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human reproduction ORIGINAL ARTICLE **Reproductive endocrinology**

Effect of acupuncture and metformin on insulin sensitivity in women with polycystic ovary syndrome and insulin resistance: a three-armed randomized controlled trial

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STUDY QUESTION: Does acupuncture improve insulin sensitivity more effectively than metformin or sham acupuncture in women with polycystic ovary syndrome (PCOS) and insulin resistance (IR)?

SUMMARY ANSWER: Among women with PCOS and IR, acupuncture was not more effective than metformin or sham acupuncture in improving insulin sensitivity.

WHAT IS KNOWN ALREADY: Uncontrolled trials have shown that acupuncture improved insulin sensitivity with fewer side effects compared with metformin in women with PCOS and IR. However, data from randomized trials between acupuncture and metformin or sham acupuncture are lacking.

STUDY DESIGN, SIZE, DURATION: This was a three-armed randomized controlled trial enrolling a total of 342 women with PCOS and IR from three hospitals between November 2015 and February 2018, with a 3-month follow-up until October 2018.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women aged from 18 to 40 years with PCOS and homeostasis model assessment of insulin resistance (HOMA-IR) ≥2.14 were randomly assigned (n = 114 per group) to receive true acupuncture plus placebo (true acupuncture), metformin plus sham acupuncture (metformin, 0.5 g three times daily) or sham acupuncture plus placebo (sham acupuncture) for 4 months, with an additional 3-month follow-up. True or sham acupuncture was given three times per week, and 0.5 g metformin or placebo was given three times daily. The primary outcome was change in HOMA-IR from baseline to 4 months after baseline visit. Secondary outcomes included changes in the glucose AUC during an oral glucose tolerance test, BMI and side effects at 4 months after baseline visit.

[†]The first two authors contributed equally to this work.

MAIN RESULTS AND THE ROLE OF CHANCE: After 4 months of treatment, the changes of HOMA-IR were –0.5 (decreased 14.7%) in the true acupuncture group, –1.0 (decreased 25.0%) in the metformin group and –0.3 (decreased 8.6%) in the sham acupuncture group, when compared with baseline. True acupuncture is not as effective as metformin in improving HOMA-IR at 4 months after baseline visit (difference, 0.6; 95% CI, 0.1–1.1). No significant difference was found in change in HOMA-IR between true and sham acupuncture groups at 4 months after baseline visit (difference, –0.2; 95% CI, –0.7 to 0.3). During the 4 months of treatment, gastrointestinal side effects were more frequent in the metformin group, including diarrhea, nausea, loss of appetite, fatigue, vomiting and stomach discomfort (31.6%, 13.2%, 11.4%, 8.8%, 14.0% and 8.8%, respectively). Bruising was more common in the true acupuncture group (14.9%).

LIMITATIONS, REASONS FOR CAUTION: This study might have underestimated the sample size in the true acupuncture group with 4 months of treatment to enable detection of statistically significant changes in HOMA-IR with fixed acupuncture (i.e. a non-personalized protocol). Participants who withdrew because of pregnancy did not have further blood tests and this can introduce bias.

WIDER IMPLICATIONS OF THE FINDINGS: True acupuncture did not improve insulin sensitivity as effectively as metformin in women with PCOS and IR, but it is better than metformin in improving glucose metabolism (which might reduce the risk of type 2 diabetes) and has less side effects. Metformin had a higher incidence of gastrointestinal adverse effects than acupuncture groups, and thus acupuncture might be a non-pharmacological treatment with low risk for women with PCOS. Further studies are needed to evaluate the effect of acupuncture combined with metformin on insulin sensitivity in these women.

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Key words: polycystic ovary syndrome / acupuncture / metformin / sham acupuncture / insulin resistance / homeostasis model assessment of insulin / insulin sensitivity / glucose metabolism

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder in women of reproductive age and has a prevalence of 5–20% (Azziz *et al.*, 2016). It is characterized by ovulatory dysfunction, polycystic ovarian morphology and hyperandrogenism. Approximately 50–75% of women with PCOS also suffer from insulin resistance (IR) (Dunaif, 1997; Ovalle and Azziz, 2002; Carmina and Lobo, 2004; Tosi *et al.*, 2017). IR and compensatory hyperinsulinemia exacerbate hyperandrogenemia and increase the risk of type 2 diabetes in women with PCOS by aggravating ovarian dysfunctions and metabolic disorders while suppressing the hepatic production of sex hormone-binding globulin (Bhathena, 2011; Conway *et al.*, 2014; Wu *et al.*, 2014; Ruth *et al.*, 2020).

Metformin is commonly prescribed for improving the metabolic complications and reproductive dysfunctions in women with PCOS and IR (Palomba *et al.*, 2009). Metformin reduces body weight, plasma insulin levels and blood pressure. It inhibits excess androgen output, and improves menstrual cycles and ovulation in women with PCOS (Lord *et al.*, 2003; Naderpoor *et al.*, 2015; Jin *et al.*, 2020). However, the use of metformin may be limited by gastrointestinal side effects, and chronic metformin treatment may cause lactic acidosis (Chang *et al.*, 2002; Lord *et al.*, 2003; Moll *et al.*, 2006).

Acupuncture is an important part of traditional Chinese medicine, but the efficacy of acupuncture in women with PCOS remains debatable. The findings of Wu *et al.* (2017) did not support acupuncture as an infertility treatment in anovulatory women with PCOS. Systematic reviews and some studies have demonstrated that electroacupuncture has the potential to increase whole-body glucose uptake and to improve insulin sensitivity through the activation of the sympathetic and, partly, the parasympathetic nervous systems in women with PCOS or in animal models (Liang and Koya, 2010; Benrick *et al.*, 2017; Zheng *et al.*, 2021). Our prospective pilot studies showed that acupuncture also has a significant effect on the homeostatic model assessment of insulin resistance (HOMA-IR) in women with PCOS and IR after 5 weeks, and after 6 months of treatment (Zheng *et al.*, 2015; Stener-Victorin *et al.*, 2016; Li *et al.*, 2020). There is a need, however, for well-designed randomized controlled trials to confirm the effects of acupuncture in women with PCOS and IR.

