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Effect of acupuncture on insulin sensitivity in women with polycystic ovary syndrome and insulin resistance: Study protocol of a prospective observational study

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ABSTRACT

INTRODUCTION: Hyperinsulinemia and insulin resistance are key features of polycystic ovary syndrome (PCOS) and metabolic syndrome. The effect of five-weeks of acupuncture treatment has been investigated in a completed prospective pilot trial (Clinicaltrials.gov: NCT01457209), and acupuncture with electrical stimulation applied to insulin-resistant rats with dihydrotestosterone-induced PCOS was shown to improve insulin sensitivity. Therefore, we now aim to conduct a prospective observational study to evaluate whether using the same acupuncture treatment protocol given over a longer period of time (6 months) than in the previous pilot trial will improve insulin sensitivity in women with PCOS and insulin resistance (IR). Our hypothesis is that acupuncture with combined manual and low-frequency electrical stimulation of the needles will improve insulin sensitivity in women with PCOS and IR.

METHODS/ANALYSIS: This is a prospective observational trial. A total of 112 women with PCOS and IR will be recruited and categorized according to their body mass index (BMI) as normal weight (BMI = 18.5–23 kg/m^2) or as overweight/obese (BMI > 23 kg/m^2). Acupuncture will be applied three times per week for 6 months at 30 minutes per treatment. The primary outcome will be the change in insulin sensitivity before and after 6 months of acupuncture treatment as measured by an oral glucose tolerance test.

ETHICS/DISSEMINATION: Ethical approval of this study has been granted from the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (No. 2013039). Written and informed consent will be obtained from each patient before any study procedure is performed according to good clinical practice. The results of this trial will be disseminated in a peer-reviewed journal and presented at international congresses.

TRIAL REGISTRATION: ClinicalTrials.gov (NCT02026323) and the Chinese Clinical Trials Registry (ChiCTR-OCH-13003921).

KEY WORDS: acupuncture, insulin resistance, polycystic ovary syndrome

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Background

Features of polycystic ovary syndrome (PCOS) include ovulatory dysfunction, clinical and biochemical signs of hyperandrogenism, and the presence of polycystic ovaries upon pelvic scanning [1 2]. PCOS is the most common endocrine and metabolic disorder in reproductive-age women [2] and is found in 5.6% of Chinese women aged 19–45 years as shown in a recent community-based study [1]. The main metabolic phenotype is hyperinsulinemia and insulin resistance (IR), which is observed in about 50%–70% of all women with PCOS [3 4]. Importantly, IR can be present in both lean and obese women with PCOS [5 6], especially in those with hyperandrogenism and ovulatory dysfunction [7 8].

PCOS causes significant menstrual and fertility issues, but over the past few decades its additional metabolic and cardiovascular risks have become apparent. Women with PCOS display both IR and reduced insulin responsiveness [9] and have at least a 3-fold increased risk of developing type 2 diabetes mellitus [10]. IR in women with PCOS has been attributed to post-binding defects in adipocytes and skeletal muscle insulin signaling [9 11]. Furthermore, compensatory hyperinsulinemia fuels ovarian androgen production by stimulating androgen production and secretion by theca cells and by reducing sex hormone-binding globulin (SHBG) levels, which increases free androgens and further exacerbates PCOS symptoms [12]. Thus, there is a strong association between hyperinsulinemia and hyperandrogenemia.

Overweight/obesity is a common feature of PCOS [13]. A Korean study showed that 61% of women with PCOS were lean, 10.3% were overweight, and 28.4% were obese[14]. Obesity aggravates most of the metabolic dysfunctions in women with PCOS. Women with comorbid PCOS and depression have been found to have higher BMI and IR compared to women with

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PCOS without depression [15], and this increase in BMI negatively affects quality of life [16].

Despite the high prevalence of IR, impaired glucose tolerance, and/or type 2 diabetes mellitus in women with PCOS, there is no consensus on the best long-term management of these conditions. Pharmacological treatments, including metformin, are symptom oriented and usually effective but have unpleasant gastrointestinal side effects. Therefore, it is important to evaluate other non-pharmacological treatment strategies because most women with PCOS require long-term treatment.

Acupuncture, which is one of the main treatment modalities of traditional Chinese medicine, is increasingly being used in the area of reproductive endocrinology and infertility in many different parts of the world. Interestingly, increasing the treatment frequency and the number of treatments leads to higher ovulation frequency [17] indicating the importance of the correct treatment dose. In a secondary analysis of the trial by Jedel et al [18], low-frequency electro-acupuncture (EA) decreased high plasminogen activator inhibitor 1 (PAI-1) activity without affecting insulin sensitivity as measured by the euglycemic hyperinsulinemic clamp [19]. Clearly the intensity, frequency, and duration of low-frequency EA treatment in that study were too low to affect insulin sensitivity. Support for this assumption comes from our experimental studies in the dihydrotestosterone (DHT)-induced PCOS rat model in which we demonstrated that low-frequency EA improves whole-body insulin sensitivity in a dose-dependent manner [20 21].

EA induces both systemic and local effects involving intracellular insulin signaling pathways in skeletal muscle and adipose tissue [20 22 23]. Furthermore, EA has been shown to reduce plasma glucose levels by promoting insulin production and to improve insulin sensitivity by

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inducing secretion of endogenous β-endorphin in different rodent models of diabetes mellitus [24 25]. The potential role of acupuncture in the treatment of women with PCOS and IR has not yet been determined, and this is an important area to investigate because most women with PCOS require long-term treatment. There is one completed prospective pilot trial investigating the effect of five-weeks of acupuncture treatment with combined manual and low-frequency electrical stimulation of the needles (Clinicaltrials.gov: NCT01457209). Therefore, the aim of the proposed study is to investigate the clinical effectiveness of acupuncture on insulin sensitivity in women with PCOS and IR when acupuncture is given over a longer period of time (6 months) than in the previous pilot trial using the same treatment protocol. Our hypothesis is that acupuncture with combined manual and low-frequency electrical stimulation of the needles will improve insulin sensitivity in women with PCOS and IR.

Materials and Methods

Study Design

This is a single-center prospective observational study, and subjects will be enrolled from the First Affiliated Hospital of Guangzhou Medical University. Ethical approval of this study has been granted from the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (No. 2013039). Written and informed consent will be obtained from each patient before any study procedure is performed according to good clinical practice. The trial has been registered at ClinicalTrial.gov (NCT02026323) and with the Chinese Clinical Trials Registry (ChiCTR-OCH-13003921).

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Participants

Subjects will be recruited if they fulfill all of the inclusion criteria and do not meet any of the exclusion criteria.

Inclusion criteria

1) Age between 18 and 40 years.

2) BMI ≥ 18.5 kg/m^2.

3) Presence of PCOS as defined by the Rotterdam criteria and including at least two of the following three features:

- Oligomenorrhea or amenorrhea. Oligomenorrhea is defined as an intermenstrual interval >35 days or <8 menstrual bleedings in the past year. Amenorrhea is defined as complete cessation of menstrual cycles for 6 months or more when a patient has previously had regular cycles and for 12 months or more when the patient has had irregular cycles.
- Clinical or biochemical hyperandrogenism. Biochemical hyperandrogenemia is defined as a total serum testosterone concentration above 60 ng/dL [26], and clinical hyperandrogenism is defined as a Ferriman–Gallwey (FG) score ≥ 5 in mainland China [27].
- Polycystic ovary morphology. This is defined as ≥ 12 antral follicles (2–9 mm in diameter) or an ovarian volume > 10 mL upon transvaginal scanning [28].

4) Presence of IR as defined by the homeostatic model assessment (HOMA-IR: [fasting insulin (μU/mL) × fasting glucose (mmol/L)] / 22.5). A value ≥ 2.14 will be considered

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to be indicative of IR [29].

5) No desire to bear children and having used barrier methods of contraception for one year.

6) Willingness to sign the consent form.

Exclusion criteria

1) Having other endocrine disorders such as hyperprolactinemia (defined as two prolactin levels measured at least one week apart of 25 ng/mL or greater or as determined by local normative values), nonclassic congenital adrenal hyperplasia (17-hydroxyprogesterone < 3 nmol/L), or androgen-secreting tumors.

2) FSH levels > 15 mIU/mL. A normal level within the last year is adequate for entry.

3) Uncorrected thyroid disease defined as thyroid stimulating hormone (TSH) < 0.2 mIU/mL or TSH > 5.5 mIU/mL. A normal level within the last year is adequate for entry.

4) Type I diabetes mellitus or Type I and Type II patients who are receiving antidiabetic medications such as insulin, thiazolidinediones, acarbose, sulfonylureas, or other medications that are likely to confound the effects of the study. Patients currently receiving metformin for a diagnosis of Type I or Type II diabetes or for PCOS are also specifically excluded.

5) Suspected Cushing's syndrome.

6) Use of hormones or other medications in the past 3 months, including Chinese herbal prescriptions, which might affect the outcome.

7) Pregnancy within the last 6 weeks.

8) Post-abortion or postpartum within the last 6 weeks.

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9) Breastfeeding within the last 6 months.

