Acupuncture or metformin to improve insulin resistance in women with polycystic ovary syndrome: study protocol of a combined multinational cross-sectional case-control study and a randomised controlled trial

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ABSTRACT

Introduction Polycystic ovary syndrome (PCOS) is linked to hyperinsulinemia and insulin resistance with dysfunctional glucose metabolism. Pilot studies suggest that acupuncture treatment with combined manual and low-frequency electrical stimulation (electroacupuncture (EA)) of the needles decrease circulating glycated haemoglobin (HbA1c) and homeostatic model assessment-insulin resistance. Therefore, we here aim to investigate if acupuncture treatment or metformin together with lifestyle or lifestyle management alone improves insulin sensitivity and related symptoms in overweight/obese women with PCOS.

Methods and analysis This is a two-centre multinational (Sweden and China), cross-sectional case–control study combined with an open-labelled randomised controlled trial (RCT). Participants are randomised to one of three groups: (1) EA 2–3 times/week during 4 months+lifestyle management; (2) metformin, 500 mg, three/day during 4 months+lifestyle management; or (3) lifestyle management alone. The primary outcome measure in the RCT is changes in HbA1C. A total of 123 obese overweight women with PCOS will be enrolled and randomised into one of the three groups with a target power of at least 80% and 5% significance level based on two-sided tests.

Ethics and dissemination The study has been approved by the Regional Ethical Review Board of Stockholm and of Peking University Third Hospital, China. Primary outcome data of the RCT will be published in a relevant journal together with supporting secondary outcome measurements. Further, outcome measurements will be published in separate papers as well as case–control data.

Expected results We anticipate that EA and metformin, both with lifestyle management, are equally effective and superior to lifestyle management alone for improvement of glycaemic control.

Trial registration numbers NCT02647827 and EudraCT2015-004250-18.

Strengths and limitations of this study

A strength of this trial is that all patients will benefit from receiving treatment, all of which alone and/or in combination may offer an increased chance for improved metabolic function and reproductive health.

It has the potential to gain deeper insight into the pathophysiology of polycystic ovary syndrome, and to uncover new knowledge for treatment of insulin resistant in related diseases, including obesity, insulin resistance and type 2 diabetes.

The results from the present study have the potential to immediately be implemented into the healthcare system since it has previously been shown to be cost-effective and to have few negative side effects.

A potential limitation is that metformin might cause side effects such as diarrhoea, nausea/vomiting, flatulence, asthenia, indigestion, abdominal discomfort and headache and acupuncture local skin irritation, discomfort and vasovagal reactions during the procedure.

BACKGROUND

Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder in women of reproductive age and is characterised by anovulation, hyperandrogenism and metabolic dysfunction. Women with PCOS have a threefold to sevenfold increased risk of developing type 2 diabetes (T2D), and with younger onset, PCOS increases cardiovascular risk factors. Independent of body weight, insulin sensitivity is ~40% lower in women with PCOS than in healthy women, and impaired glucose regulation, insulin resistance and reduced insulin responsiveness
have been attributed to defects in insulin signalling in adipocytes and skeletal muscle.\textsuperscript{7,8} Of note, obesity aggravates all symptoms related to PCOS.

Despite detrimental impact on women’s health, the aetiology of PCOS is not well understood. Genetic, epigenetic and environmental factors have all been implicated in its development. Emerging evidence suggests that PCOS originates, at least in part, in fetal life,\textsuperscript{9,10} and elevated maternal androgens have been implicated to play a role; however, the mechanisms are largely unknown.\textsuperscript{11,12} Of interest is that we have found that women with PCOS have multiple transcriptional and epigenetic changes in adipose tissue that are relevant for development of the disease.\textsuperscript{13} Further, twin studies suggest that genetic influences explain >70\% of PCOS pathogenesis.\textsuperscript{14} However, whether genetic or epigenetic alterations in target tissues, for example, adipose tissue, skeletal muscle and endometrium, contribute to development of metabolic disease requires further investigation.

