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# BMJ Open

## Acupuncture or metformin for insulin resistance in women with polycystic ovary syndrome: Study protocol of a combined multinational case-control and a randomized controlled trial

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Complete List of Authors:	<p>Stener-Victorin, Elisabet; Karolinska Institutet, Department of Physiology and Pharmacology</p> <p>Zhang, Haolin; Peking University Third Hospital, Department of Traditional Chinese Medicine (TCM)</p> <p>Rong, LI; Peking University Third Hospital, OB &amp; GYN</p> <p>Friden, Cecilia; Karolinska Institutet, 5. Department of Neurobiology, Care Sciences and Society, Division of Physiotherapy</p> <p>Li, Dong; Peking University Third Hospital, Department of Traditional Chinese Medicine (TCM)</p> <p>Wang, Wei; Peking University Third Hospital, 4. Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China</p> <p>Wang, Haining ; Peking University Third Hospital, Department of Endocrinology and Metabolism, Peking University Third Hospital, Beijing, China</p> <p>Chang , Cuiqing; Peking University Third Hospital, Institute of Sports Medicine</p> <p>Li , Shi; Peking University Third Hospital, 3. Center for Reproductive Medicine, Department of Obstetrics and Gynecolog</p> <p>Huo , ZeJun ; Peking University Third Hospital, Research Center of Clinical Epidemiology</p> <p>Zhang, Hua; Peking University Third Hospital, Research Center of Clinical Epidemiology</p> <p>Ji, Xiaolan ; Peking University Third Hospital, Department of Traditional Chinese Medicine (TCM)</p> <p>Linden-Hirschberg, Angelica; Karolinska Institutet, Department of Obstetrics and Gynecology</p> <p>Jie, Qiao; Peking University Third Hospital, Center for Reproductive Medicine, Department of Obstetrics and Gynecology and Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China</p>
Keywords:	glucose homeostasis, insulin resistance, acupuncture, metformin, life style, polycystic ovary syndrome

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3 **Acupuncture or metformin for insulin resistance in women with polycystic ovary**  
4 **syndrome: Study protocol of a combined multinational case-control and a randomized**  
5 **controlled trial**  
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8 Elisabet Stener-Victorin<sup>1#\*</sup>, Haolin Zhang<sup>2</sup>, Rong Li<sup>3,4</sup>, Cecilia Fridén<sup>5</sup>, Dong Li<sup>2</sup>, Wei  
9 Wang<sup>4</sup>, Haining Wang<sup>6</sup>, Cuiqing Chang<sup>7</sup>, Shi Li<sup>3</sup>, ZeJun Huo<sup>2</sup>, Hua Zhang<sup>8</sup>, Xiaolan Ji<sup>2</sup>,  
10 Angelica Linden Hirschberg<sup>9</sup>, Jie Qiao<sup>3,4\*</sup>  
11  
12

- 13 1. Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm,  
14 Sweden
- 15 2. Department of Traditional Chinese Medicine (TCM), Peking University Third  
16 Hospital, Beijing, China.
- 17 3. Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking  
18 University Third Hospital, Beijing, China.
- 19 4. Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing,  
20 China
- 21 5. Department of Neurobiology, Care Sciences and Society, Division of Physiotherapy,  
22 Karolinska Institutet, Huddinge, Sweden
- 23 6. Department of Endocrinology and Metabolism, Peking University Third Hospital,  
24 Beijing, China
- 25 7. Institute of Sports Medicine, Peking University Third Hospital, Beijing, China
- 26 8. Research Center of Clinical Epidemiology, Peking University Third Hospital, Beijing,  
27 China.
- 28 9. Department of Obstetrics and Gynecology, Karolinska Institutet, Stockholm, Sweden  
29  
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35 **Short title:** Acupuncture or metformin for insulin resistance in PCOS  
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37 \* Shared senior authors contributed equally to this paper  
38

39 **# Corresponding authors and reprint requests**

40 Elisabet Stener-Victorin

41 Department of Physiology and Pharmacology, Biomedicum QB5

42 Karolinska Institutet, 171 65 Stockholm, Sweden

43 Phone: +46(0)705643655

44 E-mail: [elisabet.stener-victorin@ki.se](mailto:elisabet.stener-victorin@ki.se)  
45  
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49 **Author Contributions**

50 ESV, HZ, RL, ALH and JQ conceived and designed the study. ESV and JQ sought funding  
51 and ethical approval. CF, DL, WW, HW, CC, SL, ZH, HZ, XJ and ALH recruited and  
52 screened subjects, coordinated and carried out acupuncture treatment. ESV drafted the  
53 manuscript. All authors read and approved the final manuscript.  
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## Abstract

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

**Methods and analysis:** This is a two centre multinational (Sweden and China), prospective case-control study combined with an open-labelled randomized controlled trial (RCT) with a comparative effectiveness design. Participants are randomized to one of three groups: 1) life style management alone; 2) EA 2-3 times/week during 4 months + lifestyle management; or 3) metformin, 500 mg, three/day during 4 months + lifestyle management. The primary outcome measures in the RCT are Hba1C and HOMA-IR. A total of 114 (Hba1C) or 303 (HOMA-IR) obese overweight women with PCOS will be enrolled and randomized 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 is performed according to good clinical practice and conducted in accordance with the Declaration of Helsinki and 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.

**Trial registration:** Clinicaltrials.gov: NCT02647827 and EudraCT: 2015-004250-18.

**Key words:** glucose homeostasis; insulin resistance; metformin; electroacupuncture; metformin; lifestyle

### Strength and Limitations

1. 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.
2. It has the potential to gain deeper insight into the pathophysiology of polycystic ovary syndrome (PCOS), and to uncover new knowledge for treatment of insulin resistant in related diseases, including obesity, insulin resistance, and type 2 diabetes (T2D).
3. 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.
4. 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 characterized by anovulation, hyperandrogenism and metabolic dysfunction.<sup>1</sup> Women with PCOS have a 3 to 7-fold increased risk of developing type 2 diabetes (T2D), and with younger onset, PCOS increases cardiovascular risk factors<sup>1-6</sup>. 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.<sup>7,8</sup> 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,<sup>9,10</sup> and elevated maternal androgens have been implicated to play a role, however the mechanisms are largely unknown.<sup>11,12</sup> 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.<sup>13</sup> Further, twin studies suggest that genetic influences explain >70% of PCOS pathogenesis.<sup>14</sup> However, whether genetic or epigenetic alterations in target tissues e.g. adipose tissue, skeletal muscle and endometrium contribute to development of metabolic disease requires further investigation.

Women with PCOS require long-term individualized treatment programs. 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 and metformin are the first line treatment for improving whole body glucose homeostasis and preventing type 2 diabetes (T2D).<sup>1</sup> Interestingly, five 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.<sup>15</sup>

Whilst pharmacological treatment strategies have shown efficacy, importantly, there is a need for Comparative Effectiveness Research (CER) to strengthen the evidence base for clinical and policy decision-making<sup>16</sup>. Therefore we aim to compare the effect of pharmacological first-line treatment, metformin, with a non-pharmacological treatment strategy, acupuncture, with lifestyle management for improvement and prevention of metabolic dysfunction and related symptoms in insulin resistant women with PCOS.

We hypothesize that acupuncture is equally effective as metformin (both treatments combined with lifestyle management) in improving whole body glucose homeostasis in insulin resistant women with PCOS, and that both are superior to lifestyle management alone. Although equally effective (acupuncture and metformin), we hypothesize that acupuncture is associated with less negative side-effects. We also hypothesize that these treatments have the potential 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.

Therefore, the purpose of this study is twofold, first we aim to gain deeper insight into the pathophysiology of PCOS in a case-control study by comparing women with PCOS with women without PCOS matched for age, weight and BMI in primary and secondary outcome variables, and secondly we aim to perform a prospective RCT of women with PCOS, comparing the effectiveness of lifestyle management alone, and in combination with acupuncture or metformin treatment on whole body glucose homeostasis, with the ultimate goal to prevent the development of type 2 diabetes.

## **Study Objectives**

### ***Primary Objective***

1. To determine the clinical effectiveness of 4 months of 1) electroacupuncture + lifestyle management and 2) metformin + lifestyle management, compared to 3) lifestyle management only for improvement of insulin sensitivity as measured by homeostatic model assessment (HOMA)-IR, and by glucose regulation (assessed by analyzing 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 ( $AUC_{\text{insulin}}$ ) during the oral glucose tolerance test (OGTT), fasting insulin, glucose, and , calculation of HOMA-B (*i.e.* the Islet  $\beta$ -cell function) and the assessment of the and 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 (e.g. sex steroids, AMH, gonadotropins), and excretion of metabolites of sex steroids in blood
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.

## Methods and Analyses

### Study design

This is a two centre multinational prospective trial with a prospective 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.

### Patient and Public Involvement

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

### 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 randomized and followed-up by research staff. The case-control part of the study equals baseline measurements for women with PCOS and controls. If a patient do 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. Body mass index (BMI)  $\geq 25$  to  $\leq 40$  given that 95% of all women with PCOS with a BMI  $\geq 25$  are insulin resistant.<sup>17 18</sup>
3. PCOS diagnosis according to Rotterdam criteria 2003<sup>19</sup>, with at least two of the following three symptoms: Clinical and/or biochemical signs of hyperandrogenism (hirsutism or acne); oligo/amenorrhea; and/or polycystic ovaries (PCOS).  
Biochemical hyperandrogenism is defined by total testosterone  $>1.2$  nmol/L or a free

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3 androgen index (FAI) $>5$ .<sup>20</sup> Hirsutism is defined as a self-reported Ferriman-Gallwey  
4 (FG) score  $\geq 8$  ( $\geq 5$  Asian).<sup>21 22</sup> Acne is defined by a positive response to the question  
5 *Do you have acne?* Oligomenorrhea is defined as an intermenstrual interval  $>35$  days  
6 and  $<8$  menstrual bleedings in the past year. Amenorrhea as  $<3$  cycles per year. PCO  
7 is defined by transvaginal ultrasound with  $\geq 12$  follicles 2–9 mm and/or ovarian  
8 volume  $\geq 10$  ml in one or both ovaries.  
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13 4. Willing to sign the consent form.  
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### 15 ***Inclusion criteria – controls***

16 Controls should have BMI  $\geq 25$  to  $\leq 40$ , regular cycles with 28 days  $\pm 2$  days, and no signs of  
17 hyperandrogenism. They are excluded if they have menstrual irregularities, signs of  
18 hyperandrogenism (FG  $>4$ ), or evidence of PCO morphology on ultrasound.  
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### 20 ***Exclusion criteria for all women***

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24 1. Age  $>40$   
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26 2. Exclusion of other endocrine disorders such as non-classic congenital adrenal  
27 hyperplasia (17-hydroxyprogesterone  $< 3$ nmol/L), androgen secreting tumors or  
28 suspected Cushing's syndrome.  
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30 3. Having known renal disease (creatinine clearance  $< 60$  mL/min), hepatic  
31 insufficiency, autoimmune disorders or cancer.  
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33 4. Any acute condition with potential to alter renal function or cause tissue hypoxia.  
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35 5. Type I diabetes.  
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37 6. Pharmacological treatment (cortizon, antidepressant, other antidiabetic treatment such  
38 as insulin and acarbose, hormonal contraceptives, hormonal ovulation induction or  
39 other drugs judged by discretion of investigator) within 12 weeks. Depo Provera or  
40 similar within 6 months.  
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42 7. Hypersensitivity to metformin hydrochloride or to any of the excipients.  
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44 8. Blood pressure  $>160 / 100$  mmHg  
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46 9. Pregnancy or breastfeeding the last 6 months  
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48 10. Acupuncture the last 2 months  
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50 11. Daily smoking and alcoholic intake  
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52 12. Language barrier or disabled person with reduced ability to understand the  
53 information given.  
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3 In total 50 controls will be matched at baseline (age, weight and BMI) to women with PCOS.  
4 Controls will undergo screening and baseline visit, but will not be randomized to any  
5 treatment.  
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### 8 9 **Interventions**

10 Participants fulfilling the inclusion criteria will be randomized to one of three groups after  
11 baseline measurements:  
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- 13 1. Electroacupuncture 2-3 times/week during 4 months + lifestyle management
- 14 2. Metformin, 500 mg, three times/day during 4 months + lifestyle management
- 15 3. Lifestyle management alone which will be available for participants in all three  
16 groups.  
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21 ***Lifestyle management:*** All women will receive lifestyle management instructions at the  
22 baseline visit, before randomization. The lifestyle management involves one initial  
23 counselling session in connection with the baseline visit, which includes information about  
24 the importance of weight management, healthy diet and physical activity. Focus will be on  
25 the importance of physical activity. Each participant will receive a book with lifestyle advice  
26 about weight reduction, maintenance and physical activity. All participants will receive a text  
27 message once weekly to respond number of step during the last week and if they have had  
28 any menstrual bleedings. Once every fourth week, study coordinator will call to the  
29 participant and ask about number of step last week, menstruation and compliance and side-  
30 effects.  
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33 ***Electroacupuncture:*** Women randomized to receive acupuncture will start their treatment  
34 within one week after baseline measurements. The rationale of the acupuncture protocol is  
35 based on Western Medical Acupuncture theories. We will use a fixed acupuncture protocol  
36 following the two pilot studies: ClinicalTrial.gov NCT01457209 and NCT02026323 with two  
37 modifications. First, the treatment period will be 16 weeks (i.e. 4 months) compared to 5  
38 weeks and 6 months in the previous pilot studies. Second, the treatment frequency will be 2  
39 to 3 times per week during 16 weeks, i.e. in total 32 to 48 acupuncture treatments over 16  
40 weeks. The rationale for these changes is that the procedure is time-consuming for the patients  
41 and this will increase the feasibility and likely reduce the number of dropouts. Acupuncture  
42 treatment will be given by registered physiotherapists or medical doctors educated in  
43 theoretical and practical acupuncture and trained to follow the fixed protocol.  
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3 Disposable, single-use, sterilized CE marked needles made of stainless steel, 0.25 x 30 mm  
4 and 0.30 x 40/50 mm (XENO, HEGU Svenska AB, Landsbro, Sweden and in China Huatuo,  
5 Suzhou Medical Co Ltd, China) will be inserted to a depth of 15–40 mm in segmental  
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7 acupuncture points located in abdominal and leg muscles, with innervations corresponding to  
8 the ovaries and the pancreas. Two sets of acupuncture points will be alternated every second  
9 treatment (Table 1). The first set of acupuncture points include points located in abdominal  
10 muscles: conception vessel (CV)4, CV12, stomach (ST)29 bilaterally, and in quadriceps  
11 muscle, ST32 and ST34, and in the muscles below the knee, spleen (SP)6, and ST36  
12 bilaterally. In addition, needles are placed in the hand, large intestinal (LI)4, bilateral. All  
13 needles will be stimulated manually when inserted. CV4 and 12, ST29 bilateral, ST32 and  
14 ST34 bilateral will be connected to an electrical stimulator and stimulated with low-  
15 frequency EA of 2 Hz (Stimulators used in Sweden: Export Abteilung, Schwa-Medico GmbH,  
16 Wetzlarer Str. 41-43;35630 Ehringshausen and in China: Shanghai Huayi Electric  
17 Acupuncture Instrument: G6805-1A) for 30 min at each treatment. The intensity will be  
18 adjusted by the physiotherapist to produce local muscle contractions without pain or  
19 discomfort, and thereafter will the patient monitor the stimulation intensity. Six additional  
20 points are selected to strengthen the effect: LI4, ST36, and SP6, and they will be stimulated  
21 manually by rotating the needle to evoke needle sensation every 10 min.

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23 The second set of acupuncture points include abdominal points: ST27 bilaterally (EA), CV6  
24 to CV10 (EA); and leg points: SP10 to a non-acupuncture point located 6 cun proximal of  
25 patellas medial border (EA) are all connected to an electrical stimulator and stimulated as in  
26 the first set of acupuncture points. Six additional points; ST38, liver (LR)3 and pericardium  
27 (PC)6, all bilateral, are stimulated manually by rotating the needle to evoke needle sensation  
28 every 10 min.

