:

- To compare the effects of professional supportive dietary and/or physical exercise intervention on hormone levels, menstrual regularity and ovarian function.

- To evaluate the effects of these three different lifestyle interventions on metabolic outcome variables in relation to changes in food intake and dietary components in overweigh/obese women with PCOS.

3 MATERIALS AND METHODS

3.1 SUBJECTS

The study was performed between January 2003 and December 2008 at the Women's Health Research Unit, Karolinska University Hospital, Stockholm, Sweden. Potential study participants were recruited by clinical referral or advertisement in the local newspaper and initially screened by a nurse employing a standardized telephone questionnaire, after which those who seemed eligible were scheduled for a visit to a gynecologist. Eighty-four women were screened in this manner (Figure 4), and 57 fulfilled the inclusion criteria: a diagnosis of PCOS including all three criteria according to the Rotterdam Consensus (i.e., oligo-or anovulation, hyperandrogenism, and PCO on ultrasound) (Rotterdam, 2004); age 18-40 years; BMI > 27 kg/m^2; absence of hormonal treatment for the last three months; no pregnancy, lactation or change in weight during the past year. Furthermore, they did not have any of the exclusion criteria: the presence of other disease or another endocrine disorder; eating disorder; smoking; or continuous medication including insulin sensitizing drugs.
The study was approved by the Ethics Committee at Karolinska University Hospital (IRB 02-243), and the clinical trial registry number was ISRCTN48342048. Written informed consent was obtained from each participant.

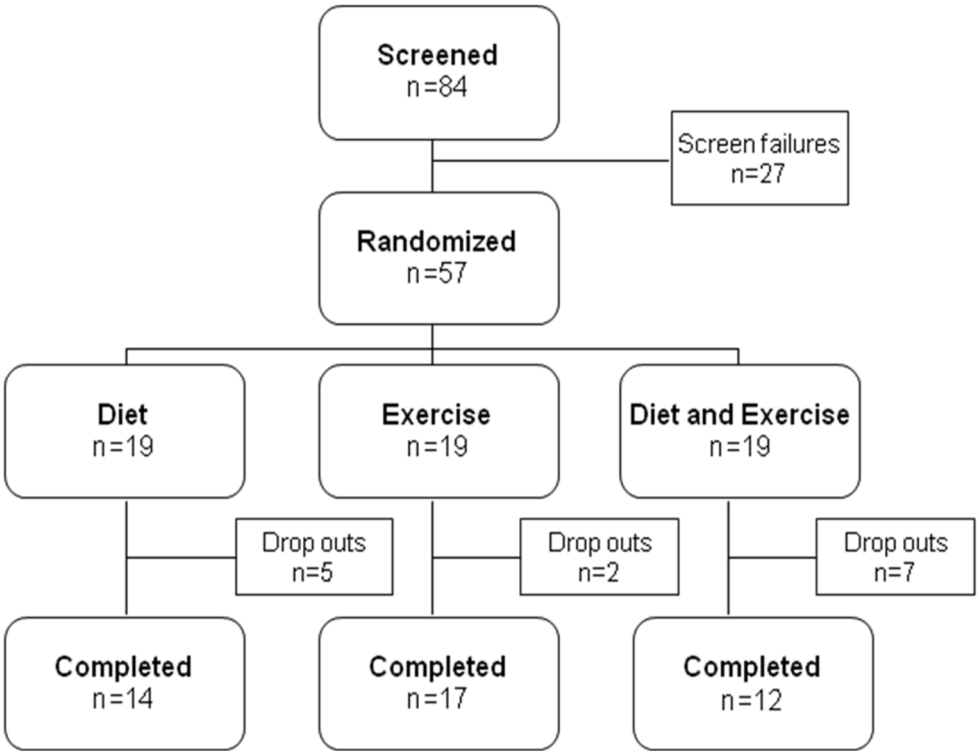

Figure 4. Study design.

3.2 EXPERIMENTAL DESIGN AND INTERVENTIONS

The study was designed as a Randomized Clinical Trial (RCT), in which the 57 women were randomized to three different interventions: a) dietary management, b) exercise or c) combined diet and exercise (Figure 4). The randomization was carried out with the permuted-block randomization method using ten blocks and a block size of six.

The interventions lasted for four months with monthly visits. A follow-up visit was scheduled at least one year after termination of the interventions. Those who did not respond were reminded by another letter, followed by a telephone call.

3.2.1 Diet

The diet program was designed individually under the close supervision of a dietician. It was recommended that total daily caloric intake be reduced by at least 600 kcal/day in comparison with meals before the intervention, while maintaining a well-balanced diet containing 55-60% carbohydrates, 25-30% fat (10% saturated) and 10-15% proteins, according to Swedish nutritional recommendations (SNO) in 2005 (Enghardt et al 2005). The goal was to reduce at least 5% of body weight. A strict schedule of three main meals and two or three snacks was also introduced. Food intake was assessed by self-reporting once every 24 hours during the 4 days both immediately before and after four months of treatment (three working days and one weekend day). The individual intake of energy and nutrients was computed using a food database (Dietist XP, version 3.2, Kost- och Näringsdata AB, Bromma, Sweden). Energy consumption of macronutrients and fiber in the diet composition was evaluated.

3.2.2 Exercise

The exercise program, which was individually adjusted and overseen by a physiotherapist, was designed to enhance both the type (endurance, aerobic and/or weight training depending on each of the subject's preferences), and the level of physical activity. The exercise included walking (with or without poles), aerobics, jogging, swimming, strength training, with an exertion of moderate to vigorous, performed 2–3 times a week with a duration of 45–60 minutes each time during the intervention period. Physical activity was assessed utilizing pedometers (Yamax SW-200 Tokyo, Japan) during the four days immediately before and at the end of the program.

During both types of intervention and the combination of both, monthly follow-ups with the dietician and/or physiotherapist were scheduled for discussion of the goals achieved, as well as setting up new goals for the next month. Flow diagram of the study procedures is shown in Figure 5.

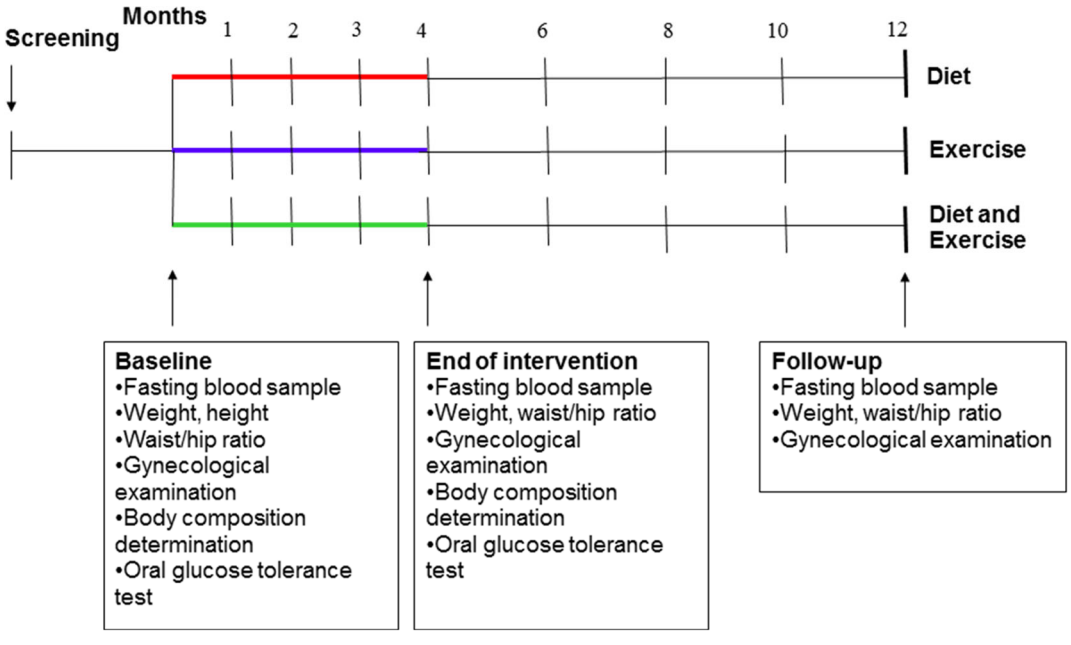

Figure 5. Flow diagram of study procedures.

