Acupuncture for ovulation induction in polycystic ovary syndrome: a randomized controlled trial

Julia Johansson,1 Leanne Redman,2 Paula P. Veldhuis,3 Antonina Sazonova,4 Fernand Labrie,5,6 Göran Holm,7 Gudmundur Johannsson,8 and Elisabet Stener-Victorin1,9

1Institute of Neuroscience and Physiology, Department of Physiology, Sahlgrenska Academy, University of Göteborg, Gothenburg, Sweden; 2Pennington Biomedical Research Center, Los Angeles, California; 3Department of Pharmacology and Center for Biomathematical Technology, University of Virginia, Charlottesville, Virginia; 4Institute of Clinical Science, Department of Obstetrics and Gynaecology, Sahlgrenska Academy, University of Göteborg, Gothenburg, Sweden; 5Laval University Research Center in Molecular Endocrinology, Oncology and Human Genomics, CHUL Research Center, Quebec, Canada; 6College of Medicine, Al Imam Mohammed Ibn Saud Islamic University, Riyadh, Saudi Arabia; 7Institute of Medicine, Department of Metabolism and Cardiovascular Research, Sahlgrenska Academy, University of Göteborg, Gothenburg, Sweden; 8Institute of Medicine, Department of Endocrinology, Sahlgrenska Academy, University of Göteborg, Gothenburg, Sweden; and 9Department of Obstetrics and Gynecology, First Affiliated Hospital, Heilongjiang University of Chinese Medicine, Harbin, China

Submitted 22 January 2013; accepted in final form 4 March 2013

Johansson J, Redman L, Veldhuis PP, Sazonova A, Labrie F, Holm G, Johannsson G, Stener-Victorin E. Acupuncture for ovulation induction in polycystic ovary syndrome: a randomized controlled trial. Am J Physiol Endocrinol Metab 304: E934–E943, 2013. First published March 12, 2013; doi:10.1152/ajpendo.00039.2013.—Acupuncture has been demonstrated to improve menstrual frequency and to decrease circulating testosterone in women with polycystic ovary syndrome (PCOS). Our aim was to investigate whether acupuncture affects ovulation frequency and to understand the underlying mechanisms of any such effect by analyzing LH and sex steroid secretion in women with PCOS. This prospective, randomized, controlled clinical trial was conducted between June 2009 and September 2010. Thirty-two women with PCOS were randomized to receive either acupuncture with manual and low-frequency electrical stimulation or to meetings with a physical therapist twice a week for 10–13 wk. Main outcome measures were changes in LH secretion patterns from baseline to after 10–13 wk of treatment and ovulation frequency during the treatment period. Secondary outcomes were changes in the secretion of sex steroids, anti-Müllerian hormone, inhibin B, and serum cortisol. Ovulation frequency during treatment was higher in the acupuncture group than in the control group. After 10–13 wk of intervention, circulating levels of estrone, estrone sulfate, estradiol, dehydroepiandrosterone, dehydroepiandrosterone sulfate, androstenedione, testosterone, free testosterone, dihydrotestosterone, androstosterone glucuronide, androstane-3α,17β-diol-3-glucuronide, and androstane-3α,17β-diol-17-glucuronide were significantly lower in the acupuncture group compared to baseline and in the control group for all of these except androstenedione. We conclude that repeated acupuncture treatments resulted in higher ovulation frequency in lean/overweight women with PCOS and were more effective than just meeting with the therapist. Ovarian and adrenal sex steroid serum levels were reduced with no effect on LH secretion.

the main characteristics of polycystic ovary syndrome (PCOS) are polycystic ovaries (34), oligo/anovulation, and elevated serum levels of sex steroid precursors, estrogens, androgens, and glucuronidated androgen metabolites (38). PCOS is related to hyperinsulinemia and insulin resistance and is exacerbated by obesity (7). Numerous studies have reported hypersecretion of luteinizing hormone (LH) in women with PCOS (3). Together with an exaggerated ovarian response, hypersecretion of LH drives excessive ovarian androgen production and causes anovulation (13). In PCOS, altered sex steroid production, metabolic dysfunction, and obesity all contribute to changes in LH secretion patterns and to anovulation (6, 12, 29, 30).

Clomiphene citrate, exogenous gonadotropin therapy, and laparoscopic ovarian drilling are commonly used to induce ovulation in women with PCOS (1). These treatments often have negative side effects, thus indicating the importance of evaluating alternative treatments such as acupuncture. Acupuncture is used worldwide to achieve fertility, but its efficacy is supported by only limited scientific evidence. In a randomized controlled trial (RCT), we previously demonstrated that acupuncture with manual and low-frequency electrical needle stimulation was superior to both exercise and no treatment for improving menstrual frequency and total testosterone levels (15). The effect of acupuncture was, at least in part, mediated by reduction in sympathetic nerve activity (39). The main limitations of that study were the inability to confirm ovulation and to control for the increased attention associated with the therapeutic meeting and needle insertion. In another RCT, true acupuncture was compared with sham acupuncture using “placebo needles” (31). The ovulation frequency did not differ between the groups, and both groups showed improved LH-to-follicle-stimulating hormone (FSH) ratios. The lack of a difference between true and sham acupuncture in that study is in line with previous studies on different pain conditions and nausea caused by chemotherapy that demonstrated that true acupuncture is not more effective than sham acupuncture. However, all of these trials found a significant effect when groups were compared with a nonintervention group (8, 22, 28). These results indicate that sham acupuncture is not an inert method and highlight the methodological difficulties in the design of acupuncture trials.
In the present study, we tested the hypothesis that frequent acupuncture treatment could improve ovulation frequency, LH secretion and pulsatility, and sex steroid secretion in lean/overweight PCOS patients. To compensate for the attention that women treated by acupuncture would receive, a control group was randomized to meet with a therapist, because sham acupuncture has been shown not to be an inert control. The primary outcome measures were changes in LH pulsatility from baseline to the end of treatment and differences in ovulation frequency during the study.

