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Am J Physiol Endocrinol Metab 300:E37-E45, 2011. First published 13 October 2010;
doi:10.1152/ajpendo.00495.2010

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Impact of electro-acupuncture and physical exercise on hyperandrogenism and oligo/amenorrhea in women with polycystic ovary syndrome: a randomized controlled trial

Elizabeth Jedel,¹ Fernand Labrie,² Anders Odén,³ Göran Holm,⁴ Lars Nilsson,⁵ Per Olof Janson,⁵ Anna-Karin Lind,⁵ Claes Ohlsson,⁶ and Elisabet Stener-Victorin^{7,8}

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Submitted 20 August 2010; accepted in final form 6 October 2010

Jedel E, Labrie F, Odén A, Holm G, Nilsson L, Janson PO, Lind A, Ohlsson C, Stener-Victorin E. Impact of electro-acupuncture and physical exercise on hyperandrogenism and oligo/amenorrhea in women with polycystic ovary syndrome: a randomized controlled trial. *Am J Physiol Endocrinol Metab* 300: E37–E45, 2011. First published October 13, 2010; doi:10.1152/ajpendo.00495.2010.—Polycystic ovary syndrome (PCOS), the most common endocrine disorder in women of reproductive age, is characterized by hyperandrogenism, oligo/amenorrhea, and polycystic ovaries. We aimed to determine whether low-frequency electro-acupuncture (EA) would decrease hyperandrogenism and improve oligo/amenorrhea more effectively than physical exercise or no intervention. We randomized 84 women with PCOS, aged 18–37 yr, to 16 wk of low-frequency EA, physical exercise, or no intervention. The primary outcome measure changes in the concentration of total testosterone (T) at week 16 determined by gas and liquid chromatography-mass spectrometry was analyzed by intention to treat. Secondary outcome measures were changes in menstrual frequency; concentrations of androgens, estrogens, androgen precursors, and glucuronidated androgen metabolites; and acne and hirsutism. Outcomes were assessed at baseline, after 16 wk of intervention, and after a 16-wk follow-up. After 16 wk of intervention, circulating T decreased by –25%, androsterone glucuronide by –30%, and androstane-3 α ,17 β -diol-3-glucuronide by –28% in the EA group ($P = 0.038$, 0.030, and 0.047, respectively vs. exercise); menstrual frequency increased to 0.69/month from 0.28 at baseline in the EA group ($P = 0.018$ vs. exercise). After the 16-wk follow-up, the acne score decreased by –32% in the EA group ($P = 0.006$ vs. exercise). Both EA and exercise improved menstrual frequency and decreased the levels of several sex steroids at week 16 and at the 16-wk follow-up compared with no intervention. Low-frequency EA and physical exercise improved hyperandrogenism and menstrual frequency more effectively than no intervention in women with PCOS. Low-frequency EA was superior to physical exercise and may be useful for treating hyperandrogenism and oligo/amenorrhea.

acupuncture; androgens; estrogens; exercise; glucuronidated androgen metabolites; oligomenorrhea; sex steroid precursors

POLYCYSTIC OVARY SYNDROME (PCOS), the most common endocrine disorder in women of reproductive age, is characterized

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by clinical or biochemical hyperandrogenism, oligo/amenorrhea, and polycystic ovaries with or without increased ovarian volume (4). The most constant and prominent feature is hyperandrogenism, manifested by hirsutism, persistent acne, and biochemical abnormalities (4), including elevated levels of androgens, sex steroid precursors, and glucuronidated androgen metabolites as well as estrogens (22).

There is no gold standard for the long-term treatment of women with PCOS who do not attempt to conceive (1). Treatments for hyperandrogenism and menstrual disturbances include oral contraceptives, insulin sensitizers, and lifestyle interventions. Combined low-dose oral contraceptives are recommended as the primary treatment for long-term management of these symptoms despite a paucity of studies (32). The insulin-sensitizing agent metformin has beneficial effects in women with PCOS but does not improve fertility (15). However, when evaluating interventions, it is important to consider adverse events, and pharmacological treatments, while effective, may induce unfavorable metabolic and gastrointestinal effects (15).

Lifestyle intervention, focusing predominantly on diet and physical exercise, is considered the first-line treatment for metabolic complications in overweight and obese women with PCOS and may have the potential to improve ovulatory function and fertility (12, 13, 18, 27). Reproductive function is improved by a low-carbohydrate diet combined with physical exercise (18). In a recent, nonrandomized study, aerobic physical exercise resulted in lower sex steroid levels, a higher ovulation rate, and greater menstrual frequency than a hypocaloric high-protein diet (19).

Complementary and alternative therapies, including various forms of acupuncture, are widely used by patients for infertility problems, although their efficacy has not yet been established (24). In women with PCOS or undefined ovulatory dysfunction, repeated acupuncture treatments decrease total testosterone (T) and other sex steroid levels, reduce the luteinizing hormone-to-follicle-stimulating hormone (LH/FSH) ratio, and improve menstrual frequency without adverse effects (8, 25, 31). However, a randomized controlled trial (RCT) has not been performed to evaluate the effects of low-frequency electro-acupuncture (EA) on hyperandrogenism and oligo/amenorrhoea in women with PCOS who do not attempt to conceive.

Case-control studies suggest that acupuncture has stronger effects than physical exercise on these variables (8, 25, 31).

In this study, we investigated whether low-frequency EA would decrease hyperandrogenism and improve oligo/amenorrhea more effectively than physical exercise or no active intervention in women with PCOS. All sex steroids were measured by liquid chromatography-tandem mass spectrometry (LC-MS-MS) and gas chromatography-mass spectrometry (GC-MS) as recommended for clinical research.

MATERIAL AND METHODS

Study Design and Ethics

This study was a prospective RCT comparing low-frequency EA, physical exercise, and no active intervention. After randomization, each participant underwent a 12-wk observation period followed by a baseline assessment, 16 wk of intervention, and 16 wk of follow-up. Women who met the inclusion criteria were randomly allocated in a 2:2:1 ratio to low-frequency EA, physical exercise, or no active intervention. To ensure equal proportions of age and body mass index (BMI) in each study arm, randomization was stratified by those variables. Computer-generated randomization within each stratum was conducted using permuted blocks of five and was concealed until interventions were assigned. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee, University of Göteborg, Sweden. The trial is registered at ClinicalTrials.gov with Identifier no. NCT00484705. The trial protocol is found as a supplementary file (supplementary materials are found with the online version at the Journal website).