10) Receiving acupuncture treatment related to PCOS within the past 2 months.

11) Having undergone a bariatric surgery procedure within the past 12 months or being in a period of acute weight loss.

12) Having known congenital adrenal hyperplasia.

13) Lack of written consent to participate in the study.

Eligible subjects will be recruited and categorized according to their BMI as normal weight (BMI = 18.5–23 kg/m^2) or overweight/obese (BMI ≥ 23 kg/m^2) [30].

Interventions

The acupuncture protocol is based on Western medical theories, and the study protocol follows the CONSORT [31] and STRICTA [32] recommendations with detailed descriptions of the treatment, including the number of needles used, depth of needle insertion, how needles will be stimulated (manual or electrical), frequency of sessions, and length of the treatment period. Acupuncture will be given according to a fixed protocol by traditional Chinese medicine practitioners educated in theoretical and practical acupuncture. The protocol is based on experimental studies elucidating the effect of acupuncture in rodent models of diabetes mellitus and PCOS [20-22] and in women with PCOS [19 33].

Acupuncture protocol

Acupuncture will be given three times per week over 6 months for a total of 80 sessions.

Disposable, sterilized needles for single use made of stainless steel (0.20 mm × 30 mm and

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0.20 mm × 40/50 mm; Hwoto, Suzhou Medical Appliance Fact., 215005 Suzhou, China) will be inserted to a depth of 15 mm to 35 mm in segmental acupuncture points located in the abdominal and leg muscles with innervations corresponding to the ovaries and pancreas that have been shown to improve insulin sensitivity in rodents. Two sets of acupuncture points will be alternated for every other treatment session. The two acupuncture protocols follow the STRICTA recommendations and are presented in Table 1. The rationale for using the traditional nomenclature of acupuncture points is that they are well described in the literature, and acupuncturists trained according to medical acupuncture theories or trained in classic traditional Chinese medicine theories all know the locations of such points and how the needles should be inserted. Thus, using the acupuncture name/number makes it easier for all practitioners to know where the needles were placed. All needles will be stimulated manually by rotation until needle sensation is evoked, which is indicative of activation of a-delta and c-fibers when the needles are inserted. Needles placed in the abdominal muscles and leg muscles will be attached to an electrical stimulator (Export Abteilung, Schwa-Medico GmbH, Germany) and electrically stimulated at 2 Hz for 30 min during each treatment session. Needles not connected to the electrical stimulator will be stimulated manually 4 times during each 30 min treatment session (Table 1).

Study Procedures

The trial has two phases. The first phase is the 6 months of acupuncture treatment, and the second phase is 6 months of follow-up (Figure 1). Each specific visit and measurement is summarized in Table 2.

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The women in the study will be screened for IR. Those with a HOMA-IR ([fasting insulin (μU/mL) × fasting glucose (mmol/L)] / 22.5) ≥ 2.14 will be included and divided into the normal weight (BMI = 18.5–23 kg/m^2) group and the overweight/obese (BMI ≥ 23 kg/m^2) group. After inclusion and baseline measurements, acupuncture treatment will start and last for 6 months followed by 6 months of follow-up.

Outcome measurements

Primary outcome

The primary outcome will be the changes in HOMA-IR between baseline and after 6 months of acupuncture treatment and between baseline and the 6-month follow-up. The oral glucose tolerance test (OGTT) with 75 g glucose will be performed in all subjects after an overnight fast. Blood samples will be obtained to measure plasma glucose and serum insulin at 0, 60, and 120 min during the OGTT.

Secondary outcomes

1. HOMA-B: Islet β-cell function will be evaluated by the formula (20 × fasting insulin (mU/mL) / (fasting plasma glucose (mmol/L) − 3.5)) [34] and by the C-peptide index (CPI), which is measured as (fasting C-peptide (nmol/L) / fasting plasma glucose (mmol/L) × 100) [35].
2. The insulin response to glucose will be assessed by calculating the area under the curve during the OGTT for glucose (AUC_{glu}) and insulin (AUC_{ins}) using the trapezoidal rule[36].
3. Menstrual bleeding patterns will be evaluated.
4. Body composition will be determined as weight, height, waist-to-hip circumference, and

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BMI.

5. Metabolic measures will include HbA1c, C-peptide, adiponectin, fasting cholesterol, fasting triglycerides (TG), ApoA1, ApoB, and blood pressure.
6. Endocrine measures will include hirsutism as assessed by Ferriman–Gallwey (FG) score, the presence of acne, and serum concentrations of testosterone, sex hormone-binding globulin (SHBG), follicle stimulating hormone (FSH), luteinizing hormone (LH), and dehydroepiandrosterone sulfate (DHEAS).
7. Questionnaires will include the short form-36 (SF36) [37], the PCOS questionnaire (PCOSQ) [38], generic and diagnosis-specific health-related quality of life questionnaires, and the Chinese Quality of Life (ChQOL) questionnaire [39]. Symptoms of anxiety and depression will be assessed by the Zung Self-Rating Anxiety Scale (Zung SAS) and the Zung Self-Rating Depression Scale (Zung SDS) [40]. In addition, the International Physical Activity Questionnaire (IPAQ) [41] written in Chinese will be used to measure the frequency and duration of moderate physical activity every week.
8. Adverse events will be recorded.

Data entry and quality control of data

Case report forms will be developed for data entry, and quality control of the data will be handled at two different levels. The investigators will be required to ensure the accuracy of the data as the first level of control, and the second level will include data monitoring and validation that will be carried out on a regular basis throughout the study.

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Sample size calculations and statistical analysis

Our previous study on treating obese PCOS women with abdominal acupuncture showed that the HOMA-IR was significantly reduced after treatment (3.9 ± 1.4) compared to baseline measurements (2.5 ± 1.7) [33]. A HOMA-IR ≥ 2.14 was considered to be abnormal [29]. If we assume a more moderate 20% reduction in HOMA-IR, i.e., a reduction by 0.78, we will have to recruit 40 overweight or obese subjects and 40 normal-weight subjects. With an estimated 40% drop out rate, we plan to recruit 56 normal weight and 56 overweight/obese women with PCOS.

One sample of the Kolmogorov-Smirnov test will be used to test the normal distribution of continuous variables. Continuous variables will be shown as means ± standard deviations if they are normally distributed or as medians with interquartile ranges if they are not normally distributed. Statistical comparisons will be carried out according to the intention to treat by Student's t-test, Mann–Whitney U-test, and Wilcoxon signed ranks test for continuous variables and by χ^2 tests for categorical variables where appropriate. All statistical analyses of the data will be performed using the SPSS program version 21.0 (SPSS Inc., Chicago, IL, USA), and a *P*-value < 0.05 will be considered statistically significant.

Safety and ethical considerations

Acupuncture is a safe procedure, and few side effects have been reported. The major risks of acupuncture are local skin irritation, discomfort, and vasovagal reactions during the procedure. The women who agree to participate in the study will sign a consent form. The study has been

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approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (No. 2013-039).

Discussion

Acupuncture has been used in the treatment of women with PCOS [17-19 33], but the effect of acupuncture on IR in women with PCOS is still unknown. This prospective observational study has been designed, therefore, to evaluate whether acupuncture improves insulin sensitivity in women with PCOS and IR. The research seeks to add significantly to the clinical evidence base and to allow conclusions to be made on the role of acupuncture in the treatment of PCOS.

A limitation of the present study is that it is a single-center study without comparison groups, and it can be argued that it lacks scientific rigor because of this. To increase the validity of the study, we will have experts controlling the quality of study procedure through regular site visits. The experts will monitor the recruitment of study subjects, the measurement and treatment procedures, and the data analysis. We plan to conduct a randomized controlled trial at a later data using standard care as a control.

This study was conceived and designed in 2013, and the first subject was recruited on February 18, 2014. At the time of this manuscript submission, 368 women with PCOS and IR had been recruited, and at the time of revision and resubmission 83 women had been recruited.