MATERIALS AND METHODS

Study Population
PCOS patients were recruited by advertisements in local newspapers between June 2009 and September 2010. PCOS was diagnosed according to the Rotterdam criteria: ultrasound-verified polycystic ovaries (≥12 follicles of 2–9 mm and/or ovarian volume ≥10 ml in one or both ovaries), oligo/amenorrhea, or clinical signs of hyperandrogenism (hirsutism or acne) (34). A self-reported Ferriman-Gallwey (FG) score of ≥8 defined the presence of hirsutism, and the presence of acne was defined by an affirmative answer to being asked if they had excessive acne. An intermenstrual interval of >35 days or <6 menstrual bleedings in the previous year was used as the definition for oligomenorrhea, and amenorrhea was defined as a total absence of menstrual bleeding in the previous 90 days. Patients were excluded if they were younger than 18 or older than 38 yr of age, had a body mass index (BMI) over 30, had taken any pharmacological treatments in the previous 3 mo, or had breastfed or received acupuncture during the previous 90 days. Patients were excluded if they were confirmed by weekly progesterone measurements throughout the study. Participants were randomized before baseline assessments to either receiving acupuncture or to receiving similar attention by meeting with a therapist. Randomization was computer generated (http://www.randomization.com), and investigators were blinded until statistical analyses. After randomization, each participant underwent a 10- to 13-wk intervention period after which the baseline measurements were repeated. Baseline assessments were made at the Endocrine and Metabolic Research Centre at Sahlgrenska during an overnight stay.

Power Calculation and Sample Size
Power calculation and sample size were determined on the basis of changes in the 12-h LH pulse frequency in women with PCOS from baseline to end of treatment. We expected a 10% change in the number of pulses with a mean change (Δ) of −0.8 and standard deviation (SD) of 1.81 within the groups (32). A total of 14 patients per group was required for a statistical power of 80% at a 5% significance level. We enrolled 16 subjects per group to account for dropouts.

Interventions
Acupuncture. Women in the acupuncture group were treated for 30 min twice weekly for 10–13 wk by two therapists educated in Western medical acupuncture. Subjects rested and listened to relaxing music during treatment. The acupuncture protocol was based on a previous study of acupuncture for ovulation induction in PCOS (15), an experimental study (37), and clinical experience. Sterile stainless steel needles (Hegu Xeno, Hegu Svenska; length 30 or 50 mm, diameter 0.30 mm) were inserted to a depth of 15–35 mm at acupuncture points located in abdominal and leg muscles that have innervations corresponding to the ovaries and in acupuncture points that do not innervate the ovaries. Two sets of 11 and 13 acupuncture points were alternated every other treatment due to the intensity of the treatment (Table 1). All needles were rotated manually to evoke needle sensation (de qi) when inserted. Needles in the leg and abdominal muscles were then connected to an electrical stimulator (CEFAR ACUS 4; Cefar-Compex Scandinavia, Landsbro, Sweden) and stimulated with low-frequency (2 Hz) bursts. The intensity was adjusted to produce local muscle contractions without pain or discomfort.

Table 1. Two sets of acupuncture points and their anatomic positions, needle stimulations, and muscle innervations

<table>
<thead>
<tr>
<th>Points</th>
<th>Location</th>
<th>Stimulation</th>
<th>Innervation</th>
<th>Muscle Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV3</td>
<td>Midline</td>
<td>EA, 2Hz</td>
<td>L1</td>
<td>Fibrous tissue, linea alba</td>
</tr>
<tr>
<td>CV6</td>
<td></td>
<td></td>
<td>Th11</td>
<td></td>
</tr>
<tr>
<td>ST29</td>
<td>Bilateral</td>
<td>EA, 2Hz</td>
<td>Th6-12</td>
<td>M. rectus abdominis</td>
</tr>
<tr>
<td>SP6</td>
<td>Bilateral</td>
<td>EA, 2Hz</td>
<td>L4-5, S1-2</td>
<td>Mn. flexor digitorum longus, tibialis posterior</td>
</tr>
<tr>
<td>SP9</td>
<td>S1-2</td>
<td></td>
<td></td>
<td>M. gastrocnemius</td>
</tr>
<tr>
<td>LI4</td>
<td>Bilateral</td>
<td>Manual</td>
<td>C8, Th1</td>
<td>Mn. interosseus dorsalis I, lumbricalis II, adductor pollicis</td>
</tr>
<tr>
<td>GV20</td>
<td>Midline</td>
<td>Manual</td>
<td>C2-3</td>
<td>Aponeurosis epicanii</td>
</tr>
<tr>
<td>Set 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV3</td>
<td>Midline</td>
<td>Manual</td>
<td>L1</td>
<td>Fibrous tissue, linea alba</td>
</tr>
<tr>
<td>CV6</td>
<td></td>
<td></td>
<td>Th11</td>
<td></td>
</tr>
<tr>
<td>ST25</td>
<td>Bilateral</td>
<td>EA, 2Hz</td>
<td>Th6-12</td>
<td>M. rectus abdominis</td>
</tr>
<tr>
<td>ST29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP6</td>
<td>Bilateral</td>
<td>EA, 2Hz</td>
<td>L4-5, S1-2</td>
<td>Mn. flexor digitorum longus, tibialis posterior</td>
</tr>
<tr>
<td>LR3</td>
<td>S2-3</td>
<td></td>
<td></td>
<td>M. interosseus dorsalis I</td>
</tr>
<tr>
<td>PC6</td>
<td>Bilateral</td>
<td>Manual</td>
<td>C8, Th1</td>
<td>M. flexor digitorum superficialis</td>
</tr>
<tr>
<td>GV20</td>
<td>Midline</td>
<td>Manual</td>
<td>C2-3</td>
<td>Aponeurosis epicanii</td>
</tr>
</tbody>
</table>