Participants

Participants were recruited between November 2005 and January 2008 by newspaper advertisements in the local community, and the study took place at Sahlgrenska University Hospital and Sahlgrenska Academy at University of Göteborg. To be eligible, participants had to have ultrasound-verified polycystic ovaries with ≥ 12 follicles 2–9 mm and/or an ovarian volume of ≥ 10 ml in one or both ovaries, together with either oligo/amenorrhea and/or clinical signs of hyperandrogenism (hirsutism or acne) (28). Hirsutism was defined as a Ferriman Gallwey (FG) score of ≥ 8 (11). The presence of acne was defined by a positive response to the question “Do you have acne?” Oligomenorrhea was defined as an intermenstrual interval >35 days and fewer than eight menstrual bleedings in the previous year. Amenorrhea was defined as absent menstrual bleeding or no menstrual bleeding in the previous 90 days. The study objective and all procedures were explained to women who met the inclusion criteria. All participants gave written informed consent. Exclusion criteria were age ≥ 38 yr and any pharmacological treatment within 12 wk or breast feeding within 24 wk of entering the study. Other exclusion criteria were cardiovascular disease, diabetes mellitus, and endocrine or neoplastic causes of hyperandrogenemia, including androgen-secreting tumors, Cushing’s syndrome, congenital adrenal hyperplasia, and hyperprolactinemia.

Interventions

All participants received general information concerning the benefits of regular physical exercise and were instructed to complete a physical exercise diary during weeks 1–32 of the study.

Low-frequency EA. Western medical acupuncture (16) was given twice weekly for 2 wk, once weekly for 6 wk, and once every other week for 8 wk, for a total of 14 treatments over 16 wk, by a registered physical therapist (E. Jedel) educated in theoretical and practical acupuncture at Karolinska Institutet, Stockholm, Sweden. The acupuncture protocol was based on our studies of acupuncture in PCOS (8, 25), experimental studies (21), and our clinical experience in

treating women with PCOS. Acupuncture points and electrical stimulation were the same for all women in the low-frequency EA group, as described previously (23). Disposable, single-use, sterilized needles made of stainless steel (Hegu Xeno, Hegu, Landsbro, Sweden; length 30/50 mm, diameter 0.32 mm) were inserted to a depth of 15–35 mm in segmental acupuncture points located in abdominal muscles with innervations corresponding to the ovaries (7), conception vessel (CV) 3, CV 6, and stomach (ST) 29 bilaterally and in the muscles below the knee, spleen (SP) 6, and SP 9 bilaterally. Needles were also placed in extrasegmental acupuncture points that do not innervate the ovaries, alternating between muscles in the hand and/or lower arm; large intestine (LI) 4, or pericardium (PC) 6 bilaterally. All needles were stimulated manually (de qi) once when inserted. CV 3, CV 6, ST 29, SP 6, and SP 9 were stimulated electrically with low-frequency EA of 2 Hz (CEFAR ACUS 4; Cefar-Compex Scandinavia, Malmö, Sweden) for 30 min at each treatment; the intensity was adjusted to produce local muscle contractions without pain or discomfort. LI 4/PC 6 was stimulated manually by rotating the needle to evoke needle sensation every 10 min.

Physical exercise. The physical exercise program consisted of 16 wk of regular exercise, including brisk walking, cycling, or any other aerobic exercise at a self-selected pace described as “faster than normal walking at a pace that could be sustained for at least 30 min at least 3 days per week” (20). Physical exercise was self-monitored with a heart rate monitor (ECG2, Sports Instruments) to ensure a heart rate of ≥ 120 beats/min. Participants met E. Jedel once to decide how to perform their physical exercise and were thereafter supervised through weekly telephone calls to provide guidance on how to continue their physical exercise. All exercise was in addition to daily physical activity.

No active intervention. Like the other participants, women in the no-intervention group received oral information about the benefits of regular physical exercise. The participants could call the study coordinator at any time.

Outcome Measurements

After randomization but before baseline assessment, participants were asked to document their menstrual bleeding pattern for 12 wk. Baseline assessments included anthropometric measurements and calculation of BMI (kg/m^2), hyperandrogenism measured by FG scores, and presence of acne (determined by the response to the question “Do you have acne?”). Fasting blood samples for endocrine analyses were drawn between 0730 and 0830 and immediately aliquoted on ice and stored at -80°C until assay. Blood samples were taken independently of the follicular phase of the menstrual cycle, as most participants had oligo/amenorrhea. Physical fitness was assessed with Åstrand’s submaximal bicycle test. Outcome measurements were repeated after 16 wk of intervention (i.e., within 1 wk after the last treatment) and again after 32 wk (i.e., 16 wk after the last treatment).

GC-MS. Dehydroepiandrosterone (DHEA), 5-androstene-3 β , 17 β -diol (5-DIOL), androstenedione (4-DIONE), total T, 5 α -dihydrotestosterone (DHT), estrone (E1), and estradiol (E2) were measured with a validated GC-MS system, which uses a 50% phenyl-methyl polysiloxane (DB-17HT) capillary column (30 m \times 0.25 mm internal diameter; 0.15 μm film thickness) and helium as the carrier gas. The analyses and internal standard were detected with an HP5973 quadrupole mass spectrometer equipped with a chemical ionization source. The limits of detection were 0.10 ng/ml for DHEA, 30 pg/ml for 5-DIOL, 0.05 ng/ml for 4-DIONE, 0.02 ng/ml for T, 5.00 pg/ml for DHT, 5.00 pg/ml for E1, and 1.00 pg/ml for E2.

LC-MS-MS. A validated LC-MS-MS system with a TurboIonSpray source was used to analyze DHEA sulfate (DHEAS; limit of detection, 0.075 $\mu\text{g}/\text{ml}$), E1 sulfate (E1-S; 0.075 ng/ml), androsterone glucuronide (ADT-G; 2.00 ng/ml), androstane-3 α , 17 β -diol-3-glucuronide (3G; 0.50 ng/ml), and 17 β -diol-17-glucuronide (17G; 0.50 ng/ml).

