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POLYCYSTIC OVARY SYNDROME

Studies of affective symptoms in association with sex steroids and evaluation of electroacupuncture and physical exercise

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To women with polycystic ovary syndrome

ABSTRACT

Polycystic ovary syndrome (PCOS) is a common heterogeneous condition in women of reproductive age, relating to both physical and mental health. Thoughts on primary pathological origins shift from the ovary to the adrenal gland. Insulin signaling pathways and sympathetic nerve activity are thought to be involved. PCOS features include signs of high androgen concentrations and oligo/amenorrhea. Affective symptoms such as depression and anxiety are prevalent among women with the syndrome and have been associated with body mass index (BMI). PCOS interventions are symptom-oriented and should include physical exercise as part of lifestyle management. Effective pharmacological interventions have side-effects to be taken in consideration. Previous studies suggest electroacupunture (EA) to modulate features commonly observed in women with PCOS. The general aims of this thesis were to explore self-reported depression and anxiety-related symptoms in women with PCOS; and to evaluate low-frequency EA and physical exercise on muscle sympathetic nerve activity (MSNA), biochemical hyperandrogenism and oligo/amenorrhea.

Study I investigated depression and anxiety-related symptoms in women with PCOS and noted several anxiety symptoms to distinguish women with PCOS from controls matched on age, body weight and BMI. Symptoms of worry, phobias and sleep disturbances were overrepresented among women with PCOS and may be an indication of an increased arousal.

Study II explored associations between depression and anxiety symptoms, and estrogens, sex steroid precursors, androgens, glucuronidated androgen metabolites, sex hormone-binding globulin (SHBG) and insulin sensitivity in drug-naïve women with PCOS. Women with depression-related symptoms had lower testosterone (T), free testosterone (FT) and androstane-3α 17β-diol-3glucuronide (3G). There were inverse associations between circulating FT, 3G and symptoms of depression.

Study III aimed to evaluate low-frequency EA and physical exercise on MSNA in women with PCOS. Twenty women with PCOS were allocated to low-frequency EA, physical exercise, or no intervention. From baseline to week 16, MSNA burst frequency decreased by -39.4% for low-frequency EA; -39.0 % for physical exercise; and -8.7% for no intervention.

Study IV investigated if low-frequency EA would decrease hyperandrogenism and improve oligo/amenorrhea more effectively than physical exercise or no active intervention in women with PCOS. Eighty four women with PCOS were randomized to 16 weeks of low-frequency EA, physical exercise, or no intervention. Circulating T, androsterone glucuronide (ADT-G) and 3G decreased from baseline measurements to week 16 for low-frequency EA compared to physical exercise. Circulating T, FT, estrone-sulfate (E1-S), ADT-G, 3G and androstane-3α 17β-diol-17glucuronide (17G) decreased from baseline measurements to week 16 for low-frequency EA compared to no intervention. The monthly menstrual bleeding frequency increased for low-frequency EA compared to physical exercise and no intervention from baseline measurements to week 16.

Conclusions: This thesis supports the effects of low-frequency EA and physical exercise to be partly mediated via the sympathetic nervous system. Low-frequency EA may be a complement for treatment of hyperandrogenism in women with PCOS. With few minor adverse events of short duration for repeated low-frequency EA and no adverse events for physical exercise, a combination of low-frequency EA and physical exercise is recommended for treatment of oligo/amenorrhea in women with PCOS. With an unmet need for assessment and treatment of affective symptoms in women with PCOS further studies are warranted.

LIST OF PUBLICATIONS

I. Jedel E, Waern M, Gustafson D, Landén M, Eriksson E, Holm G, Nilsson L, Lind AK, Janson PO, Stener-Victorin E. Anxiety and depression symptoms in women with polycystic ovary syndrome compared with controls matched for body mass index. Hum Reprod 2010,25:450-456.

II. Jedel E, Waern M, Gustafson D, Landén M, Janson PO, Labrie F, Ohlsson C, Stener-Victorin E. Sex steroids and insulin sensitivity in relation to affective symptoms in women with polycystic ovary syndrome. Manuscript.

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IV. Jedel E, Labrie F, Odén A, Holm G, Nilsson L, Jansson PO, Lind AK, Ohlsson C, Stener-Victorin E. Impact of electroacupuncture and physical exercise on hyperandrogenism and oligo/amenorrhea in women with polycystic ovary syndrome: a randomized controlled trial. Am J Physiol Endocrinol Metab 2010 Oct 13. [Epub ahead of print].

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LIST OF ABBREVIATIONS

ADT-G	Androsterone glucuronide
ACTH	Adrenocorticotrophic hormone
AE-PCOS	Androgen Excess and Polycystic ovary syndrome Society
ASRM	American Society for Reproductive Medicine
BMI	Body mass index
BSA-S	Self-reported version of the Brief Scale for Anxiety
CC	Clomiphene citrate
COCs	Combined oral contraceptives
CPRS-S-A	Comprehensive Psychopathological Rating Scale for Affective Syndromes
CV	Conception vessel
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DHT	5α-dihydrotestosterone
EA	Electroacupuncture
ESHRE	European Society for Human Reproduction and Embryology
E1	Estrone
E1-S	Estrone-sulfate
E2	Estradiol
FSH	Follicle-stimulating hormone
FT	Free testosterone
GC-MS	Gas chromatography mass spectrometry
GDR	Glucose disposal rate
GnRH	Gonadotropin-releasing hormone
HDL	High density lipoprotein
HOMA-IR	Homeostasis model assessment of insulin resistance
HRQL	Health related quality of life
IGF-1	Insulin growth factor
ITT	Intention to treat
LI	Large intestine
LC-MS/MS	Liquid chromatography tandem-mass spectrometry
LDL	Low density lipoprotein
LH	Luteinizing hormone

MADRS-S	Self-reported version of the Montgomery Åsberg Depression Rating Scale
MSNA	Muscle sympathetic nerve activity
NGF	Nerve growth factor
NIH	National Institutes of Health
PASW	Prediction Application Software
PCOS	Polycystic ovary syndrome
PC	Pericardium
RCTs	Randomized controlled trials
RIA	Radioimmunoassay
SAS	Statistical Analysis Software
SHBG	Sex hormone-binding globulin
SSRIs	Serotonin reuptake inhibitors
ST	Stomach
T	Testosterone
T4	Thyroxine
TG	Triglycerides
TSH	Thyroid stimulating hormone
WHR	Waist-to-hip ratio
3G	Androstane-3α 17β-diol-3glucuronide
4-DIONE	Androstenedione
5-DIOL	5-androstene-3β 17β-diol
17G	Androstane-3α 17β-diol-17glucuronide

1 INTRODUCTION- POLYCYSTIC OVARY SYNDROME

1.1 Historical aspects

Bilateral polycystic ovaries accompanied by oligo/amenorrhea were first given attention with a report published in 1935 [1]. Women with the chief complaints oligo/amenorrhea and infertility had enlarged ovaries containing several cystic structures [1]. Ten years after the original report a follow-up study reported of male-pattern hair growth and obesity among women with oligo/amenorrhea and infertility [2]. As was discovered over time, women may have polycystic ovaries without conforming to other clinical signs and the condition became known as more encompassing condition called polycystic ovary syndrome (PCOS).

1.2 Current definitions

At present there are three main definitions for PCOS. One was applied in 1990, after the first international conference on PCOS, held by the National Institutes of Health (NIH) [3]. The NIH criteria require anovulation and biochemical or clinical signs of hyperandrogenism [3]. In 2003 an expanded PCOS definition was suggested at a conference sponsored by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) [4]. The 2003 Rotterdam criteria added ovarian morphology to the criteria and require presence of two or more of oligo/anovulation, biochemical or clinical signs of hyperandrogenism and polycystic ovaries. The polycystic ovaries are defined as either 12 or more follicles measuring 2-9 mm in diameter, and/or increased ovarian volume of > 10 ml [4]. In 2006 the Androgen Excess and PCOS Society (AE-PCOS) published a report emphasizing PCOS to be regarded primarily as a condition of androgen excess and defines the syndrome as follows; hyperandrogenism together with oligo/anovulation and/or polycystic ovaries [5]. The current definitions include the exclusion criteria congenital adrenal hyperplasia, Cushing´s syndrome and androgen secreting tumors [3-5].

