Low-frequency electroacupuncture and physical exercise decrease high muscle sympathetic nerve activity in polycystic ovary syndrome

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Stener-Victorin E, Jedel E, Janson PO, Sverrisdottir YB. Low-frequency electroacupuncture and physical exercise decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. Am J Physiol Regul Integr Comp Physiol 297: R387–R395, 2009. First published June 3, 2009; doi:10.1152/ajpregu.00197.2009.—We have recently shown that polycystic ovary syndrome (PCOS) is associated with high muscle sympathetic nerve activity (MSNA). Animal studies support the concept that low-frequency electroacupuncture (EA) and physical exercise, via stimulation of ergoreceptors and somatic afferents in the muscles, may modulate the activity of the sympathetic nervous system. The aim of the present study was to investigate the effect of these interventions on sympathetic nerve activity in women with PCOS. In a randomized controlled trial, 20 women with PCOS were randomly allocated to one of three groups: low-frequency EA ($n=9$), physical exercise ($n=5$), or untreated control ($n=6$) during 16 wk. Direct recordings of multiunit efferent postganglionic MSNA in a muscle fascicle of the peroneal nerve before and following 16 wk of treatment. Biometric, hemodynamic, endocrine, and metabolic parameters were measured. Low-frequency EA ($P=0.036$) and physical exercise ($P=0.030$) decreased MSNA burst frequency compared with the untreated control group. The low-frequency EA group reduced sagittal diameter ($P=0.001$), while the physical exercise group reduced body weight ($P=0.004$) and body mass index ($P=0.004$) compared with 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 body mass index and MSNA in the exercise group. There were no differences between the groups in hemodynamic, endocrine, and metabolic variables. 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.

Despite extensive research, the primary etiology remains unknown, although both environmental and genetic factors are implicated (17). Existing evidence suggests the ovarian theca cells, the major source of androgen production and secretion (22) to play a key role in the etiology. Also, the hypothalamic-pituitary axis (50) and defective insulin activity (12) may cause the syndrome. Furthermore, altered activity in the sympathetic nervous system has been suggested in the PCOS etiology (26).

Recently, for the first time, direct intraneural 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 may 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). Furthermore, we recently demonstrated that low-frequency electroacupuncture (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 decrease 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 improve 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.

**MATERIALS 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 and approved by the Ethics Committee, University of Gothenburg, Sweden. The study is registered at http://www.ClinicalTrials.gov, Identifier: NCT00484705.

The randomization was performed by the study coordinator according to a computerized list. PCOS women were stratified by age and body mass index (BMI) and thereafter block randomized to one of three study groups in a 2:2:1 ratio: 1) low-frequency EA, 2 Hz, 2) physical exercise, and 3) an untreated control group given the same information as all women of the importance to be physically active.

After screening (see Patients) and randomization, a 12-wk observation period followed where patients were asked to document their menstrual bleeding pattern. Thereafter, a common core of measurements was performed at 1) baseline and 2) after 16 wk of treatment. Anthropometric measurement included height, body weight, BMI (ratio between weight in kilograms and the square of the height in meters); 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 (ratio between the smallest circumference at the torso and the widest circumference at the hip). Clinical hyperandrogenism was measured by Ferriman-Gallwey score > 8 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 and 8:30 AM, immediately aliquoted on ice, and stored at −20°C until assayed. Blood samples were taken independently of the follicular phase of the menstrual cycle, since most of the women had oligo- or amenorrhea. MSNA was assessed by microneurography (see Microneurography). The menstrual bleeding patterns were confirmed by daily recordings of the basal body temperature throughout the entire study period and via interviews by gynecologists.

**Patients**

All patients were consecutively recruited between November 2005 and January 2008 advertisement in the local community, Sahlgrenska University Hospital, 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 > 35 days, and/or clinical and/or biochemical signs of hyperandrogenism (hirsutism or acne), according to the Rotterdam consensus report (68).