3.2.3 Physical examination

Immediately before the intervention, after four months of intervention, and at the time of the long-term follow-up, each patient underwent a general health control involving measurement of blood pressure, weight, height and waist/hip ratio (WHR), blood sampling, gynecological examination and determination of body composition. In a resting and fasting state at 8:00 AM, a blood sample was collected from a peripheral vein and the serum separated by centrifugation and stored at – 70°C, pending analysis for hormones, binding proteins and glucose. Menstruating women were examined in early follicular phase of the menstrual cycle (cycle days 1–5), whereas women with amenorrhea (AM) were investigated on an arbitrary day. Amenorrhea was defined as no bleeding for the last three months and oligomenorrhea (OM) as periods with intervals exceeding six weeks and five to nine periods during the previous 12 months.
All gynecological examinations including transvaginal ultrasound using Sonoline SI-250 equipment (Siemens Healthcare Diagnostics, Deerfield, IL, USA) were performed by the same investigator (ALH). The ovarian parameters evaluated were the maximal number of follicles in one plane and the volumes of the largest follicle of the entire ovary (Goswamy et al 1983). Menstrual bleedings were recorded, and ovulation confirmed based on an elevation in the serum level of progesterone (>17 nmol/L) during the luteal phase of the menstrual cycle.

3.2.4 Body composition

Body composition was examined by dual-energy X-ray absorptiometry (DXA) using a Lunar Prodigy Advance whole-body scanner (GE Healthcare Sverige AB, Stockholm, Sweden) as previously described (Hagmar et al 2009). The whole-body dual-energy X-ray absorptiometry thus obtained was divided into regions, each of which was evaluated for fat tissue, lean mass, bone mass, and total and spinal bone mineral density according to standard criteria. The Lunar software (Lunar Software Inc., Portland, OR, USA) calculates the amount of fat in the trunk and legs, the boundary between these being defined by the line drawn from the upper margin of the iliac crest to the neck of the femur. The ratio of trunk/leg fat was used as an estimate of the ratio of upper/lower body fat.

3.2.5 Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed according to standard clinical procedure by ingestion of 75 g glucose dissolved in 250 mL water. Blood samples were drawn into prechilled vacutainer tubes at 0, 30, 60, 90 and 120 min during the OGTT. The blood samples were centrifuged at 4ºC for 10 min, 2055g, and plasma aliquoted and stored at -70 ºC until assayed in the same run.

3.2.6 Biochemical measurements

Serum levels of steroid and protein hormones, growth factors and binding proteins were determined by immunoassays specified in Table 3. Serum levels of testosterone, estradiol and 17 hydroxy progesterone (17OHP) were determined by radioimmunoassay (RIA), insulin by enzyme-linked immunosorbent assay (ELISA), FSH, LH, SHBG, dehydroepiandrosterone sulphate (DHEAS) and IGF-I by chemiluminescence enzyme immunoassay (CEIA), and IGFBP-1 by immunoradiometric assay (IRMA).
FSH and LH values are expressed as U/L of 2:nd International reference preparation (IRP) FSH 78/549 and 1:st IRP LH 68/40, respectively. Apparent free concentrations

of testosterone was calculated from the concentrations of total testosterone, SHBG and a fixed albumin value of 40 g/L according to Södergård and co-workers (Södergård et al 1982). The ratio between total testosterone and SHBG (testosterone/SHBG-ratio, "free androgen index") was also calculated.
Glucose was assayed utilizing the YSI 2300 STAT PlusTM Glucose & Lactate Analyzer (YSI Inc., Life Sciences, Yellow Springs, OH, USA). As a measure of insulin resistance, the Homeostasis Model of Assessment (HOMA) index was calculated using the formula ([fasting insulin, mIU/L] x [fasting glucose, mg/dL])/405 (Turner et al 1979). HOMA index > 3 (HOMA-IR) is defined as insulin resistance.
Serum levels of total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides (TG) were determined by enzymatic clinical routine methods (DXC 800 Access, Beckman Coulter Inc, Fullerton, CA). Low density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula (Friedewald et al 1972). High-sensitive C-reactive protein (hsCRP) was analyzed immunochemically with kinetic nephelometry (Beckman – Coulter Inc, Fullerton, CA).

Table 3. Analytical methods for hormones, growth factors and binding proteins.

Analyte	Method	Manufacturer	Detection limit	Within assay CV	Between assay CV
Testosterone	RIA	Siemens/DPC	0.1 nmol/L	6%	10%
Estradiol	RIA	Orion	5 pmol/L	5.7%	8.4%
17OHP	RIA	Orion	0.1 nmol/L	7.8%	10.0%
Insulin	ELISA	DAKO	3 pmol/L	6.7%	7.5%
FSH	CEIA	Immulite® (Siemens/DPC)	0.1 U/L	8%	8%
LH	CEIA	Immulite® (Siemens/DPC)	0.7 U/L	6.0%	9%
SHBG	CEIA	Immulite® (Siemens/DPC)	0.2 nmol/L	6.5%	8.7%
DHEAS	CEIA	Immulite® (Siemens/DPC)	0.8 µmol/L	8.2%	12%
IGF-I	CEIA	Immulite® (Siemens/DPC)	20 µg/L	3.6%	6.6%
IGFBP-1	IRMA	DSL	0.33 µg/L	4.3%	4.4%

Manufacturers: Beckman Coulter Inc, Fullerton, CA; DAKO A/S, Glostrup, Denmark; Diagnostic Systems Laboratories (DSL) Inc, Webster TEX; Orion Diagnostica Ab, Esbo, Finland; Siemens Medical Solutions (former Diagnostic Products (DPC) Corp.), Los Angeles, CA.

3.2.7 Statistical analyses

All values are presented as means, standard deviations and 95% confidence intervals (95% CI) or as medians and inter-quartile (IQR) ranges (P25 – P75). Employing a Mixed model in SAS software (Cary, NC, USA), repeated measurements of the different parameters taken before and after the interventions were compared. The factors in the model were Group (Diet, Exercise and Diet + Exercise), Time (before and after) and the Group x Time interaction. Intention to treat analysis (ITT), using all available data from the 57 women in the Mixed Model analyses, was performed for paper II but not for paper I. Logistic regression analysis was utilized to evaluate the relationship between improved menstrual pattern, as well as confirmed ovulation and the measurements of body composition, endocrinological, gynecological and metabolic parameters. Within each block of parameters stepwise logistic regression analyses were performed and the significant variables thus obtained then included in a

final stepwise logistic model. Prior to these analyses, certain of the variables were log-transformed to compensate for their positively skewed distributions. A p-value <0.05 was considered statistically significant.
A power calculation revealed that 3 x 15 subjects would provide 80% power to detect a difference in means of BMI of 1.5 kg/m^2 within the groups and a difference in means of at least 2.0 kg/m^2 between the groups, assuming that the common standard deviation is 1.7, with a 0.05 two-sided significance level.

4 RESULTS

There were no significant differences in baseline values between the three groups (Table 4). Among the participants, none of them had pathologically elevated fasting blood glucose, impaired glucose tolerance or increased serum 17OHP levels, all values speaking against the presence of type 2 diabetes mellitus or adrenal 21- or 11ß-hydroxylase deficiency. However, the majority had increased HOMA index >3 indicating insulin resistance.

Table 4. Baseline characteristics of the women in the three different intervention groups.

	Diet	Exercise	Diet and exercise
n	19	19	19
Age, y	29.9 ± 5.5	31.3 ± 4.8	31.8 ± 4.9
BMI, kg/m^2	35.4 ± 4.9	34.8 ± 5.2	38.1 ± 7.0
LH/FSH ratio	2.1 ± 1.1	2.0 ± 1.1	2.0 ± 1.2
Testosterone, ng/mL	0.51 ± 0.22	0.44 ± 0.18	0.50 ± 0.21
SHBG, mg/L	2.3 ± 0.8	2.4 ± 0.8	2.3 ± 0.9
Free testosterone, pg/mL	16.3 ± 7.4	13.0 ± 6.1	17.2 ± 9.0
17OHP, ng/mL[a]	0.86 ± 0.20	0.93 ± 0.40	0.86 ± 0.33
Estradiol, pg/mL[a]	42.4 (36.8-45.9)	40.5 (33.5-53.8)	40.4 (33.4-58.5)
Insulin, mIU/L	16 (14-28)	14 (11-22)	19 (14-27)
Glucose, mg/dL	86.9 ± 11.5	84.6 ± 7.2	89.9 ± 10.2
HOMA index	3.6 (3.0-6.9)	2.9 (2.3-4.7)	4.6 (3.4-5.9)
HOMA-IR (> 3), %	72	50	81
IGF-I, µg/L[a]	171 ± 45	165 ± 48	162 ± 53
IGFBP-1, µg/L[a]	4.2 (3.3-5.6)	5.0 (2.9-9.9)	3.1 (2.4-7.6)

Values are mean and SD or median and IQR.
Conversion factor to SI: Testosterone nmol/L 3.467; SHBG nmol/L approx. 10.9; Free testosterone pmol/L 3.467; 17OHP nmol/L 3.026; Estradiol pmol/L 3.671; Insulin pmol/L 7.175; Glucose mmol/L 0.056.
[a]Baseline data based on only subjects who completed the study.