1.3 Prevalence

PCOS is a common heterogeneous condition in women of reproductive age. An estimate of PCOS in the community suggests a prevalence of 8.7 % using the NIH criteria, 17.8 % using the Rotterdam criteria and 12.0 % using the AE-PCOS criteria [6].

1.4 Pathophysiology

The pathophysiolology of PCOS is complex and highly debatable with environmental and constitutional defects [7]. Thoughts on primary pathological origins shift from the ovary with its hypothalamic-pituitary-ovarian axis activity [8]; to the adrenal gland with its hypothalamic-pituitary-adrenal axis activity [9]. Insulin signaling pathways [10] and sympathetic nerve activity [11] may also be involved. With close interactions between suggested origins there may be several different alterations contributing to PCOS.

During normal circumstances, hypothalamus secretes gonadotropin-releasing hormone (GnRH) to the anterior pituitary in a pulsatile manner [12]. The anterior pituitary gland responds to GnRH by releasing luteinizing hormone (LH) and follicle-stimulating hormone (FSH) to the ovaries. The GnRH pulse pattern vary throughout the menstrual cycle and an increased frequency of pulsatile hypothalamic GnRH release in the late follicular phase favors LH over FSH and increases the LH/FSH ratio. In the luteal and early follicular phases of the ovulatory cycle the GnRH pulse frequency is decreased which favor FSH secretion from the pituitary, necessary for recruitment and selection of the maturing cells [12]. LH acts primarily on the theca cells of the ovary to increase the production and secretion of androgens. FSH acts on the granulosa cells to promote conversion of androgens into estrogens, particularly estradiol (E2), which assists in follicular development. With normal follicle development, recruitment and maturation of primordial follicles results in antral follicles from which a dominant follicle is selected [13].

In women with PCOS the neuronal activity is increased with a predominantly high GnRH pulsatility [8]. Elevation of the LH pulse amplitude stimulates the theca cells to produce and secrete androgens. High androgen concentrations exert a direct inhibitory effect on follicular maturation in the ovary. In conjunction with diminished but steady presence of FSH, follicles continue to develop without ever maturing. This results in higher amount of primary follicles and a reciprocal decrease of primordial follicles with failed dominant follicle selection [14].

In women with PCOS the ovarian theca cells seem to have the potential to increase androgen production with and without gonadotropin stimulation, suggesting constitutional defects to be part of the pathophysiology [7]. Measurements of ovarian follicular fluid from women with PCOS and controls have revealed differences, not

only in androgen concentrations, but also in enzyme activity and estrogen concentrations [15]. Several enzymes have been recognized [16]; and down regulation of key enzymes such as 17alpha-hydroxylase/17, 20-lyase encoded by CYP17, may inhibit ovarian androgen biosynthesis [17]. High serum concentrations of estrogens, sex steroid precursors, androgens and glucuronidated androgen metabolites have been observed in women with PCOS when analyzed by gas chromatography mass spectrometry (GC-MS) and liquid chromatography tandem mass-spectrometry (LC-MS/MS) [18].

Insulin signaling pathways are part of the ovarian androgen biosynthesis regulation and insulin activity interacts directly and indirectly with androgen concentrations [7, 10]. Insulin serve as a co-gonadotropin and interacts with LH and FSH via its own receptors by stimulating enzymes to convert androgen precursors to testosterone (T) [10]. High concentrations of insulin reduce the hepatic sex hormone-binding globulin (SHBG) production, resulting in an increase of the circulating free testosterone (FT) concentrations [7].

The ovarian androgen production, together with the hypothalamic secretion of adrenocorticotrophic hormone (ACTH) to the adrenal gland, are the two main sources of sex steroid production in women with and without PCOS [9]. Excess adrenal androgens, basally and in response to ACTH stimulation, can be detected mainly by elevated dehydroepiandrosterone sulfate (DHEAS) concentrations. Intra-adrenal factors regulating cortisol metabolism, and extra-adrenal factors, such as ovarian androgens and insulin activity, may all play a role in the adrenal androgen excess observed in PCOS [9].

The sympathetic nervous system activity is thought to affect or be affected by PCOS in various ways [19]. The sympathetic innervation of the ovaries derives from the ovarian ganglion, and has been discussed as a contributing pathological factor to changes in follicular development in PCOS [11]. A greater density of catecholaminergic nerve fibers have been observed in polycystic ovaries [20], which is supported by an observed alteration of the peripheral catecholamine metabolism in adolescents with PCOS [21]. The excessive ovarian production of nerve growth factor (NGF) observed in women with PCOS supports an increased ovarian sympathetic nerve activity [22]. Studies measuring heart rate variability [23] and heart rate recovery [24-25] during physical exercise,

suggest an increased sympathetic nerve activity together with a decreased parasympathetic nerve activity in women with PCOS. Higher muscle sympathetic nerve activity (MSNA) has been observed in women with PCOS compared with controls with T as the strongest independent factor explaining the augmented MSNA activity [26].

Multiple family studies have demonstrated an increased prevalence of polycystic ovarian morphology [27], hyperandrogenism [28], and reduced insulin sensitivity in relatives of women with PCOS [29-30]; and both environmental and genetic components are likely to be important PCOS contributors.

1.5 Clinical features

Oligo/anovulation in women with PCOS evoke clinical signs of oligo/amenorrhea. Irregular menstrual cycles may be defined as oligomenorrhea when there are more than 35 days between cycles and less than eight menstrual bleedings per year [31-32]. Absent menstrual bleeding or 90 days or more between menstrual bleedings may be defined as amenorrhea [32]. Decreased rates of ovulation will lead to difficulties conceiving and subsequently to infertility.

Excessive ovarian and/or adrenal androgen production and secretion may induce dermatological signs, such as hirsutism, acne, androgenic alopecia that is scalp hair loss, and seborrhea that is scalp eczema [33-35] . Hirsutism is the presence of excess body hair of male type pattern on face and body in women [36]. The prevalence of hirsutism in PCOS varies with age, body weight and ethnic origin [33]. Acne is highly prevalent in the general population, especially among younger women, and the degree to which PCOS increase acne is not known. In a study of 627 participants, women with acne had a greater incidence of PCOS compared with women without acne [34]. The obese women, regardless of T concentrations, had a lower incidence of acne compared to non-obese women [34]. Another study, investigating hirsutism, acne, androgenic alopecia and seborrhea in relation to androgens and metabolic markers, observed positive associations for hirsutism and seborrhea; negative associations for androgenic alopecia; and no associations with acne [35]. In addition to biochemical hyperandrogenism, other factors mediate the development of acne [37]. Genetics, nutrition and environmental factors are all involved in dermatological signs. Thus, biochemical markers are thought to be more reliable for estimation of hyperandrogenism in women with PCOS [32, 35].

A long-term androgen excess may result in abdominal adiposity [38]. Inflammatory markers observed in women with PCOS have been associated with BMI, high-density lipoprotein (HDL) cholesterol, diastolic blood pressure and insulin resistance [39]. Insulin resistance with compensatory hyperinsulinemia, overweight and obesity are all metabolic factors which may lead to development of diabetes type 2 [40]. Women with PCOS and established diabetes type 2 may be at high risk for cardiovascular disease [41]. Obesity and high waist circumference in women with or without PCOS, have been observed with arterial stiffness when compared to lean women, which indicate an increased risk for cardiovascular disease in women with abdominal obesity [42].