Immunoassay. Sex hormone-binding globulin (SHBG), LH, and FSH were analyzed by chemiluminescence microparticle immunoassay (CMIA) at an accredited laboratory at the Department of Clinical Chemistry, Sahlgrenska University Hospital, using Architect reagent kits from Biokit (Barcelona, Spain) for SHBG (limit of detection, 0.1 nmol/l) and from Abbott Laboratories (Chicago, IL) for LH (0.07 IU/l) and FSH (0.05 IU/l).

Calculation of free T. Free T (FT) was calculated as described (30), using the total T concentration determined by GC-MS and SHBG determined by CMIA and assuming a fixed albumin concentration of 43 g/l.

Menstrual bleeding pattern. Menstrual bleeding was recorded and confirmed by daily measurement of basal body temperature with an oral thermometer throughout the study period and by interviews by gynecologists. Baseline was the monthly menstrual frequency during the 12-wk observation period after randomization but before intervention and was calculated by dividing the number of menstrual bleedings by 3. Monthly menstrual frequency from baseline to *week 16* and during the 16-wk follow-up period was calculated by dividing by 4. This means that the menstrual frequency was normalized to 1.00 at baseline, during treatment, and after treatment. During the study, ovulation was defined by progesterone measurements ~21 days after spontaneous bleeding or ~7 days after increase in basal body temperature.

Maximal oxygen uptake. The estimated $\dot{V}O_{2\max}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was measured with Åstrand's submaximal bicycle test, and as described, $\dot{V}O_{2\max}$ is a reliable and valid test of aerobic capacity (3). It measures the maximum amount of oxygen that can be utilized during exercise and increases with the level of fitness. The test was performed on a Monark 828E ergometer cycle (Monark, Varberg, Sweden), and heart rate (beats/min) was recorded from electrodes connected to a chest belt (Polar Favor, Kempele, Finland).

Primary outcome measure. The primary outcome measure was changes in serum T between low-frequency EA and physical exercise at *week 16*. Secondary outcome measures were changes in serum T between low-frequency EA and physical exercise at *week 32*; changes in menstrual frequency, acne and hirsutism, FT, DHT, E1, E1-S, E2, DHEA, DHEAS, 4-DIONE, 5-DIOL, ADT-G, 3G, 17G, SHBG, LH, FSH, LH/FSH ratio, BMI, and $\dot{V}O_{2\max}$ at *weeks 16* and *32* between the two intervention groups. In addition, we assessed changes in primary and secondary outcome measures at *weeks 16* and *32* between low-frequency EA and no active intervention and between physical exercise and the no active intervention.

Sample Size and Statistical Analyses

We assumed that the mean change in T from baseline to *week 16* would be 0.10 ng/ml in the low-frequency EA group and 0.05 ng/ml in the physical exercise group; no effect was expected in the no active intervention group. We assumed a standard deviation of 0.06 of the difference. We have experience from an earlier study (25), but the technique for measuring T was changed. To achieve 80% power to detect a significant difference in T between the two actively treated groups at the two-sided 5% level, we needed 24 patients for each group. In the no-intervention group, we planned to reach 12 participants. Thus, to compensate for a dropout rate of 25%, we needed 75 patients who went through all baseline measurements.

Data were analyzed according to the intention-to-treat (ITT) principle. In the evaluation of change between baseline and *week 16* and baseline and *week 32*, missing data were replaced using the method of last observation carried forward. Dropouts between randomization and baseline assessments were excluded from the ITT analyses. Most of the data were not normally distributed, so the Kruskal-Wallis test followed by a Mann-Whitney *U*-test were used to assess differences between groups; the primary comparison was between the low-frequency EA and the physical exercise groups. The χ^2 test was used to assess differences in acne (categorical variable) between groups.

The Wilcoxon rank sum test was used to analyze changes within each group. Descriptive statistics are presented as means \pm SD. $P < 0.05$ (two-sided) was considered statistically significant. Analyses were performed with Prediction Application Software (PASW) v. 17.0.

RESULTS

Flow of Participants Through the Study

Figure 1 summarizes the flow of participants through the study. Of 504 women who responded to advertisements, 404 were excluded after the initial telephone screening; 100 were interviewed and examined by two-dimensional ultrasound. Eighty-four women who met inclusion criteria agreed to participate and were randomly allocated to one of the three study arms. There were 10 dropouts between allocation and baseline assessments, 15 between baseline and *week 16*, and 9 between *weeks 16* and *32* (Fig. 1). The ITT population comprised 74 participants who went through baseline assessments (Fig. 1).

Groups were comparable at baseline (Table 1). Oligo/amenorrhea was present in 23 of 29 women allocated to low-frequency EA, in 24 of 30 allocated to physical exercise, and in 10 of 15 allocated to no active intervention. Twenty-one in the low-frequency EA group, 19 in the physical exercise group, and 10 in the no active intervention group reported their menstrual frequency at baseline. Nineteen in the low-frequency EA group, 24 in the physical exercise group, and 11 in the no active treatment group reported an FG score of ≥ 8 . Phenotypic presentation and number of women with BMI ≥ 30 are given in Table 2.

Differences Between Low-Frequency EA and Physical Exercise

Between baseline and *week 16*, serum T decreased in the low-frequency EA group and was lower than in the physical exercise group ($P = 0.038$; Fig. 2A and Table 3). ADT-G and 3G also decreased in the low-frequency EA group, and the percentage change was significantly greater than in the physical exercise group ($P = 0.030$ and $P = 0.047$, respectively; Table 3). Menstrual frequency increased from 0.28 to 0.69/month in the low-frequency EA group and was significantly higher than in the physical exercise group at *week 16* ($P = 0.018$; Fig. 3 and Table 3). Between baseline and *week 32*, the acne score decreased in the low-frequency EA group, with fewer women reporting acne (13 vs. 19 at baseline) and differed from the physical exercise group ($P = 0.006$). There were no other differences between the low-frequency EA and the physical exercise group at *week 32* (Table 4 and Figs. 2B and 3).

Differences Between Low-Frequency EA and No Active Intervention

Between baseline and *week 16*, serum T, FT, E1-S, ADT-G, 3G, and 17G decreased in the low-frequency EA group and differed significantly from the levels in the no active intervention group (Table 3 and Fig. 2A). Menstrual frequency also improved more in the low-frequency EA group than in the no active intervention group ($P < 0.001$; Table 3 and Fig. 3). Between baseline and *week 32*, menstrual frequency remained improved, and serum T, FT, and 17G levels remained decreased and differed significantly from those in the no active intervention group (Table 4 and Figs. 2B and 3).