The main objective was to evaluate the hypothesis that acupuncture improves insulin sensitivity more effectively than metformin or sham acupuncture in women with PCOS and IR.

Materials and methods

Study design

This was a randomized trial enrolling 342 women with PCOS and IR from three hospitals in China. The full protocol had previously been published (Li *et al.*, 2017). All participants gave written informed consent prior to participation, and the trial was approved by the institutional review board at each center (Ethics number: medical research ethics review 2015010. Time of the ethical review: 24 June 2015) and monitored by a data and safety monitoring board.

Participants

In short, participants were aged from 18 to 40 years with BMI $\geq$18.5 kg/m^2 and were diagnosed with PCOS and IR. The diagnosis of PCOS was based on the revised Rotterdam criteria of 2004

(Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), with at least two of the following three symptoms: oligo/amenorrhea, biochemical and/or clinical signs of hyperandrogenism and/or polycystic ovaries. IR was evaluated by the HOMA-IR index, which was calculated as fasting plasma glucose (FPG) (mmol/l) × fasting insulin (FINS) (μU/ml)/22.5, and a value ≥2.14 was considered to indicate IR (Chen *et al.*, 2006). Women who had no immediate desire to become pregnant and who were willing to use barrier contraceptive methods for 7 months were recruited. Women with endocrine diseases such as hyperprolactinemia, FSH >15 mIU/ml, thyroid dysfunction and diabetes were excluded as were women with Cushing's syndrome, androgen-secreting neoplasms, and cervical, endometrial or breast cancers.

Randomization

Participants were randomly assigned in a 1:1:1 ratio to three treatment groups, including acupuncture plus placebo (true acupuncture, n = 114), metformin plus sham acupuncture (metformin, n = 114) and sham acupuncture plus placebo (sham, n = 114). Central randomization by an online medical research management platform (ResMan, www.medresman.org.cn) was performed, which was stratified within three participating sites and individual randomization was used. The use of true or sham acupuncture was known only to the acupuncturists and the data administrators. Metformin and placebo were packed in a pre-labeled bag by a commercial pharmacy supply company (Panlongyunhai Pharmaceutical Co., Kunming, Yunnan, China) specifically for this study. These similar-looking bags were distributed to the centers and given to the women when they started treatment.

Interventions

All treatments began 2 days after the baseline examinations. Recruited women were told about the importance of having regular physical exercise and a balanced diet before they received the treatments to ensure the comparability of the three treatments during the study.

All women received true or sham acupuncture for 30 min three times a week. The treatment was given with an interval of 1–3 days for a total of 48 sessions over 4 months. Acupuncture was performed by acupuncturists who had received theoretical and practical education in acupuncture for more than 5 years and who had been trained to follow the protocol. The rationale for the acupuncture protocol was based on the theories of traditional Chinese medicine and Western medical acupuncture and followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Schulz *et al.*, 2010) and the Standards for Reporting Interventions in Clinical Trials of Acupuncture (MacPherson *et al.*, 2010) recommendations.

In the true acupuncture treatment, two sets of acupuncture points were alternated every second treatment, as described in our previous protocol (Li *et al.*, 2017). In brief, a total of 14 needles were placed and all were stimulated manually by rotating with the thumb and forefinger to evoke needle sensation (de qi) when inserted. De qi indicates the activation of afferent nerve fibers and can be described as a feeling of numbness, distension or electrical tingling. Needles placed in the knee and abdomen were connected to an electrical stimulator (Export Abteilung, Schwa-Medico GmbH, Wetzlarer Str. 41-43; 35630 Ehringshausen) with low frequency (2 Hz) electrical stimulation, and the strength was adjusted to produce local muscle contractions without pain or discomfort. Needles placed in the hand and legs were manually stimulated every 10 min for a total of four times.

In the sham acupuncture treatment, needles were inserted superficially in non-acupuncture positions on the shoulder and upper arm bilaterally with a depth of <5 mm and connected to an electrical stimulator with mimetic electricity and no manual stimulation (Li *et al.*, 2017).

Metformin (Bristol-Myers Squibb Co., Shanghai, China) or placebo (Jaden Pharmaceutical Co., Ltd., Guangzhou, China) was given to the participants at the same time as the first acupuncture treatment. A total of 336 tablets of metformin or placebo were distributed, and the oral dose was 0.5 g three times daily for 4 months. Participants with adverse side effects, such as diarrhea, vomiting or dizziness, could reduce the dose to 0.5 g twice or once per day according to the severity of the side effects, and this was recorded by the study coordinators. Empty packages and unused drugs were handed over to the study coordinator for counting the number of tablets consumed and then were destroyed.

Outcomes

The primary outcome was the change in HOMA-IR from baseline to 4 months after baseline visit. The secondary outcomes included changes in the following variables: anthropometry (BMI, waist-to-hip ratio, acne lesion counts and hirsutism (determined by Ferriman–Gallwey score)), metabolic profile (FPG, FINS, the AUC during the oral glucose tolerance test for glucose ($glucose_{AUC}$) and for insulin ($insulin_{AUC}$), homeostatic model assessment for beta-cell function (HOMA-β), C-peptide, hemoglobin A1c, hormonal profile (LH/FSH, total testosterone and free androgen index), and adverse events during the treatment and follow-up. Degree of physical activity during the study was recorded by the International Physical Activity Questionnaire (IPAQ).