4.1 BODY COMPOSITION (PAPER I)

Of the 57 initial women, 13 were lost to follow-up, excluded or dropped out for different reasons leaving a final total of 43 (Figure 4). The most common finding for dropping out was difficulties to participate in the combined diet and exercise program with limited intake of rich foods and increase in physical exercise. Other reasons for drop out during the 4 months' study period were: fertility treatment (n= 2), hypothyroidism (n=1), and ovarian cyst (n=1).
After intervention, the 24-hour caloric intake was significantly reduced in the diet and the combined groups and the number of steps per day increased significantly only in the exercise group when analyzed according to ITT. This resulted in reduced BMI in all three groups from –3% to –6% with no significant difference between the groups (Figure 6a). In line with this, the primary readout for the statistical power calculation, BMI, showed a clear decrease in the diet group and the combined diet and exercise group, while results were less pronounced in the exercise group. Loss of body weight ≥5% was obtained by one third in the combined material with no difference between groups. The percentage of total body fat measured by DXA decreased significantly only in the diet group. The upper body fat, mainly reflecting the abdominal fat mass,

decreased significantly in the exercise group (Figure 6b), but also in the total material, whereas lower body fat decreased significantly in the dietary and combined groups (Figure 7a). Lean body mass was diminished with diet, alone or in combination with exercise but not in the exercise only group (Figure 7b). The change in lean body mass was significantly different between groups ($p<0.05$).

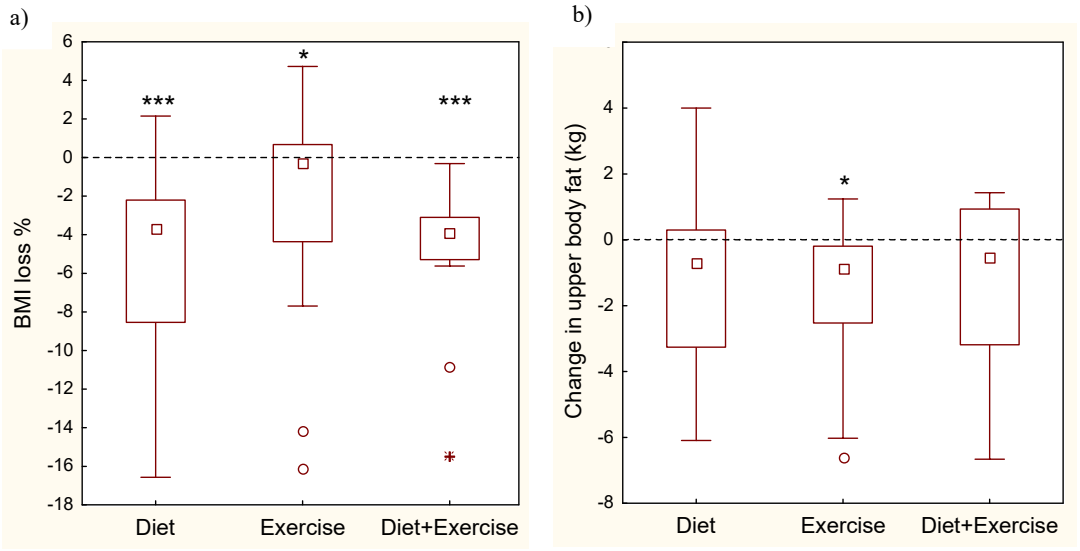

Figure 6. Change in BMI (a) and upper body fat (b) in the three different intervention groups. The values presented are medians (the symbols), the inter-quartile ranges (P25 – P75, the boxes) and the non-outlier ranges (the whiskers). * $p<0.05$, *** $p<0.001$ compared to baseline.

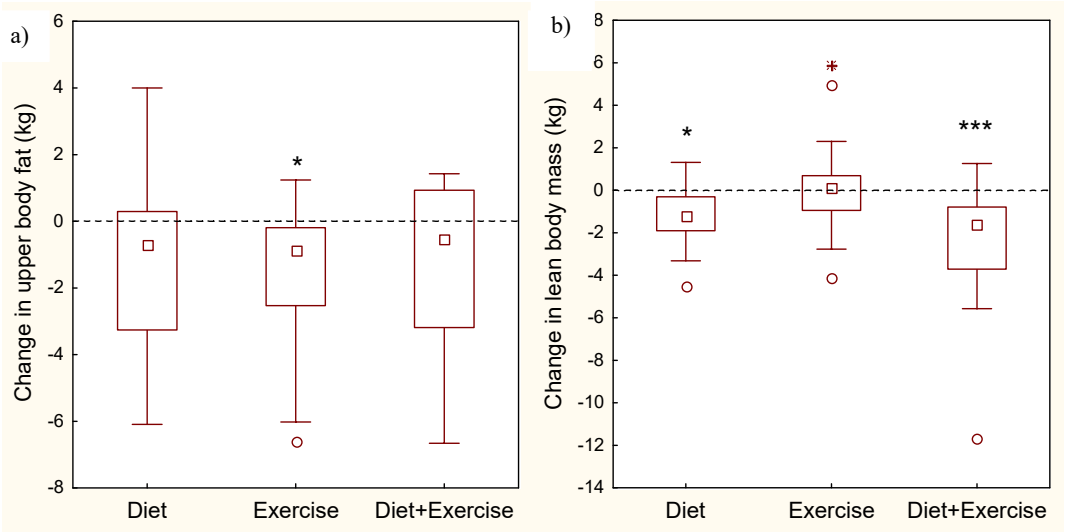

Figure 7. Change in lower body fat (a) and lean mass (b) in the three different intervention groups. The values presented are medians (the symbols), the inter-quartile ranges (P25 – P75, the boxes) and the non-outlier ranges (the whiskers). * $p<0.05$, *** $p<0.001$ compared to baseline.

4.2 ENDOCRINE AND METABOLIC SERUM BIOMARKERS (PAPER I)

Before intervention, all women had disrupted ovulatory function demonstrating either amenorrhea (n = 24) or oligomenorrhea (n = 33). Furthermore, they displayed typical hormonal changes for PCOS including high serum levels of LH, LH/FSH ratio, and

total testosterone (Table 5). At the same time, serum levels of SHBG were low, and consequently, free testosterone and the testosterone/SHBG ratio were elevated. These clinical and endocrine changes agree with the diagnostic criteria of PCOS.
After the interventions, the serum levels of many sex hormones were rectified whereas others did not change. Estradiol and SHBG were not significantly changed (Table 5). Total and free testosterone decreased significantly in the diet group only, whereas these hormones were unchanged in the exercise and diet plus exercise regimes.
Among the metabolic hormones, fasting insulin and glucose decreased significantly in the dietary group, but not in the other two groups (Table 5). Furthermore, serum IGF-I and IGFBP-1 increased significantly only in the dietary group. To conclude, the dietary regime stands out as the most effective measure in order to improve endocrine and metabolic biomarkers in blood.

Table 5. Changes in endocrine and metabolic serum biomarkers in the three different intervention groups.

	Diet	Exercise	Diet and exercise
Testosterone, ng/mL	-0.14 (-0.14 to -0.06) *	0.00 (-0.09 to 0.06)	-0.03 (-0.12 to 0.10)
SHBG, mg/L	0.20 (-0.06 to 0.74)	0.26 (-0.14 to 0.36)	0.32 (-0.12 to 0.10)
Free testosterone, pg/mL	-5.21 (-5.56 to -1.04)**	0.00 (-2.43 to 1.39)	-0.35 (-5.73 to 1.74)
Estradiol, pg/mL	12.70 (-8.92 to 27.30)	-1.35 (-11.90 to 10.54)	2.16 (-13.65 to 21.62)
Insulin, mIU/L	-1.50 (-8.50 to 0.00)*	0.00 (-2.00 to 5.00)	-3.00 (-7.00 to -1.00)
Glucose, mg/dL	-3.93 (-5.72 to -1.07)*	0.71 (-5.54 to 5.18)	-3.66 (-6.16 to 1.52)
IGF-I, µg/L	10.00 (-2.00 to 44.00)*	-2.00 (-22.00 to 13.00)	9.65 (-11.00 to 16.50)
IGFBP-1, µg/L	1.36 (0.37 to 2.24)*	0.17 (-1.16 to 4.32)	0.04 (-0.87 to 1.54)

Values are median and IQR.
Conversion factor to SI: Testosterone nmol/L 3.467; SHBG nmol/L approx. 10.9; Free testosterone pmol/L 3.467; Estradiol pmol/L 3.671; Insulin pmol/L 7.175; Glucose mmol/L 0.056. * $p<0.05$, ** $p<0.01$ compared to baseline.