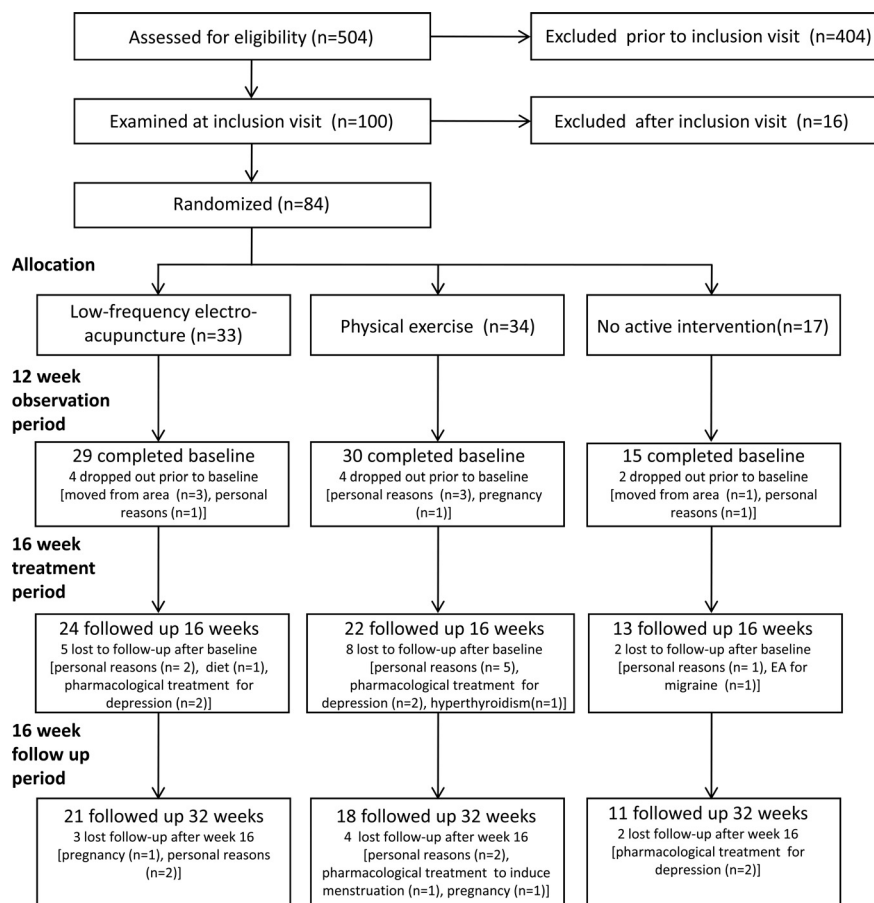


Fig. 1. Flow of participants through the study. All participants assigned to each treatment and performing baseline assessments (including those who discontinued the study) were included in the primary intent-to-treat analyses.

Differences Between Physical Exercise and No Active Intervention

Between baseline and *week 16*, serum E1-S, and 17G decreased in the physical exercise group and differed significantly from the levels in the no active intervention group (Table 3). Menstrual frequency improved more in the physical exercise group than in the no active intervention group ($P < 0.014$; Table 3 and Fig. 3). Between baseline and *week 32*, menstrual frequency remained improved, and serum T, E2, and 17G levels decreased in the physical exercise group and differed significantly from those in the no active intervention group (Table 4 and Figs. 2B and 3).

Exercise Frequency and Physical Fitness

There were no differences between the groups in self-reports of physical exercise frequency. Weekly exercise frequency was 3.0 ± 2.2 in *weeks 1–16*, and 2.9 ± 2.0 in *weeks 16–32* in the low-frequency EA group. The corresponding frequencies were 3.1 ± 1.3 and 3.0 ± 1.5 in the physical exercise group and 2.6 ± 2.0 and 3.0 ± 2.2 in the no active intervention group. The mean number of low-frequency EA treatments was 12.5 ± 3.2 . There were no differences between the groups in changes of BMI or $\dot{V}O_{2\max}$ (Tables 3 and 4). However, between baseline and *week 16*, $\dot{V}O_{2\max}$ increased by 13% in the low-frequency EA group and 13% in the physical exercise group, and between baseline and *week 32*, $\dot{V}O_{2\max}$ increased by 7% within the low-frequency EA group and by 12% within the physical

exercise group, indicating improved physical fitness in the active treatment groups (Tables 3 and 4).

Adverse Events

Three participants had adverse events (isolated redness and subsequent hematomas) after one of the 14 low-frequency EA treatments. One participant reported dizziness and one reported nausea after one low-frequency EA treatment. No long-term adverse events occurred in the low-frequency EA group. No short-term or long-term adverse events were reported by women in the physical exercise group or in the group without an active intervention.

DISCUSSION

In this RCT, repeated low-frequency EA was more effective than physical exercise at reducing serum T, ADT-G, 3G, and acne and at increasing menstrual frequency in women with PCOS who did not attempt to conceive. Importantly, both low-frequency EA and physical exercise were more effective than no active intervention at improving menstrual frequency and several of the measured sex steroids, although with a different response pattern. Thus, both treatments may be helpful and have the potential to interrupt the vicious cycle of ovarian dysfunction and androgen excess in women with PCOS. Our findings provide initial data on two alternative or complementary interventions with few negative side effects.