Women on medication(s) < 3 mo or breast feeding < 6 mo 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 (E. Jedel) who was 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), to strengthen and lengthen the effect on the central nervous systems (69, 70). Stainless steel, disposable one-time sterile needles (size 0.32 mm in diameter and the length of 30 or 50

<table>
<thead>
<tr>
<th>Points</th>
<th>Location</th>
<th>Stimulation</th>
<th>Innervation</th>
<th>Muscle Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV 3</td>
<td>Midline</td>
<td>EA, 2 Hz</td>
<td>L1</td>
<td>Fibrous tissue, linea alba</td>
</tr>
<tr>
<td>CV 6</td>
<td>Midline</td>
<td>EA, 2 Hz</td>
<td>Th11</td>
<td>Fibrous tissue, linea alba</td>
</tr>
<tr>
<td>ST 29</td>
<td>Bilateral</td>
<td>EA, 2 Hz</td>
<td>Th6–12</td>
<td>M. rectus abdominis</td>
</tr>
<tr>
<td>SP 6</td>
<td>Bilateral</td>
<td>EA, 2 Hz</td>
<td>L4–5, S1–2</td>
<td>Mm. flexor digitorum longus, tibialis posterior</td>
</tr>
<tr>
<td>SP 9</td>
<td>Bilateral</td>
<td>EA, 2 Hz</td>
<td>S1–2</td>
<td>M. gastrocnemius</td>
</tr>
<tr>
<td>LI 4*</td>
<td>Bilateral</td>
<td>Manual, 4 times</td>
<td>C8, Th1</td>
<td>Mm. interosseus dorsalis I, lumbocralis II, adductor pollicis</td>
</tr>
<tr>
<td>PC 6*</td>
<td>Bilateral</td>
<td>Manual, 4 times</td>
<td>C8, Th1</td>
<td>M. flexor digitorum superficialis</td>
</tr>
</tbody>
</table>

CV, conception vessel; LI, large intestinal; PC, pericardium; SP, spleen; ST, stomach; EA, electroacupuncture; M, muscle; Mm, muscles. *Points used alternating, every second time.
mm, Hegu Xeno; Hegu, Landsbro, Sweden) 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, Malmo, 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 degrees to evoke needle sensation every 10 min. Each treatment lasted 30 min, was given two per week during 2 wk, one per week during 6 wk, and once every second week for 8 wk, in a total of 14 treatments during 16 wk. 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 selected pace described as “faster than normal walking but a pace that could be sustained for at least 30 min” 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 wk. Once a week, women in the exercise group received a phone call for guidance on 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 RIA [Coat-A-Count Free Testosterone and Coat-A-Count DHEA-SO4, Diagnostic Products (DPC), Los Angeles, CA]. Serum total testosterone and insulin were measured by competitive immunoochemistry with chemoluminiscence technology (ADVIa Centaur TSTO Ready Pack primary reagents and ADVIa Centaur Insulin Ready Pack, Bayer Health Care, Tarrytown, NY). Serum sex hormone binding globulin, luteinizing hormone, and follicle-stimulating hormone reagent kit; Biokit, Barcelona, Spain for Abbott Laboratories Diagnostic Division, Chicago, IL and Architect luteinizing hormone reagent and follicle-stimulating hormone reagent pack, Abbott Laboratories Diagnostic Division). Free thyroxin 4 and IGF-1 were measured with immunochemoluminiscence microparticle immunoassay (Architect sex hormone-binding globulin reagent kit; Biokit, Barcelona, Spain for Abbott Laboratories Diagnostic Division). Free thyroxin 4 and IGF-1 were measured with immunochemoluminiscence technology (FT4 Free thyroxine, Roche Diagnostics, Mannheim, Germany and Immulite 2500 IG1, Euro/Diagnostic Products, UK). Thyroid-stimulating hormone (TSH) was measured with electrochemoluminiscence immunoassay, 37°C (TSH thyrotropin; Roche Diagnostics). Serum triglycerides (TG), cholesterol and HDL-cholesterol and plasma glucose were measured with an enzymatic photometric method, 37°C (TG, CHOL, and HDL-C second generation, Roche/Hitachi, Roche Diagnostics). LDL-cholesterol is a calculation according to Freivalds formula when serum-TG < 4.0; serum LDL-cholesterol = serum cholesterol – serum HDL-cholesterol – 0.45 × serum TG. Insulin sensitivity was estimated with a homeostasis model assessment for estimating insulin resistance (41), the index of which was calculated according to the formula: [fasting plasma glucose (mmol/l) × fasting plasma insulin concentration (µU/ml)]/22.5. All analyses were carried out at an accredited laboratory at the Department of Clinical Chemistry, Sahlgrensk University Hospital, Sahlgrenska, Sweden.

