Low-frequency Electro-Acupuncture and Physical Exercise Decrease High Muscle Sympathetic Nerve Activity in Polycystic Ovary Syndrome

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Running head: Acupuncture and Exercise decrease sympathetic tone in PCOS

Key Words: Polycystic ovary syndrome, sympathetic nerve activity, electro-acupuncture, physical exercise, testosterone, insulin resistance, metabolic syndrome, cardiovascular disease

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ClinicalTrials.gov Identifier: NCT00484705.

Disclosure statement
The authors have nothing to disclose

Grants
Swedish Medical Research Council (Project No. 2005-72VP-15445-01A, 2005-72VX-15276-01A, 2008-72VP-15445-01A); Novo Nordisk Foundation; Wilhelm and Martina Lundgren’s Science Fund; Hjalmar Svensson Foundation; Tore Nilson Foundation; Åke Wiberg Foundation; Adlerbert Research Foundation; Ekhaga Foundation; Swedish federal government under the LUA/ALF agreement ALFFGBG-10984 and Regional FOU agreement.
Abstract

Context: We have recently shown that polycystic ovary syndrome (PCOS) is associated with high muscle sympathetic nerve activity. Animal studies support the concept that low-frequency electro-acupuncture (EA) and physical exercise, via stimulation of ergoreceptors and somatic afferents in the muscles, may modulate the activity of the sympathetic nervous system.

Objective: The aim of the present study was to investigate the effect of these interventions on sympathetic nerve activity in women with PCOS.

Design: Randomized controlled trial.

Setting: Sahlgrenska University Hospital, Gothenburg, Sweden.

Outcome Measures and Subjects: Twenty women with PCOS were randomly allocated to one of three groups; low-frequency EA (n=9), physical exercise (n=5) or to an untreated control (n=6) group during 16 weeks. Direct recordings of multunit efferent postganglionic muscle sympathetic nerve activity (MSNA) in a muscle fascicle of the peroneal nerve before and following 16 weeks of treatment. Biometric, hemodynamic, endocrine and metabolic parameters were measured.

Results: Low-frequency EA ($P = 0.036$) and physical exercise ($P = 0.030$) decreased MSNA burst frequency compared to the untreated control group. Low-frequency EA group reduced sagittal diameter ($P = 0.001$), while physical exercise group reduced body weight ($P = 0.004$) and body mass index (BMI) ($P = 0.004$) as compared to the untreated control group. Sagittal diameter was related to MSNA burst frequency ($R_s = 0.58, P < 0.005$) in the EA group. No correlation was found for BMI and MSNA in the exercise group. There were no differences between the groups in hemodynamic, endocrine and metabolic variables.

Conclusions: For the first time we demonstrate that low-frequency EA and physical exercise lowers high sympathetic nerve activity in women with PCOS. Thus, treatment with low-frequency EA or physical exercise with the aim to reduce MSNA may be of importance for women with PCOS.
Introduction

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders affecting around 10% of women of reproductive age, and is strongly associated with hyperandrogenism and ovulatory dysfunction (8, 46). PCOS increases the risk of metabolic disturbances such as abdominal obesity, hyperinsulinaemia and insulin resistance which can lead to type 2 diabetes, and dyslipidemia (8, 46), as well as hypertension which may lead to the development of other cardiovascular diseases (35).

Despite extensive research, the primary etiology remains unknown, although both environmental and genetic factors are implicated (17). Exciting evidence suggesting the ovarian theca cells, the major source of androgen excess (22), play a key role in the etiology. Also, the hypothalamic-pituitary axis (50), and defective insulin activity (12) may cause the syndrome. Further, altered activity in the sympathetic nervous system has been suggested in the PCOS etiology (26). Recently, for the first time, direct intra-neural recordings of sympathetic nerve activity was obtained in women with PCOS by our group. We clearly demonstrated that PCOS is associated with increased sympathetic nerve activity and that the strongest independent factor explaining the augmented activity was the elevated testosterone level, characteristic for PCOS (63). We concluded that the augmented sympathetic outflow may contribute to the increased prevalence of vascular disease reported in these individuals and be involved in the etiology of the condition.

The spectrum of therapeutic options is broad and ranges from lifestyle intervention to specific pharmacologic agents. Pharmacological approaches are helpful but have adverse effects. As PCOS is a life-long disorder, these patients need long-lasting treatment due to increased risk of associated disorders.

Repeated acupuncture treatments in women with PCOS and women with undefined ovulatory dysfunction have been shown to exert long-lasting beneficial effects on endocrinological parameters and anovulation with no negative side effects (7, 44, 60). Physical exercise has also been shown to have positive effects on ovulation and cardiopulmonary function in women with PCOS (20, 28, 29, 48, 65, 78).