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

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42 ***Metformin:*** Oral metformin 500 mg three times daily, in total 1500 mg per day<sup>23 24</sup>. To  
43 reduce gastrointestinal side-effects of metformin, the dose will be slowly escalated starting  
44 with 500 mg daily during the first week, increasing to 500 mg twice per day during the  
45 second the week, and 500 mg three times daily, morning, lunch and dinner from the third  
46 week in total 16 weeks including the 3 weeks step-up phase (i.e. 4 months). Patients with  
47 negative effects can remain 500 mg during the remaining weeks.

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3 *Compliance:* Empty bottles are handed over to the study coordinator after 16 weeks of  
4 treatment and number of tablets are counted. Also, once per month, the study coordinator call  
5 the participant and ask her to count the number of tablets there are left in the bottle.  
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### 8 **Study Procedure**

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10 *Screening:* The study coordinator describes the study design in detail and written informed  
11 consent is collected. Of note, if a participant hesitates to go through tissue sampling as  
12 described below, this is not an exclusion criteria. All other outcome measures will be  
13 collected and are listed in Table 2.  
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17 In all participants, a comprehensive anamnestic interview will be conducted, including  
18 menstrual frequency, hirsutism - FG and acne determined by an affirmative answer to the  
19 question “Do you have excessive acne? yes or no”, heredity, medication or other diseases.  
20 The physical examination including gynaecological examination is performed by transvaginal  
21 ultrasound (PCO morphology: yes or no). Body weight (kg) and body height (cm) are  
22 measured in an upright position with light clothing and no shoes. BMI is calculated as body  
23 weight (kg) divided by squared body height (m<sup>2</sup>). Waist circumference is measured in  
24 centimetres at the midpoint between the iliac crest and lower rib margin at the end of  
25 expiration, while standing without clothing. Hip circumference is measured in centimetres at  
26 the widest point between waist and thighs. Waist-Hip-Ratio (WHR) is calculated as the ratio  
27 of waist and hip circumferences. Systolic (SBP) and diastolic blood pressure (DBP) is  
28 measured with a semiautomatic blood pressure monitor, and heart rate.  
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32 Each woman (PCOS and controls) is given seven questionnaires to be filled in and returned at  
33 next (baseline) visit. They are asked to start to register their bleeding periods from now until  
34 the end of study.  
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38 An appointment for body composition (lean and fat mass and bone mineral density) measure  
39 with dual energy x-ray absorptiometry (DXA) is given.  
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43 To enable measurements day 6-8 in the menstrual cycle, all women are given information on  
44 how to induce withdrawal bleeding with medroxyprogesterone acetate, 10 mg per day for 7  
45 days (participants in Sweden) or dydrogesteron, 20 mg per day for 10 days (participants in  
46 China).  
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50 *Baseline:* The baseline visit takes place in the morning after an overnight fast on day 6 – 8  
51 after induced withdrawal bleeding in all women (see above). The time point is selected as the  
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3 endometrial lining has to be thicker. The questionnaires are returned and checked. Missing  
4 information in the questionnaires is checked.  
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7 First, a gynaecological examination is performed by transvaginal ultrasound, measuring  
8 ovarian size in three dimensions, total antral follicle count (2-9 mm) and endometrial  
9 thickness (mm).  
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12 Second, if biopsies are taken, local anaesthesia will be placed and an endometrial biopsy is  
13 collected and snap frozen. Immediately after, local anaesthesia is placed close to the  
14 umbilicus and in the vastus lateralis muscle, fat and muscle biopsy are taken.  
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17 Third, a venflon will be placed and fasting blood samples will be drawn for serum and  
18 plasma analyses e.g. *genetic* (e.g. next generation sequencing, SNP, methylation), *metabolic*  
19 (e.g. lipids, adipokines, inflammatory markers) and *endocrine* (e.g. sex steroids,  
20 gonadotropins, growth factors) measures.  
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24 Fourth, an oral glucose tolerance test (OGTT) with 75 g glucose will be performed. Blood  
25 samples is collected to measure plasma glucose and serum insulin at 0, 30, 60, and 120 min  
26 during the OGTT.  
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30 At the baseline visit, after OGTT, all participants will receive lifestyle advice by the study  
31 coordinator. Patient will be told to register daily number of steps and will receive a step-  
32 counter and asked to register menstrual bleeding. If allocated to the electroacupuncture group,  
33 time will be booked and treatment started within one week. If randomized to the metformin  
34 group, the study drug will be administered and the treatment started the next day. The  
35 lifestyle management only group are given appointments for repeated measurements after 4  
36 months and follow-up 4 months later.  
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40 Women with PCOS who are randomized are informed that they should use contraception that  
41 are non-hormonal.  
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46 ***Follow-up 4 months after last treatment:*** All baseline measures are repeated after 4 months  
47 of treatment and at follow-up 4 months after last treatment.  
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#### 50 51 **Randomisation and treatment allocation**

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53 The randomization will employ a minimization algorithm to balance across the following  
54 factors: Age and BMI and are separated by centre. Each study site (Stockholm and Beijing)  
55 use the same randomization and electronic case report form (eCRF). A web-based  
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3 randomization program (<https://data.dynareg.se/pia2/Default.aspx>) has been generated to  
4 ensure allocation concealment. The study coordinators log on the web-based system to  
5 randomize eligible patients. All women who enter the study will be logged and given a  
6 unique study number. Blinding or masking of the intervention will not be possible because of  
7 the nature of the intervention. Importantly, however, the assessor will be blinded to the  
8 patients' group assignment.  
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### 15 **Outcome Measurements**

16 Outcome measures will be collected at:

- 17 1. Baseline
- 18 2. After 4 months of intervention
- 19 3. Follow-up 4 months after last treatment

### 20 **Primary Outcome**

21 At baseline in cases versus controls and in women with PCOS changes from baseline to after  
22 4 months of treatment in

- 23 • HOMA-IR [fasting insulin ( $\mu\text{U}/\text{mL}$ )  $\times$  fasting glucose (mmol/L)] / 22.5], and
- 24 • HbA1c

25 comparing 1) acupuncture + lifestyle management and 2) metformin + lifestyle management  
26 and 3) lifestyle management only.  
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### 29 **Secondary Outcome**

30 At baseline in cases versus controls and in women with PCOS changes from baseline to after  
31 4 months of treatment and from baseline to the 4-month follow-up between 1) acupuncture +  
32 lifestyle management, and 2) metformin + lifestyle management, compared to 3) lifestyle  
33 management only, in the following variables:  
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- 36 • *Detailed characterisation of body composition*: In addition to weight, height and waist  
37 circumference, all women will be examined by DXA to measure lean and fat mass and  
38 bone mineral density using a Lunar Prodigy Advance whole body scanner (GE Medical  
39 Systems)<sup>25</sup>.
- 40 • *Metabolic measures*: Insulin response to glucose during the OGTT (AUC using the  
41 trapezoidal rule), and direct analyses of fasting blood samples of insulin and glucose to  
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enable calculation of HOMA-B /islet  $\beta$ -cell function [ $20 \times$  fasting insulin (mU/mL) / (fasting plasma glucose (mmol/L) – 3.5)]<sup>26</sup>. Further, fasting blood samples are collected and saved for later analyses of e.g. C-peptide and calculation of C-peptide index [Fasting C-peptide (nmol/L)/ f-glucose (mmol/L) x 100]<sup>27</sup>, for analyses of adipokines, inflammatory markers, non-esterified fatty acids (NEFA), total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), high sensitive CRP, catecholamine's; norepinephrine (NE), adrenalin (A) and dopamine (DA).

- *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 4<sup>th</sup> 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), luteinizing hormone (LH), follicle stimulating hormone (FSH), antimüllerian hormone (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 determination of adipocyte size and distribution. Part of tissue biopsies will also be isolated for *in vitro* experiments.<sup>28</sup> Deep RNA, microRNA and/or bisulfite sequencing will be performed with the latest available technology.
- *Health related quality of life*: Will be determined by quality of life by EuroQol-5 dimension (EQ-5D),<sup>29 30</sup> short form-36 (SF36),<sup>31 32</sup> and polycystic ovary syndrome questionnaire (PCOSQ).<sup>33 34</sup>
- *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)<sup>35</sup> to assess psychiatric symptoms within a time frame of the last 3 days in Sweden. For the purpose of this study, two scales will be extracted from the CPRS-S-A,<sup>35</sup> the Brief Scale for Anxiety (BSA-S)<sup>36</sup> and the Montgomery Åsberg Depression Rating Scale (MADRS-S).<sup>37-39</sup> In China will the Zung symptom depressions score (SDS) and Zung symptom



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3 anxiety score (SAS) be used<sup>40 41</sup>. Depression symptoms of potential clinical relevance is  
4 for MADR-S  $\geq 11$  and for Zung SDS  $\geq 0.5$  (Depressive index), Anxiety symptoms of  
5 potential clinical relevance is for BSA-S  $\geq 11$  and for Zung SAS  $\geq 50$  (Standard total  
6 score),  
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- 11 • *Physical Activity*: International Physical Activity Questionnaire (IPAQ) long and short  
12 version will be used to assess physical activity.<sup>42 43</sup> In addition, one text message per week  
13 will be sent to the participants by the study coordinators asking of number of steps the last  
14 week when asking for menstrual bleeding (date).  
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  - 16 • *Eating questionnaire and eating pattern*: Only assessed at baseline using the self-reported  
17 version of the Three-Factor Eating Questionnaire (TFEQ-R21),<sup>44 45</sup> and Questionnaire of  
18 Eating and Weight Patterns-Revised (QEWPR) to measure eating behaviour (Sweden  
19 only).<sup>46</sup>  
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  - 21 • *Side-effects and adverse events* will be continuously and equally recorded in each study  
22 arm. One text message per week will be sent to all participants by the study coordinators  
23 in which they are asked to report (in addition to number of steps) any side effects or  
24 adverse events. All participants will receive a phone call every 4<sup>th</sup> week by the study  
25 coordinator and will be asked about side effects or adverse events.  
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## 34 **Statistical analysis**

### 35 ***Sample size and power calculations***

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38 Samples size calculation derives from our pilot study demonstrating that 5 weeks of treatment  
39 decreases HOMA-IR by mean $\Delta$   $-0.62$ , standard deviation (SD)  $1.21$  and HbA1c by mean $\Delta$   $-$   
40  $1.30$ , SD  $1.40$ .<sup>15</sup> Here we expect a decrease in HOMA-IR from baseline to after 4 months of  
41 treatment with metformin or acupuncture with an anticipated mean difference of  $0.43$  and a  
42 standard deviation of  $1$  compared to lifestyle management alone. With a target power of at  
43 least  $80\%$  and  $5\%$  significance level, we need to recruit  $84$  women per group. We plan to  
44 recruit  $101$  women per group estimating  $20\%$  dropout rate. If statistical power is calculated  
45 based on HbA1c, with an anticipated mean difference of  $0.86$  and a standard deviation of  $1.4$   
46 compared to lifestyle management alone, and with a target power of at least  $80\%$  and  $5\%$   
47 significance level, we would need to recruit  $23$  women ( $28$  if estimating a  $20\%$  dropout) per  
48 group.  
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3 As this is a comparative effectiveness trial we have decided to have two primary outcome  
4 variables and accordingly we have calculated the samples size for the different outcomes. The  
5 primary end-point HOMA-IR requires the highest number of participants. When the intended  
6 number of participants for HbA1c have been reached, an interim analyses will be performed.  
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9 Further, for the mechanistic studies, we estimate that successful tissue samples will be  
10 recruited from a minimum 20 participants in each group in Sweden and China respectively,  
11 giving a strong power to detect differences.  
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### 16 ***Minimizing sources of bias***

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18 Blinding is not possible given the nature of the intervention. We do not feel it is necessary or  
19 ethical to perform sham acupuncture and are confident that the primary outcome is unlikely  
20 to be affected by observer bias.  
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### 24 ***Type of analyses***

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26 The statistical analyses will be performed by qualified statisticians and biostatisticians. The  
27 data in the RCT will be analysed according to the intent-to-treat principle to investigate  
28 differences between the groups.  
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31 *Interim Analysis:* After 114 subjects (number based on samples size calculation of HbA1c)  
32 have finalized the 16 weeks of treatment with follow-up, an interim analysis will be  
33 conducted. The interim data will be used to check assumptions in the sample size calculation  
34 for both co-primary variables and samples size will be recalculated based on observed data.  
35 The stop criterion are meant for both co-primary and covers two group comparisons. The  
36 Haybittle-Peto approach is used and the trial will be ended using symmetric stopping  
37 boundaries at  $P < 0.001$ . Bonferroni will be used for correction for multiple testing. The final  
38 analyses is evaluated at the significance level  $P < 0.05$ , with posthoc corrections as given  
39 below.  
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46 *Clinical outcome measures:* Continuous variables will be presented as means  $\pm$  standard  
47 deviations and categorical variables as medians with interquartile ranges. Between group  
48 comparisons will be carried out with changes from baseline to after treatment and from  
49 baseline to follow-up by ANOVA followed by Dunnet post-hoc test for continuous and  
50 Kruskal-Wallis followed by Mann Whitney U-test or by  $\chi^2$  tests for categorical variables.  
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3 In the case-control part of the study the Student t-test will be used for continuous variables  
4 and Mann Whitney U-test or  $\chi^2$  tests for categorical variables and logistic regression when  
5 needed.  
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7 All statistical analyses of the data will be performed using the SPSS program version 23.0 or  
8 higher (SPSS Inc., Chicago, IL, USA), and a *P*-value < 0.05 will be considered statistically  
9 significant. All tests are two-sided and adjustments for multiple comparisons will be  
10 performed.  
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15 *Expression and methylation data:* These analyses will be adjusted to the technique used. In  
16 brief, raw data will be checked and processed and a quality control report will be completed.  
17 Different analysis pipelines for traceability and track-ability will be performed. Then  
18 extended data analyses, including functional analysis, GeneOntologies, Biological Pathways,  
19 Principle Component Analysis (PCA)-analysis, Clustering, Visualizations and mapping  
20 against a reference genome, will be performed, and data will be submitted to repositories (i.e.  
21 the Array Express: [www.ebi.ac.uk/arrayexpress](http://www.ebi.ac.uk/arrayexpress)).  
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26 Group comparison will be carried out with changes from baseline to after treatment and from  
27 baseline to follow-up by Kruskal-Wallis followed by Mann Whitney U-test for expression  
28 analyses. In the case-control part of the study, Mann Whitney U-test will be used for  
29 expression analyses. False discovery rate (FDR) will be used to correct for multiple testing in  
30 the analyses of gene and methylation arrays.  
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### 37 **Ethics and Dissemination**

38 The study is performed according to good clinical practice and conducted in accordance with  
39 the Declaration of Helsinki. The study has been approved by the Regional Ethical Review  
40 Board of Stockholm, Sweden Dnr: 2015/1656-31/2 and by the Regional Ethical Review  
41 Board of Peking University Third Hospital, China Dnr: 2016-212-02. In addition, the  
42 Medical Products Agency have approved the study: EudraCT: 2015-004250-18 and the trial  
43 is registered at Clinicaltrials.gov: NCT02647827. Reporting of the study results will follow  
44 the 2010 revised CONSORT statement and STRICTA.<sup>47 48</sup> Primary outcome data the RCT  
45 will be published in a relevant journal together with supporting secondary outcome  
46 measurements. Further, secondary outcome measurements will be published in separate  
47 papers as well as case-control data.  
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3 The relevance of this study is that it has potential to uncover new knowledge in the  
4 pathophysiology of the disorder and result an additional treatment strategy for insulin  
5 resistant in women with PCOS and related diseases, including obesity, insulin resistance, and  
6 T2D. Thus, it may have an impact on both genders and does not apply only to women with  
7 PCOS.  
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### 11 **Trial status**

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13 The study was conceived and designed during 2015. The first participant was recruited and  
14 randomized in February 2016 in Sweden and September 2016 in China. We anticipate that all  
15 participants are recruited by the end of 2019 with follow-up done during 2020.  
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31 role in data collection and analysis, decision to publish, or preparation of the manuscripts.  
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### 40 **Competing interest**

41  
42 The authors declare that they have no competing interests.  
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### 45 **Author contribution**

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47 The authors declare that they have no competing interests.  
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**Table 1:** Acupuncture points, stimulation, localization, tissue in which needles are inserted, and innervation areas. The two sets will be alternated for every other treatment.