4.3 OVARIAN FUNCTION (PAPER I)

The functional outcome of the interventions was significantly improved menstrual pattern shifting from amenorrhea to oligomenorrhea/regular menstruation or from oligomenorrhea to regular menstruation, however, without any significant difference between groups.
Figure 8 shows the frequency of amenorrhea (AM)/oligomenorrhea (OM)/regular menstruation (RM) before and after intervention in the three intervention groups. A lower level of total testosterone *before* intervention was the strongest factor to predict improvement in menstrual function independently of intervention group and weight loss (odds ratio (OR) 0.55, 95% CI 0.35-0.85, $p<0.01$, respectively). *After* intervention, testosterone levels correlated significantly with menstrual status (AM/OM/RM) ($r = -0.34$, $p<0.05$) (Figure 9).

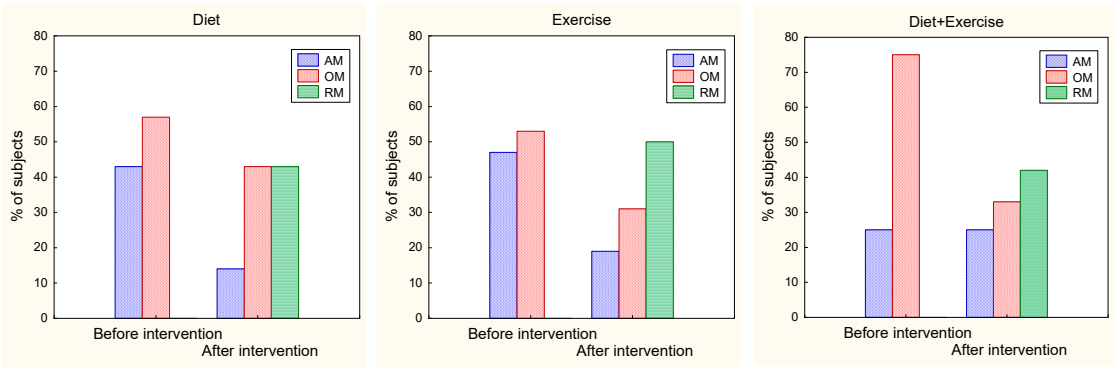

Figure 8. Frequency of amenorrhea (AM), oligomenorrhea (OM) and regular menstruation (RM) before and after intervention in the three intervention groups.

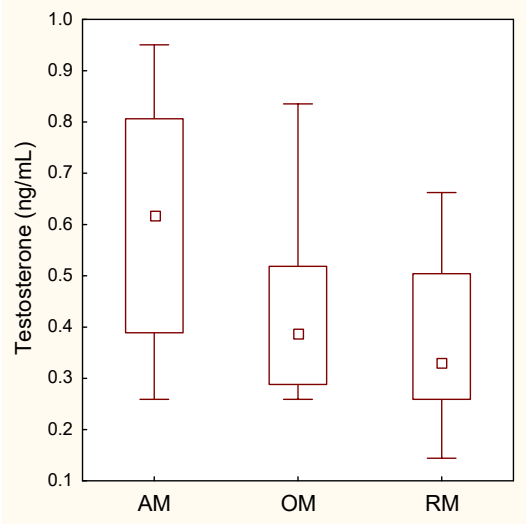

Figure 9. Serum levels of testosterone after intervention in women with amenorrhea (AM), oligomenorrhea (OM) and regular menstruation (RM).

Of all women, 15 had confirmed ovulation after intervention with no difference between groups. Ovarian volume was not altered by any of the interventions, but the overall number of follicles in both the right and left ovary was significantly reduced in all groups (data not shown). *Before* intervention, less degree of menstrual disturbance (OM instead of AM) and a lower amount of body fat mass were the strongest predictors of ovulation (OR 22, 95% CI 2.35-214, $p<0.01$ and OR 0.99, 95% CI 0.99-1.00, $p<0.05$), respectively. *After* intervention, an increase in IGFBP-1 and a decrease in total testosterone were associated with ovulation (OR 723, 95% CI 1.09-477699, $p<0.05$ and OR 1.53, 95% CI 1.06-2.20, $p<0.05$).

Taken together, improvements in endocrine and metabolic values were primarily seen in the diet group where the largest weight loss was obtained, whereas the functional reproductive result of improved menstrual function and ovulation seem possible to achieve with either form of weight-controlling regimen independently, be it in the form of diet or exercise or a combination of both.

4.4 DIETARY INTAKE AND METABOLIC OUTCOMES (PAPER II)

4.4.1 Daily dietary intake

Baseline intake of calories and dietary components were similar in the groups. After intervention, there was a general decrease in total energy intake with no significant difference between groups (all groups $p<0.01$). However, this decrease was most clearly shown in the diet and combined groups. Total fat intake decreased in the diet and combined groups where carbohydrate intake was also reduced, but exercise alone had no significant effect on macronutrient intake. Protein intake did not change significantly in any of the groups.

Diet and combined groups consistently reduced all forms of fat intake, whereas the fat reduction in the exercise group was limited to saturated and monounsaturated fats (Figure 10). The ingested amount of trans fatty acids was significantly reduced in both the diet group and in the combined group, but not at all in the exercise group. Consequently, there was a significant group difference in trans fatty intake ($p<0.05$).

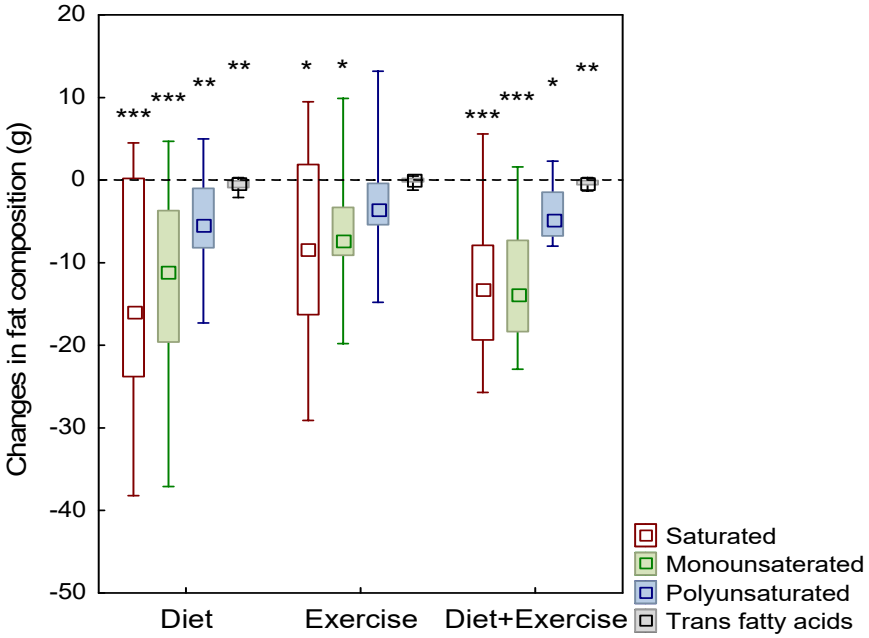

Figure 10. Changes in fat composition in the three different intervention groups.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$, all compared to baseline.

Intake of starch and sucrose decreased significantly in the combined group and starch also in the diet group, whereas these components of carbohydrates were not significantly changed in the exercise group (Figure 11). Furthermore, fiber intake increased significantly in both the diet and combined groups, but not in the exercise group, resulting in a significant group difference in this respect ($p<0.05$).

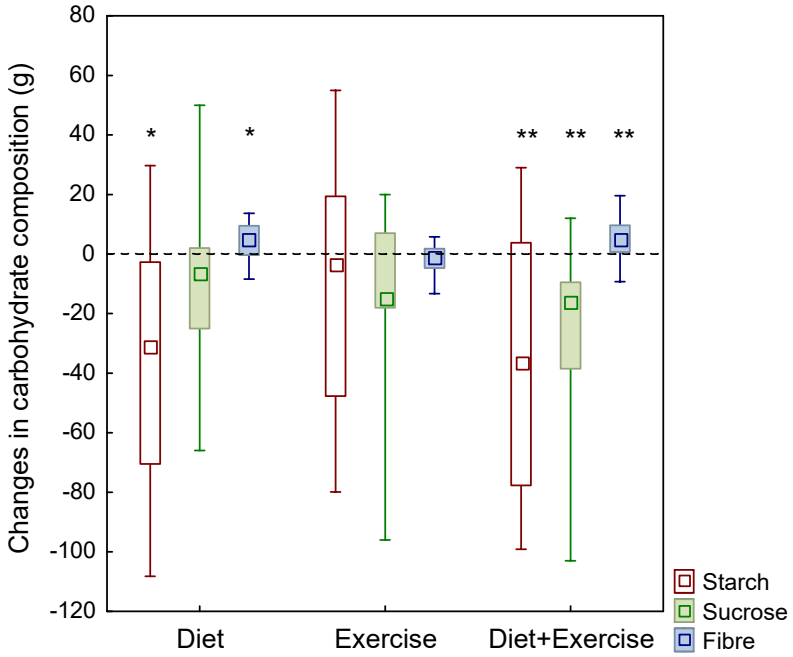

Figure 11. Changes in carbohydrate composition in the three different intervention groups. * p<0.05, ** p<0.01 compared to baseline.