Despite extensive research, the physiological mechanisms of acupuncture still remain unclear. Few investigations in humans have studied whether acupuncture modulates the activity in the autonomic
nervous system. However, data from animals support the concept that acupuncture, via stimulation of
ergoreceptors and somatic afferents in the muscles (32) which are physiologically activated during muscle
contractions, modulates the secretion of opioids in the central nervous system, which mediate analgesic,
sympatholytic and depressor effects (6, 36, 54, 80). Interestingly, the decrease in sympathetic nerve
activity and blood pressure is greatest in animal models of elevated sympathetic activity and hypertension
(80). Further, we recently demonstrated that low-frequency electro-acupuncture (EA) in rats modulates the
activity in ovarian sympathetic nerves (57, 58) and that the effect was mediated via supraspinal
mechanisms (56). This is supported from human data where acupuncture has been shown to increase heart
rate variability (HRV) in healthy subjects (51) and in patients with migraine (3) and to decrease MSNA in
patients with advanced heart failure (43).

Cross-sectional and longitudinal studies evaluating physical exercise in healthy subjects indicate
alterations in resting MSNA (49). Despite few studies in healthy humans showing a possible increase (55)
or decrease (23) in MSNA, there is no convincing evidence that exercise alters resting MSNA. Heart
failure and type 2 diabetes are associated with high sympathetic nerve activity (13). Conversely, exercise
has been shown to reduce MSNA in heart failure (16) and to alter HRV in type 2 diabetes (83). More
comprehensive exercise studies with long exercise periods to elicit adaptive sympathetic outflow in
disorders associated with high sympathetic nerve activity, as in women with PCOS, are needed to better
understand the role of exercise on sympathetic neural outflow.

Thus, both low-frequency EA and physical exercise may have the potential to decrease the high activity in
sympathetic nervous system in women with PCOS. The impact of low-frequency EA and physical
exercise on the activity in the sympathetic nervous system has, to our knowledge, not previously been
investigated in women with PCOS. The primary aim of the present study was therefore to elucidate the
effect of these interventions on sympathetic nerve activity in women with PCOS.

Material and Methods
Study Procedure

This was a randomized controlled trial with independent observers and with blind, independent analysis. All women gave oral and written consent prior to inclusion and the study was conducted in accordance with the Declaration of Helsinki, approved by the Ethics Committee, University of Gothenburg, Sweden. The study is registered at ClinicalTrials.gov Identifier: NCT00484705.

The randomization was performed by the study coordinator according to a computerized list. PCOS women were stratified by age and BMI and thereafter block randomized to one of three study groups in a 2:2:1 ratio; (i) low-frequency EA, 2 Hz, (LF EA), (ii) physical exercise, and (iii) an untreated control group given the same information as all women of the importance to be physically active.

After screening (see under patients) and randomization, a 12-week observation period followed where patients were asked to document their menstrual bleeding pattern. Thereafter, a common core of measurements was performed at A) baseline and B) after 16 w of treatment. Anthropometric measurement included height, body weight, body mass index (BMI; ratio between weight in kilograms and the square of the height in meter); Sagittal abdominal diameter was measured in the supine position as the distance between the examination table and the highest position of the abdomen at the level of the iliac crest in a recumbent position (76); waist-to-hip ratio (WHR; ratio between the smallest circumference at the torso and the widest circumference at the hip). Clinical hyperandrogenism was measured by Ferriman-Gallwey score >6 and/or acne (14). Fasting blood samples for hormone assessment, lipid profile, and fasting glucose and insulin concentrations (see Biochemical assays) were taken between 7.30 – 8.30 am, immediately aliquoted on ice and stored at −20°C until assay. Blood samples were taken independent of follicular phase of the menstrual cycle since most of the women had oligo- or amenorrhoea. Muscle sympathetic nerve activity (MSNA) was assessed by microneurography (see Microneurography). The menstrual bleeding patterns were confirmed by daily recordings of the basal body temperature (BBT) throughout the entire study period and via interviews by gynecologists.
Patients

The patients were consecutively recruited between November 2005 until January 2008 from the Department of Obstetrics and Gynecology, Sahlgrenska University Hospital in Göteborg, Sweden, and through advertisement in the local community.

PCOS women were eligible for randomization if they displayed polycystic ovaries (at least 12 follicles, 2-9 mm); and/or increased ovarian volume (>10 ml) revealed by two dimensional ultrasound examinations in one or both ovaries, together with one of the following clinical symptoms; oligomenorrhea with intermenstrual interval greater than 35 d, and/or clinical and/or biochemical signs of hyperandrogenism (hirsutism or acne), according to the Rotterdam consensus report (68).

Women on medication(s) less than 3 months or breast feeding less than 6 months prior to inclusion were excluded. Other exclusion criteria were known endocrine or neoplastic causes of hyperandrogenemia including androgen-secreting tumors, Cushing’s syndrome, congenital adrenal hyperplasia and hyperprolactinemia.