Women with PCOS require long-term individualised treatment programmes. Pharmacological treatments, including the glucose-reducing drug metformin, have limitations related to adverse effects and patient compliance. Therefore, there is a need for inexpensive and easily administered treatments with few negative side effects. Lifestyle management is the first-line treatment eventually with addition of metformin for improving whole-body glucose homeostasis and preventing T2D.\textsuperscript{1,15-17} Interestingly, 5 weeks of acupuncture with combined manual and low-frequency electrical stimulation has in a pilot study been shown to improve whole-body glucose homeostasis in insulin-resistant women with PCOS.\textsuperscript{18} The pilot study was an uncontrolled trial, and it is therefore of importance to compare the effect of acupuncture with first-line treatment to investigate the effectiveness.

While pharmacological treatment strategies have shown efficacy, importantly, there is a need for Comparative Effectiveness Research to strengthen the evidence base for clinical and policy decision-making.\textsuperscript{19} Therefore, we aim to compare the effect of pharmacological first-line treatment, metformin, with a non-pharmacological treatment strategy, acupuncture (both together with lifestyle management), with lifestyle management for improvement and prevention of metabolic dysfunction and related symptoms in insulin-resistant women with PCOS.

Our main hypothesis is that acupuncture and metformin (both treatments combined with lifestyle management) are superior to lifestyle management alone in improving whole-body glucose regulation in insulin-resistant women with PCOS. Secondary hypotheses are that these treatments have the potential to improve metabolic and endocrine measures, quality of life and symptom of anxiety and depression, and to restore epigenetic and molecular alterations in target tissues (endometrial, adipose and skeletal muscle tissue) and thus have the potential to improve and potentially prevent the development of metabolic alterations including T2D.

Thus, the purpose of this study is twofold; first, we aim to gain deeper insight into the pathophysiology of PCOS in a cross-sectional case–control study by comparing women with PCOS with women without PCOS matched for age, weight and body mass index (BMI); and second we aim to perform a prospective randomised controlled trial (RCT) of women with PCOS.

**STUDY DESIGN**

This is a two-centre multinational prospective trial with a cross-sectional case–control part and an open-labelled RCT with a comparative effectiveness design. The interventions to be tested are (1) electroacupuncture during 4 months+lifestyle management; (2) metformin during 4 months+lifestyle management or (3) lifestyle management alone which will be available for participants in all three groups. Participants will be enrolled at Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden, and at Peking University Hospital, Beijing, China, respectively.

**RANDOMISATION AND TREATMENT ALLOCATION**

The randomisation is stratified across the factors age and BMI and is also separated by study site with a balanced allocation ratio 1:1:1. Randomisation is performed in blocks with a variable block size between 3 and 15; for example, first there is a block of 12, when it is full it is followed by a block of 9, and thereafter a block of 3. The order of the block sizes is unknown to the participating study sites and also differs among the strata’s.

Each study site (Stockholm and Beijing) uses the same randomisation and electronic case report form (eCRF). A web-based randomisation program (https://data.dynareg.se/pia2/Default.aspx) has been generated to ensure allocation concealment. The study coordinators log on the web-based system to randomise eligible patients. All women who enter the study will be logged and given a unique study number. Blinding or masking of the intervention will not be possible because of the nature of the intervention. Importantly, however, the assessor will be blinded to the patients’ group assignment.

**PATIENT AND PUBLIC INVOLVEMENT**

Patients and/or public were not involved in the design of this study.

**STUDY OBJECTIVES**

**Primary objective**

To determine the clinical effectiveness of 4 months of (1) electroacupuncture+lifestyle management and (2) metformin+lifestyle management, compared with (3) lifestyle management only for improvement of glucose regulation assessed by HbA1c levels.
Secondary objectives

1. To evaluate changes in secondary metabolic measures including the insulin response to glucose assessed by calculating the area under the curve (AUC_{insulin}) during the oral glucose tolerance test (OGTT), fasting insulin, glucose, calculation of homeostatic model assessment (HOMA-IR) and HOMA-B (ie, the Islet $\beta$-cell function) and the assessment of, for example, adipokines, lipid profile, body size and proportions and body fat distribution.

2. To determine changes in gene expression and DNA methylation profiles related to insulin sensitivity in fat, muscle and endometrial tissue biopsies, and biomarkers in whole blood.