Besides gynecological, endocrine and metabolic features of PCOS psychological correlates have been identified as clinical features of the syndrome. Psychological health related quality of life (HRQL) is reduced in women with PCOS as compared with other physical conditions [43-45]. Infertility [46], hirsutism [47] and acne [45] have been linked to mental well being. Women with overweight and obesity have concerns in regards to their body weight which have a negative effect on HRQL [44-45, 47-50]. The importance of obesity on HRQL was demonstrated in a study evaluating the impact of obesity and PCOS on HRQL [51]. Obesity impaired general HRQL to a greater extent than PCOS in women of reproductive age [51].

Affective disorders, such as major depression and bipolar disorders, have been noted among women in ambulatory treatment for PCOS [52]. Studies have shown an increased risk for depression in women with PCOS compared with controls [53-55]. One of those studies noted differences on BMI and insulin resistance between women with and without depression [53]; the second study did not observe differences on self-reported difficulties due to menstrual irregularities, infertility, hirsutism or acne between women with and without depression [54]; and the third study could not explain a higher risk for depression from selected sociodemographics, or clinical signs and biochemical markers [55]. Depression and anxiety often coexist, and a life time history of social phobia has been observed in PCOS with suggestions of possible confounding effects of obesity [56]. The effects of obesity may be enhanced if psychotropics inducing weight gain are used [57]. Eating disorders and suicidal behavior are also overrepresented among women with PCOS [56].

Focusing on psychiatric symptom burden rather than diagnosis, anxiety symptoms of clinical relevance are common in women with PCOS [58-60]. In fact, symptoms of anxiety may be underdiagnosed [60]. Both depression and anxiety symptoms have been associated with higher BMI [61-62] and waist-to-hip ratio (WHR) [61] in women with PCOS and may [63] or may not [61] be associated with hyperandrogenism and insulin resistance. With a potential negative impact of mood in women with PCOS, affective symptoms need to be further explored and addressed as part of PCOS assessment [64-65].

1.6 Pharmacological interventions

Interventions for PCOS are symptom-oriented and individualized management programs for women with the syndrome are essential. Pharmaceuticals are effective but associated with side-effects. Oral contraceptives are widely used among women with PCOS not aiming to conceive. The low-dose combined oral contraceptives (COCs), containing an estrogen component and a progestogene component, improve menstrual regularity and reduce circulating androgens by suppression of gonadotropins from the pituitary to the ovaries [66]. One reported side-effect of the COCs is weight gain, which should be considered, as many women with PCOS are overweight and obese. For women with arterial hypertension and at high thromboembolic risk, progestogene-only contraceptives can be an alternative to the COCs [67].

The associations between oligo/anovulation and insulin resistance with compensatory hyperinsulinemia in women with PCOS have inspired the use of insulin sensitizers, such as metformin, for oligo/amenorrhea [68]. Metformin acts on the reduction of glucose concentrations, exerts beneficial effects on hyperandrogenism, and has the advantage of long-term potential prevention of diabetes type 2 and cardiovascular disease [69]. Importantly, metformin has adverse events including nausea, abdominal pain, abdominal bloating, dyspepsia, watery diarrhea, flatulence, metallic taste and anorexia [69].

For women wishing to conceive, anti-estrogens, like clomiphene citrate (CC), is first-line therapy to induce ovulation [70-71]. CC is a selective estrogen receptor modulator acting on the hypothalamic GnRH release with effects promoting follicular development [72], and increased pregnancy rates have been demonstrated in randomized controlled trials (RCTs) evaluating CC on ovulation in PCOS [71]. Women who do not tolerate CC may experience hot flashes, headaches, visual changes and mood instability

[72]. Some women do not respond to CC, and if demonstrated CC resistance, the second-line therapies to induce ovulation include either exogenous gonadotropins or laparoscopic ovarian surgery [73]. Provocation of multiple follicle development by CC and exogenous gonadotropins can lead to ovarian hyperstimulation. All surgery is associated with risks, which needs to be considered prior to laparoscopic ovarian surgery [70]. In vitro fertilization is the third-line therapy for women failing second-line therapies [73].

1.7 Physical exercise as part of lifestyle management

Lifestyle management, including nutritional counseling and physical exercise, are considered treatments of choice for all women with PCOS and necessary to reduce the excess weight commonly observed in women with the syndrome [74].

Regular physical exercise promotes an increased muscle blood flow and capillarization, which facilitates insulin action and glucose transport to muscles. Reduced plasma homocysteine concentrations after a six month unsupervised walking program in young overweight or obese women with PCOS indicate positive effects of physical exercise on cardiovascular markers [75]. When comparing a three-month structured physical exercise program to no intervention, the physical exercise program improved inflammatory markers, maximal oxygen uptake and heart rate recovery, indicating an improved autonomic function, potentially reducing risk of cardiovascular disease in women with PCOS on structured physical exercise programs [76]. Several other studies have been evaluating physical exercise and/or nutritional programs on features common in PCOS [77-82]. One of the studies compared a supervised endurance and resistance exercise program plus nutritional counseling to nutritional counseling in women PCOS [77]. After 12 weeks both groups improved their hormonal profile and fasting insulin but there were no difference between groups [77]. A second study noted reduced BMI together with improved oxygen consumption and insulin sensitivity after three months enrollment in a structured physical exercise program [78]. A third study compared a structured physical exercise program to a dietary program [79]. After 24 weeks of intervention there were no differences between groups on the primary endpoint, cumulative pregnancy rate, although menstrual frequency and ovulation rate were increased for the structured physical exercise program as compared to the dietary program [79]. Sub group analysis demonstrated improvements in fasting insulin for those who ovulated in both groups [79]. When comparing a diet only intervention, a diet plus

aerobic exercise intervention, and a diet/combined aerobic-resistance exercise intervention in women with PCOS; within group analysis demonstrated improvements on metabolic markers, androgens, SHBG and reproduction for all groups after the interventions; but there were no differences between groups [80]. Another study, comparing physical exercise of moderate intensity to no active intervention, observed improvements on metabolic markers among those who exercised [81]. Evaluation of a six week structured exercise training program plus a hypo-caloric diet with one cycle of CC after the first two weeks, observed an increased probability of ovulation under CC in overweight and obese women with PCOS, previously considered non-responders to CC [82].

For treatment of depression, a combined physical exercise, nutritional counseling and cognitive-behavioral therapy program in adolescents with PCOS have indicated promising results [83]. An energy-restricted diet; diet plus an aerobic exercise program; and diet plus a combined aerobic-resistance exercise program were evaluated on depression symptoms and specific HRQL in overweight and obese women with PCOS [84]. All groups improved on both depression scores and specific HRQL scores, with no differences between groups [84].

1.8 Acupuncture

Studies have been evaluating effects of manual acupuncture on infertility [85], ovarian hyperstimulating syndrome [86] and amenorrhea [87] in women with clinical features commonly observed in PCOS. Importantly, all studies were non-randomized and have other limitations in study design. In one of those studies auricular acupuncture was compared to hormonal therapies in women wishing to conceive [85]. After 12 weeks, auricular acupuncture yielded pregnancy rates equivalent to those induced by hormonal therapies, with less adverse events and miscarriages [85]. A second study evaluated manual acupuncture as an alternative to gonadotropins in 11 women with infertility and ovarian hyperstimulating syndrome [86]. Ovulation was induced in two cases with remission of the ovarian hyperstimulating symptoms [86]. In a third study 34 women with oligo/amenorrea and infertility were treated by manual acupuncture [87]. Twenty eight women responded to treatment by improved menstrual frequency and two became pregnant [87].