Description of interventions

Low-frequency EA. Acupuncture was performed by one of the authors (EJ) who were educated and trained in the theoretical and practical knowledge of acupuncture within women’s health. The education and training include wide variations of styles and acupuncture technique. Selection of acupuncture points was based on experience of previous studies on the effects of acupuncture in PCOS (60) and climacteric vasomotor symptoms (79), and was based on clinical experience when treating women with PCOS.

Acupuncture points selected were located bilaterally in the abdominal muscles and in the muscles below the knee in somatic segments according to the innervation of the ovaries (Th12-L2, S2-S4) (5) (Table 1), with the aim of stimulation to modulate both segmental and central sympathetic nerve activity. Additionally, points were selected bilaterally, extra-segmental to the ovaries, in muscles of the arm below
the elbow (Table 1), in order to strengthen and lengthen the effect on the central nervous systems (69, 70).

Stainless steel, disposable 1-time sterile needles (Hegu Xeno: Hegu AB, Landsbro, Sweden; size 0.32 mm in diameter and the length of 30 or 50 mm) were inserted intramuscularly to a depth of 15–35 mm. Needles in abdominal muscle and in the leg were attached to an electrical stimulator (CEFAR ACUS 4, Cefar-Compex Scandinavia AB, Malmö, Sweden) and stimulated electrically with low-frequency EA of 2 Hz with square-wave burst pulses (a burst length of 0.1 s and a burst frequency of 80 Hz) with alternating polarity. The intensity of the electrical stimulation was adjusted to produce local muscle contractions, as strong as possible, without pain and discomfort. All other needles were stimulated manually by hand with rotation of the needle through an arc of at least 180° to evoke needle sensation every 10 minutes. Each treatment lasted 30 minutes, was given 2 per week during 2 weeks, 1 per week during 6 weeks, once every second week for 8 weeks, in total 14 treatments during 16 weeks. Acupuncture points and electrical stimulation were the same for all women in the low-frequency EA group.

*Physical exercise.* All women underwent cardiopulmonary exercise test on a bicycle ergometer as described previously (2). Women received a pulse watch and were instructed to take up regular exercise, comprising brisk walking, cycling or any other aerobic exercise at a self-selected pace described as “faster than normal walking but a pace that could be sustained for at least 30 minutes” as previously described (48). They were instructed to do their exercise at least 3 days per week, each of 30 to 45 min duration, with a pulse frequency above 120 / min during 16 weeks. Once a week, women in the exercise group received a phone call, for guidance how to increase the physical exercise. All exercise should be beyond the daily physical activity.

*Untreated controls.* This group was given information about the importance of physical activity and healthy diet, as women in the low-frequency EA group and the physical exercise group. They could call the study coordinator at any time point.

Biochemical assays
Serum free testosterone and dehydroepiandrosterone sulphate (DHEAS) were measured by radioimmunoassay (RIA) (Coat-A-Count Free Testosterone and Coat-A-Count® DHEA-SO4, Diagnostic Products Corporation (DPC), Los Angeles, CA, USA). Serum total testosterone and insulin were measured by competitive immunochemistry with chemoluminiscence technology (ADVIA Centaur® TSTO Ready Pack® primary reagens and ADVIA Centaur Insulin ready Pack, Bayer Health Care LLC, Tarrytown, NY, US). Serum sex hormone binding globulin (SHBG), luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured by chemoluminiscence microparticle immunoassay (CMIA) (Architect® SHBG reagent kit, Biokit S.A., Barcelona, Spain for Abbott Laboratories Diagnostic Division and Architect® LH reagent and FSH reagent pack, Abbott Laboratories Diagnostic Division, Chicago, US). Free thyroxin (T4) and IGF-1 were measured with immunochemoluminiscence technology (FT4 Free thyroxine, Roche Diagnostics GmbH, Mannheim, Germany and Immulite 2500 IG1, Euro/DPC, UK). Thyroid-stimulating hormone (TSH) was measured with electrochemiluminescence immuno assay (ECLIA), 37 °C (TSH Thyrotropin, Roche Diagnostics GmbH, Mannheim, Germany). Serum triglycerides (TG), cholesterol and high density lipoprotein (HDL)-cholesterol and plasma glucose were measured with an enzymathic photometric method, 37 °C (TG, Roche/Hitachi, CHOL, Roche/Hitachi, HDL-C 2nd generation, Roche/Hitachi, Roche Diagnostics, GmbH, Mannheim, Germany). Low density lipoprotein (LDL)-cholesterol is a calculation according to Freivalds formula when serum-TG< 4.0; serum LDL-cholesterol = serum cholesterol – serum HDL-cholesterol – 0.45x serum TG. Insulin sensitivity was estimated with a homeostasis model assessment for estimating insulin resistance (HOMA-IR) (41). HOMA-IR index was calculated according to the formula: [fasting plasma glucose (mmol/l) X fasting plasma insulin concentration (mU/ml)]/22.5. All analyses were carried out at an accredited laboratory at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Sweden.
Microneurography

Direct 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. A low impedance reference electrode was inserted subcutaneously a few centimetres away. When a muscle nerve fascicle had been identified, small electrode adjustments were made until a site was found in which spontaneous, pulse-synchronous bursts of neural activity could be recorded. Details of the nerve recording technique and criteria for MSNA have been reported previously (73). Bursts identified by inspection of the mean voltage neurogram were expressed as burst frequency (bursts per min) and burst incidence (bursts per 100 heartbeats). The nerve recordings were assigned a code and analyzed blinded.