4.4.2 Metabolic outcome variables

Waist circumference decreased significantly in all groups (Table 6). There was a tendency for an overall reduction in systolic blood pressure whereas diastolic blood pressure was unchanged. The HOMA index decreased significantly in the diet group and showed a similar but not statistically significant trend in the combined group but not in the exercise group. The change in HOMA index differed significantly between groups ($p<0.05$). However, there were no significant changes in glucose or insulin responses to OGTT or insulinogenic index. In the total material, there was an overall decrease in hsCRP, which became significant also in the exercise group. Total cholesterol was significantly reduced in the diet and combined groups, but not in the exercise group. Similarly, LDL was reduced in the diet group but not in the other groups. There was a significant group difference in the change in total cholesterol and LDL ($p<0.05$, respectively).

To conclude, the dietary regime stands out as the most effective measure in order to improve metabolic parameters. The strongest predictor of reduced BMI was increased fiber intake (-0.44, $p<0.05$), whereas a decrease in trans fatty acid intake predicted reduced insulinogenic index (0.44, $p<0.01$).

Table 6. Metabolic outcome variables in the three different intervention groups.
Values are mean and SD or median and IQR. * p<0.05, ** p <0.01, *** p<0.001 compared to baseline.

	Diet		Exercise		Diet and exercise	
Waist circumference, cm	103.8 ± 13.0	95.5 ± 13.2***	103.3± 10.9	99.3 ± 12.5*	110.3 ± 14.6	106.6 ± 14.6*
Systolic blood pressure, mm Hg	129 ± 10.7	128 ± 11	126 ± 10	122 ± 14	126 ± 14	123 ± 11
Diastolic blood pressure, mm Hg	80 ± 11	82 ± 7	81 ± 11	80 ± 11	82 ± 8	80 ± 8
HOMA index	3.6 (3.0-6.9)	2.9 (2.2-3.6)*	2.9 (2.3-4.7)	3.1 (2.4-4.5)	4.6 (3.4-5.9)	3.8 (2.3-5.0)
Insulinogenic index	46.0 (42.3-70.8)	35.8 (29.9-69.4)	33.8 (25.2-39.4)	35.9 (24.2-54.0)	35.8 (28.0-81.6)	31.6 (22.4-54.8)
hsCRP, mg/L	4.5 ± 6.1	3.1 ± 2.5	5.1 ± 6.8	3.8 ± 3.5*	5.0 ± 4.0	3.6 ± 2.7
Total cholesterol, mmol/L	4.5 ± 1.0	4.0 ± 1.4**	4.4 ± 0.7	4.5 ± 0.6	4.7 ± 1.1	4.3 ± 1.1*
LDL, mmol/L	2.8 ± 0.8	2.3 ± 0.9**	2.6 ± 0.5	2.7 ± 0.6	2.9 ± 1.0	2.6 ± 0.9
HDL, mmol/L	1.1 ± 0.2	1.0 ± 0.2	1.2 ± 0.3	1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.3
Triglycerides	1.4 ± 0.7	1.4 ± 0.7	1.4 ± 0.6	1.3 ± 0.4	1.4 ± 0.5	1.2 ± 0.4

4.5 LONG-TERM FOLLOW-UP

Twenty-one patients, seven in each group, came to long-term follow-up 33 (19-56) months after termination of the intervention. Ten women declined to come, and the others were not possible to reach. In comparison to baseline, the mean loss in BMI was 8% in the dietary group, 1% in the exercise group and 11% in the combined group (Table 7). In patients not using hormonal contraception, 11/17 menstruated regularly.

Table 7. Changes from baseline in BMI and endocrine variables at long-term follow-up.
Values are median and IQR or percentage.

	Diet	Exercise	Diet and exercise
n	7	7	7
BMI, kg/m^2	-2.6 (-5.3 to 3.9)	-0.4 (-0.8 to 1.2)	-5.9 (-6.4 to -1.5)
BMI loss, %	-8	-1	-11
Regular menstruation	3/5	4/6	4/6

5 GENERAL DISCUSSION

5.1 CLINICAL OUTCOMES

This thesis shows that out of the 57 overweight/obese women fulfilling all three Rotterdam criteria of PCOS and that were randomized to lifestyle intervention with diet, exercise, or both exercise and diet, 43 women were able to fully pursue the study until termination. The greatest dropout rate was found in the exercise plus dieting group, followed by the dieting, and exercise groups. Many participants claimed that strenuous exercise in combination with diet was a too heavy burden on their daily life in order to follow the study intervention. Despite difficulties with compliance in our lifestyle study, there were significant changes in clinical outcomes in all intervention groups.

5.1.1 Body composition

There was an overall significant decrease in BMI by the interventions with a mean weight loss of -4.1% corresponding to -3.9 kg. This is in line with what has been reported after lifestyle intervention in similar studies of obese women with PCOS (Haqq et al 2015). As expected, the most pronounced weight loss was in the two diet groups, followed by the exercise group. The weight loss remained or even improved in those women who were examined at the long-term follow-up. However, it must be noted that the drop-out rate was high and only 21 out of 57 (37%) women came to long-term follow-up. Thus, at least for some individuals it seems that a lifestyle program could be beneficial for long-term weight maintenance.

Body composition as examined by DXA showed that total body fat decreased significantly only in the diet group, whereas upper body fat, which mainly reflects the abdominal fat mass, decreased significantly only in the exercise group. In contrast, lower body fat decreased significantly in the diet and combined groups. Lean body mass decreased with diet, alone or in combination with exercise but not with exercise alone.

Diet appears to be more efficient for losing body fat than exercise, except for the abdominal fat. Previous interventional studies in obese women have shown that exercise alone results in modest reduction in body weight but more pronounced effect on abdominal fat (Irwin et al 2003). The reduction in abdominal fat should be beneficial since there are clear associations with metabolic improvements (Dunn et al 2014). It has been suggested that the mechanism is reduced inflammation by physical training (Cavicchia et al 2009). Furthermore, exercise prevents the loss of lean body mass secondary to dietary restriction, as seen in our study. However, considering that women with PCOS usually have increased amount of lean mass, the clinical significance of some loss of lean mass is not clear. Based on our results, no intervention seems to be superior than the other in terms of body composition.

5.1.2 Endocrine and metabolic serum biomarkers

Before intervention, endocrine and metabolic serum biomarkers were comparable between the three groups. The women demonstrated endocrine patterns characteristic of PCOS, including increased LH, LH/FSH ratio and testosterone, whereas SHBG was low resulting in increased testosterone/SHBG ratio and high levels of free testosterone. Estradiol levels were in the normal range corresponding to early follicular phase. Serum levels of 17OHP were normal, and thus excluding mild forms of congenital adrenal hyperplasia. Furthermore, the frequency of HOMA index >3 was high in all three groups. However, none had increased fasting blood glucose levels indicating type 2 diabetes.

After intervention, serum testosterone decreased and thereby also free testosterone but only significant in the diet group. Furthermore, several metabolic serum biomarkers improved in the diet group, including reduction in fasting glucose and insulin, and increase in IGF-I and IGFBP-1. However, all these variables were unchanged in the other intervention groups. Thus, the diet intervention was more effective in reducing androgen levels and improving serum metabolic markers than exercise and combined intervention.
There is systematic review evidence for improvement of total testosterone, free androgen index and fasting insulin but not for SHBG or glucose by lifestyle intervention (combined diet and exercise or exercise only) in women with PCOS when compared to minimal treatment (Moran et al 2011, Lim et al 2019). Our results for the diet group are in line with these results.