Microneurography

Direct recordings of multifunit effenter 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 centimeters 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/min) and burst incidence (bursts/100 heartbeats). The nerve recordings were assigned a code and analyzed blinded.

During the microneurographic recording, finger arterial blood pressure was measured noninvasively by the volume-clamp method (Finapress 2300; Ohmeda, Louisville, KY) (47), heart rate was monitored via 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 analyses were performed with SPSS software (version 17.0; SPSS, Chicago, IL). Descriptive results are presented as means ± SD. The significance of the difference between groups, calculated on baseline vs. after 16 wk of treatment, was assessed by Kruskal-Wallis. If significant, differences between the treatment groups and the untreated control group were tested with the Mann-Whitney U-test. Wilcoxon’s matched-pairs signed-rank sum test was used to compare baseline values with values obtained after 16 wk of treatment within each group. Correlation analyses were performed using Spearman’s rank correlation coefficient (Rs) in bivariate analyses. Significance was accepted as a two-sided value of P = 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 were 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 (four patients in the low-frequency EA with the following reasons: moved from area n = 3, personal reasons n = 1; four patients in the physical exercise group with these reasons: personal reasons n = 3, pregnancy n = 1; and two patients in the untreated control group with these reasons: moved from area n = 1, personal reasons n = 1). Of the remaining patients, 23 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 wk of treatment.

Their phenotype was as follows: 14 had oligomenorrhoea (O), 19 had signs of hyperandrogenism (HA), and all (n = 20) had polycystic ovaries (PCO) according to the Rotterdam

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consensus report (68). All three criteria (O, HA, and PCO) were fulfilled by 13 patients: seven patients in the EA group, three in the physical exercise group, and three in the control group. A combination of HA and PCO was found in six patients: 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, and this subject was found in the physical exercise group.

Baseline Characteristics and Treatment

The study groups did not deviate in terms of age (means ± SD: 29.9 ± 4.5, 30.4 ± 5.5, and 31.0 ± 3.2, respectively) or other measured variables at baseline (Tables 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 MSNA

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

While there were no differences between the groups in hemodynamic, endocrine, and metabolic variables (Table 3),

Table 2. Biometric characteristic of the study population before and after 16 wk of treatment

<table>
<thead>
<tr>
<th></th>
<th>LF Electroacupuncture, n = 9</th>
<th>Physical Exercise, n = 5</th>
<th>Untreated controls, n = 6</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>27.5±8.6</td>
<td>26.8±4.8</td>
<td>28.0±6.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Sagittal diameter, cm</td>
<td>20.1±4.7</td>
<td>19.3±4.6*</td>
<td>19.0±3.1</td>
<td>0.01</td>
</tr>
<tr>
<td>WHR</td>
<td>0.8±0.08</td>
<td>0.8±0.07</td>
<td>0.8±0.07</td>
<td>ns</td>
</tr>
<tr>
<td>Ferriman-Gallwey score</td>
<td>16.1±8.5</td>
<td>12.8±10.1</td>
<td>9.5±5.1</td>
<td>ns</td>
</tr>
</tbody>
</table>

All data are means ± SD. Group differences were assessed by Kruskal-Wallis (1) at baseline and 2) on the difference between baseline vs. after 16 wk of treatment. If significant, differences between the treatment groups and the untreated control group were tested with the Mann-Whitney U-test. LF, low-frequency; BMI, body mass index; WHR, waist-to-hip ratio; ns, not significant. P values were determined by the Kruskal Wallis test. There were no differences between the treatment groups at baseline in any variable. *P < 0.001 calculated on difference between baseline and after 16 wk treatment, LF EA vs. untreated controls (Mann-Whitney U-test). †P < 0.01 calculated on difference between baseline and after 16-wk treatment, physical exercise vs. untreated controls (Mann-Whitney U-test).