3. To evaluate endocrine measures including menstrual pattern and ovulation frequency, circulating hormones (eg, sex steroids, antimüllerian hormone (AMH), gonadotropins).

4. To determine changes in women’s health-related quality of life (HRQoL), symptoms of anxiety and depression, dieting and eating patterns, and negative side effects.

OUTCOME MEASUREMENTS

Outcome measures will be collected at
1. Baseline.
2. After 4 months of intervention.
3. Follow-up 4 months after last treatment.

Primary outcome

Changes from baseline to after 4 months of treatment in HbA1c comparing (1) acupuncture+lifestyle management and (2) metformin+lifestyle management, respectively, with (3) lifestyle management only. In the cross-sectional study, difference in HbA1c between cases and controls.

Secondary outcome

Changes from baseline to after 4 months of treatment and from baseline to the 4-month follow-up comparing (1) acupuncture+lifestyle management and (2) metformin+lifestyle management, respectively, with (3) lifestyle management only, and in the cross-sectional study, difference between cases and controls in the following variables:

- **Body composition:** In addition to weight, height and waist circumference, women will be examined by dual-energy X-ray absorptiometry (DXA) to measure lean and fat mass and bone mineral density using a Lunar Prodigy Advance whole body scanner (GE Medical Systems).

- **Metabolic measures:** Insulin response to glucose during the OGTT (AUC using the trapezoidal rule) and direct analyses of fasting blood samples of insulin/glucose to enable calculation of HOMA-IR \(\frac{\text{fasting insulin (mU/mL)} \times \text{fasting glucose (mmol/L)}}{22.5}\) and HOMA-B (Islet $\beta$-cell function \(20 \times \text{fasting insulin (mU/mL)} / \text{fasting plasma glucose (mmol/L)}\) – 3.5). Further, fasting blood samples are collected and saved for later analyses of, for example, C-peptide and calculation of C-peptide index \(\frac{\text{Fasting C-peptide (nmol/L)} / \text{fasting glucose (mmol/L)} \times 100}\) for analyses of adipokines, inflammatory markers, non-esterified fatty acids (NEFA), total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), high-sensitive C reactive protein (CRP), catecholamine’s and metabolites analysed on a split-fraction HPLC-ED system.

- **Endocrine measures:** menstrual frequency: Participants will be asked to note date of menstruation which will be reported to the study coordinator once per week by text message and every fourth week by phone. Ovarian morphology antral follicle count and ovarian volume. Blood samples will be drawn for analyses of sex steroids by the validated gas and liquid chromatography/tandem mass spectroscopy technique, as well as sex hormone binding globulin (SHBG), luteinising hormone (LH), follicle-stimulating hormone (FSH), AMH, prolactin, thyroid-stimulating hormone (TSH) and free thyroxine (T4).

- **Tissue and whole blood collection:** Whole blood will be collected for DNA and microRNA analyses. Endometrial, fat and skeletal muscle tissue biopsies will be collected at baseline (cases and controls), after 4 months of treatment and at follow-up 4 months after treatment in women with PCOS, snap frozen in liquid nitrogen within 30 s and stored at −80°C for later analyses. Fat cells will be isolated for the determination of adipocyte size and distribution. Part of tissue biopsies will also be isolated for in vitro experiments. Deep RNA, microRNA and/or bisulfite sequencing will be performed with the latest available technology.

- **HRQoL:** Will be determined by quality of life by EuroQol-5 dimension (EQ-5D), short form-36 (SF36), and polycystic ovary syndrome questionnaire (PCOSQ).

- **Symptoms of anxiety and depression** will be assessed by the self-reported version of the Comprehensive Psychopathological Rating Scale for Affective Syndromes (CPRS-S-A) to assess psychiatric symptoms within a time frame of the last three days in Sweden. For the purpose of this study, two scales will be extracted from the CPRS-S-A, the Brief Scale for Anxiety (BSA-S) and the Montgomery Åsberg Depression Rating Scale (MADRS-S). In China, the Zung symptom depression score (SDS) and Zung symptom anxiety score (SAS) will be used. Depression symptoms of potential clinical relevance are for MADRS-S ≥11 and for Zung SDS ≥0.5 (depressive index), and anxiety symptoms of potential clinical relevance are for BSA-S ≥11 and for Zung SAS ≥50 (standard total score).