5.1.3 Ovarian function

The connection between increased body weight in PCOS and clinical consequences has been studied previously resulting in a recommendation of a weight reduction of at least 5% to achieve an improved biochemical profile and, more importantly, restoration of fertility (Teede et al 2018). Weight loss through a controlled low-calorie diet and exercise should improve anthropometric indices in the PCOS patients, reduce ovarian volume and follicle number and restore ovulatory cycles, allowing spontaneous pregnancy (Kiddy et al 1992, Crosignani et al 2003, Moran et al 2003, Tolino et al 2005, Palomba et al 2008, Thomson et al 2008). This has been recommended to be achieved by conservative treatment with sustainable weight loss through dietary modification and exercise (Teede et al 2018).
In our study, the weight reduction ranged from -3% to -6% with the largest weight loss in the dietary intervention group. However, all three interventions were equally effective to normalize menstrual cycling and ovulation. Although weight loss was not the strongest factor predicting improvement in menstrual function and ovulation, a Kaplan-Meier plot demonstrated a relationship between weight reduction and ameliorated reproductive function in those who completed the intervention (Figure 12). Thus, a weight loss of 5 kg resulted in improved menstrual function in 50% of the patients and ovulation in 33%, whereas a weight loss of 10 kg improved menstrual function in 75% of the patients and restored ovulation in 60%.

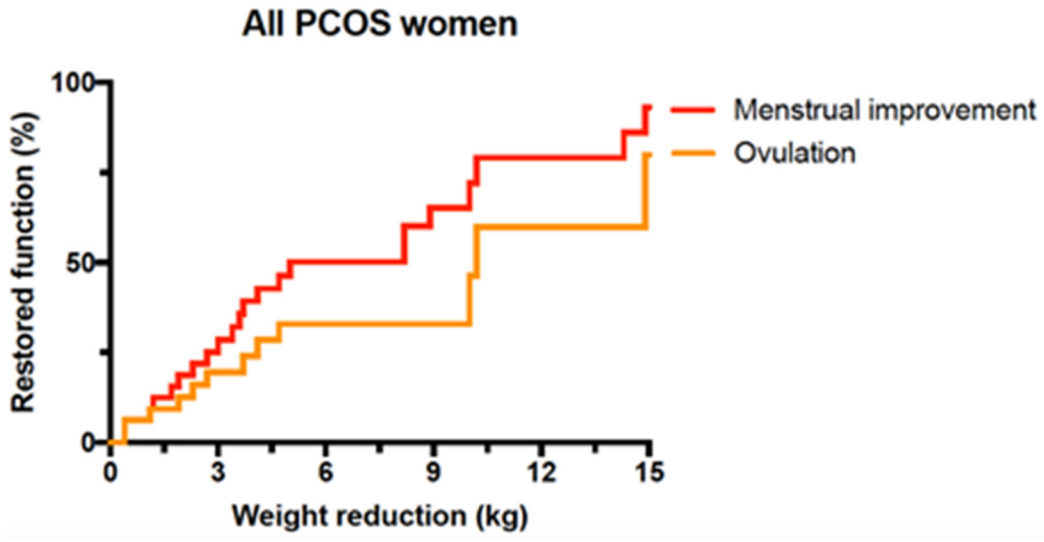

Figure 12. Kaplan-Meier plot of PCOS women showing a menstrual pattern shifting from amenorrhea to oligomenorrhea/regular menstruation or from oligomenorrhea to regular menstruation. Analysis performed by per protocol, n= 43, $p<0.07$.

The strongest factor predicting improved menstrual function was a lower testosterone level before intervention, indicating that women with a milder form of PCOS/hyperandrogenism have a greater chance of reproductive improvement. Similarly, we found that less degree of menstrual disturbance before intervention predicted restored ovulation. After intervention, we found that a decrease in serum testosterone and an increase in IGFBP-1 were associated with ovulation.
PCOS is related to low serum levels of IGFBP-1 due to inhibited production by increased levels of insulin in the circulation (Kelly et al 2011). A decrease in IGFBP-1 will in turn increase free IGF-I in serum, which together with insulin can stimulate ovarian androgen production (Kelly et al 2011). However, low levels of IGFBP-1 seem to be mainly determined by BMI and is unlikely the cause of ovarian hyperandrogenism in women with PCOS (Kelly et al 2011). Instead, IGFBP-1 is considered a marker of insulin sensitivity with low levels reflecting insulin resistance (Maddux et al 2006).
To conclude, our data suggest that both enhanced insulin sensitivity (independently of weight loss) and decreased hyperandrogenism could be underlying mechanisms for improved reproductive function by lifestyle intervention in women with PCOS.

5.1.4 Metabolic outcomes

The interventions obviously had an effect not only on body weight and BMI, but also on metabolic outcome variables such as waist circumference, a parameter that is usually encountered when relating diseases to the cardiovascular risk. According to WHO, a waist circumference > 88 cm in women is considered an increased disease risk. The majority of women had increased waist circumference at baseline. This parameter decreased significantly in all three groups but was still increased in most women. In line with this, there was also an overall decrease in hsCRP, primarily in the exercise group, as a biochemical biomarker of cardiovascular risk. However, there was no significant change in systolic or diastolic blood pressure. HOMA index decreased only in the diet group while there was no significant improvement in glucose or insulin response to OGTT. Furthermore, cholesterol and LDL decreased in the diet group, speaking of the fact that a lowered cardiovascular risk is within reach with primarily a dietary intervention directed at reduced carbohydrates and fat, and increased protein intake. With these dietary steps, also fiber intake was increased which should be beneficial in a cardiovascular context. However, although women with PCOS have an increased prevalence of risk factors for cardiovascular disease, there is no evidence of increased risk of cardiovascular disease (DeGroot et al 2011). It has therefore been speculated that these women possess protecting factors for the development of cardiovascular disease. On the other hand, they have clearly an increased risk of type 2 diabetes (Ehrmann et al 1999).
Taken together, it seems that diet, either alone or in combination with exercise, is most effective to improve metabolic disturbances in overweight/obese women with PCOS. In comparison with our results, systematic reviews in women with PCOS lend evidence for decreased weight, waist circumference and waist-hip-ratio by lifestyle intervention (combined diet and exercise or exercise only) (Moran et al 2011, Lim et al 2019), whereas there is no support for improvement in blood lipids and the effect on glucose tolerance is uncertain. Our data extend previous knowledge in the field by showing not only metabolic advantages, but also improved reproductive outcomes.

5.2 LIFESTYLE CHANGE

5.2.1 Nutrient intake

Changes in clinical outcomes were obtained by a recommended reduction of the dietary energy intake of at least 600 kcal per day in each subject in keeping with a rectified dietary composition of 55-60% carbohydrates, 25-30% fat (10% saturated) and 10-15% protein according to SNO 2005.

Over the 4-month study period, total energy intake decreased significantly in the diet and combined diet and exercise groups, but not in the exercise group. In a similar manner, total fat ingestion was reduced in the diet and combined diet and exercise groups, whereas carbohydrate intake was reduced in the combined exercise and diet group alone. None of the groups lessened the amount of protein in meal-taking. Instead, the percental change in protein intake increased in all intervention groups. This finding was encouraging in terms of balancing the foods eaten, as dietary protein plays a key role in body weight regulation, partly because of its effects on appetite. Protein intake has a well-known and even dose-dependent satiating effect (Westerterp-Plantenga et al 2012).

Dietary fiber represents a wide spectrum of polysaccharides that escape digestion in the human gastrointestinal tract, but forms substrate for microbial metabolism. The term “fiber” has been widely associated with positive health outcomes, with the fiber content of food products being a potential basis for nutritional composition claims in overweight and obesity. The impact of fiber-rich foods and isolated dietary fiber on body weight management has been studied in PCOS. Based on clinical observations, Marsh and Brand-Miller (2005) stated recommendations on nutrient intake and dietary composition for PCOS women. In their review, a diet low in saturated fat and high in fiber from predominantly low-glycemic-index-carbohydrate foods was recommended. However, their conclusions were largely based on metabolic factors such as reducing insulin levels and improving insulin sensitivity as an essential part of management of secondary long-term risks of PCOS including type 2 diabetes, cardiovascular disease and certain cancers.

In our study, fiber intake increased significantly in the two diet intervention groups but not in the exercise only group. We found a negative correlation between the change of fiber intake and change of BMI, i.e. the higher the fiber intake, the greater the loss of BMI. Surprisingly, with a daily increase of fiber with maximally about 20 grams, PCOS patients were able to reduce their BMI with about 3 kg/m^2. This should be considered as a sizable weight loss of about 10 kg. Other authors have described weight control correlation with only minute additions of fiber intake of about 5 grams per day (Qublan et al 2007). An increase of fiber at the cost of refined carbohydrates also seems beneficial (Liepa et al 2008). A previous study has shown women with PCOS to ingest about half of the recommended amount of dietary fiber (Turner-McGrievy et al 2015), which should be optimized to similar levels as used in our study to achieve weight control. However, data suggest that overweight women with PCOS-related infertility have a poor dietary intake, particularly in terms of whole grains, fiber, and iron, and eating behaviors inconsistent with achieving a healthy body weight, as well as low scores for PCOS-related quality of life. Furthermore, observations from Iran (Eslamian et al 2017) indicate that low fiber intake is significantly associated with PCOS. These data confirm our findings of negative relationship between taking fiber and change in BMI.