During the microneurographic recording, finger arterial blood pressure was measured non-invasively by the volume-clamp method (Finapress 2300, Ohmeda, Louisville, USA) (47), heart rate was monitored via echocardiography (ECG)-chest electrodes and respiration via a strain-gage strapped around the waist.

MSNA consists of baroreceptor reflex controlled vasoconstrictor impulses to the muscle vascular bed, involved in dynamic blood pressure regulation. Although MSNA only represents one subdivision of the sympathetic nervous system, at rest it correlates well with a global measure of sympathetic nerve activity such as total body norepinephrine spillover, and with regional (heart and kidney) norepinephrine spillover (74, 75).

Statistical analyses

Statistical evaluations were performed with SPSS software (version 17.0, SPSS, Chicago, IL). Descriptive results are presented as the mean ± SD. The significance of the difference between groups, calculated on base line vs. after 16 weeks of treatment, was assessed by Kruskal-Wallis. If significant, differences between the treatment groups and the untreated control group were tested with Mann-Whitney U-test. Wilcoxon’s matched pairs signed rank sum test was used to compare baseline values with values obtained
after 16 weeks of treatment within each group. Correlation analyses were performed using Spearman rank correlation coefficient (Rs) in bivariate analyses. Significance was accepted as a two-sided value of $\leq 0.05$.

Results

Patient characteristic

A total of 504 women were recruited for this study. Of these, 404 were excluded as they did not meet the inclusion criteria. The remaining 100 were examined at the first visit, after which 84 women rendered eligible for the study. They were randomized to low-frequency EA (n=33), physical exercise (n=34) or untreated control (n=17) group. Prior to baseline measurements 10 patients dropped-out; 4 patients in the low-frequency EA with the reasons: moved from area n=3, personal reasons n=1; 4 patients in the physical exercise group with the reasons: personal reasons n=3, pregnancy n=1; and 2 patients in the untreated control group with the reasons: moved from area n=1, personal reasons n=1. Of the remaining patients, twenty three were randomly recruited for microneurography. Nerve recordings were successfully performed in 20 women; low-frequency EA group (n=9), physical exercise (n=5) control group (n=6), prior to and following 16 weeks of treatment.

Their phenotype was as follows: 14 had oligomenorrhea (O), 19 had signs of hyperandrogenism (HA) and all (n=20) had polycystic ovaries (PCO) according to Rotterdam consensus report (68). All three criteria (O, HA and PCO) were fulfilled by 13 patients (Rotterdam and NIH criteria); 7 patients in the EA group, 3 in the physical exercise group and 3 in the control group. A combination of HA and PCO was found in 6 patients (Rotterdam criteria); two patients in the EA group, one in the physical exercise and three in the untreated control group. In the whole cohort of subjects, only one had the combination of PCO and O (Rotterdam criteria), this subject was found in the physical exercise group.

Baseline characteristics and treatment
The study groups did not deviate in terms of age (mean ± SD, 29.9 ± 4.5, 30.4 ± 5.5 and 31.0 ± 3.2 respectively) or other measured variables at baseline (Table 2 and 3). Women in the low-frequency EA group received 14 treatments and exercise frequency was 3.0 ± 0.8 per week in the physical exercise group.

Low-frequency EA and physical exercise decrease muscle sympathetic nerve activity

Low-frequency EA and physical exercise significantly decreased MSNA burst frequency compared to the untreated control group after 16 weeks of treatment (Figure 1A) and the same holds for MSNA burst incidence. Figure 1B illustrates within group analyses of MSNA burst frequency. Low-frequency EA reduced sagittal diameter (Figure 2), while physical exercise reduced body weight and BMI compared to the untreated control group (Figure 3).

While there were no differences between the groups in hemodynamic, endocrine and metabolic variables (Table 3) interestingly, within group analyses revealed that free testosterone was significantly decreased, ($P < 0.05$) in the low-frequency EA group but not in the physical exercise and the untreated controls groups.

A positive correlation was observed between the sagittal diameter and MSNA (Rs = 0.58, $P < 0.01$) in the low-frequency EA group following 16 weeks of treatment, while MSNA was not related to changes in body weight or BMI in the physical exercise group.