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Abbreviations

Apoa1: Apolipoprotein A1; ApoB: Apolipoprotein B; C: Cervical vertebra; CV: Conception vessel; DHEAS: Dehydroepiandrosterone sulfate; E2: Estradiol; EA: Electro-acupuncture; FSH: Follicle-stimulating hormone; FG: Ferriman–Gallwey score; GDR: Glucose disposal rate; IR: insulin resistance; L: Lumbar vertebra; LH: Luteinizing hormone; LI: Large intestine; LR: Liver; PC: Pericardium; PCOS: Polycystic ovary syndrome; S: Sacral vertebra; SP: Spleen; ST: Stomach; SHBG: Sex hormone-binding globulin; T: Total testosterone; TC: Total cholesterol; TG: Triglyceride; Th: Thoracic vertebra; OGTT: Oral glucose tolerance test.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YHZ, ESV, and EHNG contributed equally to this work. ESV, EHNG, and HMX conceived and designed the study. YHZ, EHNG, and ESV drafted and critically revised the manuscript for important intellectual content. HXM sought funding and ethical approval. All authors contributed to the further writing of the manuscript and approved the 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
Set 1				
Zhongji; CV3	EA	4 cun caudal to the umbilicus	Fibrous tissue, *linea alba*	L1
Zhongwan; CV12	EA	On the midline, 4 cun superior to the umbilicus	Fibrous tissue, *linea alba*	Th7–8
Guilai; ST29 Bilateral	EA	1 cun cranial to the pubic bone and 2 cun lateral of the midline	*M. rectus abdominis*	Th6–12
Liangqiu; ST34 Bilateral	EA	2 cun above the superior lateral border of the patella on the line connecting the anterior superior iliac spine found with the knee flexed	*M. quadriceps femoris*	femoral nerve
Yinshi; ST33 Bilateral	EA	3 cun above the superior lateral border of the patella on the line connecting the anterior superior iliac spine found with the knee flexed	*M. quadriceps femoris*	femoral nerve
Sanyinjiao; SP6	DeQi four times	3 cun proximal to the *medial malleolus*	*Mm. flexor digitorum longus*, *tibialis posterior*	L4–5, S1–2
Zusanli; ST36	DeQi four times	On the anterior lateral side of the leg, 3 cun below *Dubi* (ST35), one finger width (middle finger) from the anterior crest of the tibia	*musculi tibialis anterior*	L4–5, S1
Hegu; LI4	DeQi four times	On the highest point at *m. interosseus dorsalis*	*Mm. interosseus dorsalis I*, *lumbricalis II*, *adductor pollicis*	C8, Th1

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Set 2				
Daju; ST27	EA	3 cun cranial to the pubic bone and 2 cun lateral to the midline	*M. rectus abdominis*	Th6–12
Qihai; CV6	EA	1.5 cun caudal to the umbilicus	Fibrous tissue, *linea alba*	Th11
Xiawan; CV10	EA	2 cun cranial to the umbilicus	Fibrous tissue, *linea alba*	Th8
Extra meridian point	EA	6 cun above the patella in line with SP10	*M. quadriceps femoris*	L2–L4
Xuehai; SP10	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
Sanyinjiao; SP6	DeQi four times	3 cun proximal to the medial malleolus	*Mm. flexor digitorum longus*, *tibialis posterior*	L4–5, S1–2
Taichong; LR3	DeQi four times	Between metatarsal I & II, just distal to the caput	*M. interosseus dorsalis I*	S2–3
Neiguan; PC6	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)

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Table 2. Overview of study visits, including screening, baseline measurements, after-treatment measurements, and follow-up measurements. Months indicate when each specific measurement takes place.

	Screening and base line visit	Month						Follow-up 6th month
		1st	2nd	3rd	4th	5th	6th	
Body composition (weight, height, waist circumference, hip circumference)	√	√	√	√	√	√	√	√
Menstrual cycle diary	√	√	√	√	√	√	√	√
Fasting blood samples for Apoa1, ApoB, TC, TG, C-peptide, HbA1c	√						√	√
Fasting blood samples for FSH, LH, SHBG, T, E2, P, DHEAS	√						√	√
Transvaginal ultrasound	√						√	√
Questionnaire	√						√	√
FG/acne	√	√	√	√	√	√	√	√
OGTT	√			√			√	√

(Apoa1: Apolipoprotein A1; ApoB: Apolipoprotein B; DHEAS: Dehydroepiandrosterone sulfate; E2: Estradiol; FSH: Follicle-stimulating hormone; FG: Ferriman–Gallwey score; LH: Luteinizing hormone; SHBG: Sex hormone-binding globulin; T: Total testosterone; TC: Total cholesterol; TG: Triglyceride; OGTT: Oral glucose tolerance test.)

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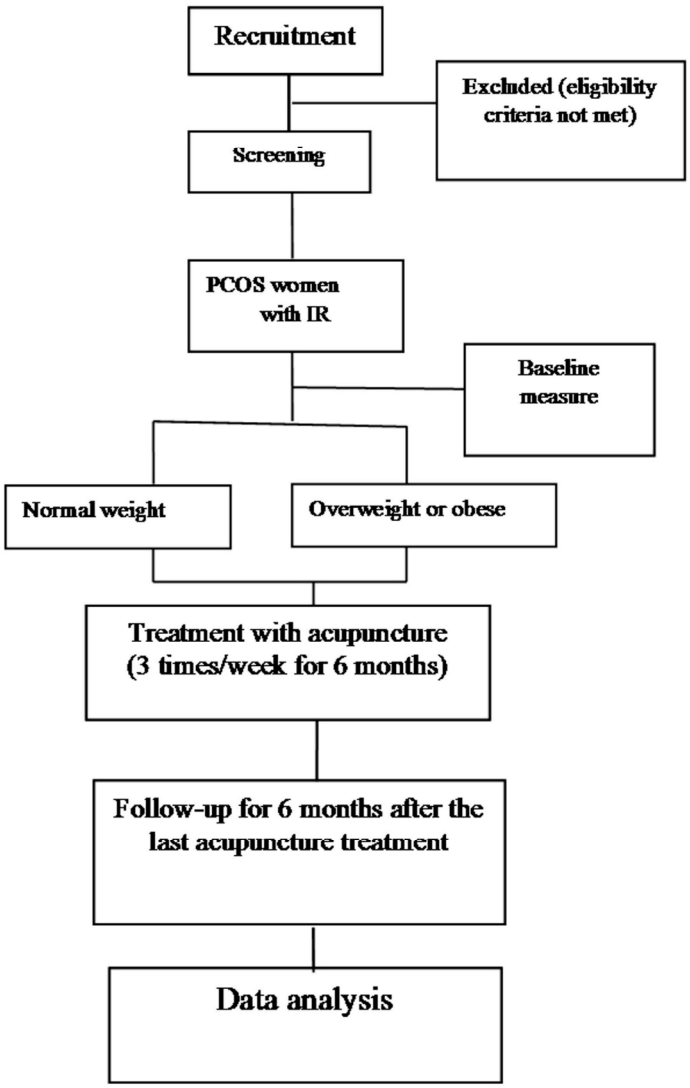

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How does acupuncture affect insulin sensitivity in women with polycystic ovary syndrome and insulin resistance? Study protocol of a prospective pilot study

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ABSTRACT

INTRODUCTION: Hyperinsulinemia and insulin resistance are key features of polycystic ovary syndrome (PCOS) and metabolic syndrome. The effect of five-weeks of acupuncture treatment has been investigated in a completed prospective pilot trial (Clinicaltrials.gov: NCT01457209), and acupuncture with electrical stimulation applied to insulin-resistant rats with dihydrotestosterone-induced PCOS was shown to improve insulin sensitivity. Therefore, we now aim to conduct a prospective pilot study to evaluate whether using the same acupuncture treatment protocol given over a longer period of time (6 months) than in the previous pilot trial will improve insulin sensitivity in women with PCOS and insulin resistance (IR). Our hypothesis is that acupuncture with combined manual and low-frequency electrical stimulation of the needles will improve insulin sensitivity in women with PCOS and IR.

METHODS/ANALYSIS: This is a prospective pilot trial. A total of 112 women with PCOS and IR will be recruited and categorized according to their body mass index (BMI) as normal weight (BMI = 18.5–23 kg/m^2) or as overweight/obese (BMI > 23 kg/m^2). Acupuncture will be applied three times per week for 6 months at 30 minutes per treatment. The primary outcome will be the change in insulin sensitivity before and after 6 months of acupuncture treatment as measured by an oral glucose tolerance test.

ETHICS/DISSEMINATION: Ethical approval of this study has been granted from the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (No. 2013039). Written and informed consent will be obtained from each patient before any study procedure is performed according to good clinical practice. The results of this trial will be disseminated in a peer-reviewed journal and presented at international congresses.

TRIAL REGISTRATION: ClinicalTrials.gov (NCT02026323) and the Chinese Clinical Trials Registry (ChiCTR-OCH-13003921).

KEY WORDS: acupuncture, insulin resistance, polycystic ovary syndrome

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Background

Features of polycystic ovary syndrome (PCOS) include ovulatory dysfunction, clinical and biochemical signs of hyperandrogenism, and the presence of polycystic ovaries upon pelvic scanning [1 2]. PCOS is the most common endocrine and metabolic disorder in reproductive-age women [2] and is found in 5.6% of Chinese women aged 19–45 years as shown in a recent community-based study [1]. The main metabolic phenotype is hyperinsulinemia and insulin resistance (IR), which is observed in about 50%–70% of all women with PCOS [3 4]. Importantly, IR can be present in both lean and obese women with PCOS [5 6], especially in those with hyperandrogenism and ovulatory dysfunction [7 8].

PCOS causes significant menstrual and fertility issues, but over the past few decades its additional metabolic and cardiovascular risks have become apparent. Women with PCOS display both IR and reduced insulin responsiveness [9] and have at least a 3-fold increased risk of developing type 2 diabetes mellitus [10]. IR in women with PCOS has been attributed to post-binding defects in adipocytes and skeletal muscle insulin signaling [9 11]. Furthermore, compensatory hyperinsulinemia fuels ovarian androgen production by stimulating androgen production and secretion by theca cells and by reducing sex hormone-binding globulin (SHBG) levels, which increases free androgens and further exacerbates PCOS symptoms [12]. Thus, there is a strong association between hyperinsulinemia and hyperandrogenemia.