Low-frequency electroacupuncture (EA) has been evaluated in women with PCOS and women with oligo/amenorrhea [88-89]. None of the studies were RCTs; one reported ovulation induction measured by daily basal body temperature and eosinocyte index after low-frequency EA in nine women with PCOS, one woman with oligomenorrhea without PCOS and one woman with amenorrhea without PCOS [88]. Ovulation was measured by menstrual cycles, and induced in six of 13 cycles [88]. Observed changes on plasma β-endorphin concentrations and hand skin temperature was suggested to reflect modulation of sympathetic nervous activity after low-frequency EA [88]. The second study evaluated low-frequency EA on menstrual frequency, endocrine and neuroendocrine markers in 24 women with PCOS [89]. After 14 low-frequency EA treatments the monthly menstrual frequency increased from 0.15 to 0.66 in 38% of the women [89]. T concentrations and LH/FSH ratio decreased for low-frequency EA and remained decreased at a three month follow-up [89].

The underlying physiological mechanisms of low-frequency EA share similarities with those of physical exercise and include modulation of the sympathetic nervous system [19]. The modulation may be direct or via the release of opioids and other peptides in the peripheral and central nervous system [90-91]. Similar to the activation of afferents from dynamic muscle exercise [92], muscular needle insertion and stimulation activate ergoreceptors innervated by thin myelinated, group III; Aδ fibers, and thinner unmyelinated group IV; C nerve fibers [93]. There is a peripheral neuropeptide release resulting in an increased microcirculation. Needle placement in areas with the same somatic innervaton as the ovaries increases ovarian blood flow, indicating a decreased ovarian sympathetic activity by supraspinal reflexes [94-95]. Changes in ovarian morphology, insulin sensitivity, adipose tissue gene expression, inflammation markers and sympathetic activity after low-frequency EA and physical exercise in PCOS rat models further suggest modulation of the sympathetic outflow [96-98]. After spinal cord transmission the signaling ascends to centers in the central nervous system. Of special interest are the potential acupuncture effects on hypothalamic opioid β-endorphin centers [99]. Changes in circulating LH, ovulation and T concentrations after naltrexone, a μ-receptor antagonist, indicate that the β-endorphins influence GnRH secretion [100]. Normalized estrous cyclicity, restored hypothalamic GnRH and restored androgen receptor protein expression following low-frequency EA in experimental settings, suggest those mechanisms to exert beneficial effects for women with PCOS [101].

1.9 Aims

The general aims of this thesis were:

To explore self-reported depression and anxiety-related symptoms in women with PCOS; and to evaluate low-frequency EA and physical exercise on MSNA, biochemical hyperandrogenism and oligo/amenorrhea

The specific aims of this thesis were:

To explore depression and anxiety-related symptoms in drug-naïve women with PCOS, and to avoid potential obesity-related confounding, cases and controls were matched on age, body weight and BMI (Study I)

To explore associations between depression and anxiety symptoms, and estrogens, sex steroid precursors, androgens, glucuronidated androgen metabolites, SHBG and insulin sensitivity in drug-naïve women with PCOS (Study II)

To elucidate the effect of low-frequency EA and physical exercise on MSNA in women with PCOS (Study III)

To investigate if low-frequency EA would decrease hyperandrogenism and improve oligo/amenorrhea more effectively than physical exercise or no active intervention in women with PCOS (Study IV)

2 MATERIALS AND METHODS

2.1 Participants

Data for studies I-IV was collected from participants recruited from the community between November 2005 and September 2008 by advertising for women aged 18-37 years. Potential cases with PCOS and/or excess body hair and/or irregular menstrual periods were asked if interested in participating in an RCT aiming to evaluate low-frequency EA and physical exercise. Data collection for studies I and II ended with completion baseline measurements; data collection for study III ended with completion 16 weeks; and data collection for study IV ended with completion 32 weeks (Figure 1).

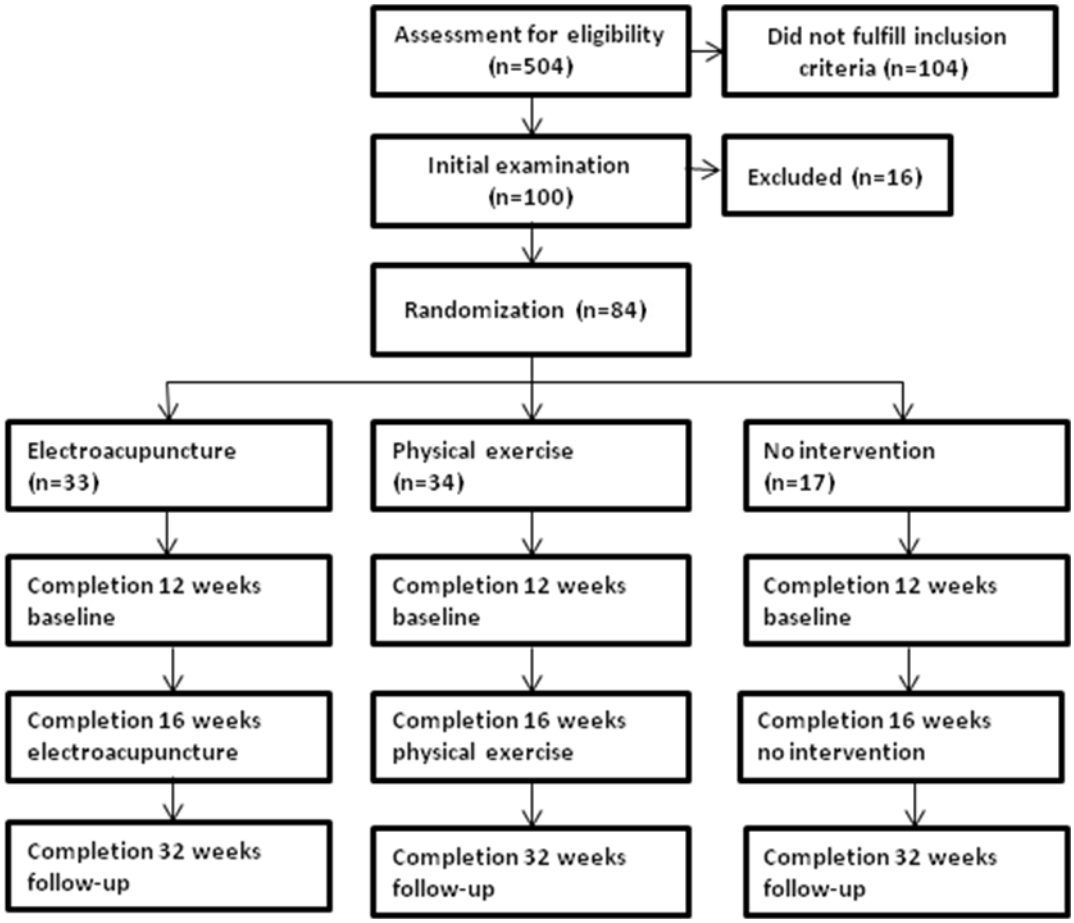

Figure 1. Flow chart of the randomized controlled trial

Polycystic ovarian morphology was part of the PCOS diagnostic inclusion criteria, and potential PCOS cases were included in studies I-IV if the following criteria were fulfilled:

- Twelve or more 2-9 mm ovarian follicles and/or ovarian volume exceeding 10 ml in one or two ovaries
- Hyperandrogenism and/or oligo/amenorrhea
- The condition was not related to congenital adrenal hyperplasia, Cushing´s syndrome, androgen secreting tumors or other related disorders

Potential controls for study I were recruited by advertising for women without PCOS or any of the symptoms listed for PCOS cases. The women failed to fulfill inclusion criteria if not matched on age, body weight and BMI to any of the women in the RCT aiming to evaluate low-frequency EA and physical exercise. The women were excluded on the basis of a gynecological examination if they had polycystic ovaries; menstrual irregularities with cycles < 28 days or > 35 days; excess body hair; acne (Figure 2).