Set 1 and Set 2 were alternated in every other treatment due to the intensity of the treatment and were the same for all women in the acupuncture group CV, conception vessel; EA, electro-acupuncture; GV, governor vessel; LI, large intestine; LR, liver; M., muscle; Mn., muscles; PC, pericardium; SP, spleen; ST, stomach.
fort. Needles not connected to the electrical stimulator were stimu-
lated by manual rotations every 10 min to evoke de qi.

Attention control. Women in the control group visited the same
physical therapists. The therapeutic meeting was held for an equal
amount of time (twice weekly for 10–13 wk) to control for the
attention involved in meeting with the therapists. Time was spent
resting and listening to relaxing music, with the therapist entering the
room every 10 min ensuring comfort and well-being in a manner
similar to the acupuncture group.

Outcome Measurements

All measurements were taken at baseline and were repeated within
1 wk after the last treatment (referred to as end of treatment in
RESULTS). Measurements were taken at menstrual cycle days 8–10 if
ovulation had occurred. If no ovulation or bleeding had occurred
during the period between screening for the study and the taking of the
baseline measurements, the measurements were taken on an arbitrary
day of the cycle.

Outcome measurements. Anthropometrics included measurements
of height, weight, waist and hip circumferences, sagittal diameter, and
calculations of waist-to-hip ratio (WHR) and BMI (kg/m²). A foot-
to-foot bioelectrical impedance system (Tanita, Middlesex, UK) was
used to measure the percent body fat. Hirsutism was assessed by the
FG score. Ovarian volume, endometrial thickness, and antral follicle
(<9 mm) count were measured by ultrasound. Bleeding and proges-
terone measurements determined the number of ovulations per month
that occurred during the study. Each 12-h sampling period started at
1930 with a sample taken to measure the level of sex hormone-
binding globulin (SHBG). Samples were then drawn every 10 min for
measurement of LH and cortisol, every hour for measurement of FSH,
and every 4 h for measurement of sex steroids. The LH:FSH ratio
calculated and expressed as a mean of the 12-h sampling
period. The final blood sample at 0730 allowed measurements of
fasting glucose, insulin, anti-Müllerian hormone (AMH), inhibin B,
and cholesterol [total, high-density lipoprotein (HDL), and low-
density lipoprotein (LDL)], and calculation of the homeostatic model
assessment (HOMA) index.

Immunoassay

LH, FSH, AMH, inhibin B, glucose, cholesterol (total, HDL, and
LDL), insulin, progesterone, and SHBG were analyzed at an accred-
ited laboratory at the Department of Clinical Chemistry, Sahlgrenska
University Hospital. Plasma glucose [detection limit (DL) = 0.11
mmol/l], cholesterol (DL = 0.10 mmol/l), HDL-cholesterol (DL = 0.08
mmol/l), and LDL-cholesterol (DL = 0.10 mmol/l) were mea-
sured at 37°C with enzymatic photometric methods. Insulin (DL = 0.2
mU/l) was measured with immunometric methods (two-step
sandwich) and chemiluminescence technology, and cortisol (DL = 0.5
mmol/l) was measured with competitive methods and chemilumi-
nescence technology. All of these were measured with kits from
Roche Diagnostics (Mannheim, Germany) on a Cobas instrument.
SHBG (DL = 0.33 nmol/l) was measured using the Access Immu-
noassay System (Beckman-Coulter, Brea, CA), and FSH (DL = 0.05
IU/l), LH (DL = 0.07 IU/l), and progesterone (DL = 0.5 nmol/l) were
measured by chemiluminescence on an Architect instrument (Abbott
Laboratories, Wiesbaden, Germany) with kits from the same com-
pany. AMH (DL = 0.2 μg/ml) was measured in an enzymatic
amplified double-epitope immunoassay, and inhibin B (DL = 4
ng/ml) was measured with a sandwich immunoassay, both from
Beckman-Coulter and measured on a multiscan flow cytometry appa-
ratus (Thermo Scientific Oy, Vantaa, Finland).

Mass Spectrometry

Circulating concentrations of sex steroids, androgen precursors,
and glucuronidated androgen metabolites were analyzed by mass
spectrometry. Analyses were performed by the Endoceutics Bioanaly-
tical Laboratory (Quebec, Canada) essentially as described (18, 19).
Free testosterone (free T) was calculated with the spreadsheet de-
veloped by Mazer et al. (27) where cortisol and cortisol-binding globulin
were set to zero. The area under the curve (AUC) for all sex steroid
concentrations, measured every 4th hour from 0 to 12 h, was calcu-
lated using the linear trapezoidal method.

Gas Chromatography with Detection by Tandem Mass
Spectrometry

Total deuterated estradiol (E2), total testosterone (T), androstened-
dione (4-DIONE), dehydroepiandrosterone (DHEA), total estrone
(E1), and total dihydrotestosterone (DHT) were added as internal
standards (CDN Isotopes, Essex, UK) to 0.5-ml serum samples to
enable quantification of their respective free steroids. The standards
and serum samples were extracted twice by liquid-liquid and solid-
phase extraction on a 500 mg/ml silica column (Phenomenex,
Torrance, CA) and underwent two derivatization procedures to improve
selectivity and specificity. Samples were then separated by GC-
MS/MS using negative ion chemical ionization.