Menstrual dysfunction and hyperandrogenism are typically treated with oral contraceptives, which have been associated

Table 1. Baseline characteristics

Variable	Low-Frequency EA (n = 29)	Physical Exercise (n = 30)	No Active Intervention (n = 15)	P
Anthropometry				
Age, yr	29.7 ± 4.3	30.2 ± 4.7	30.1 ± 4.2	NS
BMI, kg/m ²	29.1 ± 8.83	27.7 ± 6.44	26.8 ± 5.56	NS
$\dot{V}O_{2max}$, ml·kg ⁻¹ ·min ⁻¹	32.7 ± 12.1	33.9 ± 8.5	35.2 ± 9.3	NS
Clinical hyperandrogenism				
Ferriman Gallwey score	12.1 ± 8.06	13.1 ± 7.99	10.1 ± 5.20	NS
Acne (Yes/No), n (%)	19/28 (67.8)	15/29 (51.7)	11/15 (73.3)	NS
Menstrual frequency (month)#	0.28 ± 0.28	0.26 ± 0.33	0.23 ± 0.28	NS
Mass spectrometry				
T, ng/ml (GC)	0.40 ± 0.16	0.45 ± 0.19	0.47 ± 0.21	NS
FT, pg/ml	7.27 ± 4.05	7.81 ± 3.74	7.58 ± 4.04	NS
DHT, pg/ml (GC)	110 ± 40.6	132 ± 62.9	120 ± 46.1	NS
E1, pg/ml (GC)	80.4 ± 32.1	73.7 ± 24.6	72.1 ± 23.3	NS
E1-S, ng/ml (LC)	1.49 ± 0.99	1.38 ± 0.87	1.14 ± 0.55	NS
E2, pg/ml (GC)	83.0 ± 36.7	76.0 ± 45.3	66.9 ± 36.8	NS
DHEA, ng/ml (GC)	6.51 ± 2.96	7.74 ± 3.09	6.82 ± 2.81	NS
DHEAS, μg/ml (LC)	1.72 ± 0.67	1.93 ± 0.99	1.70 ± 0.67	NS
4-DIONE, ng/ml (GC)	1.57 ± 0.68	2.00 ± 0.79	2.06 ± 0.92	NS
5-DIOL, pg/ml (GC)	656 ± 219	739 ± 313	658 ± 234	NS
ADT-G, ng/ml (LC)	60.7 ± 23.0	52.6 ± 32.0	49.9 ± 27.3	NS
3G, ng/ml (LC)	2.35 ± 0.89	2.15 ± 1.38	2.01 ± 1.00	NS
17G, ng/ml (LC)	2.16 ± 0.99	2.25 ± 1.60	1.94 ± 1.67	NS
Immunoassay				
SHBG, nmol/l (CMIA)	42.4 ± 25.3	40.5 ± 18.8	45.3 ± 18.96	NS
LH, IU/l	9.21 ± 13.6	7.19 ± 5.08	9.01 ± 7.54	NS
FSH, IU/l	4.30 ± 1.98	4.02 ± 1.47	3.95 ± 1.26	NS
LH/FSH ratio	2.04 ± 1.58	1.82 ± 0.92	2.26 ± 1.41	NS

Values are means ± SD. #Number of participants reporting menstrual frequency in the low-frequency electro-acupuncture (EA; n = 21), physical exercise (n = 19) and no active treatment (n = 10) groups. BMI, body mass index; $\dot{V}O_{2max}$, maximal oxygen uptake; CMIA, chemoluminescence microparticle immunoassay; GC, gas chromatography-mass spectrometry; LC, liquid chromatography-tandem mass spectrometry; T, testosterone; FT, free T; DHT, 5 α -dihydrotestosterone; E1, estrone; E1-S, E1 sulfate; E2=estradiol; DHEA=dehydroepiandrosterone; DHEAS, DHEA sulfate; 4-DIONE=androstenedione; 5-DIOL, 5-androstene-3 β ,17 β -diol; ADT-G, androsterone glucuronide; 3G, androstane-3 α , 17 β -diol-3 glucuronide; 17G, 17 β -diol-17 glucuronide; SHBG, sex hormone-binding globulin; LH, luteinizing hormone; FSH, follicle stimulating hormone; NS, not significant. FT was calculated using the total T concentration determined by GC-MS. SHBG was determined by CMIA, assuming a fixed albumin concentration of 43 g/l (30).

with side effects. The potential worsening of metabolic function by the use of oral contraceptives is debatable. Other reasons not to use oral contraceptives include family history of venous thromboembolism and patient preference. The present study highlights effects of alternative interventions for the management of hyperandrogenism and oligo/amenorrhea. Repeated low-frequency EA treatments showed improvements superior to that afforded by physical exercise.

The hyperandrogenism in women with PCOS is thought to reflect contributions primarily from the ovaries; however, most women with PCOS have high levels of the sex steroid precursors DHEA, DHEAS, 4-DIONE, and 5-DIOL, which may indicate a role of the adrenals as well (33). Androgens are eliminated by inactivation to androsterone and 3 α -diol and their subsequent glucuronidation into ADT-G, 3G, and 17G. DHEAS is a stronger independent predictor of glucuronidated

androgen metabolites than T (22). The levels of T, FT, and the glucuronidated androgen metabolites ADT-G, 3G, and 17G were lower after EA and also DHT, but not ADT-G and 3G, at follow-up. Interestingly, acne score was significantly decreased at follow-up. The three androgen glucuronides are made specifically in different tissues; however, it is impossible to know which tissue(s) is (are) responsible for this change, although it is tempting to relate the decrease in 17G with the pilosebaceous unit and the decrease in acne score in the EA group at follow-up. Consistent with the decrease in androgen activity, E1-S levels were lower after EA treatment than at baseline but did not persist throughout follow-up.

Low-frequency EA afforded long-lasting improvements in monthly menstrual frequency. In our previous uncontrolled study (25), 38% of participants had increased menstrual frequency after 14 sessions of low-frequency EA. Interestingly, 19 women in that study were clomiphene resistant, suggesting use of EA as an alternative or complementary intervention to induce ovulation in women attempting to conceive. Two other uncontrolled studies have suggested that EA or manual acupuncture increases menstrual frequency and regulates endocrine variables; however, participant selection was made on heterogeneous grounds (8, 31).

No previous study has compared the efficacy of low-frequency EA with that of physical exercise. A structured, supervised exercise training program three times per week for 24 wk is more effective than a diet program at improving menstrual

Table 2. Phenotypic distribution and number of obese (BMI ≥ 30) women with PCOS in each group

	Low-Frequency EA (n = 29)	Physical Exercise (n = 30)	No Intervention (n = 15)
Phenotype, n (%)			
PCO + HA + O	21 (72)	20 (67)	9 (60)
PCO + HA	6 (21)	6 (20)	5 (33)
PCO + O	2 (7)	4 (13)	1 (7)
BMI ≥ 30 , n (%)	11 (38)	11 (37)	4 (27)

HA, hyperandrogenism; O, oligo/amenorrhea; PCO, polycystic ovaries.