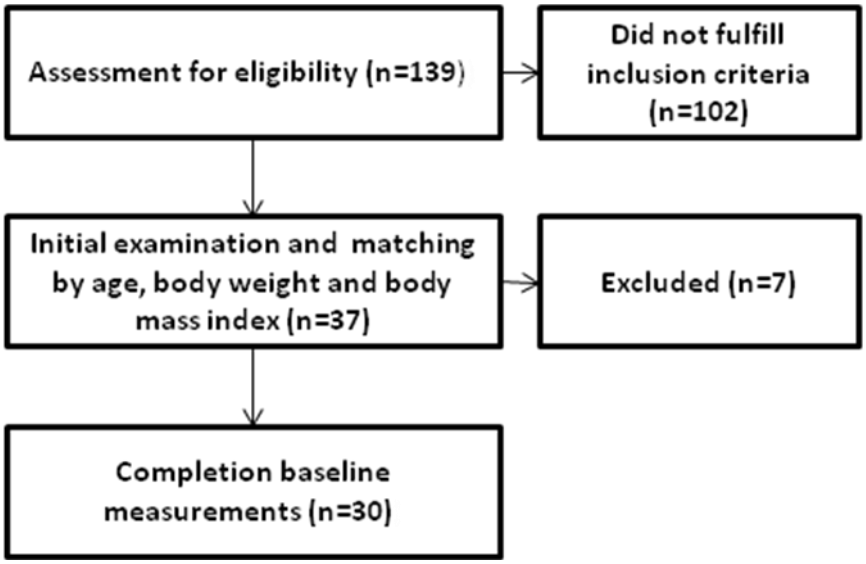

Figure 2. Flow chart of recruitment of controls

Exclusion criteria for all cases and controls were:

- Difficulties reading or writing the Swedish language
- Self-reported current physical/psychiatric disease
- Any pharmacological treatment within the past 12 weeks
- Breastfeeding within 24 weeks preceding study enrollment

All participants gave their oral and written consent and studies I-IV were conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee, University of Gothenburg, Gothenburg, Sweden.

2.2 Procedure

Initial examination of all participants in studies I-IV included a case history and gynecological examination, including two-dimensional vaginal ultrasound (HDI 5000, ATL, Bothell, Washington, USA). For participants fulfilling the inclusion criteria, the initial examination was followed by collection of serum blood samples and anthropometric measurements. Fasting blood samples for endocrine measures were obtained between 7.30 and 8.30 am and stored at –80°C. Body weight and body height were measured while standing with light clothing and no shoes and BMI was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured in centimeters at the midpoint between the iliac crest and lower rib margin at the end of expiration. Hip circumference was measured in centimeters at the widest point between waist and thighs. WHR was calculated as the ratio of waist and hip circumferences. Menstrual cycles were reported in days and oligomenorrhea was defined as more than 35 days between cycles with fewer than eight menstrual bleedings in the past year [31-32]. Amenorrhea was defined as absent menstrual bleeding or no menstrual bleeding in the past 90 days [32]. Acne was determined by response to the question *Do you have acne?* Hirsutism was measured by the Ferriman-Gallwey instrument [102]. Facial and body hair on upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, upper arm and/or upper thigh were evaluated by asking participants to mark 0 for no excess body hair, 1 for an insignificant amount of excess body hair, 2 for a moderate amount of excess body hair, 3 for a considerable amount of excess body hair or 4 for a substantial amount of excess body hair [102]. Item ratings were summed yielding a minimum value of 0 and a maximum value of 36 and hirsutism was defined as a Ferriman-Gallwey score of ≥ 8 [103].

2.3 Biochemical analyses

Various methods are available for estimates of circulating endogenous androgens, all with strengths and limitations [33, 104]. Immunoassays (Studies I-IV), GC-MS and LC-MS/MS (Studies II and IV) were used for biochemical analyses. Reagents for immunoassays were obtained from commercial kits; convenient and inexpensive as compared to GC-MS and LC-MS/MS which provide a greater degree of accuracy [105].

T was analyzed by competitive immunochemistry with chemiluminescence technology (ADVIA Centaur® TSTO ready pack® primary reagents, Bayer Health Care, Terrytown, NY, USA) (Studies I and III).

SHBG was analyzed by chemiluminiscent microparticle immunoassay (Abbott SHBG reagent pack, Abbott Laboratories Diagnostic Division, Chicago, IL, USA) and the detection limit was 0.1 nmol/l (Studies I-IV).

Free androgen index was calculated as T/SHBG x 100 (Studies I and III).

Serum FT and DHEAS were analyzed by radioimmunoassay (RIA) (Coat-A-Count Free Testosterone and Coat-A-Count DHEA-SO4, Diagnostic Products, Los Angeles, CA, USA) (Study III).

LH and FSH were analyzed by chemiluminiscent microparticle immunoassay (Architect luteinizing hormone reagent and follicle-stimulating hormone reagent pack, Abbott Laboratories Diagnostic Division, Chicago, IL, USA) (Study III).

Free thyroxine (T4) and insulin growth factor (IGF-1) were analyzed with immunochemiluminiscence technology (T4, Roche Diagnostics, Mannheim, Germany and Immunolite 2500 IG, Euro/Diagnostic Products, UK) (Study III).

Thyroid-stimulating hormone (TSH) was analyzed by electrochemiluminiscent immunoassay (TSH, Roche Diagnostics, Mannheim, Germany) (Study III).

Triglycerides (TG), cholesterol and high density lipoprotein (HDL)-cholesterol were analyzed with an enzymatic photometric method (TG, CHOL, and HDL-C second generation, Roche/Hitachi, Roche Diagnostics, Mannheim, Germany) (Study III).

Low density (LDL)-cholesterol was calculated according to Freivalds formula (serum LDL-cholesterol = serum cholesterol – serum HDL-cholesterol – 0.45 x serum TG) (Study III).

GC-MS was used to analyze estrone (E1), E2, dehydroepiandrosterone (DHEA), androstenedione (4-DIONE), 5-androstene-3β 17β-diol (5-DIOL), T and 5α-dihydrotestosterone (DHT). The detection limits were 5.00 pg/ml for E1, 1.00 pg/ml for E2, 0.10 ng/ml for DHEA, 0.05 ng/ml for 4-DIONE, 30 pg/ml for 5-DIOL, 0.02 ng/ml for T and 5.00 pg/ml for DHT (Studies II and IV). FT was calculated from the total T concentration determined by GC-MS and SHBG using a computer program to solve the law of mass action equation [106] (Studies II and IV).

LC-MS/MS with a TurboIonSpray source was used to analyze estrone-sulfate (E1-S), DHEAS, androsterone glucuronide (ADT-G), androstane-3α 17β-diol-3glucuronide (3G) and androstane-3α 17β-diol-17glucuronide (17G) (Studies II and IV). The detection limits were 0.075 ng/ml for E1-S, 0.075 μg/ml for DHEAS, 2.00 ng/ml for ADT-G, 0.50 ng/ml for 3G and 17G (Studies II and IV).

2.4 Insulin sensitivity

Insulin sensitivity is based on the relationship between glucose and insulin and was measured with homeostasis model assessment of insulin resistance (HOMA-IR) [107] (Study III), and euglycemic hyperinsulinemic clamp [108] (Study II).

Plasma glucose was analyzed with an enzymatic photometric method (Roche/Hitachi, Roche Diagnostics, Mannheim, Germany) and insulin was analyzed by immunochemistry with chemiluminescence technology (ADVIA Centaur® Insulin ready pack®, Bayer Health Care, Terrytown, NY, USA) (Study III). HOMA-IR was defined as fasting plasma glucose (mmol/l) x fasting plasma insulin concentration (mU/ml)/22.5 [107] (Study III).

The euglycemic hyperinsulinemic clamp method was used to determine the glucose disposal rate (GDR) [108] (Study II). In brief, the participants' blood glucose concentrations were determined and adjusted before, during and after an intravenous insulin infusion. The initial 10 minute primed insulin infusion (500 mU/ml) (Actrapid, 100 IU/ml; Novo Nordisk, Bagsvaerd, Denmark) was followed by a constant infusion

(0.12 U/kg body weight/min) for 110 min. The steady state infusion rate was used to determine the GDR (mg/kg x min) [108] (Study II).