- **Physical activity:** International Physical Activity Questionnaire (IPAQ) will be used to assess degree of physical activity.

In addition, one text message per week will be sent to the participants by the study
coordinators asking of number of steps the last week when asking for menstrual bleeding (date).

► Eating questionnaire and eating pattern: Only assessed at baseline using the self-reported version of the Three-Factor Eating Questionnaire (TFEQ-R21) and Questionnaire of Eating and Weight Patterns-Revised (QEWP-R) to measure eating behaviour (Sweden only).

► Side effects and adverse events will be continuously and equally recorded in each study arm. One text message per week will be sent to all participants by the study coordinators in which they are asked to report (in addition to number of steps) any side effects or adverse events. All participants will receive a phone call every fourth week by the study coordinator and will be asked about side effects or adverse events.

PARTICIPANTS

Eligible women will be identified by their clinician, or by local newspaper advertisements and invited to participate in the trial. Each participant will be given written and oral information and asked for her signed informed consent to be randomised and followed-up by research staff. The cross-sectional case–control part of the study equals baseline measurements for women with PCOS and controls. If a patient does not adhere to the frequent treatment, this will be clearly tracked as the treatment may still be effective.

Inclusion criteria: women with PCOS

1. Age≥18 to ≤40 years.
2. BMI≥25 to ≤40 given that 95% of all women with PCOS with a BMI≥25 are insulin resistant.
3. PCOS diagnosis according to Rotterdam criteria 2003, with at least two of the following three symptoms: clinical and/or biochemical signs of hyperandrogenism (hirsutism or acne); oligo/amenorrhoea; and/or polycystic ovaries (PCOS). Biochemical hyperandrogenism is defined by total testosterone ≥1.2 nmol/L or a free androgen index ≥5. Hirsutism is defined as a self-reported Ferriman-Gallwey (FG) score ≥8 (≥5 Asian). Acne is defined by a positive response to the question Do you have acne? Oligomenorrhoea is defined as an intermenstrual interval ≥35 days and ≤8 menstrual bleedings in the past year. Amenorrhoea as <3 cycles per year. PCO is defined by transvaginal ultrasound with ≥12 follicles 2–9 mm and/or ovarian volume ≥10 mL in one or both ovaries.
4. Willing to sign the consent form.

Inclusion criteria: controls

Controls should have BMI ≥ 25 to < 40, regular cycles with 28 days±2 days and no signs of hyperandrogenism. They are excluded if they have menstrual irregularities, signs of hyperandrogenism (FG >4) or evidence of PCO morphology on ultrasound.

Exclusion criteria for all women

1. Exclusion of other endocrine disorders such as non-classic congenital adrenal hyperplasia (17-hydroxyprogesterone<3 nmol/L), androgen-secreting tumours or suspected Cushing’s syndrome.
2. Having known renal disease (creatinine clearance <60 mL/min), hepatic insufficiency, autoimmune disorders or cancer.
3. Any acute condition with potential to alter renal function or cause tissue hypoxia.
4. Type I diabetes.
5. Pharmacological treatment (cortizon, antidepressant, other anti diabetic treatment such as insulin and acarbose, hormonal contraceptives, hormonal ovulation induction or other drugs judged by discretion of investigator) within 12 weeks. Depo Provera or similar within 6 months.
6. Hypersensitivity to metformin hydrochloride or to any of the excipients.
7. Blood pressure >160/100 mm Hg.
8. Pregnancy or breast feeding the last six months.
9. Acupuncture the last two months.
10. Daily smoking and alcoholic intake.
11. Language barrier or disabled person with reduced ability to understand the information given.

In total 50 controls will be matched at baseline (age, weight and BMI) to women with PCOS. Controls will undergo screening and baseline visit, but will not be randomised to any treatment.

INTERVENTIONS

Participants fulfilling the inclusion criteria will be randomised to one of three groups after baseline measurements:

1. Electroacupuncture 2–3 times/week during 4 months+lifestyle management.
2. Metformin, 500 mg, three times/day during 4 months+lifestyle management.
3. Lifestyle management alone which will be available for participants in all three groups.