Ultracean Performance Liquid Chromatography Tandem Mass
Spectrometry

Deuterated androstan-3α,17β-diol-3-glucuronide (AD3G), an-
drostane-3α,17β-diol-17-glucuronide (AD17G), and androstenedione (DHTG) were added as internal standards to 400-μl
serum samples to enable quantification of their respective glucu-
ronidated steroids. The standards and serum samples were ex-
tracted on a 500 mg/ml silica column and reconstituted in a
methanol-H2O mixture. Deuterated DHEA sulfate (DHEA-S) and
E1 sulfate (E1-S) were added as internal standards to 75-μl serum
samples to enable quantification of their respective sulfated ste-
roids and were extracted as above. Detection and quantification
were performed by UPLC-MS/MS (API 5000; AB Sciex, Framing-
ham, MA) in negative mode. The limits of quantitation for each
analyte were as follows: DHEA, 500 pg/ml; 4-DIONE, 100 pg/ml;
T, 50 pg/ml; DHT, 10 pg/ml; E1, 4.0 pg/ml; E2, 1 pg/ml; AD3G, 100
pg/ml; AD17G, 100 pg/ml; ADTG, 4 ng/ml; DHEA-S, 100
ng/ml; and E1-S, 50 pg/ml.

Data Analysis

Hormone pulsatility. The concentrations of LH and cortisol in
blood samples taken every 10 min for 12 h were analyzed with the
Autodeon pulse detection algorithm (17) in the Pulse XP software
package to detect and quantify secretory events. Three truncated LH
data series, due to missing blood samples, were recalculated to 12 h
for the time-dependent variables before statistical analysis. This re-
calculation was applied to one baseline series in the acupuncture
group and to one end-of-treatment series in both the acupuncture
and control groups. Two truncated cortisol data series in the acupuncture
group, one baseline, and one end of treatment, were recalculated to
12 h for the time-dependent variables.

Quantification of irregularity. To quantify the regularity or ran-
domness of the LH data series, we analyzed the time courses with
the Approximate Entropy (ApEn) application in the Pulse XP
software package. ApEn is a statistical method that, in contrast to
conventional pulse-detection procedures, evaluates both dominant
and subdominant patterns in time series that are not reflected in
peak occurrences or amplitudes. The parameters m (window
length) and r (tolerance parameter) must be specified to measure the
logarithmic likelihood that m contiguous observations that are
similar (within distance r) remain close to the next incremental
comparison (for m = 1, when two points have approximately equal
values, within r, the next point is also approximately equal). Larger
values represent greater randomness or irregularity, and smaller

AJP-Endocrinol Metab • doi:10.1152/ajpendo.00039.2013 • www.ajpendo.org
numbers represent more recognizable patterns (44). Here, we used a window length of $m = 1$ and a tolerance parameter of $r = 20\%$ of the individual subject's average standard deviation in the hormone time series (ApEn: 1, 20%). One thousand Monte Carlo simulations were used to calculate the standard deviation of approximate entropy in each series.

Statistical Analyses

Between-group differences for baseline values and the change between baseline and end of treatment ($\Delta$ values) were analyzed with the Mann-Whitney U-test. The $\chi^2$ test was used to assess differences in acne and menstrual cycle pattern (categorical variables) between groups at baseline.

Data were analyzed according to the intention-to-treat (ITT) principle. In cases where the end-of-treatment assessments were missing due to dropouts after baseline assessments, the baseline observations were used as the end-of-treatment observations according to the baseline observation carried forward approach. Dropouts occurring before the measurements of baseline values were excluded from the ITT analysis due to lack of data. Variables displaying between-group differences at baseline were further analyzed by Spearman’s rank correlation to identify possible correlations between baseline and $\Delta$ values. If correlations were found, a logistic regression including both $\Delta$ values and baseline data was performed to correct for the difference at baseline and to determine whether between-group changes were due to a true treatment effect or to the difference at baseline. Within-group differences between baseline and end of treatment were analyzed with the Wilcoxon rank sum test. Bonferroni corrections were made for $\Delta$ changes between groups for measurements of sex steroids, androgen precursors, and glucuronidated androgen metabolites. Associations between ovulation frequency and all end-of-treatment variables for the entire study population (not groupwise) were estimated by Spearman’s rank correlation. Data were analyzed with the Prediction Application Software package (v. 19.0 for Windows; SPSS, Chicago, IL).

RESULTS

Participant flow through the study is summarized in Fig. 1. Thirty-two women met the inclusion criteria and were randomized to the acupuncture group ($n = 16$) or attention control group ($n = 16$). There were four dropouts in the attention control group before baseline assessments and three dropouts, one in the attention control group and two in the acupuncture group, between baseline and end-of-treatment assessments. The ITT population consisted of 28 women who had baseline measurements taken and were included in the analyses: 12 in the attention control group and 16 in the acupuncture group. In the attention control group, six subjects were identified as oligoamenorrheic and six as amenorrheic, whereas in the acupuncture group six subjects were identified as oligoamenorrheic and 10 as amenorrheic. Before start of study, 10 of 12 in the attention control group and 10 of 16 in the acupuncture group had an ovulation where baseline measurements were then performed at days 8–10. Number of treatments in the acupuncture group was 19.1 ± 4.4 (mean ± SD) and 20.2 ± 3.0 in the attention control group.

Between-Group Comparisons at Baseline

Phenotypic characterization of all participants is presented in Table 2. All participants were oligo/amenorrheic before the start of treatment, and the menstrual cycle pattern did not differ between the acupuncture and the attention control groups (Table 2). Serum LH levels ($P = 0.026$), LH:FSH ratios ($P = 0.004$), LH secretion area ($P = 0.037$), ovarian volumes ($P = 0.041$), and 4-DIONE levels ($P < 0.001$) differed between the two groups at baseline (Tables 2, 3, and 4).