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We have previously shown that PCOS is a condition associated with increased sympathetic nerve activity (63). In support of our findings, women with PCOS have greater density of ovarian cathecholaminergic nerve fibers (27, 53), altered catecholamine metabolism (18), and disturbed HRV (81) and abdominal blood flow responses in women with PCOS (57, 58). Furthermore, nerve growth factor and adrenoreceptors, both markers of the sympathetic nervous system, are downregulated in visceral adipose tissue and ovaries of women with PCOS (9, 10). Additional experiments demonstrated that the effect is mediated via supraspinal mechanisms, thus indicating that low-frequency EA is shown to increase ovarian blood flow responses by modulating ovarian sympathetic nerve activity (57, 58). The novel finding in the present study is that low-frequency EA and physical exercise decrease MSNA compared with the untreated control groups. 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). The strong 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).

Low-Frequency EA and Physical Exercise Decrease Sympathetic Nerve Activity in Women with PCOS

The novel finding in the present study is that low-frequency EA and physical exercise decrease MSNA compared with 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 modulates segmental and central sympathetic nerve activity (56). Furthermore, nerve growth factor and adrenergic receptors, both markers of the sympathetic nervous system, are downregulated in visceral adipose tissue and ovaries after low-frequency acupuncture treatments in two different groups. 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 groups.

A positive correlation was observed between the sagittal diameter and MSNA ($r_s = 0.58, P < 0.01$) in the low-frequency EA group following 16 wk 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, three of six reported oligo-, amenorrhea, and there was no change in menstrual bleeding pattern during the study period. In the low-frequency EA group, seven of nine were oligo-, or amenorrheic prior to intervention and five reported 3–4 menstruations during the intervention period. However, due to difficulties in interpreting their basal body temperature curves some patients missed to do the progesterone tests. In the physical exercise group, four of five were oligo-, or amenorrheic 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 nonpharmacological treatment strategies that hypothetically might attenuate the high sympathetic activity associated with PCOS.

This study is the first 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.

PCOS 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 of our findings, women with PCOS have greater density of ovarian cathecholaminergic nerve fibers (27, 53), altered catecholamine metabolism (18), and disturbed HRV (81) and abnormal heart rate recovery (HRR) after exercise test, two indirect markers of autonomic function (20, 67, 71). Furthermore, 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 (1a, 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 (30); however, when adjusting for age and waist circumference, WHR and BMI are predictive for MSNA in men, but not women (66).

Low-Frequency EA and Physical Exercise Decrease Sympathetic Nerve Activity in Women with PCOS

The novel finding in the present study is that low-frequency EA and physical exercise decrease MSNA compared with 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 modulates segmental and central sympathetic nerve activity (56). Furthermore, nerve growth factor and adrenergic receptors, both markers of the sympathetic nervous system, are downregulated in visceral adipose tissue and ovaries after low-frequency acupuncture treatments in two different groups. 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 groups.

A positive correlation was observed between the sagittal diameter and MSNA ($r_s = 0.58, P < 0.01$) in the low-frequency EA group following 16 wk of treatment, while MSNA was not related to changes in body weight or BMI in the physical exercise group.
ent 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). Furthermore, 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 with 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). Although 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 to 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, whereas 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 ($R_s = 0.65, P < 0.05$ and $R_s = 0.34, P < 0.05$ respectively) indicating that the 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 waist-to-hip ratio or BMI, the results in the present study show that reduction in MSNA was related to a 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 a more

### Table 3. Hemodynamic, hormones, and metabolic data at baseline and after 16 wk of treatment

<table>
<thead>
<tr>
<th></th>
<th>LF Electroacupuncture, $n = 9$</th>
<th>Physical Exercise, $n = 5$</th>
<th>Untreated Controls, $n = 6$</th>
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<tr>
<td></td>
<td>Baseline After 16 wk</td>
<td>Baseline After 16 wk</td>
<td>Baseline After 16 wk</td>
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<tr>
<td>Hemodynamic</td>
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<td></td>
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<tr>
<td>Systolic BP, mmHg</td>
<td>110.7±9.2</td>
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<tr>
<td>Diastolic BP, mmHg</td>
<td>67.2±4.8</td>
<td>67.1±5.8</td>
<td>64.6±8.5</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>
</tr>
</tbody>
</table>