Acupuncture point	Stimulation	Localization	Muscle	Muscle innervation
<b>Set 1</b>				
CV4, <i>Guan Yuan</i>	EA	3 cun caudal to the umbilicus	Fibrous tissue, linea alba	L1
CV12, <i>Zhongwan</i>	EA	On the midline, 4 cun superior to the umbilicus	Fibrous tissue, linea alba	Th7–8
ST29 Bilateral, <i>Guilai</i>	EA	1 cun cranial to the pubic bone and 2 cun lateral of the midline	M. rectus abdominis	Th6–12
ST34 Bilateral, <i>Futu</i>	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
ST32 Bilateral, <i>Liangqiu</i>	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
SP6 Bilateral, <i>Sanyinjiao</i>	DeQi, four times	3 cun proximal to the medial malleolus	Mm. flexor digitorum longus, tibialis posterior	L4–5, S1–2
ST36 Bilateral, <i>Zusanli</i>	DeQi, four times	On the anterior lateral side of the leg, 3 cun below <i>Dubi</i> (ST35), one finger width (middle finger) from the anterior crest of the tibia	Musculi tibialis anterior	L4–5, S1
LI4 <i>Hegu</i>	Bilateral, DeQi, four times	On the highest point at m. interosseus dorsalis	Mm. interosseus dorsalis I, lumbricalis II, adductor pollicis	C8, Th1

**Set 2**

CV6, <i>Qihai</i>	EA	1.5 cun caudal to the umbilicus	Fibrous tissue, linea alba	Th11
CV10, <i>Xiawan</i>	EA	2 cun cranial to the umbilicus	Fibrous tissue, linea alba	Th8
ST27 Bilateral, <i>Daju</i>	EA	3 cun cranial to the pubic bone and 2 cun lateral to the midline	M. rectus abdominis	Th6–12
Extra meridian point Bilateral	EA	6 cun above the patella in line with SP10	M. quadriceps femoris	L2–L4
SP10 Bilateral, <i>Xuehai</i>	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
ST38 Bilateral, <i>Sanyinjiao</i>	DeQi four times	Between lateral malleolus and knee joint, 1 finger from tibiae crist	Musculi tibialis anterior	L4–5, S1
LR3 Bilateral, <i>Taichong</i>	DeQi four times	Between metatarsal I & II, just distal to the caput	M. Interosseus dorsalis I	S2–3
PC6 Bilateral, <i>Neiguan</i>	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

C: Cervical vertebra; CV: Conception vessel; L: Lumbar vertebra; LI: Large intestine; LR: Liver; PC: Pericardium; S: Sacral vertebra; SP: Spleen; ST: Stomach; Th: Thoracic vertebra.

**Table 2.** Overview of the study visits.

	Screening visit	Base line visit	Month				Follow-up after 4 months of treatment	Follow-up 4 months after last treatment
			1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>		
<b>Anthropometry:</b> Body composition (weight, height, waist circumference, hip circumference), FG/acne, blood pressure	X						X	X
<b>Menstrual cycle diary</b>	X	X	X	X	X	X	X	X
<b>Questionnaires:</b> EQ-5D, SF36, PCOSQ, CPRS-SA*, Zung SAS <sup>#</sup> , Zung SDS <sup>#</sup> , IPAQ, TFEQ-R21, QEWP-R*	X						X	X
Transvaginal ultrasound		X					X	X
<b>Metabolic measures:</b> Fasting blood samples for glucose, insulin, HbA1c, c-peptide, OGTT. Adipokines, lipid profile (LDL, HDL, NEFA) and inflammatory markers		X					X	X
<b>DXA</b>		X					X	X
<b>Endocrine measures:</b> Fasting blood samples for sex steroids, SHBG, LH, FSH, AMH, prolactin, TSH, T4		X					X	X
<b>Tissue and whole blood collection</b>		X					X	X

Antimüllerian hormone (AMH); dual energy x-ray absorptiometry (DXA); EuroQol-5 dimension (EQ-5D); Ferriman–Gallwey score (FG); follicle stimulating hormone (FSH); high density lipoprotein (HDL); hsCRP, International Physical Activity Questionnaire (IPAQ); low density lipoprotein (LDL), luteinizing hormone (LH); non-esterified fatty acids (NEFA); norepinephrine (NE), oral glucose tolerance test (OGTT); polycystic ovary syndrome questionnaire (PCOSQ); Questionnaire of Eating and Weight Patterns-Revised (QEWP-R); sex hormone binding globulin

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2  
3 (SHBG); short form-36 (SF36); Three-Factor Eating Questionnaire (TFEQ-R21); thyroid  
4 stimulating hormone (TSH); thyroxine (T4); Zung Self-Rating Anxiety Scale (Zung SAS); Zung  
5 Self-Rating Depression Scale (Zung SDS).  
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7 \* only in Sweden and # only in China.  
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For peer review only

# BMJ Open

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

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Complete List of Authors:	<p>Stener-Victorin, Elisabet; Karolinska Institutet, Department of Physiology and Pharmacology</p> <p>Zhang, Haolin; Peking University Third Hospital, Department of Traditional Chinese Medicine (TCM)</p> <p>Rong, LI; Peking University Third Hospital, OB &amp; GYN</p> <p>Friden, Cecilia; Karolinska Institutet, 5. Department of Neurobiology, Care Sciences and Society, Division of Physiotherapy</p> <p>Li, Dong; Peking University Third Hospital, Department of Traditional Chinese Medicine (TCM)</p> <p>Wang, Wei; Peking University Third Hospital, 4. Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China</p> <p>Wang, Haining ; Peking University Third Hospital, Department of Endocrinology and Metabolism, Peking University Third Hospital, Beijing, China</p> <p>Chang , Cuiqing; Peking University Third Hospital, Institute of Sports Medicine</p> <p>Li , Shi; Peking University Third Hospital, 3. Center for Reproductive Medicine, Department of Obstetrics and Gynecolog</p> <p>Huo , ZeJun ; Peking University Third Hospital, Research Center of Clinical Epidemiology</p> <p>Zhang, Hua; Peking University Third Hospital, Research Center of Clinical Epidemiology</p> <p>Ji, Xiaolan ; Peking University Third Hospital, Department of Traditional Chinese Medicine (TCM)</p> <p>Linden-Hirschberg, Angelica; Karolinska Institutet, Department of Obstetrics and Gynecology</p> <p>Jie, Qiao; Peking University Third Hospital, Center for Reproductive Medicine, Department of Obstetrics and Gynecology and Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China</p>
<b>Primary Subject Heading</b>:	Diabetes and endocrinology
Secondary Subject Heading:	Complementary medicine
Keywords:	glucose homeostasis, insulin resistance, acupuncture, life style, polycystic ovary syndrome

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Manuscripts

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3 **1 Acupuncture or metformin to improve insulin resistance in women with polycystic**  
4 **2 ovary syndrome: Study protocol of a combined multinational cross sectional case-**  
5 **3 control and a randomized controlled trial**

4 Elisabet Stener-Victorin<sup>1#\*</sup>, Haolin Zhang<sup>2</sup>, Rong Li<sup>3,4</sup>, Cecilia Fridén<sup>5</sup>, Dong Li<sup>2</sup>, Wei  
5 Wang<sup>4</sup>, Haining Wang<sup>6</sup>, Cuiqing Chang<sup>7</sup>, Shi Li<sup>3</sup>, ZeJun Huo<sup>2</sup>, Hua Zhang<sup>8</sup>, Xiaolan Ji<sup>2</sup>,  
6 Angelica Linden Hirschberg<sup>9</sup>, Jie Qiao<sup>3,4\*</sup>

- 7 1. Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm,  
8 Sweden  
9 2. Department of Traditional Chinese Medicine (TCM), Peking University Third  
10 Hospital, Beijing, China.  
11 3. Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking  
12 University Third Hospital, Beijing, China.  
13 4. Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing,  
14 China  
15 5. Department of Neurobiology, Care Sciences and Society, Division of Physiotherapy,  
16 Karolinska Institutet, Huddinge, Sweden  
17 6. Department of Endocrinology and Metabolism, Peking University Third Hospital,  
18 Beijing, China  
19 7. Institute of Sports Medicine, Peking University Third Hospital, Beijing, China  
20 8. Research Center of Clinical Epidemiology, Peking University Third Hospital, Beijing,  
21 China.  
22 9. Department of Obstetrics and Gynecology, Karolinska Institutet, Stockholm, Sweden

23  
24 **Short title:** Acupuncture or metformin for insulin resistance in PCOS

25 \* Shared senior authors contributed equally to this paper

26 **# Corresponding authors and reprint requests**

27 Elisabet Stener-Victorin

28 Department of Physiology and Pharmacology, Biomedicum QB5

29 Karolinska Institutet, 171 65 Stockholm, Sweden

30 Phone: +46(0)705643655

31 E-mail: [elisabet.stener-victorin@ki.se](mailto:elisabet.stener-victorin@ki.se)

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33 **Author Contributions**

34 ESV, HZ, RL, ALH and JQ conceived and designed the study. ESV and JQ sought funding  
35 and ethical approval. CF, DL, WW, HW, CC, SL, ZH, HZ, XJ and ALH recruited and  
36 screened subjects, coordinated and carried out acupuncture treatment. ESV drafted the  
37 manuscript. All authors read and approved the final manuscript.

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3 39 **Abstract**

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5 40 **Introduction:** Polycystic ovary syndrome (PCOS) is linked to hyperinsulinemia and insulin  
6 41 resistance with dysfunctional glucose metabolism. Pilot studies suggests that acupuncture  
7 42 treatment with combined manual and low-frequency electrical stimulation  
8 43 (electroacupuncture, EA) of the needles decrease circulating glycated hemoglobin (HbA1c)  
9 44 and homeostatic model assessment-insulin resistance (HOMA-IR). Therefore, we here aim to  
10 45 investigate if acupuncture treatment or metformin together with life style or life style  
11 46 management alone improve insulin sensitivity and related symptoms in overweight/obese  
12 47 women with PCOS.

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18 48 **Methods and analysis:** This is a two centre multinational (Sweden and China), cross-  
19 49 sectional case-control study combined with an open-labelled randomized controlled trial  
20 50 (RCT). Participants are randomized to one of three groups: 1) EA 2-3 times/week during 4  
21 51 months + lifestyle management; 2) metformin, 500 mg, three/day during 4 months + lifestyle  
22 52 management; or 3) life style management alone. The primary outcome measure in the RCT is  
23 53 changes in Hba1C. A total of 123 obese overweight women with PCOS will be enrolled and  
24 54 randomized into one of the three groups with a target power of at least 80% and 5%  
25 55 significance level based on two-sided tests.

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31 56 **Ethics and dissemination:** The study has been approved by the Regional Ethical Review  
32 57 Board of Stockholm and of Peking University Third Hospital, China. Primary outcome data  
33 58 of the RCT will be published in a relevant journal together with supporting secondary  
34 59 outcome measurements. Further, outcome measurements will be published in separate papers  
35 60 as well as case-control data.

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40 61 **Expected results:** We anticipate that EA and metformin, both with lifestyle management are  
41 62 equally effective and superior to lifestyle management alone for improvement of glycemic  
42 63 control.

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45 64 **Trial registration:** Clinicaltrials.gov: NCT02647827 and EudraCT: 2015-004250-18.

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49 66 **Key words:** glucose homeostasis; insulin resistance; metformin; electroacupuncture; lifestyle  
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3 68 **Strength and Limitations**  
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5 69 1. A strength of this trial is that all patients will benefit from receiving treatment, all of  
6 70 which alone and/or in combination may offer an increased chance for improved  
7 71 metabolic function and reproductive health.  
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10 72 2. It has the potential to gain deeper insight into the pathophysiology of polycystic ovary  
11 73 syndrome (PCOS), and to uncover new knowledge for treatment of insulin resistant in  
12 74 related diseases, including obesity, insulin resistance, and type 2 diabetes (T2D).  
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16 75 3. The results from the present study have the potential to immediately be implemented  
17 76 into the healthcare system since it has previously been shown to be cost-effective and  
18 77 to have few negative side effects.  
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21 78 4. A potential limitation is that metformin might cause side-effects such as diarrhoea,  
22 79 nausea/vomiting, flatulence, asthenia, indigestion, abdominal discomfort and  
23 80 headache and acupuncture local skin irritation, discomfort, and vasovagal reactions  
24 81 during the procedure.  
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## 87 **Background**

88 Polycystic Ovary Syndrome (PCOS) is the most common endocrine and metabolic disorder  
89 in women of reproductive age and is characterized by anovulation, hyperandrogenism and  
90 metabolic dysfunction.<sup>1</sup> Women with PCOS have a 3 to 7-fold increased risk of developing  
91 type 2 diabetes (T2D), and with younger onset, PCOS increases cardiovascular risk factors<sup>1-6</sup>.  
92 Independent of body weight, insulin sensitivity is ~40% lower in women with PCOS than in  
93 healthy women, and impaired glucose regulation, insulin resistance and reduced insulin  
94 responsiveness have been attributed to defects in insulin signalling in adipocytes and skeletal  
95 muscle.<sup>7,8</sup> Of note, obesity aggravates all symptoms related to PCOS.

96 Despite detrimental impact on women's health, the aetiology of PCOS is not well understood.  
97 Genetic, epigenetic, and environmental factors have all been implicated in its development.  
98 Emerging evidence suggests that PCOS originates, at least in part, in fetal life,<sup>9,10</sup> and  
99 elevated maternal androgens have been implicated to play a role, however the mechanisms  
100 are largely unknown.<sup>11,12</sup> Of interest is that we have found that women with PCOS have  
101 multiple transcriptional and epigenetic changes in adipose tissue that are relevant for  
102 development of the disease.<sup>13</sup> Further, twin studies suggest that genetic influences explain  
103 >70% of PCOS pathogenesis.<sup>14</sup> However, whether genetic or epigenetic alterations in target  
104 tissues e.g. adipose tissue, skeletal muscle and endometrium contribute to development of  
105 metabolic disease requires further investigation.

106 Women with PCOS require long-term individualized treatment programs. Pharmacological  
107 treatments, including the glucose reducing drug metformin, have limitations related to  
108 adverse effects and patient compliance. Therefore, there is a need for inexpensive and easily  
109 administered treatments with few negative side-effects. Lifestyle management is the first line  
110 treatment eventually with addition of metformin for improving whole body glucose  
111 homeostasis and preventing type 2 diabetes (T2D).<sup>15-17</sup> Interestingly, five weeks of  
112 acupuncture with combined manual and low-frequency electrical stimulation has in a pilot  
113 study been shown to improve whole body glucose homeostasis in insulin resistant women  
114 with PCOS.<sup>18</sup> The pilot study was an uncontrolled trial and it is therefore of importance to  
115 compare the effect of acupuncture with first line treatment to investigate the effectiveness.

116 Whilst pharmacological treatment strategies have shown efficacy, importantly, there is a need  
117 for Comparative Effectiveness Research (CER) to strengthen the evidence base for clinical  
118 and policy decision-making<sup>19</sup>. Therefore we aim to compare the effect of pharmacological  
119 first-line treatment, metformin, with a non-pharmacological treatment strategy, acupuncture

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3 120 (both together with lifestyle management), with lifestyle management for improvement and  
4 121 prevention of metabolic dysfunction and related symptoms in insulin resistant women with  
5 122 PCOS.

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8 123 Our main hypothesis is that acupuncture and metformin (both treatments combined with  
9 124 lifestyle management) are superior to lifestyle management alone in improving whole body  
10 125 glucose regulation in insulin resistant women with PCOS. Secondary hypotheses are that  
11 126 these treatments have the potential to improve metabolic- and endocrine measures, quality of  
12 127 life and symptom of anxiety and depression, and to restore epigenetic and molecular  
13 128 alterations in target tissues (endometrial-, adipose-, and skeletal muscle tissue) and thus have  
14 129 the potential to improve and potentially prevent the development of metabolic alterations  
15 130 including T2D.