Statistical analysis

In our pilot study (Zheng *et al.*, 2015; Li *et al.*, 2020), HOMA-IR was significantly reduced from 4.3 ± 2.5 to 3.7 ± 2.1 in women with PCOS and IR after 3 months of acupuncture treatment (an unpublished interim analysis). We thus anticipated that the HOMA-IR would decrease by 25% (mean is 1.075, and SD is 2.1) after 4 months of true acupuncture treatment, and 5% (mean is 0.215, and SD is 2.1) after sham acupuncture, because it is well known that sham acupuncture is not an inert procedure. Therefore, the difference was expected to be 20% between true and sham acupuncture after 4 months of treatment. The sample size was calculated using the software statistics toolkit supported by the Department of Obstetrics and Gynecology of the Chinese University of Hong Kong (http://www.obg.cuhk.edu.hk/Research-Support/StatTools/index.php), with two-sided α assigned to be 5% and $\beta = 20\%$ at the upper limit, and a power of 80% assuming a drop-out rate of 20%. Thus, the sample size was inflated from 95 to 114 per group, totaling 342 cases for the three treatment groups. The sample size would be able to show a 20% difference in the primary outcome between the true acupuncture and metformin groups. The outcomes were analyzed according to the intention-to-treat principle. To assess the effect of missing data at baseline, we performed the multiple imputation method with the missing-at-random assumption.

The Kolmogorov–Smirnov test was used to test the normal distribution of continuous variables. Between-group comparisons were carried out by either a χ^2 or Fisher's exact test for categorical variables and by either Student's *t*-test or Mann–Whitney *U* test for continuous variables. All *P*-values are two-sided with no adjustment made for multiple comparisons. A *P*-value <0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA).

Results

Participant flow

Between November 2015 and February 2018, 342 women were randomly assigned to the three treatment groups, and the last participant finished the follow-up in October 2018 (Fig. 1). In total, 281 (82.2%) finished the treatments and 262 (76.6%) completed the follow-up. Dropout rates were 17.5% (20 of 114) in the true acupuncture group, 28.1% (32 of 114) in the metformin group and 24.6% (28 of 114) in the sham acupuncture group at 7 months after baseline visit. The reasons for dropout are listed in Fig. 1 and women who withdrew because of pregnancy are listed in Supplementary Table SI.

Baseline characteristics are listed in Table I and were comparable among the three groups. Women who had previous acupuncture experience at baseline were similar in the three groups.

Primary outcome

The median HOMA-IR at baseline was 3.4, 4.0 and 3.5 for the true acupuncture, metformin and sham acupuncture groups, respectively (Table I). True acupuncture was less effective than metformin in improving HOMA-IR at 4 months after baseline visit (difference, 0.6; 95% CI, 0.1–1.1) (Table II and Fig. 2). The change in HOMA-IR was similar between the true acupuncture group and the sham acupuncture groups at both 4 and 7 months after baseline visit (Table II and Fig. 2). After 4 months of treatment, the changes of HOMA-IR were –0.5 (decreased 14.7%) in the true acupuncture group, –1.0 (decreased 25.0%) in the metformin group and –0.3 (decreased 8.6%) in the sham acupuncture group, when compared with the baseline.

Secondary outcomes

At 4 months after baseline visit, true acupuncture significantly decreased FPG (difference, –0.2; 95% CI, –0.4 to –0.0) compared with the sham acupuncture group, and significantly improved the $glucose_{AUC}$ (difference, –1.2; 95% CI, –1.9 to –0.5) and HOMA-β (difference, 38.6; 95% CI, –6.0 to 83.3) compared with metformin (Table III). Metformin was superior to true acupuncture in decreasing BMI (difference, 0.4; 95% CI, 0.1 to 0.8) and FINS (difference, 2.9; 95% CI, 1.0 to 4.9). At 7 months after baseline visit, true acupuncture significantly decreased BMI (difference, –0.4; 95% CI, –0.9 to –0.0) compared with the sham acupuncture group (Supplementary Table SII). No other between-group differences were found (Table III and Supplementary Table SII).

IPAQ were collected at baseline, 4 months and 7 months after baseline visit to evaluate their physical activity during the study. There was no difference in time spent on physical activity between true acupuncture and the other two groups at any time point as measured with IPAQ (Supplementary Table SIII).

The use of metformin was recorded in Supplementary Table SIV. It was shown that 71 of 114 women (62.3%) took more than 90% of the total amount of metformin, 14 of 114 women (12.3%) took 70–90%, 18 of 114 women (15.8%) took 50–70% and 11 of 114 women (9.6%) took <50%. In addition, 72 (63.2%) women took metformin three times a day (1.5 g). As stated in the protocol, some women adjusted the dose because of the side effects after taking metformin, including 10 (8.8%) adjusted to once a day (0.5 g) and 32 (28.1%) adjusted to twice a day (1.0 g). Women taking metformin placebo did not adjust the dose.

A total of 96 women (84.2%) in the true acupuncture group, 91 women (79.8%) in the metformin group and 88 women (77.2%) in the sham acupuncture group completed 48 sessions of acupuncture. There was no difference in acupuncture adherence between groups (Supplementary Table V).

Adverse events

Gastrointestinal adverse events were more frequent in the metformin group, including diarrhea, nausea, loss of appetite, fatigue, vomiting and stomach discomfort (31.6%, 13.2%, 11.4%, 8.8%, 14.0% and 8.8%, respectively). Bruising was more common in the true acupuncture group (14.9%) during the 4 months of treatment (Table IV).