Lifestyle management

All women will receive lifestyle management instructions at the baseline visit, before randomisation. The lifestyle management involves one initial counselling session in connection with the baseline visit, which includes information about the importance of weight management, healthy diet and physical activity. Focus will be on the importance of physical activity. Each participant will receive a book with lifestyle advice about weight reduction, maintenance and physical activity following WHO recommendations. All participants will receive a text message once weekly to respond number of step collected by their smart phone or step counter during the last week and if they have had any menstrual bleedings. Once every fourth week, study coordinator will call the participant and...
ask about the number of steps last week, menstruation and compliance and side effects.

**Electroacupuncture**

Women randomised to receive acupuncture will start their treatment within 1 week after baseline measurements. The rationale of the acupuncture protocol is based on Western Medical Acupuncture theories. We will use a fixed acupuncture protocol following the two pilot studies: ClinicalTrail.gov NCT01457209 and NCT02026323 with two modifications. First, the treatment period will be 16 weeks (ie, 4 months) compared with 5 weeks and 6 months in the previous pilot studies. Second, the treatment frequency will be 2–3 times per week during 16 weeks, that is, in total 32–48 acupuncture treatments over 16 weeks. The rationale for these changes is that the procedure is time-consuming for the patients and this will increase the feasibility and likely reduce the number of dropouts. Acupuncture treatment will be given by registered physiotherapists or medical doctors educated in theoretical and practical acupuncture and trained to follow the fixed protocol.

Disposable, single-use sterilised CE marked needles made of stainless steel, 0.25×30 mm and 0.30×40/50 mm (XENO, HEGU Svenska AB, Landsbro, Sweden, and in China Huatuo, Suzhou Medical, China) will be inserted to a depth of 15–40 mm in segmental acupuncture points located in abdominal and leg muscles, with innervations corresponding to the ovaries and the pancreas. Two sets of acupuncture points will be alternated every second treatment (table 1). The first set of acupuncture points includes points located in abdominal muscles: conception vessel (CV)4, CV12, stomach (ST)29 bilaterally, and in quadriceps muscle, ST32 and ST34, and in the muscles below the knee, spleen (SP)6, and ST36 bilaterally. In addition, needles are placed in the hand, large intestinal (LI)4, bilateral. All needles will be stimulated manually when inserted. CV4 and 12, ST29 bilateral, ST32 and ST34 bilateral will be connected to an electrical stimulator and stimulated with low-frequency EA of 2 Hz (stimulators used in Sweden: Export Abteilung, Schwamedico, Wetzlser Str: 41-43;35630 Ehringshausen and in China: Shanghai Huayi Electric Acupuncture Instrument: G6805-1A) for 30 min at each treatment. The intensity will be adjusted by the physiotherapist to produce local muscle contractions without pain or discomfort, and thereafter the patient will monitor the stimulation intensity. Six additional points are selected to strengthen the effect: LI4, ST36 and SP6, and they will be stimulated manually by rotating the needle to evoke needle sensation every 10 min.

The second set of acupuncture points includes abdominal points: ST27 bilaterally (EA), CV6 to CV10 (EA); and leg points: SP10 to a non-acupuncture point located 6 cun proximal of patellas medial border (EA) are all connected to an electrical stimulator and stimulated as in the first set of acupuncture points. Six additional points; ST38, liver (LR)three and pericardium (PC)6, all bilateral, are stimulated manually by rotating the needle to evoke needle sensation every 10 min.

**Compliance** If a participant in the acupuncture group deviates considerably from the study protocol, the acupuncturists should inform the study coordinator. Any negative side effects during treatment are recorded.

**Metformin**

Oral metformin 500 mg three times daily, in total 1500 mg per day. To reduce gastrointestinal side effects of metformin, the dose will be slowly escalated starting with 500 mg daily during the first week, increasing to 500 mg twice per day during the second week and 500 mg three times daily, morning, lunch and dinner from the third week in total 16 weeks including the 3 weeks step-up phase (ie, 4 months). Patients with negative effects can remain 500 mg during the remaining weeks.

**Compliance:** Empty bottles are handed over to the study coordinator after 16 weeks of treatment and number of tablets is counted. Also, once per month, the study coordinator calls the participant and asks her to count the number of tablets left in the bottle.