Effect of the Intervention

During the study period, ovulation frequency was higher in the acupuncture group than in the attention control group ($0.76 ± 0.27$ vs. $0.41 ± 0.28$ ovulations per month, $P = 0.002$; Fig. 2). There were no changes in LH or cortisol pulsatility after treatment, except for an increase in LH Log10 ApEn within the acupuncture group (Tables 3 and 5). However, there were no between-group differences in the $\Delta$ changes in any of the LH and cortisol variables between baseline and end of treatment assessments.

Significant changes in plasma glucose levels and HOMA from baseline to end of treatment occurred between groups, but no significant differences were seen within the groups (Table 2). There were no anthropometric differences between the groups, but weight, BMI, waist circumference, and WHR decreased in the attention control group, and waist circumference decreased in the acupuncture group (Table 2).

Means and AUCs of calculated free T and all measured serum sex steroids, their precursors, and their metabolites decreased from baseline to end of treatment in the acupuncture group and differed significantly between the groups. The only exceptions were mean SHGB, the mean and AUC.
In the present study, we demonstrate that repeated acupuncture treatments with manual and electrical stimulation in lean/overweight women with PCOS results in a higher ovulation frequency during the treatment period than in the control group. This effect can be separated from the attention control, which involves meeting with a therapist for the same number of visits with manual and electrical stimulation. Within the attention control group, differences remained for the means of E1-S, E2, DHEA, 4-DIONE, free T, and ADT-G, and for the AUCs of E1-S, E2, DHEA, and ADT-G (Table 6). The effect on ovulation frequency during the treatment period than in the control group. This effect can be separated from the attention control, which involves meeting with a therapist for the same number of visits with manual and electrical stimulation. Within the attention control group, differences remained for the means of E1-S, E2, DHEA, 4-DIONE, free T, and ADT-G, and for the AUCs of E1-S, E2, DHEA, and ADT-G (Table 6). The effect on ovulation frequency during the treatment period than in the control group.

**DISCUSSION**

In the present study, we demonstrate that repeated acupuncture treatments with manual and electrical stimulation in lean/overweight women with PCOS results in a higher ovulation frequency during the treatment period than in the control group. This effect can be separated from the attention control, which involves meeting with a therapist for the same number of visits with manual and electrical stimulation. Within the attention control group, differences remained for the means of E1-S, E2, DHEA, 4-DIONE, free T, and ADT-G, and for the AUCs of E1-S, E2, DHEA, and ADT-G (Table 6). The effect on ovulation frequency during the treatment period than in the control group.

**Correlations Between Ovulation Frequency During the Study and All End-Of-Treatment Variables**

Spearman's rank correlation analyses were carried out for all variables (not groupwise), with ovulation frequency as the dependent variable and end-of-treatment measurements as the independent variables. E1, E1-S, E2, free T, and AD3G were all negatively correlated with ovulation frequency (Table 6).
ACUPUNCTURE AND NEUROENDOCRINE FUNCTION

Table 3. Baseline LH, FSH, and LH pulsatility and changes from baseline after 10–13 wk of treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Attention Control</th>
<th>Acupuncture</th>
<th>*P</th>
<th>Attention Control</th>
<th>Acupuncture</th>
<th>*P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH, IU/l</td>
<td>(n = 12) Baseline</td>
<td>(n = 16)</td>
<td></td>
<td>(n = 12) Baseline</td>
<td>(n = 16)</td>
<td></td>
</tr>
<tr>
<td>3.19 ± 1.73</td>
<td>6.03 ± 3.59</td>
<td>0.026</td>
<td></td>
<td>1.11 ± 2.32</td>
<td>−0.35 ± 3.04</td>
<td>NS</td>
</tr>
<tr>
<td>FSH, IU/l</td>
<td>4.20 ± 1.70</td>
<td>3.47 ± 1.88</td>
<td>NS</td>
<td>−0.19 ± 1.42</td>
<td>0.61 ± 1.28</td>
<td>NS</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>0.94 ± 0.89</td>
<td>1.36 ± 0.60</td>
<td>0.004</td>
<td>0.28 ± 0.70</td>
<td>−0.24 ± 0.55</td>
<td>NS</td>
</tr>
<tr>
<td>LH pulsatility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-duration, min</td>
<td>7.77 ± 4.12</td>
<td>6.69 ± 3.12</td>
<td>NS</td>
<td>−2.19 ± 4.9</td>
<td>−0.92 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td>Half-life, min</td>
<td>69.60 ± 21.98</td>
<td>64.42 ± 16.29</td>
<td>NS</td>
<td>3.08 ± 29.5</td>
<td>−1.51 ± 16.9</td>
<td>NS</td>
</tr>
<tr>
<td>Secretion events, frequency/12 h</td>
<td>8.92 ± 3.73</td>
<td>8.93 ± 3.83</td>
<td>NS</td>
<td>0.54 ± 4.5</td>
<td>0.41 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Total pulsatile production, IU/l/12 h</td>
<td>21.01 ± 19.98</td>
<td>30.30 ± 16.45</td>
<td>NS</td>
<td>0.30 ± 22.6</td>
<td>−0.09 ± 14.1</td>
<td>NS</td>
</tr>
<tr>
<td>Interpulse interval, min</td>
<td>72.93 ± 21.63</td>
<td>108.39 ± 114.12</td>
<td>NS</td>
<td>4.63 ± 43.8</td>
<td>−32.3 ± 112.9</td>
<td>NS</td>
</tr>
<tr>
<td>Lg ApEn</td>
<td>0.91 ± 0.35</td>
<td>0.87 ± 0.40</td>
<td>NS</td>
<td>0.09 ± 0.4</td>
<td>0.13 ± 0.2*</td>
<td>NS</td>
</tr>
<tr>
<td>Secretion area, IU/l</td>
<td>1.78 ± 1.50</td>
<td>2.80 ± 1.38</td>
<td>0.037</td>
<td>0.30 ± 1.57</td>
<td>−0.27 ± 1.75</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. LH, luteinizing hormone; FSH, follicle-stimulating hormone; Lg ApEn, log10 approximate entropy; SHBG, sex hormone-binding globulin; T, testosterone; 4-DIONE, androstenedione. †Between-group differences were determined with the Mann-Whitney U-test. *P < 0.05 vs. baseline for within-group differences determined by the Wilcoxon rank-sum test. aAfter logistic regression with adjustment for baseline differences, NS.