Discussion

Among women with PCOS and IR, this study does not support the hypothesis that true acupuncture is more effective than metformin or sham acupuncture in improving insulin sensitivity as assessed by HOMA-IR, although true acupuncture, metformin and sham acupuncture reduced HOMA-IR over a 4-month treatment period. True acupuncture improved glucolipid homeostasis by decreasing $glucose_{AUC}$ and FPG when compared to metformin or sham acupuncture, respectively, at 4 months after baseline visit, but the effects were lost at 7 months after baseline visit. The incidence of adverse events was more frequent in the metformin group with diarrhea being the most common, while bruising was most common in the true acupuncture group. However, about 80% of the participants in each group did not have androgen excess, indicating that the findings of the present study might not be generalizable to hyperandrogenic PCOS.

The gold standard method to evaluate insulin sensitivity is the hyperinsulinemic-euglycemic clamp (DeFronzo *et al.*, 1979). However, this test is expensive and time-consuming. It requires special equipment and skilled technicians and is not readily accepted by patients. HOMA-IR is a common surrogate marker for IR assessment in clinical trials, and it is an indirect but non-invasive measurement to identify IR and islet β-cell function by FPG and FINS (Matthews *et al.*, 1985). The sensitivity, specificity and accuracy of HOMA-IR were 86.4%, 71.4% and 82.8%, respectively, when compared with the glucose disposal rate by glucose clamp (Jia *et al.*, 2001). The measurement of HOMA-IR is more acceptable to patients in the clinic for evaluating the insulin sensitivity. We therefore used HOMA-IR to investigate the effect of treatment for women with PCOS and IR.

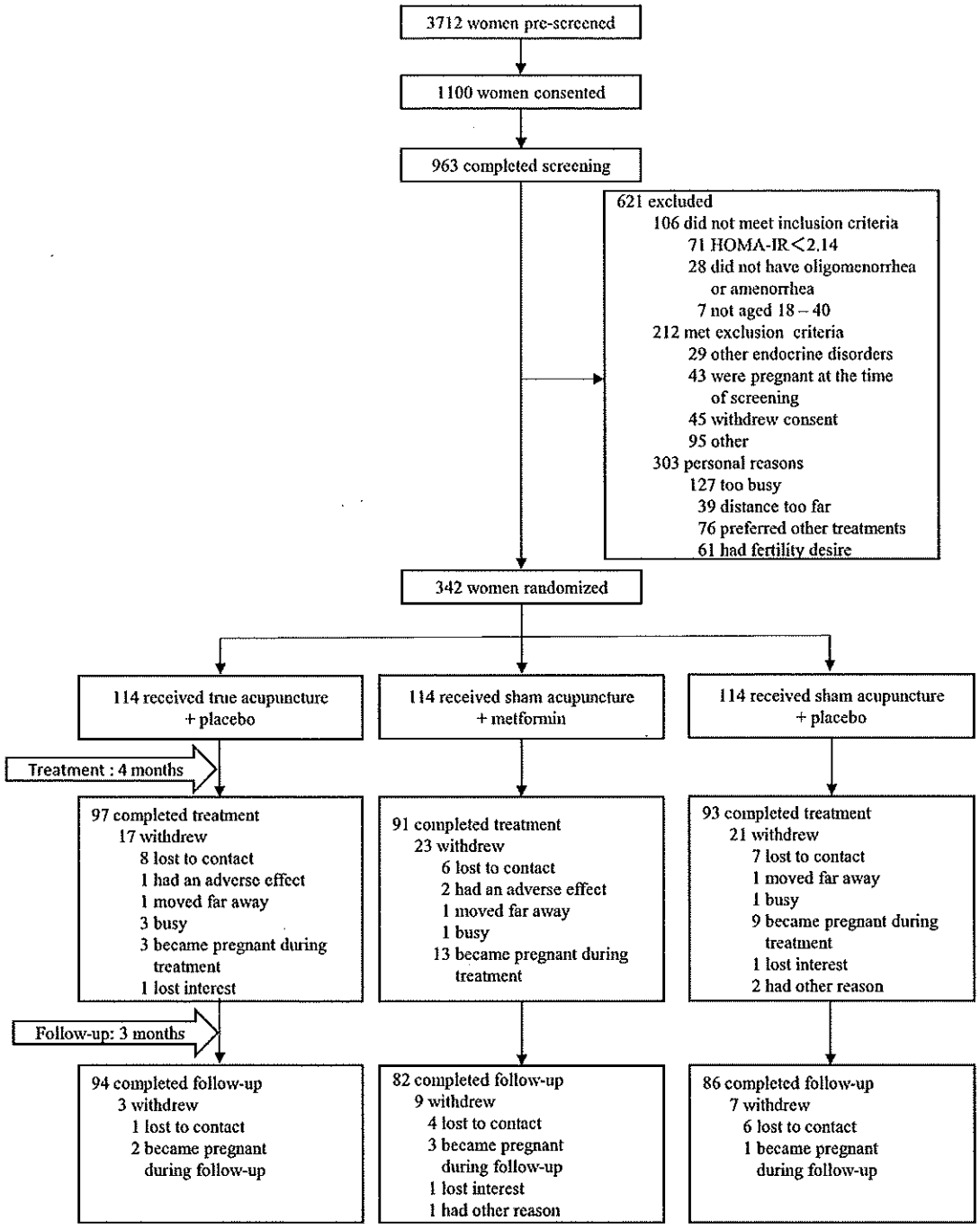

Figure 1. Flow diagram of participants in a randomized controlled trial of the effect of acupuncture and metformin on insulin sensitivity in women with polycystic ovary syndrome and insulin resistance. HOMA-IR, homeostatic model of assessment for insulin resistance.