Our results also indicate that ingestion of trans fatty acids and insulinogenic index are correlated. Women with PCOS should consider maintaining a diet that is patterned after the type 2 diabetes diet. This diet should include high amount of fibers and low refined carbohydrates, as well as low amounts of trans and saturated fatty acids (Liepa

et al 2008). The question of which type of fatty acids that should be recommended has been approached in a review (Faghfoori et al 2017), where diet therapy in PCOS should reach specific goals such as improved insulin resistance along with metabolic and reproductive functions. To achieve this goal, a reduction of saturated as well as trans fatty acids intake was claimed. In support of this, our study shows a direct relationship between the ingestion of trans fatty acids and the insulinogenic index.
Taken together, our data implies a reliable adherence to the all-over dietary instructions given, and with best outcome in the group with dietary instructions only. However, as an unwished consequence of the dietary program, lean body mass decreased. This finding may be due to an all-over reduced energy intake. Possible untoward consequences of this is presently unknown. Furthermore, even if weight reduction was the backbone of our study, women in the exercise group were not fully capable of improving their eating habits. Our data suggests that PCOS patients do have to be directly instructed about specific changes in their eating habits to comply with a healthier lifestyle which will not come by itself unless instructed so.
Few studies have investigated specific dietary composition in lifestyle interventions for women with PCOS. In one clinical trial, patients were randomized to one of two energy-restricted diets; high protein (30% protein, 40% carbohydrate, 30% fat) or high carbohydrate (15% protein, 55% carbohydrate, 30% fat), both with constant fat content (Stamets et al 2004). Over a 1-month period, weight loss was observed but neither diet was superior. In another trial, an 8-week low-starch/low-dairy diet (DASH, dietary approaches to stop hypertension) resulted in reduced waist, improved insulin sensitivity and reduced testosterone in women with PCOS compared with control diet (Asemi and Esmaillzadeh 2015). A third trial comparing a high protein diet with a normal protein diet showed similar changes in anthropometric variables and no change in metabolic outcomes (Toscani et al 2011).
It could be concluded that energy restriction and weight loss in PCOS should improve ovulation, fertlity, hyperandrogenism, as well as glucose- and insulin levels, insulin resistance and satiety hormones, whereas diet composition should be of less importance (Faghfoori et al 2017). Although it is likely that diet is not the root of PCOS, it represents a modifiable variable with potential to improve the health and quality of life in women with this condition. Lifestyle intervention including diet is therefore recommended as first line therapy to improve metabolic and reproductive health in these women (Teede et al 2018). Furthermore, the use of antiobesity pharmacological agents (e.g. orlistat and sibutramin) and bariatric surgery has been studied in PCOS and may offer additional treatment options (Hirschberg et al 2009).

5.2.2 Exercise

Aerobic exercise of moderate intensity should improve reproductive function and increase insulin sensitivity, which might reduce the risk of cardiovascular disease and type 2 diabetes in PCOS as shown in the general population. In an earlier narrative review (Harrison et al 2011), exercise therapy (aerobic and/or resistance) was evaluated as independent treatment against a comparison group. Outcomes measured included reproductive measures, such as ovulation, menstrual regularity and fertility, as well as cardiovascular risk factors. In eight studies, over a period of 12 or 24 weeks with variable duration of exercise sessions, the most consistent ameliorations included improved ovulation, increased insulin sensitivity and weight loss (4.5–10%). All over, these improvements were not dependent on the type of exercise, frequency or the length of exercise sessions. Since the studies vary considerably in design, intensity and outcome measures conclusive results seem elusive.
The exercise program we promoted was individualized by a physiotherapist in order to stimulate both intensity and interest of the activity at onset of intervention. It included

brisk walking, jogging, cycling or swimming on a moderate level performed two to three times a week with a duration of 45-60 minutes each time. Intermittent follow-ups with dietician and/or physiotherapist were scheduled for compliance both for achieving and learning goals, for the next month. The physical activity was monitored by use of pedometers for four days immediately before and four days at the end of the exercise alone, and diet plus exercise programs.

The outcome of the exercise subgroup showed improved menstrual regularity to a similar degree as diet or combined diet and exercise although weight loss was minimal. Furthermore, there were improvements in metabolic outcome variables by exercise including decreased waist circumference and hsCRP.

Already in 2001, Norman and co-workers in a position paper stated that treatment should emphasize sustainable weight loss through lifestyle modification including exercise, but without no firm evidence of its effectiveness. It was not until 2008, a pilot study showed that a structured exercise training program was shown to be effective as compared with diet (Palomba et al 2008). The frequency of menses and ovulation rate was significantly higher in the exercise training group than in the diet group, but with no difference in cumulative pregnancy rate. In parallel to this, the same research group showed physical exercise to improve autonomic function and inflammatory pattern with reduced CRP and white blood cell count in PCOS women (Giallauria et al 2008). Our findings of exercise are in agreement with those.

To date there is systematic review evidence that exercise can restore menstrual cycle regularity in obese anovulatory patients with PCOS independent of significant weight loss (Teede et al 2018). Furthermore, many review papers claim physical exercise to be effective for improved reproductive function by way of different endocrine biomarkers such as testosterone, SHBG and insulin sensitivity (Legro et al 2013, Hakimi and Cameron 2017), whereas few research papers have studied the complex interaction of exercise with fertility (Al-Eisa 2017) and pregnancy rate. In a systematic review and meta-analysis, Haqq et al (2014, 2015) reported that exercise alone improves endocrine status and biomarkers of reproductive health including testosterone, SHBG and hirsutism but also BMI and waist circumference in women with PCOS.

Although there is scientific proof of reproductive and metabolic improvements by exercise in PCOS, recent evidence-based guideline recommendations concluded that the evidence for exercise therapy in PCOS is being of low quality (Teede et al 2018, Stepto et al 2019). Therefore, the recommendations were based on current evidence for the general population. Furthermore, there is no clear evidence of which type of exercise that should be most beneficial for women with PCOS. However, the majority of studies have utilized aerobic exercise at a moderate to vigorous intensity (Stepto et al 2019).

Based on evidence in the general population and in PCOS for prevention of weight gain and maintenance of health, the recommendation is a minimum of 150 min/week of moderate intensity physical activity or 75 min/week of vigorous intensity including muscle-strengthening activities (Teede et al 2018, Stepto et al 2019). For modest weight-loss, prevention of weight-regain and greater health benefits, the recommendation is a minimum of 250 min/week of moderate intensity or 150 min/week of vigorous intensity (Teede et al 2018, Stepto et al 2019).

To summarize, employing comparable groups of diet alone, diet plus exercise, and exercise alone, we found all interventions equal in improving menstrual regularity and ovulation, whereas dieting was superior to physical exercise in achieving the metabolic treatments goals of this thesis. Most likely this is due to the fact that the energy consumption induced by exercise is outweighed by the increased appetite induced by physical activity (Gibbons et al 2017).

5.3 DIETICIAN'S CLINICAL ROLE AND CHALLENGES

Women with PCOS may present in many ways and in different settings for a dietitian. The prevalence of PCOS has been estimated to about 10% not including all women who have an undiagnosed PCOS. A great proportion of these women will most likely struggle with stressful, weight control issues. Many women with PCOS describe problems with their appetite such as 'craving' sweet foods or always being hungry and needing to eat a lot to be satisfied (Hirschberg et al 2004). Some of these issues are probably biological and hormonal, some are created by a dieting and food restriction history and some are habits.

Management of PCOS is not a one size fits all proposition. In our study, the women were of different ages, they had body image and mood concerns, and all women had menstrual disorders and metabolic risks. The treatment lens for PCOS is focused on the physiology of the syndrome (hyperandrogenism and insulin resistance), and can be at the cost of a woman's lived experience of the many challenges of this condition.

For a dietician, the Swedish Dietary Guidelines 2015 is now the basis for recommendations and there are also lifestyle recommendations from the international evidence-based guideline for PCOS (Teede et al 2018). However, advice about what food to eat and how to exercise according to established recommendations is not always helpful for women with PCOS who are sensitive for the latest diets and exercise plans. Different trends and attitudes about lifestyle in society are challenges for a dietician to offer the most effective and supportive care for clients with PCOS. Dietitians should strengthen a sound development of a clients' eating behavior and body image, by working in the borderland of medicine, culture, social attitudes, and accepted beliefs about female bodies and emotions.