is the increased treatment frequency and the attention control that does not include sham acupuncture.

The higher ovulation frequency during the treatment period in the acupuncture group confirms our previous data that showed increased menstrual frequency after 14 acupuncture treatments (15). Although we have no data on ovulation frequencies before the start of the study, there was no difference between the groups in terms of menstrual cycle pattern prior to the start of the study. This clearly indicates that the higher ovulation frequency in the acupuncture group can be attributed to a treatment effect. Importantly, ovulation frequency in the control group was also high (41%) and was comparable to clomiphene citrate stimulation and to the frequency seen in the study by Pastore et al. that used sham needles and fewer treatments (21, 31). This observation suggests a specific treatment effect by acupuncture that is separate from the less pronounced effect of meeting with a therapist. Even though ovulation frequency was here established during a treatment period of 10–13 wk, it is possible that the long-term effect is similar to our previous study, where menstrual frequency remained increased 16 wk after treatment ended (15). We previously reported that increased menstrual frequency is associated with a decrease in total T. In this study, we explored whether central gonadotropin release or changes in ovarian responsiveness are involved in the underlying mechanism behind the observed increase in menstrual frequency/ovulation with acupuncture therapy. Previous studies have indicated a role for LH in ovarian hyperandrogenism, and downregulation of LH secretion correlates with increased ovulation frequency and reduced levels of total T and 4-DIONE (4, 36). In the recent RCT by Pastore et al., they found that circulating LH levels and the LH:FSH ratio decreased, but these decreases were the same in both the true and sham acupuncture groups (31). Repeated acupuncture treatments with combined manual and electrical stimulation in our present study, however, did not affect LH levels or pulsatility pattern. However, it is possible that the

Table 4. Baseline sex steroids, precursors, and glucuronidated metabolites and changes from baseline after 10–13 wk of treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Attention Control</th>
<th>Acupuncture</th>
<th>*P</th>
<th>Attention Control</th>
<th>Acupuncture</th>
<th>*P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHBG, nmol/l^[*]</td>
<td>49.67 ± 25.5</td>
<td>40.40 ± 13.7</td>
<td>NS</td>
<td>−6.0 ± 17.4</td>
<td>4.3 ± 16.4</td>
<td>NS</td>
</tr>
<tr>
<td>E₁, pg/ml</td>
<td>59.90 ± 34.1</td>
<td>57.41 ± 19.9</td>
<td>NS</td>
<td>2.87 ± 39.5</td>
<td>−12.63 ± 15.7</td>
<td>0.008</td>
</tr>
<tr>
<td>E₂, pg/ml</td>
<td>0.96 ± 0.5</td>
<td>1.10 ± 0.9</td>
<td>NS</td>
<td>0.40 ± 5.6*</td>
<td>−0.40 ± 0.7*</td>
<td>0.001</td>
</tr>
<tr>
<td>E₃, pg/ml</td>
<td>42.84 ± 23.1</td>
<td>49.43 ± 24.9</td>
<td>NS</td>
<td>20.15 ± 30.0*</td>
<td>−21.38 ± 27.0**</td>
<td>0.001</td>
</tr>
<tr>
<td>DHEA, ng/ml</td>
<td>4.75 ± 1.8</td>
<td>6.03 ± 2.5</td>
<td>NS</td>
<td>1.30 ± 1.6*</td>
<td>−1.74 ± 1.6**</td>
<td>0.001</td>
</tr>
<tr>
<td>DHEA-S, μg/ml</td>
<td>1.72 ± 0.7</td>
<td>1.86 ± 0.7</td>
<td>NS</td>
<td>0.32 ± 0.6</td>
<td>−0.35 ± 0.7*</td>
<td>0.008</td>
</tr>
<tr>
<td>4-DIONE, ng/ml</td>
<td>1.07 ± 0.3</td>
<td>1.75 ± 0.5</td>
<td>&lt;0.001</td>
<td>0.27 ± 0.5*</td>
<td>−0.24 ± 0.4*</td>
<td>NS*</td>
</tr>
<tr>
<td>T, ng/ml</td>
<td>0.28 ± 0.04</td>
<td>0.39 ± 0.2</td>
<td>NS</td>
<td>0.26 ± 0.1</td>
<td>−0.09 ± 0.2*</td>
<td>0.018</td>
</tr>
<tr>
<td>Free T, pg/ml</td>
<td>4.34 ± 1.47</td>
<td>6.83 ± 6.0</td>
<td>NS</td>
<td>1.05 ± 1.5*</td>
<td>−2.22 ± 6.4*</td>
<td>0.004</td>
</tr>
<tr>
<td>DHT, pg/ml</td>
<td>91.40 ± 45.8</td>
<td>108.1 ± 47.0</td>
<td>NS</td>
<td>21.48 ± 38.5*</td>
<td>−14.64 ± 47.8</td>
<td>0.027</td>
</tr>
<tr>
<td>ADT-G, ng/ml</td>
<td>72.43 ± 42.8</td>
<td>56.16 ± 22.5</td>
<td>NS</td>
<td>10.49 ± 12.3*</td>
<td>−8.23 ± 15.2</td>
<td>0.001</td>
</tr>
<tr>
<td>ADT-G, ng/ml</td>
<td>0.85 ± 0.5</td>
<td>0.83 ± 0.5</td>
<td>NS</td>
<td>0.16 ± 0.5*</td>
<td>−0.14 ± 0.3</td>
<td>0.012</td>
</tr>
<tr>
<td>ADT-G, ng/ml</td>
<td>1.78 ± 1.7</td>
<td>1.82 ± 1.7</td>
<td>NS</td>
<td>0.10 ± 0.9</td>
<td>−0.29 ± 0.5</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Values are means ± SD. AD3G, androstan-3α,17β-diol-3-glucuronide; AD17G, androstan-3α,17β-diol-17-glucuronide; ADT-G, androsterone glucuronide; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; DHT, 5α-dihydrotestosterone; E₁, estrone; E₂, E₃, E₄, estradiol; SHBG, sex hormone-binding globulin; T, testosterone; 4-DIONE, androstenedione. †Between-group differences were determined with the Mann-Whitney U-test. *P and **P, < 0.05 and < 0.01, respectively, vs. baseline for within-group differences determined by the Wilcoxon rank-sum test. **Attention control, n = 12; Acupuncture, n = 16. aAfter logistic regression with adjustment for baseline differences, NS.
increased ovulation frequency induced by acupuncture did lead to a slowing of the LH pulsatility frequency during the luteal and early follicular phases followed by a normal increase in pulsatility in the late follicular phase preceding the next ovulation (33). Our timing of measurements may have been to late to discriminate possible differences in LH pulsatility between groups. Furthermore, although there were no within-group changes, we found a between-group difference in Δchange of glucose metabolism that could be due to the concurrent improvement in anthropometrics (weight, BMI, waist circumference, and WHR) within the attention control group.