2.5 Muscle sympathetic nerve activity

MSNA consists of baro-receptor reflex-controlled vasoconstrictor impulses to the muscle vascular bed involved in dynamic blood pressure regulation, and represent one subdivision of the sympathetic nervous system [109] (Study III). Microneurographic recordings of multiunit efferent postganglionic MSNA were obtained with a tungsten microelectrode with a tip diameter of 1-4 microns inserted into a muscle fascicle of the peroneal nerve, posterior to the fibular head [109]. A low impedance reference electrode was inserted subcutaneously a few centimeters from the tungsten microelectrode. After identification of a muscle nerve fascicle, small electrode adjustments were made until a site was found in which spontaneous, pulse-synchronous bursts of neural activity was recorded. Bursts identified by inspection of the mean voltage neurogram were expressed as burst frequency that is bursts/min, and burst incidence that is bursts/100 heart beats. During the microneurographic recording, finger arterial blood pressure was measured by the volume-clamp method (Finapress 2300; Ohmeda, Louisville, KY; USA); heart rate was monitored via electrocardiogram chest electrodes; and respiration was monitored via a strain-gage strapped around the waist (Study III).

2.6 Psychometrics

The Comprehensive Psychopathological Rating Scale for Affective Syndromes (CPRS-S-A) [110] was computer-administered for self-reporting of depression and anxiety symptoms (Studies I and II). CPRS-S-A assesses symptoms with a time frame of three days; is easy to administer and takes 5-30 minutes to complete [110]. The instrument is widely used in Sweden and has been noted to be clinically useful. There is good concordance between expert and self-reported versions of the instrument [111].

Preceding analyzes (Studies I and II), the subscales for depression (self-reported version of the Montgomery Åsberg Depression Rating Scale, MADRS-S) [112] and anxiety (self-reported version of the Brief Scale for Anxiety, BSA-S) [113] were extracted from the CPRS-S-A [110]. The MADRS-S is considered equivalent to the Beck Depression Inventory as a self-reported scale [114]. MADRS-S and BSA-S each include 9 individual items, two of which are present in both scales. All items are rated on a Likert scale graded from 0 to 6: 0= absence of symptoms; 2= a potentially pathological

deviation; 4= a pathological condition; and 6= an extremely pathological condition [115]. Item ratings are summed yielding a maximum value of 54 for each scale (Studies I and II).

2.7 Low-frequency electroacupuncture and physical exercise

To evaluate low-frequency EA and physical exercise, the inclusion were followed by a 12-week baseline period and baseline assessments; 16 weeks of intervention and assessment after intervention (Studies III and IV); 16-week follow-up and follow-up assessments (Study IV). Computer generated randomization was conducted after inclusion. The randomization procedure allocated the participants to low-frequency EA, physical exercise, or no active intervention in a 2:2:1 ratio. Stratification variables were age and BMI.

All participants received general information concerning the benefits of regular physical exercise and were instructed to complete diaries of their weekly physical exercise, daily basal body temperature and monthly menstrual bleeding from inclusion to follow-up assessments (Study IV). To evaluate cardiorespiratory fitness, the maximal oxygen uptake from heart rate and work load on a bicycle ergometer (Monark 828E, Varberg, Sweden) [116] was estimated at baseline, after intervention and at follow-up. The participants pedaled at 50 rates per minute for six minutes at 75, 100 or 125 watts depending on self-reported daily physical activity. Heart rate was monitored by electrodes connected to a chest belt (Polar Favor, Kempele, Finland).

Participants allocated to low-frequency EA was given treatments by a physical therapist twice a week for two weeks, once a week for 6 weeks, and once every other week for 8 weeks, with a total of 14 treatments (Studies III and IV). The acupuncture protocol was identical for all participants and based on previous studies [89, 94]. Disposable single-use sterilized needles made of stainless steel (Hegu Xeno, Hegu, Landsbro, Sweden; length 30/50 mm, diameter 0.32 mm) were inserted to a depth of 15–35 mm in points located in; abnominal muscles (conception vessel (CV) 3, CV 6 and bilateral stomach (ST) 29); musculus gastrocnemicus (bilateral spleen (SP) 9); and musculus tibialis (bilateral SP 6). In addition, needles were placed in musculus interosseus dorsalis (bilateral large intestine (LI 4) or musculus flexor digitorum superficialis (bilateral pericardium (PC) 6). Manual stimulation followed insertion to evoke an individual sensation sometimes referred to as de qi. Needles placed in CV 3, CV 6, ST 29, SP 6, and SP 9 were

attached to an EA stimulator (CEFAR ACUS 4, Cefar-Compex Scandinavia, Malmö, Sweden) set to 2 Hz for 30 minutes. The EA stimulator intensity was adjusted by the participant to maintain local muscle contractions without discomfort. Bilateral LI 4/PC 6 were stimulated by manual rotation after 10 minutes, 20 minutes and prior to needle removal at 30 minutes (Studies III and IV).

Participants allocated to physical exercise were instructed to perform regular physical exercise in addition to their daily physical activity, from baseline assessments and 16 weeks forward (Studies III and IV). Instructions included suggestion to walk, run, go for bicycle rides, or any other aerobic exercise; at a pace described as faster than normal walking that could be sustained for at least 30 minutes, three or more times a week [75]. The physical exercise was monitored by a heart rate monitor (ECG2, Sports Instruments, USA) to ensure a heart rate of ≥ 120 beats/minute. General supervision was applied by weekly telephone calls (Studies III and IV).

Participants allocated to no intervention were not supervised during the intervention but were advised to call with any questions, or if planning to go on medication and/or any other treatments (Studies III and IV).

2.8 Statistical analyses

Prediction Application Software (PASW) Statistics 17 (Studies I-IV) and Statistical Analysis Software (SAS) (Study I) were used for statistical analyses and results were considered statistically significant at $P < 0.05$.

In study I cases were matched with controls on age (± 5 years), body weight (± 5 kg) and BMI (± 2 kg/m^2) using Wilcoxon Signed Rank test, and background factors were compared using either Paired Student´s t-test or Wilcoxon Signed Rank test. Thereafter, the Wilcoxon Signed Rank test was applied to test for differences on affective symptomatology in women with and without PCOS. Categorical variables were created for MADRS-S and BSA-S total scores and for each MADRS-S and BSA-S item. A MADRS-S/BSA-S score of 11 or more was used to denote depression/anxiety symptom burden of potential clinical relevance; and an individual symptom was considered to be present with a score of two or more on MADRS-S/BSA-S items. Conditional exact logistic regression models were utilized to calculate the odds of being

a case with score ≥ 11 on the entire MADRS-S/BSA-S and score ≥ 2 on individual MADRS-S/BSA-S items (Study I).

In study II data were presented as median (min – max) or percent. Low versus high sum total MADRS-S/BSA-S scores were defined in accordance with study I, and used as outcomes to test for differences and associations between affective symptoms and endocrine and metabolic markers. To test for differences between women with and without MADRS-S/ BSA-S ≥ 11 on estrogens, sex steroid precursors, androgens, glucuronidated androgen metabolites, SHBG and GDR the Mann-Whitney U test was used. Binary logistic regression analyses with the enter method were utilized to test for potential confounding of age and BMI. Thereafter, endocrine and metabolic markers were tested as continuous variables in separate binary logistic regression models in relation to MADRS-S/BSA-S scores ≥ 11 (Study II).

In study III descriptives were presented as mean ± standard deviation. The differences between low-frequency EA, physical exercise and no intervention were assessed by Kruskal-Wallis test. If statistical significance, the Mann Whitney U-test was utilized to test for differences between low-frequency EA/physical exercise and no intervention. The Wilcoxon's matched-pairs signed-rank sum test was used for within group analyses and correlation analyses were performed using Spearman's rank correlation coefficient (Study III).