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18 131 Thus, the purpose of this study is twofold, first we aim to gain deeper insight into the  
19 132 pathophysiology of PCOS in a cross sectional case-control study by comparing women with  
20 133 PCOS with women without PCOS matched for age, weight and BMI, and secondly we aim to  
21 134 perform a prospective RCT of women with PCOS.

### 22 23 24 25 26 27 28 29 135 **Study design**

30 136 This is a two centre multinational prospective trial with a cross-sectional case-control part  
31 137 and an open-labelled RCT with a comparative effectiveness design. The interventions to be  
32 138 tested are 1) Electroacupuncture during 4 months + lifestyle management; 2) Metformin  
33 139 during 4 months + lifestyle management; or 3) Lifestyle management alone which will be  
34 140 available for participants in all three groups. Participants will be enrolled at Karolinska  
35 141 Institutet and Karolinska University Hospital, Stockholm, Sweden and at Peking University  
36 142 Hospital, Beijing China respectively.

### 37 38 39 40 41 42 43 143 **Randomisation and treatment allocation**

44 144 The randomization is stratified across the factors age and BMI and is also separated by study  
45 145 site with a balanced allocation ratios 1:1:1. Randomization is performed in blocks with a  
46 146 variable block size between 3 and 15; e.g. First there is a block of 12, when it is full it is  
47 147 followed by a block of 9 and thereafter a block of 3. The order of the block sizes are  
48 148 unknown to the participating study sites and also differs among the strata's.

49 149 Each study site (Stockholm and Beijing) use the same randomization and electronic case  
50 150 report form (eCRF). A web-based randomization program

51 151 (<https://data.dynareg.se/pia2/Default.aspx>) has been generated to ensure allocation

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3 152 concealment. The study coordinators log on the web-based system to randomize eligible  
4 153 patients. All women who enter the study will be logged and given a unique study number.  
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6 154 Blinding or masking of the intervention will not be possible because of the nature of the  
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8 155 intervention. Importantly, however, the assessor will be blinded to the patients' group  
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10 156 assignment.

## 11 157 **Patient and Public Involvement**

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13 158 Patients and or public was not involved in the design of this study.  
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## 16 159 **Study Objectives**

### 17 160 **Primary Objective**

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19 161 1. To determine the clinical effectiveness of 4 months of 1) electroacupuncture +  
20 162 lifestyle management and 2) metformin + lifestyle management, compared to 3)  
21 163 lifestyle management only for improvement of glucose regulation assessed by HbA1c  
22 164 levels.  
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### 26 165 **Secondary Objectives**

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28 166 1. To evaluate changes in secondary metabolic measures including the insulin response  
29 167 to glucose assessed by calculating the area under the curve ( $AUC_{\text{insulin}}$ ) during the oral  
30 168 glucose tolerance test (OGTT), fasting insulin, glucose, calculation of homeostatic  
31 169 model assessment (HOMA)-IR and-HOMA-B (*i.e.* the Islet  $\beta$ -cell function) and the  
32 170 assessment of e.g. adipokines, lipid profile, body size and proportions and body fat  
33 171 distribution.  
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36 172 2. To determine changes in gene expression and DNA methylation profiles related to  
37 173 insulin sensitivity in fat, muscle and endometrial tissue biopsies, and biomarkers in  
38 174 whole blood.  
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41 175 3. To evaluate endocrine measures including menstrual pattern and ovulation frequency,  
42 176 circulating hormones (e.g. sex steroids, AMH, gonadotropins).  
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45 177 4. To determine changes in women's health related quality of life (HRQoL), symptoms  
46 178 of anxiety and depression, dieting and eating patterns, and negative side-effects.  
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## 50 179 **Outcome Measurements**

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52 180 Outcome measures will be collected at:

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54 181 1. Baseline  
55 182 2. After 4 months of intervention  
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3 183 3. Follow-up 4 months after last treatment  
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5 184 **Primary Outcome**

6 185 Changes from baseline to after 4 months of treatment in HbA1c comparing 1) acupuncture +  
7 186 lifestyle management and 2) metformin + lifestyle management, respectively with 3) lifestyle  
8 187 management only. In the cross sectional study, difference in HbA1c between cases and  
9 188 controls.

10 189 **Secondary Outcome**

11 190 Changes from baseline to after 4 months of treatment and from baseline to the 4-month  
12 191 follow-up comparing 1) acupuncture + lifestyle management, and 2) metformin + lifestyle  
13 192 management, respectively, with 3) lifestyle management only, and in the cross sectional  
14 193 study, difference between cases and controls in the following variables:

- 15 194 • *Body composition*: In addition to weight, height and waist circumference, women will be  
16 195 examined by DXA to measure lean and fat mass and bone mineral density using a Lunar  
17 196 Prodigy Advance whole body scanner (GE Medical Systems)<sup>20</sup>.
- 18 197 • *Metabolic measures*: Insulin response to glucose during the OGTT (AUC using the  
19 198 trapezoidal rule), and direct analyses of fasting blood samples of insulin and glucose to  
20 199 enable calculation of HOMA-IR [fasting insulin ( $\mu\text{U}/\text{mL}$ )  $\times$  fasting glucose ( $\text{mmol}/\text{L}$ )] /  
21 200 22.5], and HOMA-B /islet  $\beta$ -cell function [ $20 \times$  fasting insulin ( $\text{mU}/\text{mL}$ ) / (fasting plasma  
22 201 glucose ( $\text{mmol}/\text{L}$ ) - 3.5)]<sup>21</sup>. Further, fasting blood samples are collected and saved for  
23 202 later analyses of e.g. C-peptide and calculation of C-peptide index [Fasting C-peptide  
24 203 ( $\text{nmol}/\text{L}$ ) / f-glucose ( $\text{mmol}/\text{L}$ )  $\times$  100]<sup>22</sup>, for analyses of adipokines, inflammatory markers,  
25 204 non-esterified fatty acids (NEFA), total cholesterol, triglycerides, high density lipoprotein  
26 205 (HDL), low density lipoprotein (LDL), high sensitive CRP, catecholamine's and  
27 206 metabolites analysed on a split-fraction HPLC-ED system<sup>23</sup>.
- 28 207 • *Endocrine measures*: Menstrual frequency: Participants will be asked to note date of  
29 208 menstruation which will be reported to the study coordinator once per week by text  
30 209 message and every 4<sup>th</sup> week by phone. Ovarian morphology antral follicle count and  
31 210 ovarian volume. Blood samples will be drawn for analyses of sex steroids by the validated  
32 211 gas- and liquid chromatography/tandem mass spectroscopy technique, as well as sex  
33 212 hormone binding globulin (SHBG), luteinizing hormone (LH), follicle stimulating  
34 213 hormone (FSH), antimüllerian hormone (AMH), prolactin, thyroid stimulating hormone  
35 214 (TSH) and free thyroxine (T4).

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3 215 • *Tissue and whole blood collection*: Whole blood will be collected for DNA and  
4 216 microRNA analyses. Endometrial, fat and skeletal muscle tissue biopsies will be collected  
5 217 at baseline (cases and controls), after 4 months of treatment and at follow-up 4 months  
6 218 after treatment in women with PCOS, snap frozen in liquid nitrogen within 30 s and stored  
7 219 at -80°C for later analyzes. Fat cells will be isolated for determination of adipocyte size  
8 220 and distribution. Part of tissue biopsies will also be isolated for *in vitro* experiments.<sup>24</sup>  
9 221 Deep RNA, microRNA and/or bisulfite sequencing will be performed with the latest  
10 222 available technology.
- 11 223 • *Health related quality of life*: Will be determined by quality of life by EuroQol-5  
12 224 dimension (EQ-5D),<sup>25 26</sup> short form-36 (SF36),<sup>27 28</sup> and polycystic ovary syndrome  
13 225 questionnaire (PCOSQ).<sup>29 30</sup>
- 14 226 • *Symptoms of anxiety and depression* will be assessed by the self-reported version of the  
15 227 Comprehensive Psychopathological Rating Scale for Affective Syndromes (CPRS-S-A)<sup>31</sup>  
16 228 to assess psychiatric symptoms within a time frame of the last 3 days in Sweden. For the  
17 229 purpose of this study, two scales will be extracted from the CPRS-S-A,<sup>31</sup> the Brief Scale  
18 230 for Anxiety (BSA-S)<sup>32</sup> and the Montgomery Åsberg Depression Rating Scale (MADR-  
19 231 S).<sup>33-35</sup> In China will the Zung symptom depressions score (SDS) and Zung symptom  
20 232 anxiety score (SAS) be used<sup>36 37</sup>. Depression symptoms of potential clinical relevance is  
21 233 for MADR-S  $\geq 11$  and for Zung SDS  $\geq 0.5$  (Depressive index), Anxiety symptoms of  
22 234 potential clinical relevance is for BSA-S  $\geq 11$  and for Zung SAS  $\geq 50$  (Standard total  
23 235 score),
- 24 236 • *Physical Activity*: International Physical Activity Questionnaire (IPAQ) will be used to  
25 237 assess degree of physical activity.<sup>38 39</sup> In addition, one text message per week will be sent  
26 238 to the participants by the study coordinators asking of number of steps the last week when  
27 239 asking for menstrual bleeding (date).
- 28 240 • *Eating questionnaire and eating pattern*: Only assessed at baseline using the self-reported  
29 241 version of the Three-Factor Eating Questionnaire (TFEQ-R21),<sup>40 41</sup> and Questionnaire of  
30 242 Eating and Weight Patterns-Revised (QEWPR) to measure eating behaviour (Sweden  
31 243 only).<sup>42</sup>
- 32 244 • *Side-effects and adverse events* will be continuously and equally recorded in each study  
33 245 arm. One text message per week will be sent to all participants by the study coordinators  
34 246 in which they are asked to report (in addition to number of steps) any side effects or

247 adverse events. All participants will receive a phone call every 4<sup>th</sup> week by the study  
248 coordinator and will be asked about side effects or adverse events.

### 249 **Participants**

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

### 256 ***Inclusion criteria – women with PCOS***

- 257 1. Age  $\geq 18$  to  $\leq 40$  years
- 258 2. Body mass index (BMI)  $\geq 25$  to  $\leq 40$  given that 95% of all women with PCOS with a  
259 BMI  $\geq 25$  are insulin resistant.<sup>43 44</sup>
- 260 3. PCOS diagnosis according to Rotterdam criteria 2003<sup>45</sup>, with at least two of the  
261 following three symptoms: Clinical and/or biochemical signs of hyperandrogenism  
262 (hirsutism or acne); oligo/amenorrhea; and/or polycystic ovaries (PCOS).  
263 Biochemical hyperandrogenism is defined by total testosterone  $> 1.2$  nmol/L or a free  
264 androgen index (FAI)  $> 5$ .<sup>46</sup> Hirsutism is defined as a self-reported Ferriman-Gallwey  
265 (FG) score  $\geq 8$  ( $\geq 5$  Asian).<sup>47 48</sup> Acne is defined by a positive response to the question  
266 *Do you have acne?* Oligomenorrhea is defined as an intermenstrual interval  $> 35$  days  
267 and  $< 8$  menstrual bleedings in the past year. Amenorrhea as  $< 3$  cycles per year. PCO  
268 is defined by transvaginal ultrasound with  $\geq 12$  follicles 2–9 mm and/or ovarian  
269 volume  $\geq 10$  ml in one or both ovaries.
- 270 4. Willing to sign the consent form.

### 271 ***Inclusion criteria – controls***

272 Controls should have BMI  $\geq 25$  to  $\leq 40$ , regular cycles with 28 days  $\pm$  2 days, and no signs of  
273 hyperandrogenism. They are excluded if they have menstrual irregularities, signs of  
274 hyperandrogenism (FG  $> 4$ ), or evidence of PCO morphology on ultrasound.

### 275 ***Exclusion criteria for all women***

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- 2
- 3 276 1. Exclusion of other endocrine disorders such as non-classic congenital adrenal
- 4 277 hyperplasia (17-hydroxyprogesterone < 3nmol/L), androgen secreting tumors or
- 5 278 suspected Cushing's syndrome.
- 6
- 7
- 8 279 2. Having known renal disease (creatinine clearance < 60 mL/min), hepatic
- 9 280 insufficiency, autoimmune disorders or cancer.
- 10
- 11 281 3. Any acute condition with potential to alter renal function or cause tissue hypoxia.
- 12
- 13 282 4. Type I diabetes.
- 14
- 15 283 5. Pharmacological treatment (cortizon, antidepressant, other antidiabetic treatment such
- 16 284 as insulin and acarbose, hormonal contraceptives, hormonal ovulation induction or
- 17 285 other drugs judged by discretion of investigator) within 12 weeks. Depo Provera or
- 18 286 similar within 6 months.
- 19
- 20
- 21 287 6. Hypersensitivity to metformin hydrochloride or to any of the excipients.
- 22
- 23 288 7. Blood pressure >160 / 100 mmHg
- 24
- 25 289 8. Pregnancy or breastfeeding the last 6 months
- 26
- 27 290 9. Acupuncture the last 2 months
- 28
- 29 291 10. Daily smoking and alcoholic intake
- 30
- 31 292 11. Language barrier or disabled person with reduced ability to understand the
- 32 293 information given.
- 33

34 294 In total 50 controls will be matched at baseline (age, weight and BMI) to women with PCOS.  
35  
36 295 Controls will undergo screening and baseline visit, but will not be randomized to any  
37  
38 296 treatment.

### 39 40 41 297 **Interventions**

42 298 Participants fulfilling the inclusion criteria will be randomized to one of three groups after  
43  
44 299 baseline measurements:

- 45
- 46 300 1. Electroacupuncture 2-3 times/week during 4 months + lifestyle management
- 47
- 48 301 2. Metformin, 500 mg, three times/day during 4 months + lifestyle management
- 49
- 50 302 3. Lifestyle management alone which will be available for participants in all three
- 51 303 groups.
- 52

53 304 **Lifestyle management:** All women will receive lifestyle management instructions at the  
54  
55 305 baseline visit, before randomization. The lifestyle management involves one initial



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3 306 counselling session in connection with the baseline visit, which includes information about  
4 307 the importance of weight management, healthy diet and physical activity. Focus will be on  
5 308 the importance of physical activity. Each participant will receive a book with lifestyle advice  
6 309 about weight reduction, maintenance and physical activity following WHO  
7  
8 310 recommendations. All participants will receive a text message once weekly to respond  
9  
10 311 number of step collected by their smart phone or step counter during the last week and if they  
11  
12 312 have had any menstrual bleedings. Once every fourth week, study coordinator will call to the  
13  
14 313 participant and ask about number of step last week, menstruation and compliance and side-  
15  
16 314 effects.