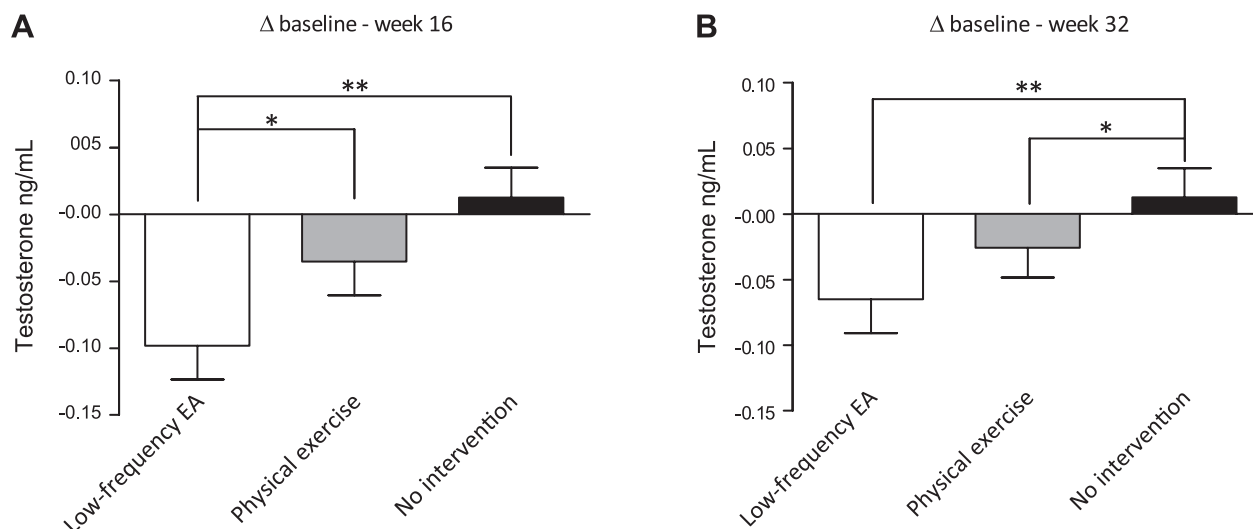


Fig. 2. Change in serum testosterone from baseline to week 16 (A) and week 32 (B) in low-frequency electro-acupuncture (EA; $n = 29$), physical exercise ($n = 30$), and no active intervention ($n = 15$) groups. * $P < 0.05$, ** $P < 0.01$.

frequency and 4-DIONE, DHEAS, and SHBG (19). In another study, diet alone or diet with aerobic or aerobic-resistance exercise lowered T and the free androgen index and increased SHBG (29). Lifestyle modification alone, including standardized lectures on nutrition and diet, behavioral support, and structured, supervised group exercise, also decreases the free androgen index and increases SHBG (12).

In the current study, physical exercise produced long-lasting improvement in menstrual frequency, and the most pronounced effect on circulating sex steroids was among estrogens, as reflected by the decrease in E1-S and E2 levels, which differed significantly from those in the no active intervention group. These findings suggest that physical exercise and EA act through different mechanisms. Results from previous exercise

Table 3. Changes in outcome measures from baseline to week 16

Variable	Δ Baseline to week 16								
	Low-frequency EA ($n = 29$)			Physical exercise ($n = 30$)			No intervention ($n = 15$)		
	Mean \pm SD	Change, %	P^a	Mean \pm SD	Change, %	P^a	Mean \pm SD	Change, %	P^a
BMI, kg/m ²	0.06 \pm 1.11	0	NS	0.01 \pm 0.70	0	NS	0.11 \pm 0.63	0	NS
$\dot{V}O_{2max}$, ml·kg ⁻¹ ·min ⁻¹	4.26 \pm 8.19	13	0.016	4.54 \pm 6.86	13	0.002	0.27 \pm 4.57	1	NS
Clinical hyperandrogenism									
FG scores	-0.18 \pm 3.32	-2	NS	0.72 \pm 3.54	5	NS	1.40 \pm 3.66	14	NS
Acne, n (%) #	17 (60.7)	-11	NS	16 (55.2%)	7	NS	11 (73%)	0	NS
Menstrual freq.##	0.41 \pm 0.33 ^{A,D}	146	0.001	0.14 \pm 0.33 ^B	58	NS	-0.04 \pm 0.007	-17	NS
Mass spectrometry									
T, ng/ml	-0.10 \pm 0.14 ^{A,C}	-25	0.002	-0.04 \pm 0.14	-7	NS	0.01 \pm 0.09	2	NS
FT, pg/ml	-2.21 \pm 2.99 ^B	-30	0.001	-1.24 \pm 2.66	-16	0.014	0.03 \pm 1.71	0	NS
DHT, pg/ml	-23.2 \pm 41.2	-20	0.007	-9.30 \pm 34.2	-7	NS	4.48 \pm 31.9	4	NS
E1, pg/ml	-16.4 \pm 32.6	-20	0.012	-8.94 \pm 30.2	-12	NS	5.50 \pm 25.5	8	NS
E1-S, ng/ml	-0.64 \pm 0.84 ^C	-42	0.001	-0.38 \pm 0.75 ^B	-28	0.005	0.06 \pm 0.46	6	NS
E2, pg/ml	-19.9 \pm 50.1	-24	0.021	-18.8 \pm 47.8	-25	0.045	11.2 \pm 51.2	17	NS
DHEA, ng/ml	-0.23 \pm 2.46	-4	NS	-0.04 \pm 3.75	-1	NS	0.66 \pm 3.40	10	NS
DHEAS, μ g/ml	-0.29 \pm 0.56	-17	0.006	-0.24 \pm 0.55	-12	0.039	-0.17 \pm 0.30	-1	NS
4-DIONE, ng/ml	-0.05 \pm 0.48	-3	NS	-0.01 \pm 0.55	0	NS	0.02 \pm 0.48	1	NS
5-DIOL, pg/ml	-64.1 \pm 145	-10	0.028	-15.7 \pm 219	-2	NS	13.9 \pm 206	2	NS
ADT-G, ng/ml	-18.3 \pm 25.8 ^{A,B}	-30	0.002	-2.33 \pm 8.91	-4	NS	0.14 \pm 10.2	0	NS
3G, ng/ml	-0.65 \pm 0.88 ^{A,B}	-28	0.001	-0.11 \pm 0.44	-6	NS	0.07 \pm 0.45	4	NS
17G, ng/ml	-0.64 \pm 1.06 ^C	-30	0.005	-0.16 \pm 0.55 ^B	-8	NS	0.35 \pm 0.74	19	NS
Immunoassay									
SHBG, nmol/l	3.52 \pm 11.8	8	0.037	7.30 \pm 22.0	18	NS	3.33 \pm 12.7	8	NS
LH, IU/l	-2.52 \pm 12.0	-27	NS	-0.45 \pm 4.33	-6	NS	-1.63 \pm 7.98	-18	NS
FSH, IU/l	-0.13 \pm 1.57	-3	NS	0.26 \pm 1.57	6	NS	-0.31 \pm 1.65	-8	NS
LH/FSH ratio	-0.34 \pm 1.58	-17	NS	-0.15 \pm 0.83	-8	NS	-0.20 \pm 1.36	-9	NS