Overweight/obesity is a common feature of PCOS [13]. A Korean study showed that 61% of women with PCOS were lean, 10.3% were overweight, and 28.4% were obese[14]. Obesity aggravates most of the metabolic dysfunctions in women with PCOS. Women with comorbid PCOS and depression have been found to have higher BMI and IR compared to women with

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PCOS without depression [15], and this increase in BMI negatively affects quality of life [16].

Despite the high prevalence of IR, impaired glucose tolerance, and/or type 2 diabetes mellitus in women with PCOS, there is no consensus on the best long-term management of these conditions. Pharmacological treatments, including metformin, are symptom oriented and usually effective but have unpleasant gastrointestinal side effects. Therefore, it is important to evaluate other non-pharmacological treatment strategies because most women with PCOS require long-term treatment.

Acupuncture, which is one of the main treatment modalities of traditional Chinese medicine, is increasingly being used in the area of reproductive endocrinology and infertility in many different parts of the world. Interestingly, increasing the treatment frequency and the number of treatments leads to higher ovulation frequency [17] indicating the importance of the correct treatment dose. In a secondary analysis of the trial by Jedel et al [18], low-frequency electro-acupuncture (EA) decreased high plasminogen activator inhibitor 1 (PAI-1) activity without affecting insulin sensitivity as measured by the euglycemic hyperinsulinemic clamp [19]. Clearly the intensity, frequency, and duration of low-frequency EA treatment in that study were too low to affect insulin sensitivity. Support for this assumption comes from our experimental studies in the dihydrotestosterone (DHT)-induced PCOS rat model in which we demonstrated that low-frequency EA improves whole-body insulin sensitivity in a dose-dependent manner [20 21].

EA induces both systemic and local effects involving intracellular insulin signaling pathways in skeletal muscle and adipose tissue [20 22 23]. Furthermore, EA has been shown to reduce plasma glucose levels by promoting insulin production and to improve insulin sensitivity by

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inducing secretion of endogenous β-endorphin in different rodent models of diabetes mellitus [24 25]. The potential role of acupuncture in the treatment of women with PCOS and IR has not yet been determined, and this is an important area to investigate because most women with PCOS require long-term treatment. There is one completed prospective pilot trial investigating the effect of five-weeks of acupuncture treatment with combined manual and low-frequency electrical stimulation of the needles (Clinicaltrials.gov: NCT01457209). Therefore, the aim of the proposed study is to investigate the clinical effectiveness of acupuncture on insulin sensitivity in women with PCOS and IR when acupuncture is given over a longer period of time (6 months) than in the previous trial using the same treatment protocol. Our hypothesis is that acupuncture with combined manual and low-frequency electrical stimulation of the needles will improve insulin sensitivity in women with PCOS and IR.

Materials and Methods

Study Design

This is a single-center prospective pilot study, and subjects will be enrolled from the First Affiliated Hospital of Guangzhou Medical University. Ethical approval of this study has been granted from the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (No. 2013039). Written and informed consent will be obtained from each patient before any study procedure is performed according to good clinical practice. The trial has been registered at ClinicalTrial.gov (NCT02026323) and with the Chinese Clinical Trials Registry (ChiCTR-OCH-13003921).

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Participants

Subjects will be recruited if they fulfill all of the inclusion criteria and do not meet any of the exclusion criteria.

Inclusion criteria

1) Age between 18 and 40 years.

2) BMI ≥ 18.5 kg/m^2.

3) Presence of PCOS as defined by the Rotterdam criteria and including at least two of the following three features:

- Oligomenorrhea or amenorrhea. Oligomenorrhea is defined as an intermenstrual interval >35 days or <8 menstrual bleedings in the past year. Amenorrhea is defined as complete cessation of menstrual cycles for 6 months or more when a patient has previously had regular cycles and for 12 months or more when the patient has had irregular cycles.
- Clinical or biochemical hyperandrogenism. Biochemical hyperandrogenemia is defined as a total serum testosterone concentration above 60 ng/dL [26], and clinical hyperandrogenism is defined as a Ferriman–Gallwey (FG) score ≥ 5 in mainland China [27].
- Polycystic ovary morphology. This is defined as ≥ 12 antral follicles (2–9 mm in diameter) or an ovarian volume > 10 mL upon transvaginal scanning [28].

4) Presence of IR as defined by the homeostatic model assessment (HOMA-IR: [fasting insulin (μU/mL) × fasting glucose (mmol/L)] / 22.5). A value ≥ 2.14 will be considered

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to be indicative of IR [29].

5) No desire to bear children and having used barrier methods of contraception for one year.

6) Willingness to sign the consent form.

Exclusion criteria

1) Having other endocrine disorders such as hyperprolactinemia (defined as two prolactin levels measured at least one week apart of 25 ng/mL or greater or as determined by local normative values), nonclassic congenital adrenal hyperplasia (17-hydroxyprogesterone < 3 nmol/L), or androgen-secreting tumors.

2) FSH levels > 15 mIU/mL. A normal level within the last year is adequate for entry.

3) Uncorrected thyroid disease defined as thyroid stimulating hormone (TSH) < 0.2 mIU/mL or TSH > 5.5 mIU/mL. A normal level within the last year is adequate for entry.

4) Type I diabetes mellitus or Type I and Type II patients who are receiving antidiabetic medications such as insulin, thiazolidinediones, acarbose, sulfonylureas, or other medications that are likely to confound the effects of the study. Patients currently receiving metformin for a diagnosis of Type I or Type II diabetes or for PCOS are also specifically excluded.

5) Suspected Cushing's syndrome.

6) Use of hormones or other medications in the past 3 months, including Chinese herbal prescriptions, which might affect the outcome.

7) Pregnancy within the last 6 weeks.

8) Post-abortion or postpartum within the last 6 weeks.

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9) Breastfeeding within the last 6 months.

10) Receiving acupuncture treatment related to PCOS within the past 2 months.

11) Having undergone a bariatric surgery procedure within the past 12 months or being in a period of acute weight loss.

12) Having known congenital adrenal hyperplasia.

13) Lack of written consent to participate in the study.

Eligible subjects will be recruited and categorized according to their BMI as normal weight (BMI = 18.5–23 kg/m^2) or overweight/obese (BMI ≥ 23 kg/m^2) [30].

Interventions

The acupuncture protocol is based on Western medical theories, and the study protocol follows the CONSORT [31] and STRICTA [32] recommendations with detailed descriptions of the treatment, including the number of needles used, depth of needle insertion, how needles will be stimulated (manual or electrical), frequency of sessions, and length of the treatment period. Acupuncture will be given according to a fixed protocol by traditional Chinese medicine practitioners educated in theoretical and practical acupuncture. The protocol is based on experimental studies elucidating the effect of acupuncture in rodent models of diabetes mellitus and PCOS [20-22] and in women with PCOS [19 33].

Acupuncture protocol

Acupuncture will be given three times per week over 6 months for a total of 80 sessions.

Disposable, sterilized needles for single use made of stainless steel (0.20 mm × 30 mm and

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0.20 mm × 40/50 mm; Hwoto, Suzhou Medical Appliance Fact., 215005 Suzhou, China) will be inserted to a depth of 15 mm to 35 mm in segmental acupuncture points located in the abdominal and leg muscles with innervations corresponding to the ovaries and pancreas that have been shown to improve insulin sensitivity in rodents. Two sets of acupuncture points will be alternated for every other treatment session. The two acupuncture protocols follow the STRICTA recommendations and are presented in Table 1. The rationale for using the traditional nomenclature of acupuncture points is that they are well described in the literature, and acupuncturists trained according to medical acupuncture theories or trained in classic traditional Chinese medicine theories all know the locations of such points and how the needles should be inserted. Thus, using the acupuncture name/number makes it easier for all practitioners to know where the needles were placed. All needles will be stimulated manually by rotation until needle sensation is evoked, which is indicative of activation of a-delta and c-fibers when the needles are inserted. Needles placed in the abdominal muscles and leg muscles will be attached to an electrical stimulator (Export Abteilung, Schwa-Medico GmbH, Germany) and electrically stimulated at 2 Hz for 30 min during each treatment session. Needles not connected to the electrical stimulator will be stimulated manually 4 times during each 30 min treatment session (Table 1).

Study Procedures

The trial has two phases. The first phase is the 6 months of acupuncture treatment, and the second phase is 6 months of follow-up (Figure 1). Each specific visit and measurement is summarized in Table 2.

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The women in the study will be screened for IR. Those with a HOMA-IR ([fasting insulin (μU/mL) × fasting glucose (mmol/L)] / 22.5) ≥ 2.14 will be included and divided into the normal weight (BMI = 18.5–23 kg/m^2) group and the overweight/obese (BMI ≥ 23 kg/m^2) group. After inclusion and baseline measurements, acupuncture treatment will start and last for 6 months followed by 6 months of follow-up.