In accordance with study III, study IV presented descriptives as mean ± standard deviation. The mean change in T from baseline to week 16 was assumed to be 0.10 ng/ml for low-frequency EA; 0.05 ng/ml for physical exercise; and no changes were expected for no intervention. Seventy five women at a 2:2:1 ratio was assumed to detect differences on T. The Kruskal-Wallis test was utilized to test for differences between low-frequency EA, physical exercise and no intervention from baseline to completing week 16; and from baseline to completing week 32. All participants completing baseline measurements were included in intention to treat (ITT) analyses. If the Kruskal-Wallis test detected differences between groups, the Mann Whitney U-test was utilized to test for differences between low-frequency EA versus physical exercise and low-frequency EA versus no intervention. The Wilcoxon's matched-pairs signed-rank sum test was used for within group analyses. The chi-square test was used for analyzing categorical variables.

3 RESULTS

3.1 Study I

Self-reported affective symptoms differed between women with PCOS and controls matched by age, body weight and BMI. Higher median scores were observed among women with PCOS on several individual anxiety-related symptoms including sleep, worry about unimportant matters, phobias and pain. The only depression-related item that differentiated cases and controls was sleep. In the categorical analysis regarding individual items $\geq$ 2; sleep (57% versus 3%), phobias (43% versus 7 %) and pain (47 % versus 7 %) were more often observed in women with PCOS compared to controls. Fifty three percent of the women with PCOS versus 20% of controls had a score of $\geq$ 11 on the sum total MADRS-S and 63% of women with PCOS versus 13% of controls had a score of $\geq$ 11 on the sum total BSA-S.

3.2 Study II

Anthropometrics, PCOS characteristics, mass spectrometry, immunoassays and insulin sensitivity were measured in 72 women with ultrasound-verified PCOS. Forty seven percent (n=35) of the women scored 11 or more on MADRS-S and circulating T (P=0.026), FT (P=0.025), and 3G (P=0.029) were lower in women with MADRS-S $\geq$ 11. There were inverse associations between FT, 3G and depression symptoms. Sixty one percent of the women (n=44) scored 11 or more on BSA-S. There were no differences in estrogens, sex steroid precursors, androgens, glucuronidated androgen metabolites, SHBG or GDR between women with and without BSA-S $\geq$ 11. No associations were observed between sex steroids, SHBG, insulin sensitivity and anxiety symptoms.

3.3 Study III

Eighty four women with PCOS were randomly allocated to low-frequency EA (n=33), physical exercise (n=34), or no intervention (n=17) (Figure I). Seventy four women completed baseline measurements of which 23 were recruited for MSNA. Nerve recordings were successful before and after interventions in 20 women; low-frequency EA (n=9), physical exercise (n=5) and no intervention (n=6). Women allocated to low-frequency EA received 14 treatment sessions for 16 weeks; and among the women allocated to physical exercise the mean physical exercise frequency was 3.0 ± 0.8 per week.

There were no differences in burst frequency and burst incidence among groups with low-frequency EA, physical exercise and no intervention at baseline. From baseline to week 16, MSNA burst frequency decreased by -39.4% for low-frequency EA; -39.0 % for physical exercise; and -8.7% for no intervention. Sagittal diameter measures decreased from baseline measurements and week 16 for low-frequency EA compared to no intervention ($P < 0.01$); and there were correlations between sagittal diameter and MSNA. Physical exercise reduced BMI by -1.4 % ($P < 0.01$) from baseline measurements to week 16. There were no correlations between BMI and MSNA.

3.4 Study IV

Study compliance

Of the 84 women randomized to low-frequency EA (n=33), physical exercise (n=34), or no intervention (n=17) (Figure 1); ten left the study between randomization and baseline measurements and were excluded from the ITT analyses comprising of low-frequency EA (n=29), physical exercise (n=30), or no intervention (n=15). Eight of the women initializing low-frequency EA, 12 of the women initializing physical exercise, and four of the women initializing no intervention left the study before follow-up.

Low-frequency electroacupuncture and physical exercise

Circulating T, ADT-G and 3G decreased from baseline measurements to week 16 for low-frequency EA compared to physical exercise. Low-frequency EA decreased circulating T by -25% versus -7%, ADT-G by -30% versus -4%, and 3G by -28% versus -6%. The monthly menstrual bleeding frequency increased from 0.28 to 0.69 for low-frequency EA compared with 0.26 to 0.41 for physical exercise ($P< 0.05$). The self-reported acne scores decreased by -32% from baseline measurements to week 32 for low-frequency EA compared with 7% the physical exercise group. At week 32, there were no other observed differences between those receiving low-frequency EA and those with the physical exercise intervention.

Low-frequency electroacupuncture and no intervention

Circulating T, FT, E1-S, ADT-G, 3G and 17G decreased from baseline measurements to week 16 for low-frequency EA compared to no intervention. Low-frequency EA decreased circulating T by -25% versus 2%, FT by -30% versus 0%, E1-S by -42% versus 6%, ADT-G by -30% versus 0%, 3G by -28% versus 4%, and 17G by -30%

versus 19%. The monthly menstrual bleeding frequency increased for low-frequency EA compared to no intervention from baseline measurements to week 16 ($P< 0.001$), and from baseline measurements to week 32 ($P< 0.01$). Circulating T, FT and 17G decreased from baseline measurements to week 32 for low-frequency EA compared to no intervention. Circulating T decreased by -18% versus 9%, FT by -25% versus 5%, and 17G by -28% versus 21%.

Physical exercise and no intervention

Circulating E1-S and 17G decreased from baseline measurements to week 16 for physical exercise compared to no intervention. Physical exercise decreased circulating E1-S by -12% versus 8% and 17G by -8% versus 19%. The monthly menstrual bleeding frequency increased from 0.26 to 0.41 for physical exercise and decreased from 0.23 to 0.19 for no intervention. Circulating T, E2 and 17G decreased from baseline measurements to week 32 for physical exercise compared to no intervention. Physical exercise decreased T by -4% versus 9%, E2 by -23% versus 21%, and 17G by -1% versus 21%. The monthly menstrual frequency remained improved at the 32-week follow-up for physical exercise compared to no intervention.

Subgroup analyses

Among women with BMI < 30, those allocated to low-frequency EA had lower circulating T compared with physical exercise ($P< 0.012$) from baseline measurements to week 16. Among women with BMI > 30, there were no observed differences between those receiving low-frequency EA and those with the physical exercise intervention week 16. Circulating T decreased from baseline measurements to week 16 for low-frequency EA compared to no intervention ($P< 0.049$).

Adverse events

Two women allocated to low-frequency EA reported either temporary dizziness or nausea in conjunction with their low-frequency EA treatments. Isolated redness and hematomas of short duration were observed in three women after their low-frequency EA treatments. There were no reported adverse events for 16 weeks of regular physical exercise.

4 DISCUSSION

4.1 General discussion

When traditional pharmacological PCOS interventions do not help alleviate the features commonly seen in women with PCOS, safe, evaluated alternatives or complements may be utilized. Those treatments might be especially beneficial for women with unwanted side-effects of COCs or other hormonal treatments.

An evaluation of the effect of low-frequency EA compared to regular physical exercise in this thesis demonstrated lower concentrations of T, ADT-G and 3G after a 16-week treatment period. At the 32-week follow up there were no differences on sex steroids, their precursors or metabolites between low-frequency EA and physical exercise. When comparing low-frequency EA with no intervention the effect was enhanced, observing changes not only in T, ADT-G and 3G concentrations , but also in FT, E1-S and 17G concentrations after 16 weeks; and in T, FT and 17G at follow-up . Importantly, physical exercise was superior to no intervention demonstrated by decreased circulating E1-S and 17G after 16 weeks, and in T, E2 and 17G at follow-up; a somewhat similar, although weaker response pattern to that between low-frequency EA and no intervention. Physical exercise, as part of lifestyle management, is already an established intervention for women with PCOS. These findings suggest low-frequency EA to be superior to physical exercise and a complement for treatment of hyperandrogenism in women with PCOS.