Acupuncture is considered to be an insulin sensitizer that might have effects on controlling obesity and type 2 diabetes (Liang and Koya, 2010; Firouzjaei *et al.*, 2016). In this study, we found that true acupuncture decreased HOMA-IR in line with previous non-randomized studies (Liang and Koya, 2010; Johansson *et al.*, 2013; Benrick *et al.*, 2014; Li *et al.*, 2020), but it was not superior to metformin or sham acupuncture, as hypothesized (Trial Registration Identifier: NCT02491333). In addition, true acupuncture improved glucose metabolism by reducing $glucose_{AUC}$ and FPG when compared to metformin or sham acupuncture, respectively. The improved glucose metabolism observed with acupuncture is important because this might reduce the risk of type 2 diabetes.

Table I Baseline characteristics of participants.

Characteristics*	True acupuncture + Placebo (True acupuncture) n = 114	Sham acupuncture + Metformin (Metformin) n = 114	Sham acupuncture + Placebo (Sham acupuncture) n = 114
Biometric features			
Age, median (IQR), years	27.0 (25.0 to 31.0)	27.0 (25.0 to 30.0)	27.0 (24.0 to 29.0)
BMI, mean (SD), kg/m^2†	25.9 (4.3)	26.4 (5.0)	26.4 (4.7)
WHR, median (IQR)	0.9 (0.8 to 0.9)	0.9 (0.8 to 0.9)	0.9 (0.8 to 0.9)
Acne score, mean (SD)	0.6 (0.8)	0.6 (0.8)	0.6 (0.8)
Hirsutism score, mean (SD)	3.6 (3.6)	3.9 (4.1)	3.9 (3.9)
Fasting serum levels			
HOMA-IR, median (IQR)‡	3.4 (2.6 to 4.9)	4.0 (2.8 to 6.3)	3.5 (2.8 to 4.9)
FPG, median (IQR), mmol/l	5.3 (5.0 to 5.6)	5.2 (5.0 to 5.5)	5.2 (4.8 to 5.4)
FINS, median (IQR), mU/l	15.5 (11.1 to 19.8)	17.1 (12.7 to 25.5)	16.0 (12.5 to 21.4)
$Glucose_{AUC}$, mean (SD), mmol/l × min§	15.5 (3.1)	15.5 (3.4)	15.0 (3.2)
$Insulin_{AUC}$, median (IQR), mU/l × min‖	221.4 (131.0 to 322.9)	256.9 (158.1 to 329.9)	215.9 (154.5 to 325.8)
HOMA-β, median (IQR), %¶	178.0 (125.2 to 234.6)	214.6 (145.2 to 305.1)	191.6 (143.2 to 278.4)
C-peptide, median (IQR), nmol/l	0.9 (0.7 to 1.1)	1.0 (0.8 to 1.2)	0.9 (0.7 to 1.2)
HbA1C, median (IQR), %	5.3 (5.0 to 5.6)	5.3 (5.1 to 5.6)	5.3 (5.1 to 5.5)
TC, median (IQR), mmol/l	4.8 (4.4 to 5.4)	4.8 (4.1 to 5.4)	4.5 (4.1 to 5.3)
TG, median (IQR), mmol/l	1.4 (1.0 to 1.9)	1.4 (0.8 to 2.0)	1.2 (0.9 to 1.7)
HDL-C, median (IQR), mmol/l	1.3 (1.1 to 1.5)	1.2 (1.0 to 1.4)	1.3 (1.1 to 1.5)
LDL-C, median (IQR), mmol/l	2.9 (2.6 to 3.4)	3.0 (2.6 to 3.5)	2.9 (2.5 to 3.4)
ApoA-1, median (IQR), mmol/l	1.2 (1.1 to 1.3)	1.2 (1.1 to 1.3)	1.2 (1.1 to 1.3)
Apo B, median (IQR), mmol/l	0.9 (0.8 to 1.1)	0.9 (0.8 to 1.1)	0.9 (0.8 to 1.0)
LH, median (IQR), IU/l	9.9 (6.5 to 13.3)	9.8 (7.0 to 13.6)	10.1 (5.6 to 13.5)
FSH, mean (SD), IU/l	5.9 (1.8)	5.8 (1.6)	6.0 (1.5)
LH/FSH, median (IQR)	1.8 (1.1 to 2.5)	1.7 (1.3 to 2.3)	1.7 (1.0 to 2.4)
Total T, median (IQR), nmol/l	2.1 (1.6 to 2.8)	2.1 (1.5 to 2.8)	2.2 (1.8 to 2.8)
FAI, median (IQR)#	7.2 (4.6 to 10.8)	7.2 (4.3 to 11.0)	7.3 (4.5 to 11.8)
Phenotypes of polycystic ovary syndrome, n (%)			
Hyperandrogenism and ovulatory dysfunction	2 (1.8%)	1 (0.9%)	0 (0.0%)
Hyperandrogenism and polycystic ovarian morphology	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ovulatory dysfunction and polycystic ovarian morphology	80 (70.2%)	80 (70.2%)	82 (71.9%)
Hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology	32 (28.1%)	33 (28.9%)	32 (28.1%)
Previous acupuncture experience, n (%)	8 (7.0%)	12 (10.5%)	7 (6.1%)

Apo B, apolipoprotein B; ApoA-1, apolipoprotein A-1; FAI, free androgen index; FINS, fasting insulin; FPG, fasting plasma glucose; $Glucose_{AUC}$, the area under the curve during the oral glucose tolerance test (OGTT) for glucose (using the trapezoidal rule); HbA1C, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model of assessment for insulin resistance; HOMA-β, homeostatic model assessment for beta cell function; $Insulin_{AUC}$, the area under the curve during the OGTT for insulin (using the trapezoidal rule); LDL-C, low-density lipoprotein cholesterol; LH/FSH, LH to FSH ratio; TC, total cholesterol; TG, triglycerides; Total T, total testosterone; WHR, waist-to-hip ratio.