Outcome measurements

Primary outcome

The primary outcome will be the changes in HOMA-IR between baseline and after 6 months of acupuncture treatment and between baseline and the 6-month follow-up. The oral glucose tolerance test (OGTT) with 75 g glucose will be performed in all subjects after an overnight fast. Blood samples will be obtained to measure plasma glucose and serum insulin at 0, 60, and 120 min during the OGTT.

Secondary outcomes

1. HOMA-B: Islet β-cell function will be evaluated by the formula (20 × fasting insulin (mU/mL) / (fasting plasma glucose (mmol/L) − 3.5)) [34] and by the C-peptide index (CPI), which is measured as (fasting C-peptide (nmol/L) / fasting plasma glucose (mmol/L) × 100) [35].
2. The insulin response to glucose will be assessed by calculating the area under the curve during the OGTT for glucose (AUC_{glu}) and insulin (AUC_{ins}) using the trapezoidal rule[36].
3. Menstrual bleeding patterns will be evaluated.
4. Body composition will be determined as weight, height, waist-to-hip circumference, and

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BMI.

5. Metabolic measures will include HbA1c, C-peptide, adiponectin, fasting cholesterol, fasting triglycerides (TG), ApoA1, ApoB, and blood pressure.
6. Endocrine measures will include hirsutism as assessed by Ferriman–Gallwey (FG) score, the presence of acne, and serum concentrations of testosterone, sex hormone-binding globulin (SHBG), follicle stimulating hormone (FSH), luteinizing hormone (LH), and dehydroepiandrosterone sulfate (DHEAS).
7. Questionnaires will include the short form-36 (SF36) [37], the PCOS questionnaire (PCOSQ) [38], generic and diagnosis-specific health-related quality of life questionnaires, and the Chinese Quality of Life (ChQOL) questionnaire [39]. Symptoms of anxiety and depression will be assessed by the Zung Self-Rating Anxiety Scale (Zung SAS) and the Zung Self-Rating Depression Scale (Zung SDS) [40]. In addition, the International Physical Activity Questionnaire (IPAQ) [41] written in Chinese will be used to measure the frequency and duration of moderate physical activity every week.
8. Adverse events will be recorded.

Data entry and quality control of data

Case report forms will be developed for data entry, and quality control of the data will be handled at two different levels. The investigators will be required to ensure the accuracy of the data as the first level of control, and the second level will include data monitoring and validation that will be carried out on a regular basis throughout the study.

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Sample size calculations and statistical analysis

Our previous study on treating obese PCOS women with abdominal acupuncture showed that the HOMA-IR was significantly reduced after treatment (3.9 ± 1.4) compared to baseline measurements (2.5 ± 1.7) [33]. A HOMA-IR ≥ 2.14 was considered to be abnormal [29]. If we assume a more moderate 20% reduction in HOMA-IR, i.e., a reduction by 0.78, we will have to recruit 40 overweight or obese subjects and 40 normal-weight subjects. With an estimated 40% drop out rate, we plan to recruit 56 normal weight and 56 overweight/obese women with PCOS.

One sample of the Kolmogorov-Smirnov test will be used to test the normal distribution of continuous variables. Continuous variables will be shown as means ± standard deviations if they are normally distributed or as medians with interquartile ranges if they are not normally distributed. Statistical comparisons will be carried out according to the intention to treat by Student's t-test, Mann–Whitney U-test, and Wilcoxon signed ranks test for continuous variables and by χ^2 tests for categorical variables where appropriate. All statistical analyses of the data will be performed using the SPSS program version 21.0 (SPSS Inc., Chicago, IL, USA), and a *P*-value < 0.05 will be considered statistically significant.

Safety and ethical considerations

Acupuncture is a safe procedure, and few side effects have been reported. The major risks of acupuncture are local skin irritation, discomfort, and vasovagal reactions during the procedure. The women who agree to participate in the study will sign a consent form. The study has been

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approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (No. 2013-039).

Discussion

Acupuncture has been used in the treatment of women with PCOS [17-19 33], but the effect of acupuncture on IR in women with PCOS is still unknown. This prospective pilot study has been designed, therefore, to evaluate whether acupuncture improves insulin sensitivity in women with PCOS and IR. The research seeks to add significantly to the clinical evidence base and to allow conclusions to be made on the role of acupuncture in the treatment of PCOS.

A limitation of the present study is that it is a single-center study without comparison groups, and it can be argued that it lacks scientific rigor because of this. To increase the validity of the study, we will have experts controlling the quality of study procedure through regular site visits. The experts will monitor the recruitment of study subjects, the measurement and treatment procedures, and the data analysis. We plan to conduct a randomized controlled trial at a later data using standard care as a control.

This study was conceived and designed in 2013, and the first subject was recruited on February 18, 2014. At the time of this manuscript submission, 368 women with PCOS and IR had been recruited, and at the time of revision and resubmission 83 women had been recruited.

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Abbreviations

Apoa1: Apolipoprotein A1; ApoB: Apolipoprotein B; C: Cervical vertebra; CV: Conception vessel; DHEAS: Dehydroepiandrosterone sulfate; E2: Estradiol; EA: Electro-acupuncture; FSH: Follicle-stimulating hormone; FG: Ferriman–Gallwey score; GDR: Glucose disposal rate; IR: insulin resistance; L: Lumbar vertebra; LH: Luteinizing hormone; LI: Large intestine; LR: Liver; PC: Pericardium; PCOS: Polycystic ovary syndrome; S: Sacral vertebra; SP: Spleen; ST: Stomach; SHBG: Sex hormone-binding globulin; T: Total testosterone; TC: Total cholesterol; TG: Triglyceride; Th: Thoracic vertebra; OGTT: Oral glucose tolerance test.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YHZ, ESV, and EHNG contributed equally to this work. ESV, EHNG, and HMX conceived and designed the study. YHZ, EHNG, and ESV drafted and critically revised the manuscript for important intellectual content. HXM sought funding and ethical approval. All authors contributed to the further writing of the manuscript and approved the 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
Set 1				
Zhongji; CV3	EA	4 cun caudal to the umbilicus	Fibrous tissue, *linea alba*	L1
Zhongwan; CV12	EA	On the midline, 4 cun superior to the umbilicus	Fibrous tissue, *linea alba*	Th7–8
Guilai; ST29 Bilateral	EA	1 cun cranial to the pubic bone and 2 cun lateral of the midline	*M. rectus abdominis*	Th6–12
Liangqiu; ST34 Bilateral	EA	2 cun above the superior lateral border of the patella on the line connecting the anterior superior iliac spine found with the knee flexed	*M. quadriceps femoris*	femoral nerve
Yinshi; ST33 Bilateral	EA	3 cun above the superior lateral border of the patella on the line connecting the anterior superior iliac spine found with the knee flexed	*M. quadriceps femoris*	femoral nerve
Sanyinjiao; SP6	DeQi four times	3 cun proximal to the *medial malleolus*	*Mm. flexor digitorum longus*, *tibialis posterior*	L4–5, S1–2
Zusanli; ST36	DeQi four times	On the anterior lateral side of the leg, 3 cun below *Dubi* (ST35), one finger width (middle finger) from the anterior crest of the tibia	*musculi tibialis anterior*	L4–5, S1
Hegu; LI4	DeQi four times	On the highest point at *m. interosseus dorsalis*	*Mm. interosseus dorsalis I*, *lumbricalis II*, *adductor pollicis*	C8, Th1

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Set 2				
Daju; ST27	EA	3 cun cranial to the pubic bone and 2 cun lateral to the midline	*M. rectus abdominis*	Th6–12
Qihai; CV6	EA	1.5 cun caudal to the umbilicus	Fibrous tissue, *linea alba*	Th11
Xiawan; CV10	EA	2 cun cranial to the umbilicus	Fibrous tissue, *linea alba*	Th8
Extra meridian point	EA	6 cun above the patella in line with SP10	*M. quadriceps femoris*	L2–L4
Xuehai; SP10	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
Sanyinjiao; SP6	DeQi four times	3 cun proximal to the medial malleolus	*Mm. flexor digitorum longus*, *tibialis posterior*	L4–5, S1–2
Taichong; LR3	DeQi four times	Between metatarsal I & II, just distal to the caput	*M. interosseus dorsalis I*	S2–3
Neiguan; PC6	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)

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Table 2. Overview of study visits, including screening, baseline measurements, after-treatment measurements, and follow-up measurements. Months indicate when each specific measurement takes place.

	Screening and base line visit	Month						Follow-up 6th month
		1st	2nd	3rd	4th	5th	6th	
Body composition (weight, height, waist circumference, hip circumference)	√	√	√	√	√	√	√	√
Menstrual cycle diary	√	√	√	√	√	√	√	√
Fasting blood samples for Apoa1, ApoB, TC, TG, C-peptide, HbA1c	√						√	√
Fasting blood samples for FSH, LH, SHBG, T, E2, P, DHEAS	√						√	√
Transvaginal ultrasound	√						√	√
Questionnaire	√						√	√
FG/acne	√	√	√	√	√	√	√	√
OGTT	√			√			√	√

(Apoa1: Apolipoprotein A1; ApoB: Apolipoprotein B; DHEAS: Dehydroepiandrosterone sulfate; E2: Estradiol; FSH: Follicle-stimulating hormone; FG: Ferriman–Gallwey score; LH: Luteinizing hormone; SHBG: Sex hormone-binding globulin; T: Total testosterone; TC: Total cholesterol; TG: Triglyceride; OGTT: Oral glucose tolerance test.)