**STUDY PROCEDURE**

**Screening**

The study coordinator describes the study design in detail and written informed consent is collected. Of note, if a participant hesitates to go through tissue sampling as described below, this is not an exclusion criteria. All other outcome measures will be collected and are listed in table 2.

In all participants, a comprehensive anamnestic interview will be conducted, including menstrual frequency, hirsutism-FG and acne determined by an affirmative answer to the question ‘Do you have excessive acne? Yes or no’, heredity, medication or other diseases. The physical examination including gynaecological examination is performed by transvaginal ultrasound (PCO morphology: yes or no). Bodyweight (kg) and body height (cm) are measured in an upright position with light clothing and no shoes. BMI is calculated as bodyweight (kg) divided by squared body height (m²). Waist circumference is measured in centimetres at the midpoint between the iliac crest and lower rib margin at the end of expiration, while standing without clothing. Hip circumference is measured in centimetres at the widest point between waist and thighs. Waist-hip-ratio is calculated as the ratio of waist and hip circumferences. Systolic blood pressure and diastolic blood pressure are measured with a semiautomatic blood pressure monitor and heart rate.

Each woman (PCOS and controls) is given seven questionnaires to be filled in and returned at next (baseline) visit. They are asked to start to register their bleeding periods from now until the end of study.
An appointment for body composition (lean and fat mass and bone mineral density) measure with DXA is given. To enable measurements of days 6–8 in the menstrual cycle, all women were given information on how to induce withdrawal bleeding with medroxyprogesterone acetate, 10 mg per day for 7 days (participants in Sweden) or dydrogesterone, and 20 mg per day for 10 days (participants in China).

**Baseline**
The baseline visit takes place in the morning after an overnight fast on days 6–8 after induced withdrawal bleeding.

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**Table 1** Acupuncture points, stimulation, localisation, tissue in which needles are inserted and innervation areas (the two sets will be alternated for every other treatment)

| Acupuncture point | Stimulation | Localisation | Muscle | Muscle innervation |
|-------------------|-------------|--------------|--------|-------------------|
| **Set 1**         |             |              |        |                   |
| CV4, Guan Yuan    | EA          | 3 cun caudal to the umbilicus | Fibrous tissue, linea alba | L1 |
| CV12, Zhongwan    | EA          | On the midline, 4 cun superior to the umbilicus | Fibrous tissue, linea alba | Th7–8 |
| ST29 Bilateral,  | EA          | 1 cun cranial to the pubic bone and 2 cun lateral of the midline | M. rectus abdominis | Th6–12 |
| Guiligai          |             |              |        |                   |
| ST34 Bilateral,  | EA          | 2 cun above the superior lateral border of the patella on the line connecting the anterior superior iliac spine found | M. quadriceps femoris | femoral nerve |
| Futu              |             |              |        |                   |
| ST32 Bilateral,  | EA          | 6 cun above the superior lateral border of the patella on the line connecting the anterior superior iliac spine found | M. quadriceps femoris | femoral nerve |
| Liangqiu          |             |              |        |                   |
| SP6 Bilateral,    | DeQi, four times | 3 cun proximal to the medial malleolus | Mm. flexor digitorum longus, tibialis posterior | L4–5, S1–2 |
| Sanyinjiao        |             |              |        |                   |
| ST36 Bilateral,   | DeQi, four times | On the anterior lateral side of the leg, 3 cun below Dubi (ST35), one finger width (middle finger) from the anterior crest of the tibia | Musculi tibialis anterior | L4–5, S1 |
| Zusani             |             |              |        |                   |
| LI4 Bilateral,    | DeQi, four times | On the highest point at m. interosseus dorsalis | Mm. interosseus dorsalis I, lumbricalis II, adductor pollicis | C8, Th1 |
| Hegu              |             |              |        |                   |
| **Set 2**         |             |              |        |                   |
| CV6, Qihai        | EA          | 1.5 cun caudal to the umbilicus | Fibrous tissue, linea alba | Th11 |
| CV10, Xiawan      | EA          | 2 cun cranial to the umbilicus | Fibrous tissue, linea alba | Th8 |
| ST27 Bilateral,   | EA          | 3 cun cranial to the pubic bone and 2 cun lateral to the midline | M. rectus abdominis | Th6–12 |
| Daju              |             |              |        |                   |
| Extra meridian    | EA          | 6 cun above the patella in line with SP10 | M. quadriceps femoris | L2–L4 |
| point Bilateral   |             |              |        |                   |
| SP10 Bilateral,   | EA          | With the knee flexed, on the medial side of the thigh 2 cun above the superior medial corner of the patella on the prominence of the medial head of the quadriceps muscle of the thigh | M. quadriceps femoris | L2–L4 |
| Xuehai            |             |              |        |                   |
| ST38 Bilateral,   | DeQi four times | Between lateral malleolus and knee joint, 1 finger from tibiae crista | Musculi tibialis anterior | L4–5, S1 |
| Sanyinjiao        |             |              |        |                   |
| LR3 Bilateral,    | DeQi four times | Between metatarsal I and II, just distal to the caput | M. Interosseus dorsalis | S2–3 |
| Taichong          |             |              |        |                   |
| PC6 Bilateral,    | DeQi four times | 2 cun proximal to the processus styloideus radii, between the tendons of the palmaris longus and the flexor carpi radialis | M. flexor digitorum superficialis | C8, Th1 |
| Neiguan           |             |              |        |                   |