*Values are expressed as mean (SD) or median (25th to 75th percentile).

†Calculated as weight in kilograms divided by the square of the height in meters.

‡Calculated as fasting plasma glucose (mmol/l) × fasting insulin (mU/l)/22.5.

§Calculated according to the formula: fasting plasma glucose (mmol/l)/2 + 1-h glucose (mmol/l) + 2-h glucose (mmol/l)/2.

‖Calculated according to the formula: fasting insulin (mU/l)/2 + 1-h insulin (mU/l) + 2-h insulin (mU/l)/2.

¶Calculated as (20 × fasting insulin (m U/l))/(fasting plasma glucose (mmol/l) − 3.5).

#Calculated according to the formula: (total testosterone (nmol/l)/sex hormone-binding globulin (nmol/l)) × 100.

Table II Changes in HOMA-IR between groups.

Parameter	True acupuncture + Placebo (True acupuncture)	Sham acupuncture + Metformin (Metformin)	Sham acupuncture + Placebo (Sham acupuncture)	Absolute difference between groups (95% CI, *P*-value*)	
				True acupuncture versus Sham acupuncture	True acupuncture versus Metformin
HOMA-IR at 4 months after baseline visit, median (95% CI)	3.3 (3.2 to 4.3)	3.2 (3.4 to 4.4)	3.4 (3.7 to 4.8)		
HOMA-IR at 7 months after baseline visit, median (95% CI)	3.1 (3.0 to 5.2)	3.3 (3.5 to 4.8)	3.6 (3.4 to 4.4)		
Change from baseline to 4 months after baseline visit, median (95% CI)	−0.5 (−0.6 to 0.1)	−1.0 (−1.3 to −0.5)	−0.3 (−0.5 to 0.3)	−0.2 (−0.7 to 0.3)	0.6 (0.1 to 1.1)
No. of subjects	97	96	98	0.38	0.03
Changes from baseline to 7 months after baseline visit, median (95% CI)	−0.5 (−0.9 to 1.0)	−0.6 (−0.8 to 0.1)	−0.3 (−0.9 to 0.0)	0.5 (−0.6 to 1.6)	0.4 (−0.7 to 1.5)
No. of subjects	94	82	86	0.89	0.96

*Between-group comparisons were carried out by Mann–Whitney *U* test. All tests were two-sided, and a *P*-value <0.05 was considered significant.

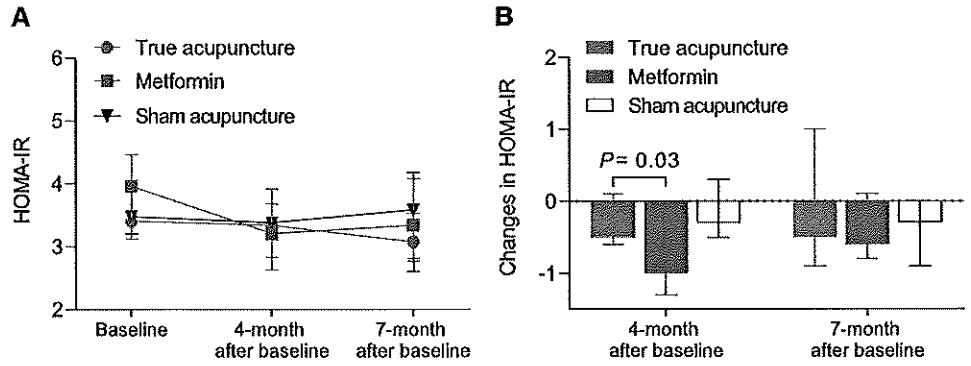

Figure 2. Changes in the HOMA-IR of treatment groups. **(A)** The levels of HOMA-IR at baseline, 4 months after baseline and 7 months after baseline. **(B)** The delta changes in HOMA-IR from baseline to 4 or 7 months. Values are expressed as median with 95% CI. HOMA-IR: homeostatic model of assessment for insulin resistance.

In this randomized trial, the true acupuncture protocol followed those used in the randomized trials registered in ClinicalTrials.gov, 'Acupuncture to Treat Insulin Resistance in Women With and Without Polycystic Ovary Syndrome', NCT01457209 (Stener-Victorin *et al.*, 2012), 'Acupuncture and Clomiphene Citrate on Live Birth in Anovulatory Women With Polycystic Ovary Syndrome', NCT01573858 (Kuang *et al.*, 2013) and our prospective study of 'Effect of Acupuncture on Insulin Sensitivity Polycystic Ovary Syndrome', NCT02026323 (Zheng *et al.*, 2015). The sham acupuncture protocol was consistent with the method used in the randomized trial published in *JAMA* (Wu *et al.*, 2017), but the treatment frequency was increased from twice a week to three times a week. Unlike the true acupuncture, the needles in the sham acupuncture treatment were inserted superficially in non-acupuncture positions on the shoulder and upper arm bilaterally with a depth of <5 mm and connected to an electrical stimulator with mimetic electricity and no manual stimulation (Kuang *et al.*, 2013). Women who had previous acupuncture experience at the baseline were similar between groups in this study. Additionally, acupuncturists in this study were skilled and had at least 5 years of experience in acupuncture. All acupuncturists were specially trained for the trial. Therefore, we consider that there was no concern about true and sham acupuncture in this study.

We recorded IPAQ at baseline, 4 and 7 months after baseline visit to evaluate physical activity during the study. The results showed that the IPAQ was similar at baseline, and 4 and 7 months after baseline visit in the true acupuncture and the other two groups, and there was no significant difference in the changes of the IPAQ between groups, which means that there was no difference in physical activity between groups during the study.

The bruising caused by subcutaneous hemorrhage was the commonest adverse effect in women who received true acupuncture, but no long-term adverse events occurred, which is in line with previous

Table III The changes in secondary outcomes at 4 months after baseline visit.