Our results suggest that the effects of acupuncture are mediated at the peripheral level. This is based on the observed reductions in most circulating estrogens, androgens, and glucuronidated androgen metabolites after correction for multiple comparisons. These changes involve steroids of mainly ovarian but also of adrenal origin and, along with a lack of effect on LH pulsatility, were all accompanied by a higher ovulation frequency in the acupuncture group. However, we cannot exclude that, due to the timing of the measurements discussed above, we missed an effect on LH pulsatility earlier in the follicular phase that could explain the reduction in the ovarian part of sex steroids. The higher ovulation frequency in the acupuncture group is probably not explained by improved anthropometrics, although waist circumference was decreased. Additionally, when associating ovulation frequency with the endpoint values measured in all of the participants regardless of treatment group, we found an association with a reduction in the levels of estrogens, free T, and AD3G. This is an indication that reductions in sex steroids are important for the induction of ovulation.

Other factors that may affect ovarian function and sex steroid secretion are ovarian AMH and inhibin B. ΔChanges in AMH and inhibin B did not differ between the groups despite a significant reduction in inhibin B within the acupuncture group. Inhibin B is associated with FSH levels, correlates to total follicle number, and may be a marker of follicle health (5, 43). The decrease in circulating sex steroids and the reduction of inhibin B within the acupuncture group suggests that the effect of acupuncture is of ovarian origin even though no changes were seen in ovarian morphology. Furthermore, adrenal sex steroid production was decreased by acupuncture, but we saw no changes in cortisol pulsatility. This suggests a peripheral adrenal effect, although a suppression of the hypothalamic-pituitary-adrenal axis cannot be excluded because we did not measure adrenocorticotropic hormone levels. Thus, the effect of acupuncture at both the ovarian and adrenal levels suggests a central underlying control.

Women with PCOS have generally high muscular sympathetic nerve activity that is associated with high circulating T (42). This is supported by data showing dense ovarian innervation by catecholaminergic nerve fibers in women with PCOS (14, 20, 42). We suggest that the effect of acupuncture is at least in part mediated via modulation of both general and ovarian (peripheral) sympathetic activity (37, 39, 40). The acupuncture needles in the present study were placed in the same somatic innervation as the sympathetic innervation of the ovaries. This placement was similar to that used in previous RCTs (15, 31), where we demonstrated a decrease in sympathetic nerve activity (39). Data obtained in rats also show that acupuncture with low-frequency electrical stimulation decreases ovarian expression of nerve growth factor (40, 41) and increases ovarian blood flow. These effects are mediated via ovarian sympathetic nerves (37) and controlled via the central nervous system (37). Additional acupuncture studies have demon-