Lifestyle intervention improves all symptoms of PCOS, although affecting change in dietary and activity habits can be difficult. Unfortunately, there is not one single scientifically supported dietary approach that is best for PCOS. Generally, strategies studied include reduced calories regardless of macronutrient content, low carbohydrate, low fat, or high protein (Teede et al 2018). Furthermore, the dietary recommendations may be altered based on the patients personalized goals and co-morbidity risk factors. But as an initial intervention, decrease in total calories is recommended, especially calories from sugar-sweetened beverages, saturated fat and trans fatty acids. In addition, fiber intake should be increased as a starting goal.

In terms of exercise, the intensity, duration, and type of exercise that is best for weight loss in women with PCOS is not clear, and again may vary based on the individual. In most cases, the best strategy is to understand the patient's baseline activity and to guide the woman to devise a personalized strategy to increase her activity and exercise through a realistic approach (Stepto et al 2019). Thus, the individual's nature of lifestyle changes and lack of clear strategies will hugely impact the treatment result.

The challenge to all practitioners is to bring about improvements in their own clinics. This includes clinics of family medicine, internal medicine, gynecology, and endocrinological specialties. Such efforts take time and coordination but are sorely needed. To establish a multidisciplinary PCOS clinic for the purpose of patient education, lifestyle changes and treatment, would bring together specialists of gynecology, physiotherapy, and nutrition in order to provide coordinated and comprehensive care with set goals of therapy. This type of multidisciplinary clinic is increasing across the globe and offers a true patient-centered care approach (Teede et al 2018).

Goals with lifestyle intervention for women with PCOS and obesity (BMI>30)

Normalized menstrual function and restored ovulation
Improved metabolic profile and reduced risk of metabolic complications
Weight reduction of at least 3% for improved menstrual function and at least 6% for improved metabolic profile. Normal weight may not be a realistic goal to obtain by lifestyle intervention for all women with obesity.

Diet recommendations

Total daily caloric intake reduced by at least 600 kcal
Fat percentage reduced to at least 25 E%, carbohydrates 55-60 E%, and protein at least to 15 E%.
Changes in diet composition should be focused on reduction in fat and particularly saturated fat and trans fatty acids. Furthermore, increase in daily fiber intake of at least 25 grams.

Exercise recommendations

For prevention of weight gain and maintenance of health: a minimum of 150 min/week of moderate intensity physical activity or 75 min/week of vigorous intensity including muscle-strengthening activities.
For modest weight-loss, prevention of weight-regain and greater health benefits: a minimum of 250 min/week of moderate intensity or 150 min/week of vigorous intensity including muscle-strengthening activities.

6 A CRITICAL EVALUATION

This thesis is based on one of the first randomized lifestyle intervention trials performed in overweight/obese women with PCOS. The randomization provided well comparable groups. However, one shortcoming with the trial is the limited number of participants in the three treatment arms. Although the power calculation based on changes in BMI revealed that 15 women in each group should be an adequate sample size, this may have been insufficient considering the drop-out rate.

Another possible limitation with the study could be the selection of the women fulfilling all three Rotterdam criteria instead of only the two necessary criteria for diagnosis. Thus, our results may not be applicable for all women with PCOS but rather those with the most outspoken features. Furthermore, in the present trial there was no control group of obese women with PCOS without treatment or minimal intervention. Therefore, we cannot evaluate efficacy of the different interventions but compare changes between and within our study groups.

The dietary recommendations in our trial was based on SNO 2005, i.e. 55-60% carbohydrates, 25-30% fat and 15-10% protein (Enghardt et al 2005). In 2012, the 5th edition of the Nordic Nutrition Recommendations (NNR) were published (http://norden.diva-portal.org/smash/record). According to NNR 2012, energy density should be decreased by improved carbohydrate quality and dietary fat quality, as well as by increased fiber intake. Furthermore, the salt intake should be decreased by reduction of processed meat and fast food. The recommended intake of macronutrients in energy% is about the same as previous recommendations. We believe that using the new recommendations would not have changed our results in a significant way.

In view of today´s public statistics, the BMI of women has steadily increased by 2% in the age group 20-29 years of age, maintained in the age group 30-39 but increased by 10% in the age group 40-49 over the period 2001-2017(www.scb.se). This speaks for an evolving bodyweight problem in fertile age, that seem to be as relevant to study today.

Since this project started, the use of analytical methods for determination of steroid hormones has gradually changed from immunological methods to the today golden standard method of liquid chromatography tandem mass spectrometry. However, this was not the standard ten years ago. At the time when the testosterone assays were performed in our project, the method used (Diagnostic Products Coat-a-Count® radioimmunoassay) turned out to be the best out of ten clinical routine methods when compared with gas chromatography - mass spectrometry (Taieb et al 2003). As far as we know from the literature, there is no corresponding comparison published between immunoassay and methods including mass spectrometry for the estradiol assay.

When we analyzed data in paper I we used per protocol analysis but changed to ITT analysis in paper II, which we found more appropriate. For some variables, this changed the results to be non-significant or vice versa. However, it did not change our main results and have therefore no impact on the overall conclusions.

7 CONCLUSIONS

- Lifestyle interventions with diet, exercise or diet and exercise in combination are equally effective in improving menstrual pattern and ovulation in overweight/obese women with PCOS, despite only a minor weight loss in the exercise group. Ameloriation in markers of insulin sensitivity and reduced hyperandrogenism seem to be important underlying mechanisms.

- The summarized effects of lifestyle interventions with diet, exercise or diet and exercise in combination show that dieting is the most effective and feasible way to control body weight excess, endocrinological and metabolic disturbances in overweight/obese women with PCOS. Similar effects can be obtained by also diet in combination with exercise but this type of intervention often seems to be too demanding to achieve proper treatment goals. Exercise alone, is less effective to achieve those endocrinological and metabolic goals. However, exercise is beneficial to reduce upper body fat and to maintain lean body mass.

- The most important dietary change for overweight/obese women with PCOS is to reduce the total daily energy intake. However, dietary components could also have a significant role. We identified increased fibre intake and decreased trans fatty acid intake as the strongest predictors of metabolic improvements and reduced body weight. It seems therefore not advisable to recommend low-carbohydrate high fat diet to these women.

- Lifestyle intervention including diet and/or exercise is the first-line treatment for all overweight/obese women with PCOS to improve reproductive and metabolic health. The type of intervention should be adapted to the individual woman's conditions and preferencies and the goals should be realistic and achievable. Health professionals should support long-term maintenance of healthy lifestyle behaviors in these women.

8 ACKNOWLEDGEMENTS

As the years have passed by, there are many that deserve a thank you, but I would like to mention some of the people that have been extra supportive, helpful, loving and caring.

All the days of ups and downs, but always caring and giving hope, love and a lot of knowledge, the greatest of thanks to my main supervisor **Angelica Lindén Hirschberg** an artist and perfectionist in the same person and my other supervisor **Per M Hellström** whom I met in the staircase at Karolinska University Hospital my first day at work, and who instantly talked about research and how research is a way to develop health care.

My expressions of gratitude
- to the late **Kjell Carlström** for guiding me among the mysteries of chemistry,
- to **Astrid Häggblad** for giving me a helping hand with administration,
- to **Kristina Gemzell Danielsson**, Head of the Department of the Women's and Children's Health, for answering questions and always making me feel welcome,
- to Elisabeth Berg for statistical support throughout the work.

Many thanks to **Lotta Blomberg**, **Berit Legerstam** and **Siv Rödin Andersson** at the Women's Health Research Unit, who took care of the study patients and extended their good spirit to all of us.

Thanks to the nurses at the Gastroenterology unit, Karolinska University Hospital for caring and helping with the oral glucose tolerance testing.

Thank you,
-**Agneta Ståhle** who made the training programs and helped with her great knowledge in physical activity.
-**Sven Nyrén** for his great knowledge in DXA examinations, and all his nurses at the X-ray-unit for always helping the patients.
-**Olle Söder**, the former Head of the Department of the Women's and Children's Health, for support and encouragement to attend international courses.

-**Linda Mann Knutas**, for beeing a great mentor, **Helen Rosenlund** for support and great ideas.
- **Stefan Nybacka** for support and caring through the years.

-The Dietitian unit at Karolinska University Hospital, Solna, and the GHP Surgery Clinic at Sophiahemmet where I work now for giving me time for research.

-The antenatal unit at Karolinska University Hospital and at the South Hospital, for support, love and interest in my work.

-All my relatives and dear friends in Västerbotten, especially my siblings Mikael, Andreas and Maria, and my extra mother Birgit, my dear friends Kicki and Sussie, always helping, loving and caring.
And all other family members and friends in the north of Sweden that always are supportive and caring.

My late Mother and Father. My mother said:" Always stay true to yourself. Never forget that we love you just as you are no matter what."

My lovely three daughters Linnea, Isabelle and Jasmine, You are the best, I will always love you, and be grateful for how blessed I am for having you in my life.

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