The monthly menstrual bleeding frequency increased from baseline measurements to week 16 for low-frequency EA compared to physical exercise. Both low-frequency EA and physical exercise improved the monthly bleeding frequency during treatments when compared to no intervention; and the improvements remained at 32-week follow-up. These observations give an indication of improved oligo/amenorrhea among women allocated to both low-frequency EA and physical exercise. There were few minor adverse events of short duration for repeated low-frequency EA and no adverse events for physical exercise suggesting a combination of low-frequency EA and physical exercise for treatment of oligo/amenorrhea in women with PCOS.

Observations of decreased hyperandrogenism and improved menstrual frequency after a 16-week physical exercise program of 30 minutes three days a week or more, add

reasons to emphasize the importance of promoting a healthy lifestyle that comes with regular physical exercise. Although the women were supervised by weekly telephone support they were more or less left to do any aerobic exercise, as long as they kept the intensity, duration and frequency in accordance with the study protocol. An assumption would be that a more intense, well structured and stringent monitored exercise program would increase the effects. A change of lifestyle need to be long-term to make a difference, which is an important reason to gradually work towards a healthier lifestyle, instead of looking at enhanced short-term effects.

It has been suggested that EA improves exercise capacity [117], which may be one explanation why there were no observed differences on cardiorespiratory fitness between low-frequency EA and physical exercise. Other contributing factors may be that all women received general information concerning the benefits of regular physical exercise and were instructed to complete diaries of their weekly physical exercise.

The underlying physiological mechanisms by which treatments mediate their effects are important to study in order to optimize symptom-oriented and individualized management programs for women with PCOS. In this thesis, low-frequency EA and physical exercise decreased sympathetic nerve activity in women with PCOS measured by microneurographic recordings of multiunit efferent postganglionic MSNA. The results support previous studies which have suggested the effects of both low-frequency EA and physical exercise to be partly mediated via the sympathetic nervous system [94-98]. These observations are promising and need further investigation since women with PCOS have been observed with high sympathetic nerve activity, which may contribute to an increased risk for cardiovascular disease [26].

Observations of high percentages of depression and anxiety-related symptoms among drug-naïve women with PCOS in this thesis suggest an unmet need for assessment and treatment of affective symptoms. Poor psychosocial functioning is associated with higher health care utilization and health care cost and pilot investigations suggest management programs including physical exercise, nutritional counseling and cognitive-behavioral therapy [83]. Interventions aiming towards depression and anxiety symptoms might improve specific HRQL including concerns related to excess body hair, emotions, body weight, infertility, menstrual irregularities. A study evaluating the effect of diet and physical exercise on depression scores and specific HRQL noted

improvements on depression scores together with specific HRQL domains, with the exception of concerns related to body hair [84].

This thesis observed lower T, FT and 3G among women with PCOS and depression symptoms of potential clinical significance. Circulating FT and 3G were associated with worse self-reported depression symptoms when T was analyzed by GC-MC, 3G by LC-MC/MC and FT was calculated from T and SHBG using a computer program to solve the law of mass action equation [106]. Divergent or contradictory results with other studies may in part be due to the underestimation or overestimation of direct assays. By analyzing sex steroids with mass spectrometry the relatively low concentrations of T in women can be detected [106]. For an improved understanding the relationship between mental health and sex hormones with corresponding metabolites in women with PCOS need further study.

4.2 Strengths and limitations

The community-based recruitment process in this thesis included women without previous health care contact, and was not limited to health care seeking women with PCOS. Rigorous inclusion and exclusion criteria add strengths to the studies. In order to evaluate the effect of interventions without interference by other treatments, women on current pharmaceuticals were excluded. It should be stressed that women with PCOS often present with affective symptoms, and are on current psychotropics. As such, the studies did not use a representative population with PCOS.

The diagnostic criteria for PCOS included presence of ultrasound verified polycystic ovaries plus oligo-/amenhorrea or clinical hyperandrogenism, and did thereby not adhere strictly to any of the current PCOS definitions [3-5], which is a limitation factor. Not all women with polycystic ovaries conform to other criteria for the syndrome and there is controversy in regards to whether polycystic ovaries and oligo/anovulation without biochemical and clinical hyperandrogenism should be included as one of the PCOS phenotypes. Biochemical hyperandrogenism was not utilized at time of inclusion since circulating androgen concentrations were measured 12 weeks later, together with other baseline assessments. Instead, the diagnostic criteria relied on clinical hyperandrogenism.

Current PCOS definitions use oligo/anovulation and not oligo/amenorrhea [3-5]. To define oligo/anovulation serum progesterone concentrations have to be measured prior to diagnosis. This was not feasible for the studies of this thesis and thereby the reason for using oligo/amenhorrea instead of oligo/anovulation.

Power was calculated to detect changes in T concentrations for low-frequency EA and physical exercise. Unfortunately, in line with many other clinical studies, there was a high drop-out rate, which may have affected the results. By using an ITT approach the last value were carried forward and all baseline measures of women allocated to interventions without completing the study were used in analysis.

The selected location and depth of acupuncture points; together with frequency and duration of the low-frequency EA treatments, were based on earlier protocols suggesting needle placement in areas innervating the ovaries [89]. Theories of specific acupuncture points to be true and other points to be false have been discussed and there is lack of evidence for using true points [118]. From a physiological perspective it is important to place needles in certain areas according to innervations; the points do not have to be specific, considering anatomical variations and physiological factors.

Acupuncture studies have several other methodological concerns including sham methods, such as superficial needling and placebo needles. Sham methods have been suggested for use to blind acupuncture participants[119] [120]. For naïve acupuncture participants single blinding of the participant might be successful [120], but this type of blinding is not applicable to evaluate the effect of repeated low-frequency EA treatments. Double-blinded acupuncture studies, where both participant and practitioner are unaware of the interventions are impossible to conduct in clinical settings. Given the uncertainties about the physiological effects of sham controls and the question of enhanced placebo effects, it is crucial to conduct direct comparisons of acupuncture and standard treatment. Therefore, in the studies of this thesis, low-frequency EA were compared with physical exercise as part of lifestyle management.

4.3 Future directions

Most studies have limitations and require further investigation. The approach of excluding women with ongoing psychopathology in the studies of this thesis might be one explanation for non-observed associations between depression and anxiety-related symptoms and insulin sensitivity. Others have observed insulin resistance in women with depressive disorders and PCOS [53]. Future studies are encouraged to investigate the relationship between mental health and insulin sensitivity in an unselected PCOS population.

The parasympathetic nervous system and the sympathetic nervous system are thought to contribute to anxiety, and high sympathetic nerve activity have been associated with affective symptoms [121]. Higher MSNA burst incidence was associated with higher anxiety scores and the affective component of depression symptoms [121]. If these associations are similar among women with PCOS remain to be elucidated.

Interventions for diagnosed depression include selective serotonin reuptake inhibitors (SSRIs) and prolonged SSRI use results in increased body weight, suggesting non-pharmacological alternatives and complements to be thoroughly investigated [122]. Women with PCOS have been observed with unspecific anxiety and depression-related symptoms, and to alleviate or diminish symptom burden without gaining body weight, lifestyle management, including physical exercise have been evaluated [83-84]. The underlying physiological mechanisms of acupuncture share similarities with those of physical exercise, and acupuncture interventions have been evaluated in studies of depression [123]. Future directions will aim towards investigating whether women with PCOS with depression and anxiety symptoms go into remission after low-frequency EA.

In summary, this thesis supports the effects of low-frequency EA and physical exercise to be partly mediated via the sympathetic nervous system. Low-frequency EA may be a complement for treatment of hyperandrogenism in women with PCOS. With few minor adverse events of short duration for repeated low-frequency EA and no adverse events for physical exercise, a combination of low-frequency EA and physical exercise is recommended for treatment of oligo/amenorrhea in women with PCOS. With an unmet need for assessment and treatment of affective symptoms in women with PCOS further studies are warranted.

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