C, cervical vertebra; CV, conception vessel; EA, electroacupuncture; L, lumbar vertebra; LI, large intestine; LR, liver; PC, pericardium; S, sacral vertebra; SP, spleen; ST, stomach; Th, thoracic vertebra.
in all women (see above). The time point is selected as the endometrial lining has to be thicker. The questionnaires are returned and checked. Missing information in the questionnaires is checked.

First, a gynaecological examination is performed by transvaginal ultrasound, measuring ovarian size in three dimensions, total antral follicle count (2–9 mm) and endometrial thickness (mm).

Second, if biopsies are taken, local anaesthesia will be placed and an endometrial biopsy is collected and snap frozen. Immediately after, local anaesthesia is placed close to the umbilicus and in the vastus lateralis muscle, fat and muscle biopsy are taken.

Third, a venflon will be placed and fasting blood samples will be drawn for serum and plasma analyses, for example, genetic (eg, next-generation sequencing, SNP, methylation), metabolic (eg, lipids, adipokines, inflammatory markers) and endocrine (eg, sex steroids, gonadotropins, growth factors) measures.

Fourth, an OGTT with 75 g glucose will be performed. Blood samples are collected to measure plasma glucose and serum insulin at 0, 30, 60 and 120 min during the OGTT.

At the baseline visit, after OGTT, all participants will receive lifestyle advice by the study coordinator. Patients will be told to register daily number of steps and will receive a step counter and asked to register menstrual bleeding. If allocated to the electroacupuncture group, time will be booked and treatment started within 1 week. If randomised to the metformin group, the study drug will be administered and the treatment started the next day. The lifestyle management-only group is given appointments for repeated measurements after 4 months and follow-up 4 months later.

Women with PCOS who are randomised are informed that they should use contraception that are non-hormonal.

Follow-up 4 months after last treatment
All baseline measures are repeated after 4 months of treatment and at follow-up 4 months after last treatment.

### STATISTICAL ANALYSIS
#### Sample size and power calculations
Sample size calculations are based on t-test between two groups. This is due to the fact that it is the pairwise comparisons that are of main interest (not overall F-test/
analysis of variance (ANOVA)). The result shows that 41 women per group, in total 123 women, is enough to prove a difference in HbA1c compared with acupuncture+lifestyle management and metformin+lifestyle management, respectively, to lifestyle management alone (repeated pairwise t-test) on −1.7 units (effect size 1.7/2.7 SD=0.63) with 80% power (significance, p=0.05, unadjusted pairwise comparisons).18,51

Further, for the mechanistic studies, we estimate that successful tissue samples will be recruited from a minimum of 20 participants in each group in Sweden and China, respectively, giving a strong power to detect differences.

Minimising sources of bias
Blinding is not possible given the nature of the intervention. We do not feel it is necessary or ethical to perform sham acupuncture and are confident that the primary outcome is unlikely to be affected by observer bias.