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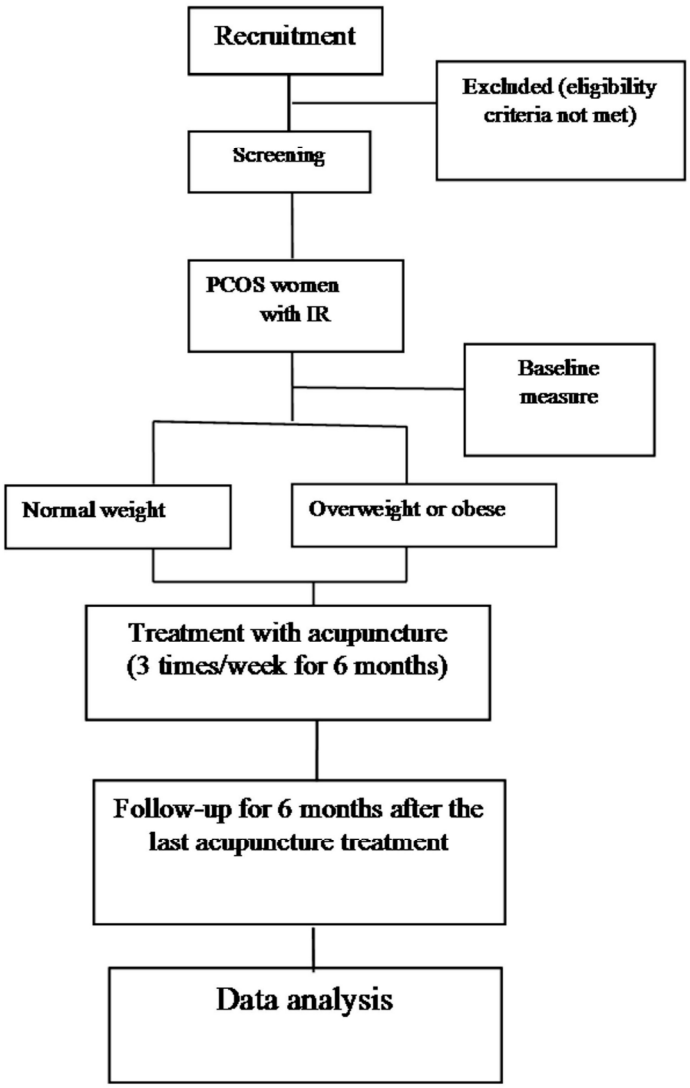

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How does acupuncture affect insulin sensitivity in women with polycystic ovary syndrome and insulin resistance? Study protocol of a prospective pilot study

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How does acupuncture affect insulin sensitivity in women with polycystic ovary syndrome and insulin resistance? Study protocol of a prospective pilot study

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ABSTRACT

INTRODUCTION: Hyperinsulinemia and insulin resistance are key features of polycystic ovary syndrome (PCOS) and metabolic syndrome. The effect of five-weeks of acupuncture treatment has been investigated in a completed prospective pilot trial (Clinicaltrials.gov: NCT01457209), and acupuncture with electrical stimulation applied to insulin-resistant rats with dihydrotestosterone-induced PCOS was shown to improve insulin sensitivity. Therefore, we now aim to conduct a prospective pilot study to evaluate whether using the same acupuncture treatment protocol given over a longer period of time (6 months) than in the previous pilot trial will improve insulin sensitivity in women with PCOS and insulin resistance (IR). Our hypothesis is that acupuncture with combined manual and low-frequency electrical stimulation of the needles will improve insulin sensitivity in women with PCOS and IR.

METHODS/ANALYSIS: This is a prospective pilot trial. A total of 112 women with PCOS and IR will be recruited and categorized according to their body mass index (BMI) as normal weight (BMI = 18.5–23 kg/m^2) or as overweight/obese (BMI > 23 kg/m^2). Acupuncture will be applied three times per week for 6 months at 30 minutes per treatment. The primary outcome will be the change in insulin sensitivity before and after 6 months of acupuncture treatment as measured by an oral glucose tolerance test.

ETHICS/DISSEMINATION: Ethical approval of this study has been granted from the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (No. 2013039). Written and informed consent will be obtained from each patient before any study procedure is performed according to good clinical practice. The results of this trial will be disseminated in a peer-reviewed journal and presented at international congresses.

TRIAL REGISTRATION: ClinicalTrials.gov (NCT02026323) and the Chinese Clinical Trials Registry (ChiCTR-OCH-13003921).

KEY WORDS: acupuncture, insulin resistance, polycystic ovary syndrome

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Background

Features of polycystic ovary syndrome (PCOS) include ovulatory dysfunction, clinical and biochemical signs of hyperandrogenism, and the presence of polycystic ovaries upon pelvic scanning [1 2]. PCOS is the most common endocrine and metabolic disorder in reproductive-age women [2] and is found in 5.6% of Chinese women aged 19–45 years as shown in a recent community-based study [1]. The main metabolic phenotype is hyperinsulinemia and insulin resistance (IR), which is observed in about 50%–70% of all women with PCOS [3 4]. Importantly, IR can be present in both lean and obese women with PCOS [5 6], especially in those with hyperandrogenism and ovulatory dysfunction [7 8].

PCOS causes significant menstrual and fertility issues, but over the past few decades its additional metabolic and cardiovascular risks have become apparent. Women with PCOS display both IR and reduced insulin responsiveness [9] and have at least a 3-fold increased risk of developing type 2 diabetes mellitus [10]. IR in women with PCOS has been attributed to post-binding defects in adipocytes and skeletal muscle insulin signaling [9 11]. Furthermore, compensatory hyperinsulinemia fuels ovarian androgen production by stimulating androgen production and secretion by theca cells and by reducing sex hormone-binding globulin (SHBG) levels, which increases free androgens and further exacerbates PCOS symptoms [12]. Thus, there is a strong association between hyperinsulinemia and hyperandrogenemia.

Overweight/obesity is a common feature of PCOS [13]. A Korean study showed that 61% of women with PCOS were lean, 10.3% were overweight, and 28.4% were obese[14]. Obesity aggravates most of the metabolic dysfunctions in women with PCOS. Women with comorbid PCOS and depression have been found to have higher BMI and IR compared to women with

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PCOS without depression [15], and this increase in BMI negatively affects quality of life [16].

Despite the high prevalence of IR, impaired glucose tolerance, and/or type 2 diabetes mellitus in women with PCOS, there is no consensus on the best long-term management of these conditions. Pharmacological treatments, including metformin, are symptom oriented and usually effective but have unpleasant gastrointestinal side effects. Therefore, it is important to evaluate other non-pharmacological treatment strategies because most women with PCOS require long-term treatment.

Acupuncture, which is one of the main treatment modalities of traditional Chinese medicine, is increasingly being used in the area of reproductive endocrinology and infertility in many different parts of the world. Interestingly, increasing the treatment frequency and the number of treatments leads to higher ovulation frequency [17] indicating the importance of the correct treatment dose. In a secondary analysis of the trial by Jedel et al [18], low-frequency electro-acupuncture (EA) decreased high plasminogen activator inhibitor 1 (PAI-1) activity without affecting insulin sensitivity as measured by the euglycemic hyperinsulinemic clamp [19]. Clearly the intensity, frequency, and duration of low-frequency EA treatment in that study were too low to affect insulin sensitivity. Support for this assumption comes from our experimental studies in the dihydrotestosterone (DHT)-induced PCOS rat model in which we demonstrated that low-frequency EA improves whole-body insulin sensitivity in a dose-dependent manner [20 21].

EA induces both systemic and local effects involving intracellular insulin signaling pathways in skeletal muscle and adipose tissue [20 22 23]. Furthermore, EA has been shown to reduce plasma glucose levels by promoting insulin production and to improve insulin sensitivity by

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inducing secretion of endogenous β-endorphin in different rodent models of diabetes mellitus [24 25]. The potential role of acupuncture in the treatment of women with PCOS and IR has not yet been determined, and this is an important area to investigate because most women with PCOS require long-term treatment. There is one completed prospective pilot trial investigating the effect of five-weeks of acupuncture treatment with combined manual and low-frequency electrical stimulation of the needles (Clinicaltrials.gov: NCT01457209). Therefore, the aim of this pilot study is to investigate the clinical effectiveness of acupuncture on insulin sensitivity in women with PCOS and IR when acupuncture is given over a longer period of time (6 months) than in the previous pilot trial using the same treatment protocol. Our hypothesis is that acupuncture with combined manual and low-frequency electrical stimulation of the needles will improve insulin sensitivity in women with PCOS and IR.