Intervention and menstrual bleeding pattern

In the untreated control group, 3 of 6 reported oligo-, amenorrhea and there was no change in menstrual bleeding pattern during the study period. In the low-frequency EA group, 7 of 9 were oligo-, amenorrhoic prior to intervention and 5 reported 3 – 4 menstruations during the intervention period. However, due to difficulties in interpreting their BBT curves some patients missed to do the progesterone tests. In the
physical exercise group, 4 of 5 were oligo-, amenorrhoic and the intervention did not affect the menstrual bleeding pattern.

Discussion

As PCOS is a life-long condition with increased risk of associated disorders (46) and with pharmacological treatments exerting adverse effects (11), it is of importance to investigate the efficacy of non-pharmacological treatment strategies that hypothetically might attenuate the high sympathetic activity associated with PCOS.

This study is the first one to demonstrate that repeated low-frequency EA and physical exercise can reduce high sympathetic nerve activity seen in women with PCOS (63). Furthermore, both therapies decreased measures of obesity while only low-frequency EA improved menstrual bleeding pattern.

Polycystic ovary syndrome is associated with high sympathetic nerve activity

We have previously shown that PCOS is a condition associated with increased sympathetic nerve activity (63). In support for our findings, women with PCOS have greater density of ovarian cathecholaminergic nerve fibers (27, 53), altered catecholamine metabolism (18), disturbed heart rate variability (HRV) (81) and abnormal heart rate recovery (HRR) after exercise test, two indirect markers of autonomic function (20, 67, 71). Further, PCOS is associated with risk factors thought to be related to altered activity of the sympathetic nervous system, such as hyperinsulinemia, insulin resistance, obesity, and cardiovascular disease (20, 31, 67, 72).

In our previous study we found that androgens were the strongest predictive factor for MSNA in women with PCOS (63). Androgens are known important determinants of body composition and high testosterone concentration is associated with high bone mass density (82) and contributes to peripheral muscle mass distribution in women with PCOS (9, 10). Whether androgens contribute to abdominal fat mass
distribution is however disputable. Recently, for example, it was demonstrated that abdominal fat distribution in PCOS women does not differ from BMI and age matched controls (4).

Although the sympathetic nervous system is thought to be affected by obesity, the relationship between MSNA and measures of obesity has yielded different results. A positive correlation between MSNA and BMI has been found in some (1, 24, 25, 52), but not all (15, 34, 61, 62, 64) previous studies. Abdominal fat mass is suggested to be a better correlate with MSNA than percentage body fat (BF %) (30), however, when adjusting for age, waist circumference, WHR and BMI are predictive for MSNA in men, but not women (66).

Low-frequency electro acupuncture and physical exercise decrease sympathetic nerve activity in women polycystic ovary syndrome

The novel finding in the present study is that low-frequency EA and physical exercise decrease MSNA compared to the untreated control group. No previous study has investigated the effect of low-frequency EA or physical exercise on sympathetic nerve activity in women with PCOS. These findings are supported by our experimental animal data where low-frequency EA is shown to increase ovarian blood flow responses by modulating ovarian sympathetic nerve activity (57, 58). Additional experiments demonstrated that the effect is mediated via supraspinal mechanisms, thus indicating that low-frequency EA modulate segmental and central sympathetic nerve activity (56). Furthermore, nerve growth factor and adrenergic receptors, both markers of the sympathetic nervous system, are down regulated in visceral adipose tissue and ovaries after low-frequency acupuncture treatments in two different rat PCOS models (37, 40, 59). In our experimental animal data acupuncture did not affect weight and body composition (38). Thus, the mechanism seems to be mediated via other pathways. Support for the hypothesis that acupuncture decreases MSNA derives from patients with advanced heart failure, a condition associated with high sympathetic nerve activity, where acupuncture decreased MSNA (43). In migraine, a neurovascular disorder associated with dysregulation of the autonomic nervous system, acupuncture
modulates HRV (3). Further, acupuncture has been shown to decrease heart rate in healthy subjects, where administration of atropine and propranolol attenuated the effect of acupuncture, thus indicating a reciprocal coordination of an increase in cardiac vagal activity and a decrease in cardiac sympathetic activity (45).

In the physical exercise group, MSNA was decreased compared to untreated controls. Physical exercise has previously been shown to modulate HRV and HRR, both indirect markers of cardiac autonomic function in women with PCOS (19-21, 78). Evidence from our animal studies indicates that physical exercise reduces nerve growth factor and adrenergic receptors in both adipose tissue and in the ovary in our rat PCOS models (37, 39). Though the effect of physical exercise on sympathetic outflow is still a matter of controversy, physical exercise has been shown to reduce MSNA in heart failure (16) and to alter HRV in type 2 diabetes (83) which lend further support the findings in the present study. Though the underlying mechanisms remain largely unknown the reduction in MSNA seen in response to physical exercise has been proposed to originate from a central effect of training (32).