Type of analyses
The statistical analyses will be performed by qualified statisticians and biostatisticians. The data in the RCT will be analysed according to the intent-to-treat principle to investigate the differences between the groups.

Clinical outcome measures: Continuous variables will be presented as means±SD and categorical variables as medians with IQRs. Between-group comparisons will be carried out with changes from baseline to after treatment and from baseline to follow-up by ANOVA followed by Mann-Whitney U-test or by χ² tests for categorical variables.

In the cross-sectional case–control part of the study, the Student’s t-test will be used for continuous variables and Mann-Whitney U test or χ² tests for categorical variables and logistic regression when needed.

All statistical analyses of the data will be performed using the SPSS program V.23.0 or higher, and a p value<0.0167 will be considered statistically significant in the RCT and p value<0.05 in the cross-sectional part. All tests are two-sided and adjustments for multiple comparisons will be performed.

Expression and methylation data: These analyses will be adjusted to the technique used. In brief, raw data will be checked and processed and a quality control report will be completed. Different analysis pipelines for traceability and trackability will be performed. Then extended data analyses, including functional analysis, GeneOntologies, biological pathways, principal component analysis, clustering, visualisations and mapping against a reference genome, will be performed and data will be submitted to repositories (ie, the Array Express: http://www.ebi.ac.uk/arrayexpress).

Group comparison will be carried out with changes from baseline to after treatment and from baseline to follow-up by Kruskal-Wallis followed by Mann-Whitney U test for expression analyses. In the case–control part of the study, Mann-Whitney U test will be used for expression analyses. False discovery rate will be used to correct for multiple testing in the analyses of gene and methylation arrays.

SAFETY ANALYSIS
Adverse events will be categorised and the percentage of patients experiencing adverse events and serious adverse events during the treatment period and follow-up period will be documented and reported to the Data and Safety Monitoring Board. These are reviewed every fourth month, and serious adverse events will be immediately handled.

DATA MANAGEMENT AND QUALITY CONTROL OF DATA
We use both paper CRF and web-based eCRF to manage individual participant data. Quality control is handled at two levels. First, the investigators are required to ensure the accuracy when imputing data into the eCRF. Second, data monitoring and validation will be carried out by an independent person not involved in data collection.

ETHICS AND DISSEMINATION
The study is performed according to good clinical practice and conducted in accordance with the Declaration of Helsinki. The study has been approved by the Regional Ethical Review Board of Stockholm, Sweden Dnr: 2015/1656-31/2 and by the Regional Ethical Review Board of Peking University Third Hospital, China Dnr: 2016-212-02. In addition, the Medical Products Agency have approved the study: EudraCT: 2015-004250-18 and the trial is registered at Clinicaltrials.gov: NCT02647827. Reporting of the study results will follow the 2010 revised Consolidated Standards of Reporting Trials statement and STRICTA.52,53 Primary outcome data of the RCT will be published in a relevant journal together with supporting secondary outcome measurements. Further, secondary outcome measurements will be published in separate papers as well as cross-sectional case–control data.

The relevance of this study is that it has potential to uncover new knowledge in the pathophysiology of the disorder and result in an additional treatment strategy for insulin-resistant women with PCOS and related diseases, including obesity, insulin resistance and T2D. Thus, it may have an impact on both genders and does not apply only to women with PCOS.

TRIAL STATUS
The study was conceived and designed during 2015. The first participant was recruited and randomised in February 2016 in Sweden and September 2016 in China. The number of participants randomised in Sweden is 26 and in China 48 in August 2018. We anticipate that
all participants are recruited by the end of 2019 with follow-up done during 2020.

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Contributors ES-V conceived and designed the study, drafted the manuscript for important intellectual content and sought funding and ethical approval in Sweden and registered the trial in EudraCT and Clinicaltrials.gov. JO sought funding and ethical approval in China. HZ, RL, CF, AL-H and JQ was involved in the planning and design of the study and critically revised the manuscript and protocols. HZ, DL, WW, HW, CC, SL, ZJH and XJ are involved in the screening, randomisation and treatment of participants. All authors read and approved the final manuscript.

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Competing interests None declared.

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