### Hemodynamic

|                      |                               |                           |                             |
| LH, IU/l             | 13.8±24.0                     | 61.3±7.1                  | 68.3±3.3                    |
| FSH, IU/l            | 5.1±2.3                       | 4.6±1.5                   | 4.6±1.8                     |
| LH/FSH ratio         | 2.1±2.2                       | 1.3±0.6^A                 | 2.1±1.2                     |
| Total testosterone, nmol/l | 1.9±0.5                  | 1.9±0.3                   | 2.0±0.9                     |
| Free testosterone, pmol/l | 4.6±2.2                  | 4.0±2.3                   | 5.3±3.4                     |
| SHBG, nmol/l         | 38.8±27.3                     | 44.3±25.8                 | 32.6±9.7                    |
| FAI                  | 7.2±4.6                       | 5.8±3.4                   | 7.1±4.3                     |
| DHEA-S, μmol/l       | 4.6±1.6                       | 5.0±1.0                   | 5.4±2.4                     |
| Free T4, pmol/l      | 15.3±1.2                      | 15.2±1.3                  | 14.8±3.6                    |
| TSH mlU/l            | 3.3±1.1^A                     | 3.1±1.2                   | 2.7±2.0                     |
| IGF-1, μg/l          | 168.9±44.8                    | 159.4±42.7                | 176.6±36.4                  |

### Insulin sensitivity

|                      |                               |                           |                             |
| Glucose, mmol/l      | 4.6±0.4                       | 4.5±0.3                   | 4.6±0.2                     |
| Insulin, μU/l        | 9.2±8.1                       | 9.1±10.1                  | 8.0±4.5                     |
| HOMA-IR              | 2.0±2.0                       | 1.9±2.3                   | 1.6±1.0                     |

### Blood lipids

|                      |                               |                           |                             |
| Cholesterol, mmol/l  | 3.9±0.7                       | 3.9±0.8                   | 4.0±0.6                     |
| Triglycerides, mmol/l| 0.8±0.2                       | 0.9±0.4                   | 0.7±0.3                     |
| HDL-cholesterol, mmol/l | 1.4±0.3                  | 1.5±0.3^C                 | 1.4±0.3                     |
| LDL-cholesterol, mmol/l | 2.1±0.7                  | 2.1±0.5                   | 2.3±0.5                     |

All data are means ± SD. Group differences were assessed by Kruskal-Wallis at baseline and 2 on the difference between baseline vs. after 16 wk of treatment. BP, blood pressure; DHEAS, dehydroepiandrosterone sulfat; FAI, free androgen index (total testosterone (mmol/l)/SHBG (nmol/l)×100; FSH, follicle stimulating hormone; HDL-cholesterol HOMA-IR, homeostasis model assessment of insulin resistance; IGF-1, insulin growth factor-1; LH, luteinizing hormone; SHBG, sex hormone binding globulin; TSH, thyroid stimulating hormone. $P$ values were determined by the Kruskal Wallis test. $P < 0.05$ before vs. after 16 wk of treatment within the LF electroacupuncture group (Wilcoxon’s matched-pairs signed-rank sum test).
regular menstrual bleeding pattern, whereas 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 downregulated 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 (1). Our hypothesis is that needle insertion and manual and/or electrical stimulation of the needles activates afferent nerve fibers. As stated in MATERIALS AND METHODS, 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 hypothesized that we would obtain the same effect if nonacupuncture 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 with the physical exercise and untreated control groups. 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 whether 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 sympathetics, e.g., needle insertion with and without electrical stimulation, might 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.

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. Lifestyle 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 nonpharmacologic 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.

GRANTS

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ACUPUNCTURE AND EXERCISE DECREASE SYMPATHETIC TONE IN PCOS


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