#Acne (yes/no). ##Number of participants reporting menstrual frequency in low-frequency EA ($n = 21$), physical exercise ($n = 19$), and no intervention ($n = 10$) groups. Between-group differences for the change from baseline to week 16 were determined by Kruskal-Wallis test followed by Mann-Whitney U -test. ^aWithin-group changes were determined by Wilcoxon rank-sum test. ^A $P < 0.05$ vs. physical exercise; ^B $P < 0.05$ vs. no intervention; ^C $P < 0.01$ vs. no intervention; ^D $P < 0.001$ vs. no intervention.

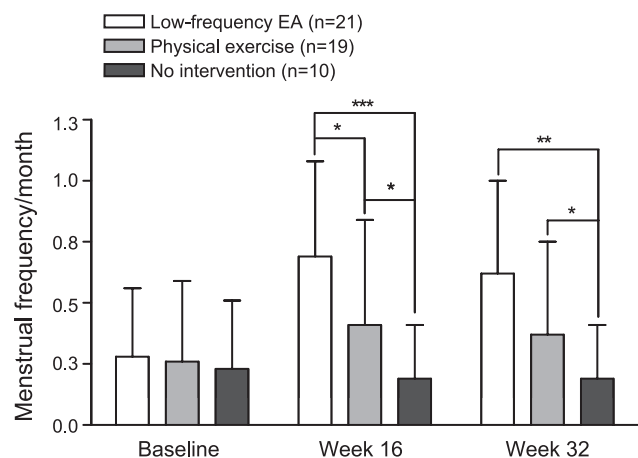


Fig. 3. Menstrual frequency in low-frequency EA, physical exercise, and no active intervention groups at baseline and at weeks 16 and 32. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

and lifestyle intervention studies support, in part, the present study. However, the response was less pronounced than in previous studies, and we found no improvement in SHBG.

The relatively weak response in the physical exercise group may reflect the fact that information on the benefits of regular physical exercise, provided to all women at the first visit, affected their physical exercise behavior. This speculation is supported by the increased $\dot{V}O_{2max}$, indicating improved fitness, in the active-intervention groups. The low-frequency EA group also increased $\dot{V}O_{2max}$, most likely because they exercised more frequently during the study than before. Neverthe-

less, there is a considerable difference between self-directed exercise and the structured, supervised exercise in many other studies. Such exercise may further enhance the physical fitness and response rate in the physical exercise group and is recommended in future studies. Notably, in the current study, low-frequency EA and physical exercise improved menstrual cyclicity and hyperandrogenism without affecting BMI. Combined treatment with acupuncture, exercise, and diet, especially in overweight and obese women, with the additional aim of reducing weight and BMI, may further enhance the response to treatment.

The effects of low-frequency EA and physical exercise may be mediated by modulation of β -endorphin production and secretion, which in turn may affect gonadotropin-releasing hormone (GnRH) and LH secretion. Women with PCOS display persistently rapid LH pulsatility and increased amplitude, which further augment ovarian androgen production (6). Evidence that β -endorphin contributes to the dysregulation of GnRH and LH secretion comes from studies in which the μ -receptor antagonists naltrexone or naloxone improved menstrual frequency, induced ovulation, and lowered T and LH levels and the LH/FSH ratio (2). We did not observe differences in gonadotropin concentration between groups, perhaps because blood samples were drawn independently of menstrual cycle day, which is critical for gonadotropins. Dysregulation of β -endorphin may also affect sympathetic nerve activity. Recently, we (26) showed that women with PCOS have high sympathetic nervous system activity and that FT, measured by radioimmunoassay, is the strongest independent factor explaining the high activity. Interestingly, in a small experimental