Materials and Methods

Study Design

This is a single-center prospective pilot study, and subjects will be enrolled from the First Affiliated Hospital of Guangzhou Medical University. Ethical approval of this study has been granted from the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (No. 2013039). Written and informed consent will be obtained from each patient before any study procedure is performed according to good clinical practice. The trial has been registered at ClinicalTrial.gov (NCT02026323) and with the Chinese Clinical Trials Registry (ChiCTR-OCH-13003921).

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Participants

Subjects will be recruited if they fulfill all of the inclusion criteria and do not meet any of the exclusion criteria.

Inclusion criteria

1) Age between 18 and 40 years.
2) BMI ≥ 18.5 kg/m^2.
3) Presence of PCOS as defined by the Rotterdam criteria and including at least two of the following three features:
   - Oligomenorrhea or amenorrhea. Oligomenorrhea is defined as an intermenstrual interval >35 days or <8 menstrual bleedings in the past year. Amenorrhea is defined as complete cessation of menstrual cycles for 6 months or more when a patient has previously had regular cycles and for 12 months or more when the patient has had irregular cycles.
   - Clinical or biochemical hyperandrogenism. Biochemical hyperandrogenemia is defined as a total serum testosterone concentration above 60 ng/dL [26], and clinical hyperandrogenism is defined as a Ferriman–Gallwey (FG) score ≥ 5 in mainland China [27].
   - Polycystic ovary morphology. This is defined as ≥ 12 antral follicles (2–9 mm in diameter) or an ovarian volume > 10 mL upon transvaginal scanning [28].
4) Presence of IR as defined by the homeostatic model assessment (HOMA-IR: [fasting insulin (μU/mL) × fasting glucose (mmol/L)] / 22.5). A value ≥ 2.14 will be considered to be indicative of IR [29].

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5) No desire to bear children and having used barrier methods of contraception for one year.

6) Willingness to sign the consent form.

Exclusion criteria

1) Having other endocrine disorders such as hyperprolactinemia (defined as two prolactin levels measured at least one week apart of 25 ng/mL or greater or as determined by local normative values), nonclassic congenital adrenal hyperplasia (17-hydroxyprogesterone < 3 nmol/L), or androgen-secreting tumors.

2) FSH levels > 15 mIU/mL. A normal level within the last year is adequate for entry.

3) Uncorrected thyroid disease defined as thyroid stimulating hormone (TSH) < 0.2 mIU/mL or TSH > 5.5 mIU/mL. A normal level within the last year is adequate for entry.

4) Type I diabetes mellitus or Type I and Type II patients who are receiving antidiabetic medications such as insulin, thiazolidinediones, acarbose, sulfonylureas, or other medications that are likely to confound the effects of the study. Patients currently receiving metformin for a diagnosis of Type I or Type II diabetes or for PCOS are also specifically excluded.

5) Suspected Cushing's syndrome.

6) Use of hormones or other medications in the past 3 months, including Chinese herbal prescriptions, which might affect the outcome.

7) Pregnancy within the last 6 weeks.

8) Post-abortion or postpartum within the last 6 weeks.

9) Breastfeeding within the last 6 months.

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10) Receiving acupuncture treatment related to PCOS within the past 2 months.

11) Having undergone a bariatric surgery procedure within the past 12 months or being in a period of acute weight loss.

12) Having known congenital adrenal hyperplasia.

13) Lack of written consent to participate in the study.

Eligible subjects will be recruited and categorized according to their BMI as normal weight (BMI = 18.5–23 kg/m^2) or overweight/obese (BMI ≥ 23 kg/m^2) [30].

Interventions

The acupuncture protocol is based on Western medical theories, and the study protocol follows the CONSORT [31] and STRICTA [32] recommendations with detailed descriptions of the treatment, including the number of needles used, depth of needle insertion, how needles will be stimulated (manual or electrical), frequency of sessions, and length of the treatment period. Acupuncture will be given according to a fixed protocol by traditional Chinese medicine practitioners educated in theoretical and practical acupuncture. The protocol is based on experimental studies elucidating the effect of acupuncture in rodent models of diabetes mellitus and PCOS [20-22] and in women with PCOS [19 33].

Acupuncture protocol

Acupuncture will be given three times per week over 6 months for a total of 80 sessions. Disposable, sterilized needles for single use made of stainless steel (0.20 mm × 30 mm and 0.20 mm × 40/50 mm; Hwoto, Suzhou Medical Appliance Fact., 215005 Suzhou, China) will

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be inserted to a depth of 15 mm to 35 mm in segmental acupuncture points located in the abdominal and leg muscles with innervations corresponding to the ovaries and pancreas that have been shown to improve insulin sensitivity in rodents. Two sets of acupuncture points will be alternated for every other treatment session. The two acupuncture protocols follow the STRICTA recommendations and are presented in Table 1. The rationale for using the traditional nomenclature of acupuncture points is that they are well described in the literature, and acupuncturists trained according to medical acupuncture theories or trained in classic traditional Chinese medicine theories all know the locations of such points and how the needles should be inserted. Thus, using the acupuncture name/number makes it easier for all practitioners to know where the needles were placed. All needles will be stimulated manually by rotation until needle sensation is evoked, which is indicative of activation of a-delta and c-fibers when the needles are inserted. Needles placed in the abdominal muscles and leg muscles will be attached to an electrical stimulator (Export Abteilung, Schwa-Medico GmbH, Germany) and electrically stimulated at 2 Hz for 30 min during each treatment session. Needles not connected to the electrical stimulator will be stimulated manually 4 times during each 30 min treatment session (Table 1).

Study Procedures

This pilot study has two phases. The first phase is the 6 months of acupuncture treatment, and the second phase is 6 months of follow-up (Figure 1). Each specific visit and measurement is summarized in Table 2.

The women in the study will be screened for IR. Those with a HOMA-IR ([fasting insulin

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(μU/mL) × fasting glucose (mmol/L)] / 22.5) ≥ 2.14 will be included and divided into the normal weight (BMI = 18.5–23 kg/m^2) group and the overweight/obese (BMI ≥ 23 kg/m^2) group. After inclusion and baseline measurements, acupuncture treatment will start and last for 6 months followed by 6 months of follow-up.

Outcome measurements

Primary outcome

The primary outcome will be the changes in HOMA-IR between baseline and after 6 months of acupuncture treatment and between baseline and the 6-month follow-up. The oral glucose tolerance test (OGTT) with 75 g glucose will be performed in all subjects after an overnight fast. Blood samples will be obtained to measure plasma glucose and serum insulin at 0, 60, and 120 min during the OGTT.

Secondary outcomes

1. HOMA-B: Islet β-cell function will be evaluated by the formula (20 × fasting insulin (mU/mL) / (fasting plasma glucose (mmol/L) − 3.5)) [34] and by the C-peptide index (CPI), which is measured as (fasting C-peptide (nmol/L) / fasting plasma glucose (mmol/L) × 100) [35].
2. The insulin response to glucose will be assessed by calculating the area under the curve during the OGTT for glucose (AUC_{glu}) and insulin (AUC_{ins}) using the trapezoidal rule[36].
3. Menstrual bleeding patterns will be evaluated.
4. Body composition will be determined as weight, height, waist-to-hip circumference, and BMI.

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5. Metabolic measures will include HbA1c, C-peptide, adiponectin, fasting cholesterol, fasting triglycerides (TG), ApoA1, ApoB, and blood pressure.
6. Endocrine measures will include hirsutism as assessed by Ferriman–Gallwey (FG) score, the presence of acne, and serum concentrations of testosterone, sex hormone-binding globulin (SHBG), follicle stimulating hormone (FSH), luteinizing hormone (LH), and dehydroepiandrosterone sulfate (DHEAS).
7. Questionnaires will include the short form-36 (SF36) [37], the PCOS questionnaire (PCOSQ) [38], generic and diagnosis-specific health-related quality of life questionnaires, and the Chinese Quality of Life (ChQOL) questionnaire [39]. Symptoms of anxiety and depression will be assessed by the Zung Self-Rating Anxiety Scale (Zung SAS) and the Zung Self-Rating Depression Scale (Zung SDS) [40]. In addition, the International Physical Activity Questionnaire (IPAQ) [41] written in Chinese will be used to measure the frequency and duration of moderate physical activity every week.
8. Adverse events will be recorded.

Data entry and quality control of data

Case report forms will be developed for data entry, and quality control of the data will be handled at two different levels. The investigators will be required to ensure the accuracy of the data as the first level of control, and the second level will include data monitoring and validation that will be carried out on a regular basis throughout the study.

Sample size calculations and statistical analysis

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Our previous study on treating obese PCOS women with abdominal acupuncture showed that the HOMA-IR was significantly reduced after treatment (3.9 ± 1.4) compared to baseline measurements (2.5 ± 1.7) [33]. A HOMA-IR ≥ 2.14 was considered to be abnormal [29]. If we assume a more moderate 20% reduction in HOMA-IR, i.e., a reduction by 0.78, we will have to recruit 40 overweight or obese subjects and 40 normal-weight subjects. With an estimated 40% drop out rate, we plan to recruit 56 normal weight and 56 overweight/obese women with PCOS.