Parameter	True acupuncture + Placebo (True acupuncture)	Sham acupuncture + Metformin (Metformin)	Sham acupuncture + Placebo (Sham acupuncture)	Absolute difference between groups (95% CI, *P*-value*)	
				True acupuncture versus Sham acupuncture	True acupuncture versus Metformin
BMI					
Median (95% CI), kg/m^2	−0.5 (−0.9 to −0.4)	−1.2 (−1.4 to −0.8)	−0.4 (−0.8 to −0.4)	−0.1 (−0.4 to 0.2)	0.4 (0.1 to 0.8)
No. of subjects	96	96	98	0.43	0.002
WHR					
Median, (95% CI)	−0.0 (−0.0 to 0.0)	−0.0 (−0.0 to 0.0)	−0.0 (−0.0 to 0.0)	0.0 (−0.0 to 0.0)	0.0 (−0.0 to 0.0)
No. of subjects	97	96	98	0.84	0.71
FPG					
Median, (95% CI), mmol/l	−0.2 (−0.4 to −0.1)	−0.1 (−0.2 to 0.0)	0.0 (−0.1 to 0.1)	−0.2 (−0.4 to −0.0)	−0.1 (−0.3 to 0.1)
No. of subjects	97	96	98	0.01	0.24
FINS					
Median, (95% CI), mU/l	−1.3 (−2.0 to 0.8)	−3.5 (−4.9 to −2.1)	−1.6 (−2.3 to 0.8)	0.2 (−1.8 to 2.2)	2.9 (1.0 to 4.9)
No. of subjects	97	96	98	0.88	0.005
$Glucose_{AUC}$					
Mean (95% CI), mmol/l × min	−1.0 (−1.5 to −0.4)	0.2 (−0.3 to 0.6)	−0.4 (−1.0 to 0.1)	−0.5 (−1.3 to 0.2)	−1.2 (−1.9 to −0.5)
No. of subjects	97	95	96	0.17	0.001
$Insulin_{AUC}$					
Median (95% CI), uU/ml × min	−28.9 (−73.9 to −27.9)	−44.7 (−76.7 to −28.5)	−16.3 (−48.8 to 10.4)	−31.7 (−69.1 to 5.7)	1.7 (−31.4 to 34.8)
No. of subjects	95	95	98	0.15	0.32
HOMA-β					
Median (95% CI), %	5.7 (−7.8 to 40.1)	−21.2 (−60.6 to 15.6)	−8.5 (−39.3 to 24.2)	23.7 (−15.8 to 63.3)	38.6 (−6.0 to 83.3)
No. of subjects	97	96	98	0.10	0.004
C-peptide					
Median (95% CI), ng/ml	−0.0 (−0.2 to −0.0)	−0.1 (−0.1 to −0.0)	−0.0 (−0.1 to 0.0)	−0.1 (−0.2 to 0.0)	−0.0 (−0.2 to 0.1)
No. of subjects	96	96	97	0.48	0.65
HbA1C					
Median (95% CI), %	0.0 (−0.1 to 0.1)	0.0 (−0.1 to 0.0)	0.0 (−0.0 to 0.0)	−0.0 (−0.1 to 0.1)	0.1 (−0.0 to 0.1)
No. of subjects	97	95	96	0.95	0.10
LH/FSH					
Median (95% CI)	0.1 (−0.1 to 0.6)	−0.0 (−0.2 to 0.3)	0.1 (−0.0 to 0.5)	0.0 (−0.4 to 0.4)	0.2 (−0.2 to 0.6)
No. of subjects	96	91	93	0.85	0.40
Total T					
Median (95% CI), ug/l	0.1 (−0.1 to 0.2)	−0.2 (−0.3 to 0.1)	0.1 (−0.1 to 0.2)	0.0 (−0.2 to 0.3)	0.2 (−0.1 to 0.4)
No. of subjects	95	93	92	0.64	0.09
FAI					
Median (95% CI)	−0.3 (−1.0 to 0.6)	−0.9 (−2.4 to −0.2)	−0.4 (−1.4 to 0.3)	0.4 (−0.7 to 1.5)	1.1 (−0.2 to 2.5)
No. of subjects	93	91	89	0.45	0.13
Acne score					
Mean (95% CI)	−0.2 (−0.3 to −0.0)	−0.1 (−0.3 to 0.1)	−0.2 (−0.3 to −0.0)	0.0 (−0.2 to 0.2)	−0.0 (−0.3 to 0.2)
No. of subjects	97	97	98	0.92	0.69
Hirsutism score					
Mean (95% CI)	−0.2 (−0.3 to 0.0)	−0.1 (−0.2 to 0.0)	−0.1 (−0.3 to 0.0)	−0.0 (−0.3 to 0.2)	−0.1 (−0.3 to 0.1)
No. of subjects	97	97	98	0.78	0.43

*Between-group comparisons were carried out by either Student's *t*-test or Mann–Whitney *U* test. All tests were two-sided, and a *P*-value <0.05 was considered significant.

Table IV Adverse events experienced by the participants.