17  
18 315 **Electroacupuncture:** Women randomized to receive acupuncture will start their treatment  
19  
20 316 within one week after baseline measurements. The rationale of the acupuncture protocol is  
21  
22 317 based on Western Medical Acupuncture theories. We will use a fixed acupuncture protocol  
23  
24 318 following the two pilot studies: ClinicalTrial.gov NCT01457209 and NCT02026323 with two  
25  
26 319 modifications. First, the treatment period will be 16 weeks (i.e. 4 months) compared to 5  
27  
28 320 weeks and 6 months in the previous pilot studies. Second, the treatment frequency will be 2  
29  
30 321 to 3 times per week during 16 weeks, i.e. in total 32 to 48 acupuncture treatments over 16  
31  
32 322 weeks. The rationale for these changes is that the procedure is time-consuming for the patients  
33  
34 323 and this will increase the feasibility and likely reduce the number of dropouts. Acupuncture  
35  
36 324 treatment will be given by registered physiotherapists or medical doctors educated in  
37  
38 325 theoretical and practical acupuncture and trained to follow the fixed protocol.  
39  
40 326 Disposable, single-use, sterilized CE marked needles made of stainless steel, 0.25 x 30 mm  
41  
42 327 and 0.30 x 40/50 mm (XENO, HEGU Svenska AB, Landsbro, Sweden and in China Huatuo,  
43  
44 328 Suzhou Medical Co Ltd, China) will be inserted to a depth of 15–40 mm in segmental  
45  
46 329 acupuncture points located in abdominal and leg muscles, with innervations corresponding to  
47  
48 330 the ovaries and the pancreas. Two sets of acupuncture points will be alternated every second  
49  
50 331 treatment (Table 1). The first set of acupuncture points include points located in abdominal  
51  
52 332 muscles: conception vessel (CV)4, CV12, stomach (ST)29 bilaterally, and in quadriceps  
53  
54 333 muscle, ST32 and ST34, and in the muscles below the knee, spleen (SP)6, and ST36  
55  
56 334 bilaterally. In addition, needles are placed in the hand, large intestinal (LI)4, bilateral. All  
57  
58 335 needles will be stimulated manually when inserted. CV4 and 12, ST29 bilateral, ST32 and  
59  
60 336 ST34 bilateral will be connected to an electrical stimulator and stimulated with low-  
337  
338 frequency EA of 2 Hz (Stimulators used in Sweden: Export Abteilung, Schwa-Medico GmbH,  
Wetzlarer Str. 41-43;35630 Ehringshausen and in China: Shanghai Huayi Electric

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2  
3 339 Acupuncture Instrument: G6805-1A) for 30 min at each treatment. The intensity will be  
4 340 adjusted by the physiotherapist to produce local muscle contractions without pain or  
5 341 discomfort, and thereafter will the patient monitor the stimulation intensity. Six additional  
6 342 points are selected to strengthen the effect: LI4, ST36, and SP6, and they will be stimulated  
7 343 manually by rotating the needle to evoke needle sensation every 10 min.

8 344 The second set of acupuncture points include abdominal points: ST27 bilaterally (EA), CV6  
9 345 to CV10 (EA); and leg points: SP10 to a non-acupuncture point located 6 cun proximal of  
10 346 patellas medial border (EA) are all connected to an electrical stimulator and stimulated as in  
11 347 the first set of acupuncture points. Six additional points; ST38, liver (LR)3 and pericardium  
12 348 (PC)6, all bilateral, are stimulated manually by rotating the needle to evoke needle sensation  
13 349 every 10 min.

14 350 Compliance: If a participant in the acupuncture group deviate considerably form the study  
15 351 protocol, the acupuncturists should inform the study coordinator. Any negative side effects  
16 352 during treatment are recorded.

17 353 Metformin: Oral metformin 500 mg three times daily, in total 1500 mg per day<sup>49 50</sup>. To  
18 354 reduce gastrointestinal side-effects of metformin, the dose will be slowly escalated starting  
19 355 with 500 mg daily during the first week, increasing to 500 mg twice per day during the  
20 356 second the week, and 500 mg three times daily, morning, lunch and dinner from the third  
21 357 week in total 16 weeks including the 3 weeks step-up phase (i.e. 4 months). Patients with  
22 358 negative effects can remain 500 mg during the remaining weeks.

23 359 Compliance: Empty bottles are handed over to the study coordinator after 16 weeks of  
24 360 treatment and number of tablets are counted. Also, once per month, the study coordinator call  
25 361 the participant and ask her to count the number of tablets there are left in the bottle.

## 26 362 **Study Procedure**

27 363 Screening: The study coordinator describes the study design in detail and written informed  
28 364 consent is collected. Of note, if a participant hesitates to go through tissue sampling as  
29 365 described below, this is not an exclusion criteria. All other outcome measures will be  
30 366 collected and are listed in Table 2.

31 367 In all participants, a comprehensive anamnestic interview will be conducted, including  
32 368 menstrual frequency, hirsutism - FG and acne determined by an affirmative answer to the  
33 369 question “Do you have excessive acne? yes or no”, heredity, medication or other diseases.  
34 370 The physical examination including gynaecological examination is performed by transvaginal

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3 371 ultrasound (PCO morphology: yes or no). Body weight (kg) and body height (cm) are  
4 372 measured in an upright position with light clothing and no shoes. BMI is calculated as body  
5 373 weight (kg) divided by squared body height (m<sup>2</sup>). Waist circumference is measured in  
6 374 centimetres at the midpoint between the iliac crest and lower rib margin at the end of  
7  
8 375 expiration, while standing without clothing. Hip circumference is measured in centimetres at  
9 376 the widest point between waist and thighs. Waist-Hip-Ratio (WHR) is calculated as the ratio  
10 377 of waist and hip circumferences. Systolic (SBP) and diastolic blood pressure (DBP) is  
11 378 measured with a semiautomatic blood pressure monitor, and heart rate.

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16 379 Each woman (PCOS and controls) is given seven questionnaires to be filled in and returned at  
17 380 next (baseline) visit. They are asked to start to register their bleeding periods from now until  
18 381 the end of study.

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22 382 An appointment for body composition (lean and fat mass and bone mineral density) measure  
23 383 with dual energy x-ray absorptiometry (DXA) is given.

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26 384 To enable measurements day 6-8 in the menstrual cycle, all women are given information on  
27 385 how to induce withdrawal bleeding with medroxyprogesterone acetate, 10 mg per day for 7  
28 386 days (participants in Sweden) or dydrogesteron, 20 mg per day for 10 days (participants in  
29 387 China).

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33 388 **Baseline:** The baseline visit takes place in the morning after an overnight fast on day 6 – 8  
34 389 after induced withdrawal bleeding in all women (see above). The time point is selected as the  
35 390 endometrial lining has to be thicker. The questionnaires are returned and checked. Missing  
36 391 information in the questionnaires is checked.

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39  
40 392 First, a gynaecological examination is performed by transvaginal ultrasound, measuring  
41 393 ovarian size in three dimensions, total antral follicle count (2-9 mm) and endometrial  
42 394 thickness (mm).

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44  
45 395 Second, if biopsies are taken, local anaesthesia will be placed and an endometrial biopsy is  
46 396 collected and snap frozen. Immediately after, local anaesthesia is placed close to the  
47 397 umbilicus and in the vastus lateralis muscle, fat and muscle biopsies are taken.

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51 398 Third, a venflon will be placed and fasting blood samples will be drawn for serum and  
52 399 plasma analyses e.g. *genetic* (e.g. next generation sequencing, SNP, methylation), *metabolic*  
53 400 (e.g. lipids, adipokines, inflammatory markers) and *endocrine* (e.g. sex steroids,  
54 401 gonadotropins, growth factors) measures.

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3 402 Fourth, an oral glucose tolerance test (OGTT) with 75 g glucose will be performed. Blood  
4 403 samples is collected to measure plasma glucose and serum insulin at 0, 30, 60, and 120 min  
5  
6 404 during the OGTT.  
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8 405 At the baseline visit, after OGTT, all participants will receive lifestyle advice by the study  
9 406 coordinator. Patient will be told to register daily number of steps and will receive a step-  
10 407 counter and asked to register menstrual bleeding. If allocated to the electroacupuncture group,  
11 408 time will be booked and treatment started within one week. If randomized to the metformin  
12 409 group, the study drug will be administered and the treatment started the next day. The  
13 410 lifestyle management only group are given appointments for repeated measurements after 4  
14 411 months and follow-up 4 months later.  
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20 412 Women with PCOS who are randomized are informed that they should use contraception that  
21 413 are non-hormonal.  
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24 414 ***Follow-up 4 months after last treatment:*** All baseline measures are repeated after 4 months  
25 415 of treatment and at follow-up 4 months after last treatment.  
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## 29 417 **Statistical analysis**

### 30 418 ***Sample size and power calculations***

31 419 Sample size calculations are based on t-test between two groups. This is due to the fact that it  
32 420 is the pairwise comparisons that are of main interest (not overall F-test/ANOVA). The result  
33 421 show that 41 women per group, in total 123 women, is enough to prove a difference in HbA1c  
34 422 compared acupuncture + lifestyle management and metformin + lifestyle management  
35 423 respectively, to lifestyle management alone (repeated pairwise t-test) on -1.7 unites (effect  
36 424 size  $1.7/2.7SD=0.63$ ) with 80 % power (significance,  $p = 0.05$ , unadjusted pairwise  
37 425 comparisons).<sup>18 51</sup>  
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40 426 Further, for the mechanistic studies, we estimate that successful tissue samples will be  
41 427 recruited from a minimum 20 participants in each group in Sweden and China respectively,  
42 428 giving a strong power to detect differences.  
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### 51 429 ***Minimizing sources of bias***

52 430 Blinding is not possible given the nature of the intervention. We do not feel it is necessary or  
53 431 ethical to perform sham acupuncture and are confident that the primary outcome is unlikely  
54 432 to be affected by observer bias.  
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### 433 ***Type of analyses***

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

437 *Clinical outcome measures:* Continuous variables will be presented as means  $\pm$  standard  
438 deviations and categorical variables as medians with interquartile ranges. Between group  
439 comparisons will be carried out with changes from baseline to after treatment and from  
440 baseline to follow-up by ANOVA followed by Dunnet post-hoc test for continuous and  
441 Kruskal-Wallis followed by Mann Whitney U-test or by  $\chi^2$  tests for categorical variables.  
442 In the cross sectional case-control part of the study the Student t-test will be used for  
443 continuous variables and Mann Whitney U-test or  $\chi^2$  tests for categorical variables and  
444 logistic regression when needed.

445 All statistical analyses of the data will be performed using the SPSS program version 23.0 or  
446 higher (SPSS Inc., Chicago, IL, USA), and a *P*-value  $< 0.0167$  will be considered statistically  
447 significant in the RCT and *P*-value  $< 0.05$  in the cross sectional part.. All tests are two-sided  
448 and adjustments for multiple comparisons will be performed.

449 *Expression and methylation data:* These analyses will be adjusted to the technique used. In  
450 brief, raw data will be checked and processed and a quality control report will be completed.  
451 Different analysis pipelines for traceability and track-ability will be performed. Then  
452 extended data analyses, including functional analysis, GeneOntologies, Biological Pathways,  
453 Principle Component Analysis (PCA)-analysis, Clustering, Visualizations and mapping  
454 against a reference genome, will be performed, and data will be submitted to repositories (i.e.  
455 the Array Express: [www.ebi.ac.uk/arrayexpress](http://www.ebi.ac.uk/arrayexpress)).

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

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### 462 **Ethics and Dissemination**

463 The study is performed according to good clinical practice and conducted in accordance with  
464 the Declaration of Helsinki. The study has been approved by the Regional Ethical Review

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3 465 Board of Stockholm, Sweden Dnr: 2015/1656-31/2 and by the Regional Ethical Review  
4 466 Board of Peking University Third Hospital, China Dnr: 2016-212-02. In addition, the  
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6 467 Medical Products Agency have approved the study: EudraCT: 2015-004250-18 and the trial  
7  
8 468 is registered at Clinicaltrials.gov: NCT02647827. Reporting of the study results will follow  
9  
10 469 the 2010 revised CONSORT statement and STRICTA.<sup>52 53</sup> Primary outcome data the RCT  
11  
12 470 will be published in a relevant journal together with supporting secondary outcome  
13  
14 471 measurements. Further, secondary outcome measurements will be published in separate  
15  
16 472 papers as well as cross sectional case-control data.

17  
18 473 The relevance of this study is that it has potential to uncover new knowledge in the  
19  
20 474 pathophysiology of the disorder and result an additional treatment strategy for insulin  
21  
22 475 resistant in women with PCOS and related diseases, including obesity, insulin resistance, and  
23  
24 476 T2D. Thus, it may have an impact on both genders and does not apply only to women with  
25  
26 477 PCOS.

#### 26 478 **Trial status**

27  
28 479 The study was conceived and designed during 2015. The first participant was recruited and  
29  
30 480 randomized in February 2016 in Sweden and September 2016 in China. Number of  
31  
32 481 participants randomized in Sweden: 26 and in China 48 in August 2018. We anticipate that  
33  
34 482 all participants are recruited by the end of 2019 with follow-up done during 2020.

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47  
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49  
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51  
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53  
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55  
56 494 role in data collection and analysis, decision to publish, or preparation of the manuscripts.

#### 56 496 **Competing interest**

1  
2  
3 497 The authors declare that they have no competing interests.

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5  
6 499 **Author contribution**

7  
8 500 ESV conceived and designed the study, drafted the manuscript for important intellectual  
9 501 content and sought funding and ethical approval in Sweden and registered the trial in  
10 502 EudraCT and Clinicaltrials.gov. JQ sought funding and ethical approval in China. HZ, RL,  
11 503 CF, ALH and JQ was involved in the planning and design of the study and critically revised  
12 504 the manuscript and protocols. HZ, DL, WW, HW, CC, SL, ZJH and XJ are involved in the  
13 505 screening, randomization and treatment of participants. All authors read and approved the  
14 506 final manuscript.  
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**Table 1:** Acupuncture points, stimulation, localization, tissue in which needles are inserted, and innervation areas. The two sets will be alternated for every other treatment.

Acupuncture point	Stimulation	Localization	Muscle	Muscle innervation
<b>Set 1</b>				
CV4, <i>Guan Yuan</i>	EA	3 cun caudal to the umbilicus	Fibrous tissue, linea alba	L1
CV12, <i>Zhongwan</i>	EA	On the midline, 4 cun superior to the umbilicus	Fibrous tissue, linea alba	Th7–8
ST29 Bilateral, <i>Guilai</i>	EA	1 cun cranial to the pubic bone and 2 cun lateral of the midline	M. rectus abdominis	Th6–12
ST34 Bilateral, <i>Futu</i>	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
ST32 Bilateral, <i>Liangqiu</i>	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
SP6 Bilateral, <i>Sanyinjiao</i>	DeQi, four times	3 cun proximal to the medial malleolus	Mm. flexor digitorum longus, tibialis posterior	L4–5, S1–2
ST36 Bilateral, <i>Zusanli</i>	DeQi, four times	On the anterior lateral side of the leg, 3 cun below <i>Dubi</i> (ST35), one finger width (middle finger) from the anterior crest of the tibia	Musculi tibialis anterior	L4–5, S1
LI4 <i>Hegu</i>	Bilateral, DeQi, four times	On the highest point at m. interosseus dorsalis	Mm. interosseus dorsalis I, lumbricalis II, adductor pollicis	C8, Th1

**Set 2**

CV6, <i>Qihai</i>	EA	1.5 cun caudal to the umbilicus	Fibrous tissue, linea alba	Th11
CV10, <i>Xiawan</i>	EA	2 cun cranial to the umbilicus	Fibrous tissue, linea alba	Th8
ST27 Bilateral, <i>Daju</i>	EA	3 cun cranial to the pubic bone and 2 cun lateral to the midline	M. rectus abdominis	Th6–12
Extra meridian point Bilateral	EA	6 cun above the patella in line with SP10	M. quadriceps femoris	L2–L4
SP10 Bilateral, <i>Xuehai</i>	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
ST38 Bilateral, <i>Sanyinjiao</i>	DeQi four times	Between lateral malleolus and knee joint, 1 finger from tibiae crist	Musculi tibialis anterior	L4–5, S1
LR3 Bilateral, <i>Taichong</i>	DeQi four times	Between metatarsal I & II, just distal to the caput	M. Interosseus dorsalis I	S2–3
PC6 Bilateral, <i>Neiguan</i>	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

1 C: Cervical vertebra; CV: Conception vessel; L: Lumbar vertebra; LI: Large intestine; LR: Liver;  
2 PC: Pericardium; S: Sacral vertebra; SP: Spleen; ST: Stomach; Th: Thoracic vertebra.