We have demonstrated that free testosterone is the strongest independent predictor of high MSNA in PCOS (63). Although there was no significant difference between the treatment groups regarding androgen level, free testosterone was significantly decreased within the low-frequency EA group ($P < 0.05$) while it remained unchanged within the physical exercise group. In our androgen induced rat PCOS model, low-frequency EA decreased androgen receptor mRNA expression in adipose tissue while physical exercise did not (37). Interestingly, the decrease in sagittal diameter and MSNA in the low-frequency EA group, were related to the reduction in free testosterone ($Rs = 0.65$, $P < 0.05$ and $Rs = 0.34$, $P < 0.05$ respectively) indicating that androgen level may affect body composition and also play a role in regulating sympathetic outflow.

Despite the recent observation showing that MSNA activity in women is not dependent on WHR or BMI, the results in the present study show that reduction in MSNA was related to decrease in sagittal diameter
in the low-frequency EA group, while it was not related to the decrease in BMI or body weight in the physical exercise group, indicating that a reduction in abdominal fat mass is more important for sympathetic outflow than changes in BMI or body weight, which do not discriminate between lean and adipose tissue *per se*. However, in a recent study HRR was shown to be improved after body weight loss in women with PCOS (71).

Interestingly, even though the reporting of menstrual bleeding pattern is not optimal, 5 out of 7 women with oligo-amenorrhea in the low-frequency EA group reported more regular menstrual bleeding pattern while there was no difference in menstrual bleeding pattern in the physical exercise group or the untreated control group. Most likely, in the EA group this is due to the decrease in circulating androgens which is supported by our animal research where androgen receptor mRNA expression was down regulated and menstrual cyclicity was improved after low-frequency EA treatment (37).

**Limitations**

The small number of patients in each group is a limitation of the present study. Also, lack of an appropriate control group is a weakness of the present study. Evidence shows that, for several conditions, acupuncture is as effective or even more effective than standard conventional care (42). Our hypothesis is that needle insertion and manual and/or electrical stimulation of the needles activates afferent nerve fibers. As stated in the material and method section, the selected acupuncture points were in the abdominal muscles and in the muscles below the knee in somatic segments according to the innervation of the ovaries. Classical acupuncture points are not the only places where the nervous system can be stimulated. The reason for the use of classical acupuncture points in the present study is that they are well described regarding anatomical location and innervation and we hypothesised that we would obtain the same effect if non acupuncture points were stimulated in the same segmental innervation. Thus, needling of “incorrect” sites is not a valid placebo control. In the present study, the low-frequency EA group received more attention compared to the physical exercise- and untreated control group. Therefore, in another study
it is of importance to compare the effect of low-frequency EA with a group that receives the same amount of attention. The effect of low-frequency EA and “sham procedure” with superficial needle insertion without needle manipulation, on sympathetic nerve traffic (MSNA) has previously been investigated in healthy subjects (33). In this study, low-frequency EA was shown to elicit acute changes in MSNA, while superficial needle insertion had no effect (33). Thus, the next step in a future study is to elucidate if there is a different response on MSNA between different stimulation techniques e.g. manual acupuncture and low-frequency EA, and to find out optimal dose e.g. number of needles, stimulation intensity and frequency. Comparison with low-frequency EA and manual acupuncture, in which the needles are inserted and manually but not electrically stimulated, will be very important, in addition to an "attention control" without needles (77). When comparing different stimulation modalities, e.g. needle insertion with and without electrical stimulation, the microneurographic technique for evaluating efferent sympathetic outflow, may provide a powerful tool for studying underlying mechanisms of action.

Another weakness in this study, is that the documentation of menstrual bleeding pattern is not complete, the reports however show clear differences between the groups (42).

**Perspectives and Significance**

Treatment of PCOS has so far encompassed weight loss and various pharmacological regimens with clear-cut benefits but also inherent adverse effects. Life style modifications are widely suggested as an additional treatment but scientific evidence for its beneficial effects is still sparse. The present study on possible cardiovascular risk for PCOS patients in the form of an increased sympathetic activity was performed in the perspective of our findings from earlier human studies and recent data from our PCOS animal models. The findings that low-frequency EA and exercise decrease sympathetic nerve activity in women with PCOS, indicate a possible alternative non-pharmacologic approach to reduce cardiovascular risk in these patients.
Conclusions

For the first time we demonstrate that low-frequency EA and physical exercise decrease high sympathetic nerve activity in women with PCOS. Thus, since MSNA may per se increase the cardiovascular risk, treatment with low-frequency EA or physical exercise with the aim to reduce MSNA may be of importance for women with PCOS.
References


71. Thomson RL, Buckley JD, Noakes M, Clifton PM, Norman RJ, and Brinkworth GD. Heart rate recovery improves after weight loss in overweight and obese women with polycystic ovary syndrome. *Fertil Steril* 2009.