Event	True acupuncture + Placebo (True acupuncture) No. of women (%)	Sham acupuncture + Metformin (Metformin) No. of women (%)	Sham acupuncture + Placebo (Sham acupuncture) No. of women (%)	*P*-value*	
				True acupuncture versus Sham acupuncture	True acupuncture versus Metformin
At 4 months after baseline visit					
Total no. of subjects	114	114	114		
Serious adverse event†					
Calculous cholecystitis‡	0 (0.0%)	0 (0.0%)	1 (0.9%)	1.00	1.00
High fever syncope§	0 (0.0%)	1 (0.9%)	0 (0.0%)	1.00	1.00
Tuberculosis‖	1 (0.9%)	0 (0.0%)	0 (0.0%)	1.00	1.00
Other adverse event					
Diarrhea	2 (1.8%)	36 (31.6%)	2 (1.8%)	1.00	0.000
Nausea	4 (3.5%)	15 (13.2%)	2 (1.8%)	0.68	0.008
Loss of appetite	0 (0.0%)	13 (11.4%)	0 (0.0%)	1.00	0.000
Fatigue	1 (0.9%)	10 (8.8%)	1 (0.9%)	1.00	0.005
Vomiting	1 (0.9%)	16 (14.0%)	0 (0.0%)	1.00	0.000
Stomach discomfort	1 (0.9%)	10 (8.8%)	1 (0.9%)	1.00	0.005
Dizziness	3 (2.6%)	8 (7.0%)	3 (2.6%)	1.00	0.12
Abnormal vaginal bleeding	1 (0.9%)	3 (2.6%)	0 (0.0%)	1.00	0.61
Hyperthyroidism¶	0 (0.0%)	1 (0.9%)	0 (0.0%)	1.00	1.00
Bruising	17 (14.9%)	5 (4.4%)	5 (4.4%)	0.007	0.007
At 7 months after baseline visit					
Total no. of subjects	97	91	93		
Diarrhea	2 (2.1%)	2 (2.2%)	4 (4.3%)	0.68	1.00
Stomach discomfort	0 (0.0%)	1 (1.1%)	3 (3.2%)	0.25	1.00
Abnormal vaginal bleeding	0 (0.0%)	1 (1.1%)	1 (1.1%)	1.00	1.00
Hypertension#	0 (0.0%)	1 (1.1%)	0 (0.0%)	1.00	1.00

*Between-group comparisons were carried out by either the χ^2 test or Fisher's exact test. All tests were two-sided, and a *P*-value <0.05 was considered significant.
†A serious adverse event was defined as any event that was fatal, immediately life-threatening, or permanently disabling; any event that required hospitalization; or any event that was considered to be serious by the principal investigator at each center.
‡This event led to hospitalization and surgery.
§A subject in the metformin group developed high fever and syncope owing to cervical lymph node enlargement, which led to hospitalization, and the subject withdrew after discharge because she refused to continue treatment.
‖In the true acupuncture group, a patient withdrew during the treatment because of tuberculosis.
¶In the metformin group, one subject lost 12.5 kg of weight during treatment and then dropped out after a diagnosis of hyperthyroidism.
#The subject was diagnosed with hypertension during the follow-up period and treated with antihypertensive drugs.

studies (Jedel *et al.*, 2011; Pastore *et al.*, 2011; Stener-Victorin *et al.*, 2016). Metformin had a higher incidence of gastrointestinal adverse effects than acupuncture groups, and thus acupuncture might be a non-pharmacological treatment with low risk for women with PCOS.

This study had several limitations. First, the sample size might be underestimated. After 4 months of intervention, the changes of HOMA-IR were −0.5 (14.7%) in the true acupuncture group, −1.0 (25.0%) in the metformin group and −0.3 (8.6%) in the sham acupuncture group. However, we estimated that the true acupuncture could reduce HOMA-IR by 25% with 4 months of treatment, and the difference between true and sham acupuncture after 4 months of treatment was expected to be 20%. However, the difference between true and sham acupuncture was about 6% and this study was underpowered. Second, although women were advised to use contraception during the study period, 31 became pregnant and did not have further blood tests: this can introduce bias because women with improved metabolic status may resume ovulation and achieve a pregnancy, but they were excluded from the analysis. In addition, some of the participants received progestin for withdrawal bleeding if they did not menstruate after 2 months, since the focus of this study is not on reproductive indicators, and therefore the menstrual and ovulation patterns could not be assessed. Moreover, although the statistical approach was in line with the objective of this study using between-group comparison, the deviation is that the analysis of variance or Kruskal–Wallis test were prespecified in the protocol. Next, in view of the findings of the study, a two-by-two factorial design would have been a stronger method for the analysis. Furthermore, women with PCOS were usually treated with personalized acupuncture and moxibustion, based on

the theory of Chinese medicine in China. However, the acupuncture protocol in this study was fixed, and it might be more effective to use a personalized protocol as reported in the treatment of other disease conditions (Cherkin *et al.*, 2009; Ko *et al.*, 2016; Zhao *et al.*, 2019).

Conclusion

In conclusion, among women with PCOS and IR, this study does not support acupuncture to be more effective than metformin or sham acupuncture in improving HOMA-IR.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article are available in Dryad, Dataset, at https://datadryad.org/stash/share/VfulGsc3AmeRZulmAz_e2BbLreFUKoRw2v7nbPcMy4.

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Authors' roles

H.-X.M. and E.S.-V. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Q.-D.W., M.H., E.H.Y.N. and E.S.-V. drafted the paper. M.-H.L., J.L., T.-X.W., E.H.Y.N., E.S.-V. and H.-X.M. designed the study. Q.-D.W., M.H., M.-H.L., J.L., Z.-X.H., K.-W.Q., J.L., H.L., Y.-B.M., S.-L.W., X.-H.W., C.-Y.Y., S.-N.L., S.-Y.H., Y.-H.Z., H.L., X.-Y.L., L.-J.L., Z.-F.M., C.-R.Z. and H.-X.M. acquired the data. Q.-D.W., M.H. and E.S.-V. did the statistical analysis. M.H., E.H.Y.N., E.S.-V. and H.-X.M. give the critical revision for important intellectual content. T.-X.W. was responsible for randomization and monitoring. All authors interpreted the data, revised the paper critically for important intellectual content and approved the final version.

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Conflict of interest

No potential conflicts of interest relevant to this article were reported.

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