1 **Table 2.** Overview of the study visits.  
2

	Screening visit	Base line visit	Month				Follow-up after 4 months of treatment	Follow-up 4 months after last treatment
			1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>		
<b>Anthropometry:</b> Body composition (weight, height, waist circumference, hip circumference), FG/acne, blood pressure	X						X	X
<b>Menstrual cycle diary</b>	X	X	X	X	X	X	X	X
<b>Questionnaires:</b> EQ-5D, SF36, PCOSQ, CPRS-SA*, Zung SAS <sup>#</sup> , Zung SDS <sup>#</sup> , IPAQ, TFEQ-R21, QEWP-R*	X						X	X
Transvaginal ultrasound		X					X	X
<b>Metabolic measures:</b> Fasting blood samples for glucose, insulin, HbA1c, c-peptide, OGTT. Adipokines, lipid profile (LDL, HDL, NEFA) and inflammatory markers		X					X	X
<b>DXA</b>		X					X	X
<b>Endocrine measures:</b> Fasting blood samples for sex steroids, SHBG, LH, FSH, AMH, prolactin, TSH, T4		X					X	X
<b>Tissue and whole blood collection</b>		X					X	X

3 Antimüllerian hormone (AMH); dual energy x-ray absorptiometry (DXA); EuroQol-5 dimension  
4 (EQ-5D); Ferriman–Gallwey score (FG); follicle stimulating hormone (FSH); high density  
5 lipoprotein (HDL); hsCRP, International Physical Activity Questionnaire (IPAQ); low density  
6 lipoprotein (LDL), luteinizing hormone (LH); non-esterified fatty acids (NEFA); norepinephrine  
7 (NE), oral glucose tolerance test (OGTT); polycystic ovary syndrome questionnaire (PCOSQ);  
8 Questionnaire of Eating and Weight Patterns-Revised (QEWP-R); sex hormone binding globulin

1 (SHBG); short form-36 (SF36); Three-Factor Eating Questionnaire (TFEQ-R21); thyroid  
stimulating hormone (TSH); thyroxine (T4); Zung Self-Rating Anxiety Scale (Zung SAS); Zung  
Self-Rating Depression Scale (Zung SDS).

\* only in Sweden and # only in China.

For peer review only

# BMJ Open

## 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 randomized controlled trial

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Complete List of Authors:	<p>Stener-Victorin, Elisabet; Karolinska Institutet, Department of Physiology and Pharmacology  Zhang, Haolin; Peking University Third Hospital, Department of Traditional Chinese Medicine (TCM)  Rong, LI; Peking University Third Hospital, OB &amp; GYN  Friden, Cecilia; Karolinska Institutet, 5. Department of Neurobiology, Care Sciences and Society, Division of Physiotherapy  Li, Dong; Peking University Third Hospital, Department of Traditional Chinese Medicine (TCM)  Wang, Wei; Peking University Third Hospital, 4. Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China  Wang, Haining ; Peking University Third Hospital, Department of Endocrinology and Metabolism, Peking University Third Hospital, Beijing, China  Chang , Cuiqing; Peking University Third Hospital, Institute of Sports Medicine  Li , Shi; Peking University Third Hospital, 3. Center for Reproductive Medicine, Department of Obstetrics and Gynecolog  Huo , ZeJun ; Peking University Third Hospital, Research Center of Clinical Epidemiology  Zhang, Hua; Peking University Third Hospital, Research Center of Clinical Epidemiology  Ji, Xiaolan ; Peking University Third Hospital, Department of Traditional Chinese Medicine (TCM)  Linden-Hirschberg, Angelica; Karolinska Institutet, Department of Obstetrics and Gynecology  Jie, Qiao; Peking University Third Hospital, Center for Reproductive Medicine, Department of Obstetrics and Gynecology and Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China</p>
<b>Primary Subject Heading</b>:	Diabetes and endocrinology
Secondary Subject Heading:	Complementary medicine
Keywords:	glucose homeostasis, insulin resistance, acupuncture, life style, polycystic ovary syndrome



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

4 Elisabet Stener-Victorin<sup>1#\*</sup>, Haolin Zhang<sup>2</sup>, Rong Li<sup>3,4</sup>, Cecilia Fridén<sup>5</sup>, Dong Li<sup>2</sup>, Wei  
5 Wang<sup>4</sup>, Haining Wang<sup>6</sup>, Cuiqing Chang<sup>7</sup>, Shi Li<sup>3</sup>, ZeJun Huo<sup>2</sup>, Hua Zhang<sup>8</sup>, Xiaolan Ji<sup>2</sup>,  
6 Angelica Linden Hirschberg<sup>9</sup>, Jie Qiao<sup>3,4\*</sup>

- 7 1. Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm,  
8 Sweden
- 9 2. Department of Traditional Chinese Medicine (TCM), Peking University Third  
10 Hospital, Beijing, China.
- 11 3. Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking  
12 University Third Hospital, Beijing, China.
- 13 4. Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing,  
14 China
- 15 5. Department of Neurobiology, Care Sciences and Society, Division of Physiotherapy,  
16 Karolinska Institutet, Huddinge, Sweden
- 17 6. Department of Endocrinology and Metabolism, Peking University Third Hospital,  
18 Beijing, China
- 19 7. Institute of Sports Medicine, Peking University Third Hospital, Beijing, China
- 20 8. Research Center of Clinical Epidemiology, Peking University Third Hospital, Beijing,  
21 China.
- 22 9. Department of Obstetrics and Gynecology, Karolinska Institutet, Stockholm, Sweden

23  
24 **Short title:** Acupuncture or metformin for insulin resistance in PCOS

25 \* Shared senior authors contributed equally to this paper

26 **# Corresponding authors and reprint requests**

27 Elisabet Stener-Victorin

28 Department of Physiology and Pharmacology, Biomedicum QB5

29 Karolinska Institutet, 171 65 Stockholm, Sweden

30 Phone: +46(0)705643655

31 E-mail: [elisabet.stener-victorin@ki.se](mailto:elisabet.stener-victorin@ki.se)

## 34 **Abstract**

35 **Introduction:** Polycystic ovary syndrome (PCOS) is linked to hyperinsulinemia and insulin  
36 resistance with dysfunctional glucose metabolism. Pilot studies suggests that acupuncture  
37 treatment with combined manual and low-frequency electrical stimulation  
38 (electroacupuncture, EA) of the needles decrease circulating glycated hemoglobin (HbA1c)  
39 and homeostatic model assessment-insulin resistance (HOMA-IR). Therefore, we here aim to  
40 investigate if acupuncture treatment or metformin together with life style or life style  
41 management alone improve insulin sensitivity and related symptoms in overweight/obese  
42 women with PCOS.

43 **Methods and analysis:** This is a two centre multinational (Sweden and China), cross-  
44 sectional case-control study combined with an open-labelled randomized controlled trial  
45 (RCT). Participants are randomized to one of three groups: 1) EA 2-3 times/week during 4  
46 months + lifestyle management; 2) metformin, 500 mg, three/day during 4 months + lifestyle  
47 management; or 3) life style management alone. The primary outcome measure in the RCT is  
48 changes in Hba1C. A total of 123 obese overweight women with PCOS will be enrolled and  
49 randomized into one of the three groups with a target power of at least 80% and 5%  
50 significance level based on two-sided tests.

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

56 **Expected results:** We anticipate that EA and metformin, both with lifestyle management are  
57 equally effective and superior to lifestyle management alone for improvement of glyceic  
58 control.

59 **Trial registration:** Clinicaltrials.gov: NCT02647827 and EudraCT: 2015-004250-18.

60  
61 **Key words:** glucose homeostasis; insulin resistance; metformin; electroacupuncture; lifestyle

## Strength and Limitations

1. 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.
2. It has the potential to gain deeper insight into the pathophysiology of polycystic ovary syndrome (PCOS), and to uncover new knowledge for treatment of insulin resistant in related diseases, including obesity, insulin resistance, and type 2 diabetes (T2D).
3. 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.
4. 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.

## 82 **Background**

83 Polycystic Ovary Syndrome (PCOS) is the most common endocrine and metabolic disorder  
84 in women of reproductive age and is characterized by anovulation, hyperandrogenism and  
85 metabolic dysfunction.<sup>1</sup> Women with PCOS have a 3 to 7-fold increased risk of developing  
86 type 2 diabetes (T2D), and with younger onset, PCOS increases cardiovascular risk factors<sup>1-6</sup>.  
87 Independent of body weight, insulin sensitivity is ~40% lower in women with PCOS than in  
88 healthy women, and impaired glucose regulation, insulin resistance and reduced insulin  
89 responsiveness have been attributed to defects in insulin signalling in adipocytes and skeletal  
90 muscle.<sup>7,8</sup> Of note, obesity aggravates all symptoms related to PCOS.

91 Despite detrimental impact on women's health, the aetiology of PCOS is not well understood.  
92 Genetic, epigenetic, and environmental factors have all been implicated in its development.  
93 Emerging evidence suggests that PCOS originates, at least in part, in fetal life,<sup>9,10</sup> and  
94 elevated maternal androgens have been implicated to play a role, however the mechanisms  
95 are largely unknown.<sup>11,12</sup> Of interest is that we have found that women with PCOS have  
96 multiple transcriptional and epigenetic changes in adipose tissue that are relevant for  
97 development of the disease.<sup>13</sup> Further, twin studies suggest that genetic influences explain  
98 >70% of PCOS pathogenesis.<sup>14</sup> However, whether genetic or epigenetic alterations in target  
99 tissues e.g. adipose tissue, skeletal muscle and endometrium contribute to development of  
100 metabolic disease requires further investigation.

101 Women with PCOS require long-term individualized treatment programs. Pharmacological  
102 treatments, including the glucose reducing drug metformin, have limitations related to  
103 adverse effects and patient compliance. Therefore, there is a need for inexpensive and easily  
104 administered treatments with few negative side-effects. Lifestyle management is the first line  
105 treatment eventually with addition of metformin for improving whole body glucose  
106 homeostasis and preventing type 2 diabetes (T2D).<sup>15-17</sup> Interestingly, five weeks of  
107 acupuncture with combined manual and low-frequency electrical stimulation has in a pilot  
108 study been shown to improve whole body glucose homeostasis in insulin resistant women  
109 with PCOS.<sup>18</sup> The pilot study was an uncontrolled trial and it is therefore of importance to  
110 compare the effect of acupuncture with first line treatment to investigate the effectiveness.

111 Whilst pharmacological treatment strategies have shown efficacy, importantly, there is a need  
112 for Comparative Effectiveness Research (CER) to strengthen the evidence base for clinical  
113 and policy decision-making<sup>19</sup>. Therefore we aim to compare the effect of pharmacological  
114 first-line treatment, metformin, with a non-pharmacological treatment strategy, acupuncture

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3 115 (both together with lifestyle management), with lifestyle management for improvement and  
4 116 prevention of metabolic dysfunction and related symptoms in insulin resistant women with  
5 117 PCOS.

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9 118 Our main hypothesis is that acupuncture and metformin (both treatments combined with  
10 119 lifestyle management) are superior to lifestyle management alone in improving whole body  
11 120 glucose regulation in insulin resistant women with PCOS. Secondary hypotheses are that  
12 121 these treatments have the potential to improve metabolic- and endocrine measures, quality of  
13 122 life and symptom of anxiety and depression, and to restore epigenetic and molecular  
14 123 alterations in target tissues (endometrial-, adipose-, and skeletal muscle tissue) and thus have  
15 124 the potential to improve and potentially prevent the development of metabolic alterations  
16 125 including T2D.

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23 126 Thus, the purpose of this study is twofold, first we aim to gain deeper insight into the  
24 127 pathophysiology of PCOS in a cross sectional case-control study by comparing women with  
25 128 PCOS with women without PCOS matched for age, weight and BMI, and secondly we aim to  
26 129 perform a prospective RCT of women with PCOS.

### 30 130 **Study design**

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33 131 This is a two centre multinational prospective trial with a cross-sectional case-control part  
34 132 and an open-labelled RCT with a comparative effectiveness design. The interventions to be  
35 133 tested are 1) Electroacupuncture during 4 months + lifestyle management; 2) Metformin  
36 134 during 4 months + lifestyle management; or 3) Lifestyle management alone which will be  
37 135 available for participants in all three groups. Participants will be enrolled at Karolinska  
38 136 Institutet and Karolinska University Hospital, Stockholm, Sweden and at Peking University  
39 137 Hospital, Beijing China respectively.

### 44 138 **Randomisation and treatment allocation**

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47 139 The randomization is stratified across the factors age and BMI and is also separated by study  
48 140 site with a balanced allocation ratios 1:1:1. Randomization is performed in blocks with a  
49 141 variable block size between 3 and 15; e.g. First there is a block of 12, when it is full it is  
50 142 followed by a block of 9 and thereafter a block of 3. The order of the block sizes are  
51 143 unknown to the participating study sites and also differs among the strata's.

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54 144 Each study site (Stockholm and Beijing) use the same randomization and electronic case  
55 145 report form (eCRF). A web-based randomization program  
56 146 (<https://data.dynareg.se/pia2/Default.aspx>) has been generated to ensure allocation

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3 147 concealment. The study coordinators log on the web-based system to randomize eligible  
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5 148 patients. All women who enter the study will be logged and given a unique study number.  
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7 149 Blinding or masking of the intervention will not be possible because of the nature of the  
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9 150 intervention. Importantly, however, the assessor will be blinded to the patients' group  
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11 151 assignment.

## 12 152 **Patient and Public Involvement**

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14 153 Patients and or public was not involved in the design of this study.

## 15 16 17 154 **Study Objectives**

### 18 155 ***Primary Objective***

- 19  
20 156 1. To determine the clinical effectiveness of 4 months of 1) electroacupuncture +  
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22 157 lifestyle management and 2) metformin + lifestyle management, compared to 3)  
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24 158 lifestyle management only for improvement of glucose regulation assessed by HbA1c  
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26 159 levels.

### 27 28 160 ***Secondary Objectives***

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30 161 1. To evaluate changes in secondary metabolic measures including the insulin response  
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32 162 to glucose assessed by calculating the area under the curve ( $AUC_{\text{insulin}}$ ) during the oral  
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34 163 glucose tolerance test (OGTT), fasting insulin, glucose, calculation of homeostatic  
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36 164 model assessment (HOMA)-IR and-HOMA-B (*i.e.* the Islet  $\beta$ -cell function) and the  
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38 165 assessment of e.g. adipokines, lipid profile, body size and proportions and body fat  
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40 166 distribution.
- 41 167 2. To determine changes in gene expression and DNA methylation profiles related to  
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43 168 insulin sensitivity in fat, muscle and endometrial tissue biopsies, and biomarkers in  
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45 169 whole blood.
- 46 170 3. To evaluate endocrine measures including menstrual pattern and ovulation frequency,  
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48 171 circulating hormones (e.g. sex steroids, AMH, gonadotropins).
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50 172 4. To determine changes in women's health related quality of life (HRQoL), symptoms  
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52 173 of anxiety and depression, dieting and eating patterns, and negative side-effects.

## 53 54 174 **Outcome Measurements**

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56 175 Outcome measures will be collected at:

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58 176 1. Baseline  
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60 177 2. After 4 months of intervention

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3 178 3. Follow-up 4 months after last treatment  
4

5 179 **Primary Outcome**

6  
7 180 Changes from baseline to after 4 months of treatment in HbA1c comparing 1) acupuncture +  
8  
9 181 lifestyle management and 2) metformin + lifestyle management, respectively with 3) lifestyle  
10  
11 182 management only. In the cross sectional study, difference in HbA1c between cases and  
12  
13 183 controls.