Table 4. Changes in outcome measures from baseline to week 32

Variable	Δ Baseline to week 32								
	Low-frequency EA (n = 29)			Physical exercise (n = 30)			No intervention (n = 15)		
	Mean \pm SD	Change, %	P^a	Mean \pm SD	Change, %	P^a	Mean \pm SD	Change, %	P^a
BMI, kg/m ²	0.07 \pm 0.89	0	NS	0.06 \pm 1.19	0	NS	0.19 \pm 0.66	1	NS
$\dot{V}O_{2max}$, ml·kg ⁻¹ ·min ⁻¹	2.37 \pm 8.25	7	0.041	4.11 \pm 5.20	12	0.001	2.27 \pm 5.11	7	NS
Clinical hyperandrogenism									
FG scores	0.71 \pm 4.65	6	NS	0.90 \pm 2.47	7	NS	2.07 \pm 5.84	20	NS
Acne, n (%)#	13 (46.4) ^A	-32	0.014	16 (55.2)	7	NS	11 (73)	0	NS
Menstrual freq.#	0.33 \pm 0.37 ^C	121	0.003	0.11 \pm 0.36 ^B	42	NS	-0.04 \pm 0.07	-17	NS
Mass spectrometry									
T, ng/ml	-0.06 \pm 0.14 ^C	-18	0.020	-0.03 \pm 0.12 ^B	-4	NS	0.04 \pm 0.0	9	0.049
FT, pg/ml	-1.82 \pm 3.50 ^B	-25	0.004	-0.78 \pm 2.10	-10	0.024	0.36 \pm 1.34	5	NS
DHT, pg/ml	-22.0 \pm 38.8 ^B	-19	0.009	-1.99 \pm 46.2	-2	NS	5.73 \pm 19.1	5	NS
E1, pg/ml	-5.79 \pm 40.5	-7	NS	-4.55 \pm 37.6	-6	NS	6.94 \pm 14.3	10	0.041
E1-S, ng/ml	-0.29 \pm 1.06	-19	NS	-0.09 \pm 1.86	-7	NS	0.09 \pm 0.45	8	NS
E2, pg/ml	-2.32 \pm 67.4	-3	NS	-17.3 \pm 60.3 ^B	-23	NS	14.1 \pm 32.0	21	NS
DHEA, ng/ml	-0.50 \pm 2.88	-8	NS	0.01 \pm 3.85	0	NS	0.48 \pm 2.03	7	NS
DHEAS, μ g/ml	-0.24 \pm 0.63	-14	0.032	-0.23 \pm 0.57	-12	0.040	0.05 \pm 0.33	2	NS
4-DIONE, ng/ml	-0.07 \pm 0.50	-4	NS	-0.01 \pm 0.51	-1	NS	-0.02 \pm 0.32	-1	NS
5-DIOL, pg/ml	-45.2 \pm 141	-7	NS	-21.1 \pm 221	-3	NS	20.1 \pm 164	3	NS
ADT-G, ng/ml	-16.4 \pm 26.0	-27	0.005	-3.44 \pm 11.3	-6	NS	-0.71 \pm 8.07	-1	NS
3G, ng/ml	-0.61 \pm 0.97	-26	0.009	-0.02 \pm 0.68	-1	NS	0.05 \pm 0.44	2	NS
17G, ng/ml	-0.61 \pm 1.12 ^C	-28	0.019	-0.02 \pm 0.49 ^B	-1	NS	0.41 \pm 0.70	21	0.034
Immunoassay									
SHBG, nmol/l	4.21 \pm 9.10	10	0.008	4.87 \pm 20.9	12	NS	2.60 \pm 8.19	6	NS
LH, IU/l	-1.86 \pm 12.0	-20	NS	1.20 \pm 8.99	17	NS	-1.60 \pm 8.21	-18	NS
FSH, IU/l	-0.18 \pm 1.53	-4	NS	0.26 \pm 1.37	7	NS	-0.21 \pm 1.38	-5	NS
LH/FSH ratio	-0.19 \pm 1.19	-9	NS	0.08 \pm 1.40	2	NS	-0.24 \pm 1.34	-11	NS

#Acne (yes/no). ##Number of participants reporting menstrual frequency in low-frequency EA (n = 21), physical exercise (n = 19), and no intervention (n = 10) groups. Between-group differences for the change from baseline to week 32 were determined by Kruskal-Wallis test followed by Mann-Whitney U-test. ^aWithin-group changes were determined by Wilcoxon rank-sum test. ^A $P < 0.1$ vs. physical exercise; ^B $P < 0.05$ vs. no intervention; ^C $P < 0.1$ vs. no intervention.

RCT in lean women with PCOS, low-frequency EA and physical exercise lowered high sympathetic nerve activity compared with a no active intervention group (23). That the effect of low-frequency EA and physical exercise is mediated, at least in part, by modulation of sympathetic nervous system activity is supported by experimental animal data (17, 21). Both treatments also improve ovarian morphology (17), estrous cyclicity, and hypothalamic GnRH and androgen receptor protein expression in rat models of PCOS, which may explain the beneficial effects of these treatments in women with PCOS (9).

Another potential explanation for the larger improvement in the low-frequency EA group is particularly potent placebo effects. There is evidence that complex medical interventions such as acupuncture have stronger placebo effects than pharmacological treatment (14). Also, the characteristics of acupuncture treatment are relevant in the context of placebo effects, including frequent patient-practitioner contacts and the repeated "ritual" of needling (14). Notably, placebo effects result in genuine psychobiological events and can exist in clinical practice (10). Further research will allow advances in the ethical use of placebo mechanisms that are inherent in routine clinical care and encourage the use of treatments that stimulate placebo effects (10).

Limitations

The major strengths of this study were the randomized design, no cointerventions, relatively high compliance rates, adequate sample sizes, and short- and long-term follow-up. The primary limitation was the low success rate to confirm ovulation (data not shown), which was mainly related to lack of coordination of progesterone measurements. A weakness of all acupuncture studies is the variability of sham methods (e.g., placebo needles, superficial needling), location and depth of needling, and the number and duration of treatments. Acupuncture studies in which the participant and the practitioner are unaware of the treatment are practically impossible to conduct. We chose the acupuncture points on the basis of earlier protocols suggesting needle placement in areas innervating the ovaries. We compared EA with physical exercise on the basis of the hypothesis that long-term management of PCOS should include this intervention. Given the uncertainties about the physiological effects of sham controls and the question of enhanced placebo effects, it is crucial to conduct direct comparisons of acupuncture and standard treatment.

In the present study, we observed an increase in $\dot{V}O_{2\max}$ in the EA group as well as in the physical exercise group. At baseline, all women were given information of the benefits of regular physical exercise and emphasized the importance of regular physical exercise to all included women. Future studies may acknowledge this by changing from a superficial to a more rigorous monitoring of women allocated to physical exercise. We cannot completely exclude the fact that low-frequency EA increased $\dot{V}O_{2\max}$. A recent trial demonstrated that acupuncture has a significant impact on the performance in athletes in endurance sports, thus indicating that acupuncture increases aerobic capacity (5). This suggests a future EA plus physical exercise vs. physical exercise alone trial.

In conclusion, repeated low-frequency EA and physical exercise each improves hyperandrogenism and menstrual frequency in women with PCOS more effectively than no active

intervention, with low-frequency EA being superior to physical exercise. These findings suggest that low-frequency EA may be used in the treatment of hyperandrogenism and oligo/amenorrhea in women with PCOS.

ACKNOWLEDGMENTS

We thank Carola Gustafsson for excellent technical assistance.

GRANTS

This study was financed by grants from the Osher Center for Integrative Medicine, the Swedish Medical Research Council (Project No. 2008-72VP-15445-01A), the Novo Nordisk Foundation, Wilhelm and Martina Lundgren's Science Fund, the Hjalmar Svensson Foundation, the Tore Nilson Foundation, the Åke Wiberg Foundation, the Adlerbert Research Foundation, the Ekhaga Foundation, and the Swedish Federal Government Under Letters of Understanding Agreement of Medical Education (ALFFGBG-10984) and the Regional Research and Development Agreement (VGFOUREG-5171, -11296, and -7861).

DISCLOSURES

No conflicts of interest are reported by the authors.

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