Table 5. Cortisol pulsatility in women with PCOS at baseline and the changes from baseline after 10–13 wk of attention control or acupuncture treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Attention Control (n = 12)</th>
<th>Acupuncture (n = 16)</th>
<th>Attention Control (n = 12)</th>
<th>Acupuncture (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Δ (endpoint-baseline)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>162.9 ± 51.4</td>
<td>–10.99 ± 46.8</td>
<td>3.21 ± 47.3</td>
<td>NS</td>
</tr>
<tr>
<td>Half-duration, min</td>
<td>7.85 ± 5.2</td>
<td>–0.98 ± 4.9</td>
<td>0.27 ± 5.9</td>
<td>NS</td>
</tr>
<tr>
<td>Half-life, min</td>
<td>59.25 ± 17.2</td>
<td>–2.91 ± 18.4</td>
<td>0.34 ± 23.1</td>
<td>NS</td>
</tr>
<tr>
<td>Secretion events, frequency/12 h</td>
<td>9.58 ± 1.93</td>
<td>–0.17 ± 2.04</td>
<td>0.45 ± 4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Total production, nmol/l/12 h</td>
<td>1790.1 ± 695.0</td>
<td>20.6 ± 723.7</td>
<td>89.2 ± 1020.5</td>
<td>NS</td>
</tr>
<tr>
<td>Total pulsatile production, nmol/l/12 h</td>
<td>1622.4 ± 592.5</td>
<td>–57.6 ± 495.0</td>
<td>12.6 ± 767.7</td>
<td>NS</td>
</tr>
<tr>
<td>Interpulse interval, min</td>
<td>53.58 ± 13.5</td>
<td>0.92 ± 13.1</td>
<td>–0.94 ± 20.1</td>
<td>NS</td>
</tr>
<tr>
<td>Lg ApEn</td>
<td>0.49 ± 0.11</td>
<td>0.04 ± 0.2</td>
<td>0.01 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Secretion area, nmol/l</td>
<td>112.6 ± 36.4</td>
<td>5.24 ± 61.8</td>
<td>6.32 ± 52.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. †Between-group differences were determined with the Mann-Whitney U-test.
strated a reduction of hypothalamic androgen receptor, gonadotropin-releasing hormone, steroid receptor, and opioid expression together with restored estrous cycles (10, 11). Thus, although we did not observe an effect on LH pulsatility or secretion pattern, it remains possible that the effect on sex steroid secretion could be mediated via the brain.

In PCOS, both central and peripheral opioid activities seem to be altered, although the data are conflicting (9). Experimental and clinical data indicate involvement of the central opioid system in the regulation of ovulation and sex steroid secretion by acupuncture in PCOS, although not included in this study (2, 9 –11). Acupuncture with low-frequency electrical stimulation implies a more general effect on the sympathetic nervous system, including a decrease in muscle sympathetic nerve activity and circulating levels of β-endorphins (39, 45).

In rats, the effect of electro-acupuncture seems to be dose related (10, 16, 25, 26). In the current study, we increased the number of treatments to almost twice those in our previous RCT and the study by Pastore et al. (15, 31), and this may explain the high ovulation frequency and the strong effect on circulating sex steroids.

In the present study, we did not use placebo or sham needles or minimal acupuncture, because studies suggest that these are not inert treatments (8, 22, 23, 28). Instead, our research question aimed to elucidate whether acupuncture is superior to the time and attention of meeting with the therapist.

In summary, we have shown that repeated acupuncture treatments result in a higher ovulation frequency in lean/overweight women with PCOS and are more effective than the attention and time involved in the meeting with a therapist. The reduction of both ovarian and adrenal serum sex steroids with no concomitant effect on LH and cortisol pulsatility or secretion pattern, together with the within-group reduction of inhibin B levels, indicate that the effect of acupuncture is mainly of peripheral origin. Acupuncture may represent an alternative or complementary therapy to standard pharmacological or surgical treatments, but clinical trials comparing acupuncture with these approaches need to be performed to determine the efficacy of such treatment.

ACKNOWLEDGMENT

We thank Carola Gustafsson, Anna-Lena Jönsson, and Ingrid Hansson for technical assistance and Anders Odén for statistical advice. We acknowledge the use of the Pulse XP software (mljohnson.pharm.virginia.edu) and Norman A. Mazer, Boston University School of Medicine, Boston, MA, for the use of the spreadsheet for calculating free T (27).

GRANTS

This study (ClinicalTrials.gov Identifier: NCT00921492) was financed by grants from the Swedish Research Council (Project No. K2012–55X-15276-08-3), the Jane and Dan Olsson Foundations, the Novo Nordisk Foundation, the Hjalmar Svensson Foundation, the Adlerbert Research Foundation, Wilhelm and Martina Lundgren’s Science Fund, the Swedish federal government under the LUA/ALF agreement (ALFGBG-136481), and the Regional Research and Development Agreement (VFOUREG-153221, -74721, and -17611). L. M. R is supported by NIH funding (R00 HD-060762).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.J., L.R., G.H., G.J., and E.S.-V. conception and design of research; J.J., A.S., and E.S.-V. performed experiments; J.J. and E.S.-V. interpreted results of experiments; J.J. and E.S.-V. prepared figures; J.J. and E.S.-V. drafted manuscript; J.J., L.R., P.P.V., A.S., F.L., G.H., G.J., and E.S.-V. edited and revised manuscript; J.J., L.R., P.P.V., A.S., F.L., G.H., G.J., and E.S.-V. approved final version of manuscript.

Table 6. Correlation between ovulation frequency and endpoint values of measured variables in the entire study group to explain ovulation

<table>
<thead>
<tr>
<th>Variable</th>
<th>R_s</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1 mean, pg/ml</td>
<td>−0.578</td>
<td>0.002</td>
</tr>
<tr>
<td>E2 mean, ng/ml</td>
<td>−0.456</td>
<td>0.023</td>
</tr>
<tr>
<td>AD3G mean, pg/ml</td>
<td>−0.408</td>
<td>0.043</td>
</tr>
<tr>
<td>Free T mean, pg/ml</td>
<td>−0.447</td>
<td>0.025</td>
</tr>
<tr>
<td>Free T AUC</td>
<td>−0.445</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Correlations were determined by Spearman’s rank correlation. P < 0.05 was considered significant.
REFERENCES

factor (NGF), and expression of NGF mRNA in the ovaries, the adrenal glands, and the central nervous system. Reprod Biol Endocrinol 1: 33, 2003.