14 184 **Secondary Outcome**

15  
16 185 Changes from baseline to after 4 months of treatment and from baseline to the 4-month  
17  
18 186 follow-up comparing 1) acupuncture + lifestyle management, and 2) metformin + lifestyle  
19  
20 187 management, respectively, with 3) lifestyle management only, and in the cross sectional  
21  
22 188 study, difference between cases and controls in the following variables:

- 23  
24 189 • *Body composition*: In addition to weight, height and waist circumference, women will be  
25  
26 190 examined by DXA to measure lean and fat mass and bone mineral density using a Lunar  
27  
28 191 Prodigy Advance whole body scanner (GE Medical Systems) <sup>20</sup>.
- 29 192 • *Metabolic measures*: Insulin response to glucose during the OGTT (AUC using the  
30  
31 193 trapezoidal rule), and direct analyses of fasting blood samples of insulin and glucose to  
32  
33 194 enable calculation of HOMA-IR [fasting insulin ( $\mu\text{U}/\text{mL}$ )  $\times$  fasting glucose ( $\text{mmol}/\text{L}$ )] /  
34  
35 195 22.5], and HOMA-B /islet  $\beta$ -cell function [ $20 \times$  fasting insulin ( $\text{mU}/\text{mL}$ ) / (fasting plasma  
36  
37 196 glucose ( $\text{mmol}/\text{L}$ ) - 3.5)] <sup>21</sup>. Further, fasting blood samples are collected and saved for  
38  
39 197 later analyses of e.g. C-peptide and calculation of C-peptide index [Fasting C-peptide  
40  
41 198 ( $\text{nmol}/\text{L}$ ) / f-glucose ( $\text{mmol}/\text{L}$ )  $\times$  100] <sup>22</sup>, for analyses of adipokines, inflammatory markers,  
42  
43 199 non-esterified fatty acids (NEFA), total cholesterol, triglycerides, high density lipoprotein  
44  
45 200 (HDL), low density lipoprotein (LDL), high sensitive CRP, catecholamine's and  
46  
47 201 metabolites analysed on a split-fraction HPLC-ED system <sup>23</sup>.
- 48 202 • *Endocrine measures*: Menstrual frequency: Participants will be asked to note date of  
49  
50 203 menstruation which will be reported to the study coordinator once per week by text  
51  
52 204 message and every 4<sup>th</sup> week by phone. Ovarian morphology antral follicle count and  
53  
54 205 ovarian volume. Blood samples will be drawn for analyses of sex steroids by the validated  
55  
56 206 gas- and liquid chromatography/tandem mass spectroscopy technique, as well as sex  
57  
58 207 hormone binding globulin (SHBG), luteinizing hormone (LH), follicle stimulating  
59  
60 208 hormone (FSH), antimüllerian hormone (AMH), prolactin, thyroid stimulating hormone  
209 (TSH) and free thyroxine (T4).



- 1  
2  
3 210 • *Tissue and whole blood collection:* Whole blood will be collected for DNA and  
4 211 microRNA analyses. Endometrial, fat and skeletal muscle tissue biopsies will be collected  
5 212 at baseline (cases and controls), after 4 months of treatment and at follow-up 4 months  
6 213 after treatment in women with PCOS, snap frozen in liquid nitrogen within 30 s and stored  
7 214 at -80°C for later analyzes. Fat cells will be isolated for determination of adipocyte size  
8 215 and distribution. Part of tissue biopsies will also be isolated for *in vitro* experiments.<sup>24</sup>  
9 216 Deep RNA, microRNA and/or bisulfite sequencing will be performed with the latest  
10 217 available technology.
- 11  
12 218 • *Health related quality of life:* Will be determined by quality of life by EuroQol-5  
13 219 dimension (EQ-5D),<sup>25 26</sup> short form-36 (SF36),<sup>27 28</sup> and polycystic ovary syndrome  
14 220 questionnaire (PCOSQ).<sup>29 30</sup>
- 15  
16 221 • *Symptoms of anxiety and depression* will be assessed by the self-reported version of the  
17 222 Comprehensive Psychopathological Rating Scale for Affective Syndromes (CPRS-S-A)<sup>31</sup>  
18 223 to assess psychiatric symptoms within a time frame of the last 3 days in Sweden. For the  
19 224 purpose of this study, two scales will be extracted from the CPRS-S-A,<sup>31</sup> the Brief Scale  
20 225 for Anxiety (BSA-S)<sup>32</sup> and the Montgomery Åsberg Depression Rating Scale (MADR-S-  
21 226 S).<sup>33-35</sup> In China will the Zung symptom depressions score (SDS) and Zung symptom  
22 227 anxiety score (SAS) be used <sup>36 37</sup>. Depression symptoms of potential clinical relevance is  
23 228 for MADR-S  $\geq 11$  and for Zung SDS  $\geq 0.5$  (Depressive index), Anxiety symptoms of  
24 229 potential clinical relevance is for BSA-S  $\geq 11$  and for Zung SAS  $\geq 50$  (Standard total  
25 230 score),
- 26  
27 231 • *Physical Activity:* International Physical Activity Questionnaire (IPAQ) will be used to  
28 232 assess degree of physical activity.<sup>38 39</sup> In addition, one text message per week will be sent  
29 233 to the participants by the study coordinators asking of number of steps the last week when  
30 234 asking for menstrual bleeding (date).
- 31  
32 235 • *Eating questionnaire and eating pattern:* Only assessed at baseline using the self-reported  
33 236 version of the Three-Factor Eating Questionnaire (TFEQ-R21),<sup>40 41</sup> and Questionnaire of  
34 237 Eating and Weight Patterns-Revised (QEWPR) to measure eating behaviour (Sweden  
35 238 only).<sup>42</sup>
- 36  
37 239 • *Side-effects and adverse events* will be continuously and equally recorded in each study  
38 240 arm. One text message per week will be sent to all participants by the study coordinators  
39 241 in which they are asked to report (in addition to number of steps) any side effects or  
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242 adverse events. All participants will receive a phone call every 4<sup>th</sup> week by the study  
243 coordinator and will be asked about side effects or adverse events.

## 244 **Participants**

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

### 251 ***Inclusion criteria – women with PCOS***

- 252 1. Age  $\geq 18$  to  $\leq 40$  years
- 253 2. Body mass index (BMI)  $\geq 25$  to  $\leq 40$  given that 95% of all women with PCOS with a  
254 BMI  $\geq 25$  are insulin resistant.<sup>43 44</sup>
- 255 3. PCOS diagnosis according to Rotterdam criteria 2003<sup>45</sup>, with at least two of the  
256 following three symptoms: Clinical and/or biochemical signs of hyperandrogenism  
257 (hirsutism or acne); oligo/amenorrhea; and/or polycystic ovaries (PCOS).  
258 Biochemical hyperandrogenism is defined by total testosterone  $> 1.2$  nmol/L or a free  
259 androgen index (FAI)  $> 5$ .<sup>46</sup> Hirsutism is defined as a self-reported Ferriman-Gallwey  
260 (FG) score  $\geq 8$  ( $\geq 5$  Asian).<sup>47 48</sup> Acne is defined by a positive response to the question  
261 *Do you have acne?* Oligomenorrhea is defined as an intermenstrual interval  $> 35$  days  
262 and  $< 8$  menstrual bleedings in the past year. Amenorrhea as  $< 3$  cycles per year. PCO  
263 is defined by transvaginal ultrasound with  $\geq 12$  follicles 2–9 mm and/or ovarian  
264 volume  $\geq 10$  ml in one or both ovaries.
- 265 4. Willing to sign the consent form.

### 266 ***Inclusion criteria – controls***

267 Controls should have BMI  $\geq 25$  to  $\leq 40$ , regular cycles with 28 days  $\pm$  2 days, and no signs of  
268 hyperandrogenism. They are excluded if they have menstrual irregularities, signs of  
269 hyperandrogenism (FG  $> 4$ ), or evidence of PCO morphology on ultrasound.

### 270 ***Exclusion criteria for all women***

1. Exclusion of other endocrine disorders such as non-classic congenital adrenal hyperplasia (17-hydroxyprogesterone < 3nmol/L), androgen secreting tumors 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 antidiabetic 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 mmHg
8. Pregnancy or breastfeeding the last 6 months
9. Acupuncture the last 2 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 randomized to any treatment.

### **Interventions**

Participants fulfilling the inclusion criteria will be randomized 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 randomization. The lifestyle management involves one initial

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2  
3 301 counselling session in connection with the baseline visit, which includes information about  
4 302 the importance of weight management, healthy diet and physical activity. Focus will be on  
5 303 the importance of physical activity. Each participant will receive a book with lifestyle advice  
6 304 about weight reduction, maintenance and physical activity following WHO  
7 305 recommendations. All participants will receive a text message once weekly to respond  
8 306 number of step collected by their smart phone or step counter during the last week and if they  
9 307 have had any menstrual bleedings. Once every fourth week, study coordinator will call to the  
10 308 participant and ask about number of step last week, menstruation and compliance and side-  
11 309 effects.

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19 310 ***Electroacupuncture:*** Women randomized to receive acupuncture will start their treatment  
20 311 within one week after baseline measurements. The rationale of the acupuncture protocol is  
21 312 based on Western Medical Acupuncture theories. We will use a fixed acupuncture protocol  
22 313 following the two pilot studies: ClinicalTrial.gov NCT01457209 and NCT02026323 with two  
23 314 modifications. First, the treatment period will be 16 weeks (i.e. 4 months) compared to 5  
24 315 weeks and 6 months in the previous pilot studies. Second, the treatment frequency will be 2  
25 316 to 3 times per week during 16 weeks, i.e. in total 32 to 48 acupuncture treatments over 16  
26 317 weeks. The rationale for these changes is that the procedure is time-consuming for the patients  
27 318 and this will increase the feasibility and likely reduce the number of dropouts. Acupuncture  
28 319 treatment will be given by registered physiotherapists or medical doctors educated in  
29 320 theoretical and practical acupuncture and trained to follow the fixed protocol.  
30 321 Disposable, single-use, sterilized CE marked needles made of stainless steel, 0.25 x 30 mm  
31 322 and 0.30 x 40/50 mm (XENO, HEGU Svenska AB, Landsbro, Sweden and in China Huatuo,  
32 323 Suzhou Medical Co Ltd, China) will be inserted to a depth of 15–40 mm in segmental  
33 324 acupuncture points located in abdominal and leg muscles, with innervations corresponding to  
34 325 the ovaries and the pancreas. Two sets of acupuncture points will be alternated every second  
35 326 treatment (Table 1). The first set of acupuncture points include points located in abdominal  
36 327 muscles: conception vessel (CV)4, CV12, stomach (ST)29 bilaterally, and in quadriceps  
37 328 muscle, ST32 and ST34, and in the muscles below the knee, spleen (SP)6, and ST36  
38 329 bilaterally. In addition, needles are placed in the hand, large intestinal (LI)4, bilateral. All  
39 330 needles will be stimulated manually when inserted. CV4 and 12, ST29 bilateral, ST32 and  
40 331 ST34 bilateral will be connected to an electrical stimulator and stimulated with low-  
41 332 frequency EA of 2 Hz (Stimulators used in Sweden: Export Abteilung, Schwa-Medico GmbH,  
42 333 Wetzlarer Str. 41-43;35630 Ehringshausen and in China: Shanghai Huayi Electric

334 Acupuncture Instrument: G6805-1A) for 30 min at each treatment. The intensity will be  
335 adjusted by the physiotherapist to produce local muscle contractions without pain or  
336 discomfort, and thereafter will the patient monitor the stimulation intensity. Six additional  
337 points are selected to strengthen the effect: LI4, ST36, and SP6, and they will be stimulated  
338 manually by rotating the needle to evoke needle sensation every 10 min.

339 The second set of acupuncture points include abdominal points: ST27 bilaterally (EA), CV6  
340 to CV10 (EA); and leg points: SP10 to a non-acupuncture point located 6 cun proximal of  
341 patellas medial border (EA) are all connected to an electrical stimulator and stimulated as in  
342 the first set of acupuncture points. Six additional points; ST38, liver (LR)3 and pericardium  
343 (PC)6, all bilateral, are stimulated manually by rotating the needle to evoke needle sensation  
344 every 10 min.

345 Compliance: If a participant in the acupuncture group deviate considerably from the study  
346 protocol, the acupuncturists should inform the study coordinator. Any negative side effects  
347 during treatment are recorded.

348 **Metformin:** Oral metformin 500 mg three times daily, in total 1500 mg per day<sup>49 50</sup>. To  
349 reduce gastrointestinal side-effects of metformin, the dose will be slowly escalated starting  
350 with 500 mg daily during the first week, increasing to 500 mg twice per day during the  
351 second the week, and 500 mg three times daily, morning, lunch and dinner from the third  
352 week in total 16 weeks including the 3 weeks step-up phase (i.e. 4 months). Patients with  
353 negative effects can remain 500 mg during the remaining weeks.

354 Compliance: Empty bottles are handed over to the study coordinator after 16 weeks of  
355 treatment and number of tablets are counted. Also, once per month, the study coordinator call  
356 the participant and ask her to count the number of tablets there are left in the bottle.

## 357 Study Procedure

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

362 In all participants, a comprehensive anamnestic interview will be conducted, including  
363 menstrual frequency, hirsutism - FG and acne determined by an affirmative answer to the  
364 question "Do you have excessive acne? yes or no", heredity, medication or other diseases.  
365 The physical examination including gynaecological examination is performed by transvaginal

1  
2  
3 366 ultrasound (PCO morphology: yes or no). Body weight (kg) and body height (cm) are  
4  
5 367 measured in an upright position with light clothing and no shoes. BMI is calculated as body  
6  
7 368 weight (kg) divided by squared body height (m<sup>2</sup>). Waist circumference is measured in  
8  
9 369 centimetres at the midpoint between the iliac crest and lower rib margin at the end of  
10  
11 370 expiration, while standing without clothing. Hip circumference is measured in centimetres at  
12  
13 371 the widest point between waist and thighs. Waist-Hip-Ratio (WHR) is calculated as the ratio  
14  
15 372 of waist and hip circumferences. Systolic (SBP) and diastolic blood pressure (DBP) is  
16  
17 373 measured with a semiautomatic blood pressure monitor, and heart rate.

18 374 Each woman (PCOS and controls) is given seven questionnaires to be filled in and returned at  
19  
20 375 next (baseline) visit. They are asked to start to register their bleeding periods from now until  
21  
22 376 the end of study.

23 377 An appointment for body composition (lean and fat mass and bone mineral density) measure  
24  
25 378 with dual energy x-ray absorptiometry (DXA) is given.

26  
27 379 To enable measurements day 6-8 in the menstrual cycle, all women are given information on  
28  
29 380 how to induce withdrawal bleeding with medroxyprogesterone acetate, 10 mg per day for 7  
30  
31 381 days (participants in Sweden) or dydrogesteron, 20 mg per day for 10 days (participants in  
32  
33 382 China).

34  
35 383 **Baseline:** The baseline visit takes place in the morning after an overnight fast on day 6 – 8  
36  
37 384 after induced withdrawal bleeding in all women (see above). The time point is selected as the  
38  
39 385 endometrial lining has to be thicker. The questionnaires are returned and checked. Missing  
40  
41 386 information in the questionnaires is checked.

42  
43 387 First, a gynaecological examination is performed by transvaginal ultrasound, measuring  
44  
45 388 ovarian size in three dimensions, total antral follicle count (2-9 mm) and endometrial  
46  
47 389 thickness (mm).

48  
49 390 Second, if biopsies are taken, local anaesthesia will be placed and an endometrial biopsy is  
50  
51 391 collected and snap frozen. Immediately after, local anaesthesia is placed close to the  
52  
53 392 umbilicus and in the vastus lateralis muscle, fat and muscle biopsy are taken.

54  
55 393 Third, a venflon will be placed and fasting blood samples will be drawn for serum and  
56  
57 394 plasma analyses e.g. *genetic* (e.g. next generation sequencing, SNP, methylation), *metabolic*  
58  
59 395 (e.g. lipids, adipokines, inflammatory markers) and *endocrine* (e.g. sex steroids,  
60  
396 gonadotropins, growth factors) measures.