Legends

**Figure 1:** A) Muscle sympathetic nerve activity (MSNA) expressed as burst frequency (bursts/minute) and calculated on differences before and after 16 weeks of treatment. Exact values and median is presented. Kruskal Wallis: $P < 0.05$. *$P < 0.05$ EA (low-frequency electro-acupuncture) vs. untreated control (Mann-Whitney) and *$P < 0.05$ PE (physical exercise) vs. untreated control (Mann-Whitney). B) Muscle sympathetic nerve activity (MSNA) expressed as burst frequency (bursts/minute) changes within each group. Exact values and median is presented. Wilcoxon’s matched pairs signed rank sum test was used to compare baseline values with values obtained after 16 weeks of treatment within each group. *$P < 0.05$ in the PE (physical exercise group); **$P < 0.01$ in the EA (low-frequency electro-acupuncture) group.

**Figure 2:** Sagittal diameter calculated on differences between measurements performed before and after 16 weeks of treatment. Exact values and median is presented. Kruskal Wallis: $P < 0.01$. **$P < 0.01$ EA (low-frequency electro-acupuncture) vs. untreated control (Mann-Whitney).

**Figure 3:** Body mass index (BMI) calculated on differences between measurements performed before and after 16 weeks of treatment. Exact values and median is presented. Kruskal Wallis: $P < 0.05$. **$P < 0.01$ PE (physical exercise) vs. untreated control (Mann-Whitney).
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<th>Points</th>
<th>Stimulation</th>
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<tr>
<td>CV 3</td>
<td>Midline</td>
<td>L1</td>
<td>Fibrous tissue, linea alba</td>
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<tr>
<td>CV 6</td>
<td>Midline</td>
<td>Th11</td>
<td>Fibrous tissue, linea alba</td>
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<td>Bilateral</td>
<td>Th6-12</td>
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<td>SP 6</td>
<td>Bilateral</td>
<td>L4-5, S1-2</td>
<td>Mm. flexor digitorum longus, tibialis posterior</td>
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<td>SP 9</td>
<td>Bilateral</td>
<td>S1-2</td>
<td>M. gastrocnemius</td>
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<td>LI 4 *</td>
<td>Bilateral</td>
<td>C8, Th1</td>
<td>Mm. interosseus dorsalis I, lumbricalis II, adductor pollicis</td>
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<td>PC 6 *</td>
<td>Manual, 4 times</td>
<td>C8, Th1</td>
<td>M. flexor digitorum superficialis</td>
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</table>

CV = conception vessel; LI = large intestinal, PC = pericardium; SP = spleen; ST = stomach. * these points were used alternating, every second time.
Table 2. Biometric characteristic of the study population before and after 16 weeks of treatment expressed as mean ± SD. Group differences was assessed by Kruskal-Wallis at 1) baseline and on 2) the difference between baseline versus after 16 weeks of treatment. If significant, differences between the treatment groups and the untreated control group were tested with Mann-Whitney U-test.

<table>
<thead>
<tr>
<th>Biometric</th>
<th>LF electro-acupuncture n=9</th>
<th>Physical Exercise n=5</th>
<th>Untreated controls n=6</th>
<th>Kruskal Wallis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 16 w</td>
<td>Baseline</td>
<td>After 16 w</td>
<td>Baseline</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.5 ± 8.6</td>
<td>27.6 ± 8.8</td>
<td>26.8 ± 4.8</td>
<td>26.4 ± 4.8</td>
<td>28.0 ± 6.2</td>
</tr>
<tr>
<td>Sagittal diameter (cm)</td>
<td>20.1 ± 4.7</td>
<td>19.3 ± 4.6</td>
<td>19.3 ± 2.9</td>
<td>19.1 ± 2.4</td>
<td>19.0 ± 3.1</td>
</tr>
<tr>
<td>WHR</td>
<td>0.8 ± 0.08</td>
<td>0.8 ± 0.09</td>
<td>0.8 ± 0.07</td>
<td>0.8 ± 0.07</td>
<td>0.8 ± 0.07</td>
</tr>
<tr>
<td>Ferriman-Gallwey score</td>
<td>16.1 ± 8.5</td>
<td>15.1 ± 10.1</td>
<td>12.8 ± 10.1</td>
<td>12.6 ± 9.1</td>
<td>9.5 ± 5.1</td>
</tr>
</tbody>
</table>

All data are shown as mean ± SD. BMI = body mass index; WHR = waist hip ratio.

^ There were no differences between the treatment groups at baseline in any variable

b P < 0.001: Calculated on difference between baseline and after 16 weeks treatment: LF EA vs. Untreated controls (Mann-Whitney U-test)

c P < 0.01: Calculated on difference between baseline and after 16 weeks treatment: Physical exercise vs. Untreated controls (Mann-Whitney U-test)
Table 3. Hemodynamic, hormones and metabolic data at baseline and after 16 weeks of treatment expressed as mean ± SD. Group differences was assessed by Kruskal-Wallis at 1) baseline and on 2) the difference between baseline versus after 16 weeks of treatment.