One sample of the Kolmogorov-Smirnov test will be used to test the normal distribution of continuous variables. Continuous variables will be shown as means ± standard deviations if they are normally distributed or as medians with interquartile ranges if they are not normally distributed. Statistical comparisons will be carried out according to the intention to treat by Student's t-test, Mann–Whitney U-test, and Wilcoxon signed ranks test for continuous variables and by χ^2 tests for categorical variables where appropriate. All statistical analyses of the data will be performed using the SPSS program version 21.0 (SPSS Inc., Chicago, IL, USA), and a *P*-value < 0.05 will be considered statistically significant.

Safety and ethical considerations

Acupuncture is a safe procedure, and few side effects have been reported. The major risks of acupuncture are local skin irritation, discomfort, and vasovagal reactions during the procedure. The women who agree to participate in the study will sign a consent form. This pilot study has been approved by the ethics committee of the First Affiliated Hospital of Guangzhou

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Medical University (No. 2013-039).

Discussion

Acupuncture has been used in the treatment of women with PCOS [17-19 33], but the effect of acupuncture on IR in women with PCOS is still unknown. This prospective pilot study has been designed, therefore, to evaluate whether acupuncture improves insulin sensitivity in women with PCOS and IR. The research seeks to add significantly to the clinical evidence base and to allow conclusions to be made on the role of acupuncture in the treatment of PCOS.

A limitation of the present study is that it is a single-center study without comparison groups, and it can be argued that it lacks scientific rigor because of this. To increase the validity of the study, we will have experts controlling the quality of study procedure through regular site visits. The experts will monitor the recruitment of study subjects, the measurement and treatment procedures, and the data analysis. We plan to conduct a randomized controlled trial at a later data using standard care as a control.

This pilot study was conceived and designed in 2013, and the first subject was recruited on February 18, 2014. As on December 18, out of 368 women approached, 176 did not fulfill selection criteria, 85 declined participation and 107 consented to participate the study. In total 99 women were screened and 83 were recruited.

Abbreviations

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Apoa1: Apolipoprotein A1; ApoB: Apolipoprotein B; C: Cervical vertebra; CV: Conception vessel; DHEAS: Dehydroepiandrosterone sulfate; E2: Estradiol; EA: Electro-acupuncture; FSH: Follicle-stimulating hormone; FG: Ferriman–Gallwey score; GDR: Glucose disposal rate; IR: insulin resistance; L: Lumbar vertebra; LH: Luteinizing hormone; LI: Large intestine; LR: Liver; PC: Pericardium; PCOS: Polycystic ovary syndrome; S: Sacral vertebra; SP: Spleen; ST: Stomach; SHBG: Sex hormone-binding globulin; T: Total testosterone; TC: Total cholesterol; TG: Triglyceride; Th: Thoracic vertebra; OGTT: Oral glucose tolerance test.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YHZ, ESV, and EHNG contributed equally to this work. ESV, EHNG, and HMX conceived and designed the study. YHZ, EHNG, and ESV drafted and critically revised the manuscript for important intellectual content. HXM sought funding and ethical approval. All authors contributed to the further writing of the manuscript and approved the 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
Set 1				
Zhongji; CV3	EA	4 cun caudal to the umbilicus	Fibrous tissue, *linea alba*	L1
Zhongwan; CV12	EA	On the midline, 4 cun superior to the umbilicus	Fibrous tissue, *linea alba*	Th7–8
Guilai; ST29 Bilateral	EA	1 cun cranial to the pubic bone and 2 cun lateral of the midline	*M. rectus abdominis*	Th6–12
Liangqiu; ST34 Bilateral	EA	2 cun above the superior lateral border of the patella on the line connecting the anterior superior iliac spine found with the knee flexed	*M. quadriceps femoris*	femoral nerve
Yinshi; ST33 Bilateral	EA	3 cun above the superior lateral border of the patella on the line connecting the anterior superior iliac spine found with the knee flexed	*M. quadriceps femoris*	femoral nerve
Sanyinjiao; SP6	DeQi four times	3 cun proximal to the *medial malleolus*	*Mm. flexor digitorum longus*, *tibialis posterior*	L4–5, S1–2
Zusanli; ST36	DeQi four times	On the anterior lateral side of the leg, 3 cun below *Dubi* (ST35), one finger width (middle finger) from the anterior crest of the tibia	*musculi tibialis anterior*	L4–5, S1
Hegu; LI4	DeQi four times	On the highest point at *m. interosseus dorsalis*	*Mm. interosseus dorsalis I*, *lumbricalis II*, *adductor pollicis*	C8, Th1

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Set 2				
Daju; ST27	EA	3 cun cranial to the pubic bone and 2 cun lateral to the midline	*M. rectus abdominis*	Th6–12
Qihai; CV6	EA	1.5 cun caudal to the umbilicus	Fibrous tissue, *linea alba*	Th11
Xiawan; CV10	EA	2 cun cranial to the umbilicus	Fibrous tissue, *linea alba*	Th8
Extra meridian point	EA	6 cun above the patella in line with SP10	*M. quadriceps femoris*	L2–L4
Xuehai; SP10	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
Sanyinjiao; SP6	DeQi four times	3 cun proximal to the medial malleolus	*Mm. flexor digitorum longus*, *tibialis posterior*	L4–5, S1–2
Taichong; LR3	DeQi four times	Between metatarsal I & II, just distal to the caput	*M. interosseus dorsalis I*	S2–3
Neiguan; PC6	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)

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Table 2. Overview of study visits, including screening, baseline measurements, after-treatment measurements, and follow-up measurements. Months indicate when each specific measurement takes place.

	Screening and base line visit	Month						Follow-up 6th month
		1st	2nd	3rd	4th	5th	6th	
Body composition (weight, height, waist circumference, hip circumference)	√	√	√	√	√	√	√	√
Menstrual cycle diary	√	√	√	√	√	√	√	√
Fasting blood samples for Apoa1, ApoB, TC, TG, C-peptide, HbA1c	√						√	√
Fasting blood samples for FSH, LH, SHBG, T, E2, P, DHEAS	√						√	√
Transvaginal ultrasound	√						√	√
Questionnaire	√						√	√
FG/acne	√	√	√	√	√	√	√	√
OGTT	√			√			√	√

(Apoa1: Apolipoprotein A1; ApoB: Apolipoprotein B; DHEAS: Dehydroepiandrosterone sulfate; E2: Estradiol; FSH: Follicle-stimulating hormone; FG: Ferriman–Gallwey score; LH: Luteinizing hormone; SHBG: Sex hormone-binding globulin; T: Total testosterone; TC: Total cholesterol; TG: Triglyceride; OGTT: Oral glucose tolerance test.)

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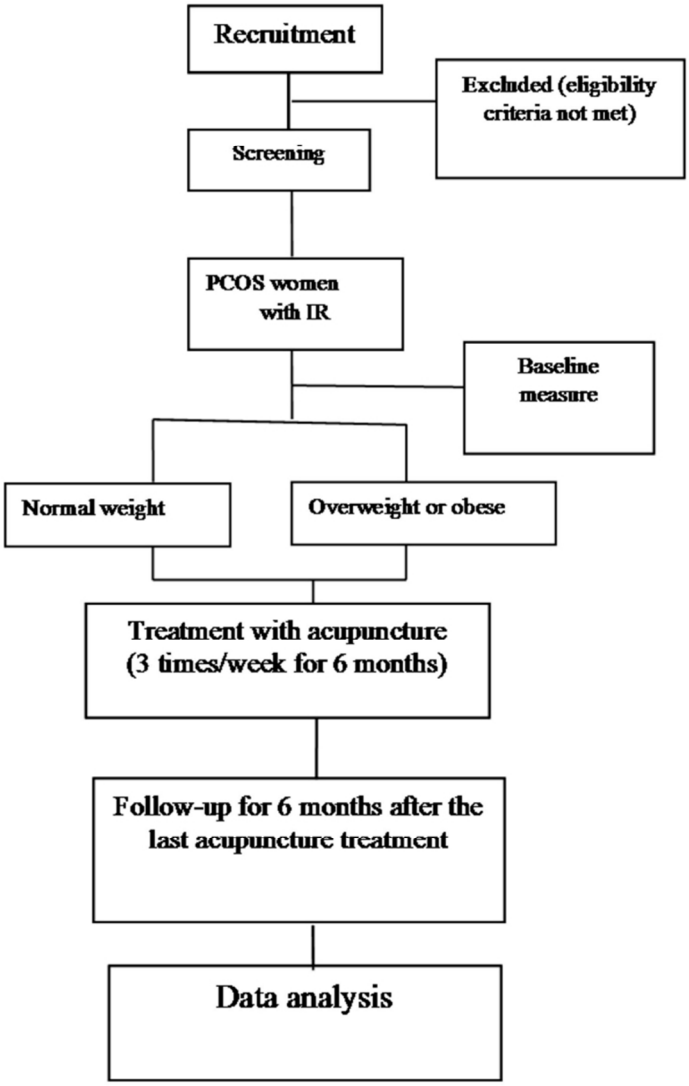

90x106mm (300 x 300 DPI)

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