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3 397 Fourth, an oral glucose tolerance test (OGTT) with 75 g glucose will be performed. Blood  
4 398 samples is collected to measure plasma glucose and serum insulin at 0, 30, 60, and 120 min  
5 399 during the OGTT.  
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8  
9 400 At the baseline visit, after OGTT, all participants will receive lifestyle advice by the study  
10 401 coordinator. Patient will be told to register daily number of steps and will receive a step-  
11 402 counter and asked to register menstrual bleeding. If allocated to the electroacupuncture group,  
12 403 time will be booked and treatment started within one week. If randomized to the metformin  
13 404 group, the study drug will be administered and the treatment started the next day. The  
14 405 lifestyle management only group are given appointments for repeated measurements after 4  
15 406 months and follow-up 4 months later.  
16  
17

18 407 Women with PCOS who are randomized are informed that they should use contraception that  
19 408 are non-hormonal.  
20  
21

22 409 ***Follow-up 4 months after last treatment:*** All baseline measures are repeated after 4 months  
23 410 of treatment and at follow-up 4 months after last treatment.  
24  
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## 26 411 27 412 **Statistical analysis**

### 28 413 ***Sample size and power calculations***

29 414 Sample size calculations are based on t-test between two groups. This is due to the fact that it  
30 415 is the pairwise comparisons that are of main interest (not overall F-test/ANOVA). The result  
31 416 show that 41 women per group, in total 123 women, is enough to prove a difference in HbA1c  
32 417 compared acupuncture + lifestyle management and metformin + lifestyle management  
33 418 respectively, to lifestyle management alone (repeated pairwise t-test) on -1.7 unites (effect  
34 419 size  $1.7/2.7SD=0.63$ ) with 80 % power (significance,  $p = 0.05$ , unadjusted pairwise  
35 420 comparisons).<sup>18 51</sup>  
36  
37

38 421 Further, for the mechanistic studies, we estimate that successful tissue samples will be  
39 422 recruited from a minimum 20 participants in each group in Sweden and China respectively,  
40 423 giving a strong power to detect differences.  
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### 43 424 ***Minimizing sources of bias***

44 425 Blinding is not possible given the nature of the intervention. We do not feel it is necessary or  
45 426 ethical to perform sham acupuncture and are confident that the primary outcome is unlikely  
46 427 to be affected by observer bias.  
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3 428 **Type of analyses**  
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5 429 The statistical analyses will be performed by qualified statisticians and biostatisticians. The  
6  
7 430 data in the RCT will be analysed according to the intent-to-treat principle to investigate  
8  
9 431 differences between the groups.

10 432 *Clinical outcome measures:* Continuous variables will be presented as means  $\pm$  standard  
11  
12 433 deviations and categorical variables as medians with interquartile ranges. Between group  
13  
14 434 comparisons will be carried out with changes from baseline to after treatment and from  
15  
16 435 baseline to follow-up by ANOVA followed by Dunnet post-hoc test for continuous and  
17  
18 436 Kruskal-Wallis followed by Mann Whitney U-test or by  $\chi^2$  tests for categorical variables.  
19  
20 437 In the cross sectional case-control part of the study the Student t-test will be used for  
21  
22 438 continuous variables and Mann Whitney U-test or  $\chi^2$  tests for categorical variables and  
23  
24 439 logistic regression when needed.

25 440 All statistical analyses of the data will be performed using the SPSS program version 23.0 or  
26  
27 441 higher (SPSS Inc., Chicago, IL, USA), and a *P*-value  $< 0.0167$  will be considered statistically  
28  
29 442 significant in the RCT and *P*-value  $< 0.05$  in the cross sectional part.. All tests are two-sided  
30  
31 443 and adjustments for multiple comparisons will be performed.

32 444 *Expression and methylation data:* These analyses will be adjusted to the technique used. In  
33  
34 445 brief, raw data will be checked and processed and a quality control report will be completed.  
35  
36 446 Different analysis pipelines for traceability and track-ability will be performed. Then  
37  
38 447 extended data analyses, including functional analysis, GeneOntologies, Biological Pathways,  
39  
40 448 Principle Component Analysis (PCA)-analysis, Clustering, Visualizations and mapping  
41  
42 449 against a reference genome, will be performed, and data will be submitted to repositories (i.e.  
43  
44 450 the Array Express: [www.ebi.ac.uk/arrayexpress](http://www.ebi.ac.uk/arrayexpress)).

45 451 Group comparison will be carried out with changes from baseline to after treatment and from  
46  
47 452 baseline to follow-up by Kruskal-Wallis followed by Mann Whitney U-test for expression  
48  
49 453 analyses. In the case-control part of the study, Mann Whitney U-test will be used for  
50  
51 454 expression analyses. False discovery rate (FDR) will be used to correct for multiple testing in  
52  
53 455 the analyses of gene and methylation arrays.

54 456 **Safety analysis**  
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56 457 Adverse events will be categorized and the percentage of patients experiencing adverse  
57  
58 458 events and serious adverse events during the treatment period and follow-up period will be  
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1  
2  
3 459 documented and reported to the Data and Safety Monitoring Board (DSMB). These are  
4  
5 460 reviewed every fourth month, and serious adverse events will be immediately handled.  
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### 8 462 **Data management and quality control of data**

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10 463 We use both paper CRF and web-based eCRF to manage individual participant data. Quality  
11  
12 464 control are handled at two levels. First the investigators are required to ensure the accuracy  
13  
14 465 when imputing data into the eCRF. Second, data monitoring and validation will be carried out  
15  
16 466 by an independent person not involved in data collection.  
17  
18 467

### 19 468 **Ethics and Dissemination**

20  
21 469 The study is performed according to good clinical practice and conducted in accordance with  
22  
23 470 the Declaration of Helsinki. The study has been approved by the Regional Ethical Review  
24  
25 471 Board of Stockholm, Sweden Dnr: 2015/1656-31/2 and by the Regional Ethical Review  
26  
27 472 Board of Peking University Third Hospital, China Dnr: 2016-212-02. In addition, the  
28  
29 473 Medical Products Agency have approved the study: EudraCT: 2015-004250-18 and the trial  
30  
31 474 is registered at Clinicaltrials.gov: NCT02647827. Reporting of the study results will follow  
32  
33 475 the 2010 revised CONSORT statement and STRICTA.<sup>52 53</sup> Primary outcome data the RCT  
34  
35 476 will be published in a relevant journal together with supporting secondary outcome  
36  
37 477 measurements. Further, secondary outcome measurements will be published in separate  
38  
39 478 papers as well as cross sectional case-control data.

40  
41 479 The relevance of this study is that it has potential to uncover new knowledge in the  
42  
43 480 pathophysiology of the disorder and result an additional treatment strategy for insulin  
44  
45 481 resistant in women with PCOS and related diseases, including obesity, insulin resistance, and  
46  
47 482 T2D. Thus, it may have an impact on both genders and does not apply only to women with  
48  
49 483 PCOS.

### 50 484 **Trial status**

51 485 The study was conceived and designed during 2015. The first participant was recruited and  
52  
53 486 randomized in February 2016 in Sweden and September 2016 in China. Number of  
54  
55 487 participants randomized in Sweden: 26 and in China 48 in August 2018. We anticipate that  
56  
57 488 all participants are recruited by the end of 2019 with follow-up done during 2020.

### 58 489 **Protocol versions**

1  
2  
3 490 Updated 1: 2015-12-16  
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5 491 Updated 2: 2016-02-11  
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7 492 Updated 3: 2016-12-12  
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21 504 role in data collection and analysis, decision to publish, or preparation of the manuscripts.  
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### 31 506 **Competing interest**

32 507 The authors declare that they have no competing interests.  
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34 508  
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36

### 37 509 **Author contribution**

38 510 ESV conceived and designed the study, drafted the manuscript for important intellectual  
39 511 content and sought funding and ethical approval in Sweden and registered the trial in  
40 512 EudraCT and Clinicaltrials.gov. JQ sought funding and ethical approval in China. HZ, RL,  
41 513 CF, ALH and JQ was involved in the planning and design of the study and critically revised  
42 514 the manuscript and protocols. HZ, DL, WW, HW, CC, SL, ZJH and XJ are involved in the  
43 515 screening, randomization and treatment of participants. All authors read and approved the  
44 516 final manuscript.  
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**Table 1:** Acupuncture points, stimulation, localization, tissue in which needles are inserted, and innervation areas. The two sets will be alternated for every other treatment.

Acupuncture point	Stimulation	Localization	Muscle	Muscle innervation
<b>Set 1</b>				
CV4, <i>Guan Yuan</i>	EA	3 cun caudal to the umbilicus	Fibrous tissue, linea alba	L1
CV12, <i>Zhongwan</i>	EA	On the midline, 4 cun superior to the umbilicus	Fibrous tissue, linea alba	Th7–8
ST29 Bilateral, <i>Guilai</i>	EA	1 cun cranial to the pubic bone and 2 cun lateral of the midline	M. rectus abdominis	Th6–12
ST34 Bilateral, <i>Futu</i>	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
ST32 Bilateral, <i>Liangqiu</i>	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
SP6 Bilateral, <i>Sanyinjiao</i>	DeQi, four times	3 cun proximal to the medial malleolus	Mm. flexor digitorum longus, tibialis posterior	L4–5, S1–2
ST36 Bilateral, <i>Zusanli</i>	DeQi, four times	On the anterior lateral side of the leg, 3 cun below <i>Dubi</i> (ST35), one finger width (middle finger) from the anterior crest of the tibia	Musculi tibialis anterior	L4–5, S1
LI4 <i>Hegu</i>	Bilateral, DeQi, four times	On the highest point at m. interosseus dorsalis	Mm. interosseus dorsalis I, lumbricalis II, adductor pollicis	C8, Th1

<b>Set 2</b>				
CV6, <i>Qihai</i>	EA	1.5 cun caudal to the umbilicus	Fibrous tissue, linea alba	Th11
CV10, <i>Xiawan</i>	EA	2 cun cranial to the umbilicus	Fibrous tissue, linea alba	Th8
ST27 Bilateral, <i>Daju</i>	EA	3 cun cranial to the pubic bone and 2 cun lateral to the midline	M. rectus abdominis	Th6–12
Extra meridian point Bilateral	EA	6 cun above the patella in line with SP10	M. quadriceps femoris	L2–L4
SP10 Bilateral, <i>Xuehai</i>	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
ST38 Bilateral, <i>Sanyinjiao</i>	DeQi four times	Between lateral malleolus and knee joint, 1 finger from tibiae crist	Musculi tibialis anterior	L4–5, S1
LR3 Bilateral, <i>Taichong</i>	DeQi four times	Between metatarsal I & II, just distal to the caput	M. Interosseus dorsalis I	S2–3
PC6 Bilateral, <i>Neiguan</i>	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

1 C: Cervical vertebra; CV: Conception vessel; L: Lumbar vertebra; LI: Large intestine; LR: Liver;  
2 PC: Pericardium; S: Sacral vertebra; SP: Spleen; ST: Stomach; Th: Thoracic vertebra.

1 **Table 2.** Overview of the study visits.  
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	Screening visit	Base line visit	Month				Follow-up after 4 months of treatment	Follow-up 4 months after last treatment
			1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>		
<b>Anthropometry:</b> Body composition (weight, height, waist circumference, hip circumference), FG/acne, blood pressure	X						X	X
<b>Menstrual cycle diary</b>	X	X	X	X	X	X	X	X
<b>Questionnaires:</b> EQ-5D, SF36, PCOSQ, CPRS-SA*, Zung SAS#, Zung SDS#, IPAQ, TFEQ-R21, QEWP-R*	X						X	X
Transvaginal ultrasound		X					X	X
<b>Metabolic measures:</b> Fasting blood samples for glucose, insulin, HbA1c, c-peptide, OGTT. Adipokines, lipid profile (LDL, HDL, NEFA) and inflammatory markers		X					X	X
<b>DXA</b>		X					X	X
<b>Endocrine measures:</b> Fasting blood samples for sex steroids, SHBG, LH, FSH, AMH, prolactin, TSH, T4		X					X	X
<b>Tissue and whole blood collection</b>		X					X	X

3 Antimüllerian hormone (AMH); dual energy x-ray absorptiometry (DXA); EuroQol-5 dimension  
4 (EQ-5D); Ferriman–Gallwey score (FG); follicle stimulating hormone (FSH); high density  
5 lipoprotein (HDL); hsCRP, International Physical Activity Questionnaire (IPAQ); low density  
6 lipoprotein (LDL), luteinizing hormone (LH); non-esterified fatty acids (NEFA); norepinephrine  
7 (NE), oral glucose tolerance test (OGTT); polycystic ovary syndrome questionnaire (PCOSQ);  
8 Questionnaire of Eating and Weight Patterns-Revised (QEWP-R); sex hormone binding globulin

1 (SHBG); short form-36 (SF36); Three-Factor Eating Questionnaire (TFEQ-R21); thyroid  
2 stimulating hormone (TSH); thyroxine (T4); Zung Self-Rating Anxiety Scale (Zung SAS); Zung  
3 Self-Rating Depression Scale (Zung SDS).  
4 \* only in Sweden and # only in China.  
5

For peer review only



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	<a href="#">Page 1</a>
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	<a href="#">Page 16</a>
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	<a href="#">Protocol version: Page 16</a>
Funding	4	Sources and types of financial, material, and other support	<a href="#">See page 16</a>
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	<a href="#">See page 17, author contributions for</a>
	5b	Name and contact information for the trial sponsor	<a href="#">5a-d.</a>
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
<b>Introduction</b>			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	
	6b	Explanation for choice of comparators	<a href="#">Page 4-5</a>
Objectives	7	Specific objectives or hypotheses	<a href="#">Page 5</a>
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	<a href="#">Page 5-6</a>

## Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained <a href="#">Page 5</a>
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) <a href="#">Page 9-10</a>
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered <a href="#">Page 10-12</a>
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) <a href="#">Page 10-12</a>
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) <a href="#">Page 10-12</a>
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial <a href="#">Page 10-12</a>
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended <a href="#">Page 7-8</a>
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) <a href="#">Table 2</a>
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations <a href="#">Page 14</a>
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size <a href="#">Page 9</a>

## Methods: Assignment of interventions (for controlled trials)

### Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions <a href="#">Page 5-6</a>
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1			
2	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central
3	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
4	mechanism		describing any steps to conceal the sequence until interventions are
5			assigned <a href="#">Page 5-6</a>
6			
7	Implementation	16c	Who will generate the allocation sequence, who will enrol participants,
8			and who will assign participants to interventions <a href="#">Page 5-6</a>
9			
10	Blinding	17a	Who will be blinded after assignment to interventions (eg, trial
11	(masking)		participants, care providers, outcome assessors, data analysts), and
12			how <a href="#">Page 5-6</a>
13			
14		17b	If blinded, circumstances under which unblinding is permissible, and
15			procedure for revealing a participant's allocated intervention during
16			the trial <a href="#">Page 5-6</a>
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20	<b>Methods: Data collection, management, and analysis</b>		
21	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other
22	methods		trial data, including any related processes to promote data quality (eg,
23			duplicate measurements, training of assessors) and a description of
24			study instruments (eg, questionnaires, laboratory tests) along with
25			their reliability and validity, if known. Reference to where data
26			collection forms can be found, if not in the protocol <a href="#">Page 5-6</a>
27			
28		18b	Plans to promote participant retention and complete follow-up,
29			including list of any outcome data to be collected for participants who
30			discontinue or deviate from intervention protocols <a href="#">Page 5-6</a>
31			
32	Data	19	Plans for data entry, coding, security, and storage, including any
33	management		related processes to promote data quality (eg, double data entry;
34			range checks for data values). Reference to where details of data
35			management procedures can be found, if not in the protocol <a href="#">Page 5-6</a>
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40	Statistical	20a	Statistical methods for analysing primary and secondary outcomes.
41	methods		Reference to where other details of the statistical analysis plan can be
42			found, if not in the protocol <a href="#">page 14-15</a>
43			
44		20b	Methods for any additional analyses (eg, subgroup and adjusted
45			analyses) <a href="#">Page 14-15</a>
46			
47		20c	Definition of analysis population relating to protocol non-adherence
48			(eg, as randomised analysis), and any statistical methods to handle
49			missing data (eg, multiple imputation) <a href="#">Page 14-15</a>
50			
51			
52	<b>Methods: Monitoring</b>		
53			
54	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role
55			and reporting structure; statement of whether it is independent from
56			the sponsor and competing interests; and reference to where further
57			details about its charter can be found, if not in the protocol.
58			Alternatively, an explanation of why a DMC is not needed <a href="#">Page 15-16</a>
59			
60			

1		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	<a href="#">Page 14</a>
2				
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6	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	<a href="#">Page 8–9</a>
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11	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	<a href="#">N/A</a>
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13				
14				

**Ethics and dissemination** [See page 16](#)

15				
16				
17	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	
18				
19				
20				
21	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	
22				
23				
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25				
26	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	
27				
28				
29		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
30				
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32				
33	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	
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37	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	
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41	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
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45	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
46				
47				
48	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	
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54		31b	Authorship eligibility guidelines and any intended use of professional writers	
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57		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
58				
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**Appendices**

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	<a href="#">See appendix</a>
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	<a href="#">See appendix</a>

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\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)" license.

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