<table>
<thead>
<tr>
<th></th>
<th>LF electro-acupuncture n=9</th>
<th>Physical Exercise n=5</th>
<th>Untreated controls n=6</th>
<th>Kruskal Wallis P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemodynamic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>110.7 ± 9.2</td>
<td>113.2 ± 10.4</td>
<td>116.0 ± 8.4</td>
<td>116.6 ± 10.3</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>67.2 ± 4.8</td>
<td>67.1 ± 5.8</td>
<td>64.6 ± 8.5</td>
<td>67.4 ± 6.3</td>
</tr>
<tr>
<td>Heart rate</td>
<td>61.2 ± 7.4</td>
<td>61.6 ± 7.3</td>
<td>67.8 ± 8.9</td>
<td>63.6 ± 9.1</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH, IU/L</td>
<td>13.8 ± 24.0</td>
<td>6.1 ± 3.7</td>
<td>6.8 ± 3.3</td>
<td>5.8 ± 1.5</td>
</tr>
<tr>
<td>FSH, IU/L</td>
<td>5.1 ± 2.3</td>
<td>4.6 ± 1.5</td>
<td>4.6 ± 1.8</td>
<td>4.1 ± 1.3</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>2.1 ± 2.2</td>
<td>1.3 ± 0.6             ^A</td>
<td>2.1 ± 1.2</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>Total testosterone, nmol/L</td>
<td>1.9 ± 0.5</td>
<td>1.9 ± 0.3</td>
<td>2.0 ± 0.9</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Free testosterone, pmol/L</td>
<td>4.6 ± 2.2</td>
<td>4.0 ± 2.3</td>
<td>5.3 ± 3.4</td>
<td>5.4 ± 2.8</td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>38.8 ± 27.3</td>
<td>44.3 ± 25.8</td>
<td>32.6 ± 9.7</td>
<td>54.6 ± 53.9</td>
</tr>
<tr>
<td>FAI</td>
<td>7.2 ± 4.6</td>
<td>5.8 ± 3.4</td>
<td>7.1 ± 4.3</td>
<td>8.8 ± 3.3</td>
</tr>
<tr>
<td>DHEA-S, µmol/L</td>
<td>4.6 ± 1.6</td>
<td>5.0 ± 1.0</td>
<td>5.4 ± 2.4</td>
<td>4.8 ± 2.2</td>
</tr>
<tr>
<td>Free T4, pmol/L</td>
<td>15.3 ± 1.2</td>
<td>15.2 ± 1.3</td>
<td>14.8 ± 3.6</td>
<td>19.0 ± 10.7</td>
</tr>
<tr>
<td>TSH mIU/L</td>
<td>3.3 ± 1.1 ^A</td>
<td>3.1 ± 1.2</td>
<td>2.7 ± 2.0</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>IGF-1, µg/L</td>
<td>168.9 ± 44.8</td>
<td>159.4 ± 42.7</td>
<td>176.6 ± 36.4</td>
<td>188.6 ± 67.7</td>
</tr>
<tr>
<td><strong>Insulin sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.6 ± 0.4</td>
<td>4.5 ± 0.3</td>
<td>4.6 ± 0.2</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>9.2 ± 8.1</td>
<td>9.1 ± 10.1</td>
<td>8.0 ± 4.5</td>
<td>6.4 ± 2.8</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.0 ± 2.0</td>
<td>1.9 ± 2.3</td>
<td>1.6 ± 1.0</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td><strong>Blood lipids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>3.9 ± 0.7</td>
<td>3.9 ± 0.8</td>
<td>4.0 ± 0.6</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.4</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.3 C ^C</td>
<td>1.4 ± 0.3</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>2.1 ± 0.7</td>
<td>2.1 ± 0.5</td>
<td>2.3 ± 0.5</td>
<td>2.2 ± 0.6</td>
</tr>
</tbody>
</table>

All data are shown as mean ± SD. BP = blood pressure; DHEAS = dehydroepiandrosterone sulfate; FAI = free androgen index (total testosterone (nmol/l)/SHBG (nmol/l)X100; FSH = follicle stimulating hormone; high density lipoprotein (HDL)-cholesterol HOMA-IR = homeostasis model assessment of insulin resistance; IGF-1 = insulin growth factor-1; LF electro-acupuncture = low-frequency electro-acupuncture; LH = luteinizing hormone; SHBG = sex hormone binding globuline; TSH = thyroid stimulating hormone.

^A P < 0.05: Before versus after 16 weeks of treatment within the LF electro-acupuncture group (Wilcoxon’s matched pairs signed rank sum test)
Figure 2
Figure 3