Study on intestinal flora of acupoint catgut embedding intervention in female patients with abdominal obesity: Study protocol for a randomized controlled trial

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Research Article

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Abstract

Background

With high morbidity, and many complications, obesity has become a global public health problem, and attracted extensive attention from the medical community. Previous studies have suggested that acupoint catgut embedding (ACE) is a safe and effective therapy for abdominal obesity (AO). However, the investigation of its mechanisms remains limited. The purpose of this study is to observe the effect of ACE intervention in AO on the intestinal flora, to reveal its site of action and therapeutic mechanisms, and provide further theoretical support for ACE in the clinical treatment of AO.

Methods/design:

A total of 60 eligible female participants diagnosed with AO will be recruited in this study. They will be blinded to group assignment and randomized to either ACE group, sham ACE group, and waiting list (WL) group, with 20 patients in each group. Each patient in the two ACE-based groups will receive one ACE treatment per week for 12 consecutive weeks. This study will focus on analyzing the intestinal flora changes elicited by ACE treatment. The body mass index (BMI), waist circumference (WC), body weight (BW), blood pressure (BP), heart rate (HR), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), visual analogue score (VAS) of appetite, and the Gastrointestinal Symptom Rating Scale (GSRS) will be used to evaluate the clinical efficacy of ACE treatment by making assessments before and after treatment. High-throughput 16S ribosomal ribonucleic acid (rRNA) gene sequencing will be used to detect changes in the intestinal flora composition before and after treatment. The repeated measures analysis of variance (3 groups × 2-time points ANOVA) will be employed to analyze numerical variables of the clinical and intestinal flora data generated in the study.

Discussion

The results of this trial are expected to further confirm that ACE can effectively relieve AO and verify the intervention mechanisms of ACE on intestinal flora in patients with AO.

Trial registration:

Chinese Clinical Trial Registry, ChiCTR2100048853. Registered on July 19, 2021. https://www.chictr.org.cn/showproj.aspx?proj=130531

Background

Obesity is a type of chronic metabolic disease characterized by the excessive accumulation or abnormal distribution of fat in the body. With the development of the social economy and changes in people's lifestyles, the morbidity of obesity is increasing annually and has become a serious global public health problem. The latest global epidemiological data indicate that more than 2.0 billion adults worldwide are overweight [1]. The global overweight and obese populations are predicted to reach more than 2.16 billion and 1.12 billion, respectively, by 2030 [2]. Especially in China, which obese population has reached the world's highest number with 34.3% for overweight and 16.4% for obesity in adults (≥ 18 years) [3]. Obesity not only brings a heavy medical burden to society but also seriously affects people's health. A meta-analysis about medical care costs of obesity found that in 2014, the US spent $1,901 on costs attributed to obese individuals, and $149.4 billion at the national level [4]. The World Health Organization (WHO) defined obesity as a chronic metabolic disease characterized by abnormal or excessive accumulation of fat in the body [5]. The excessive fat accumulation adversely increases the risk of developing multiple morbidities, such as cardiovascular disease, obstructive sleep apnea, type 2 diabetes mellitus (T2DM), psychiatric disorders,musculoskeletal disorders liver disease, and even cancers [6–10]. Obesity is also strongly associated with mortality [11]. Surveys show that about 4 million deaths worldwide were caused by obesity or overweight in 2015 [12]. AO is the more dangerous type of obesity. When the BMI is only mildly elevated but the WC is larger, the prevalence of coronary heart disease and mortality will also increase [13]. AO is more strongly correlated with most of the above-mentioned diseases, such as cardiovascular, cancer [14, 15], T2DM [16], and obstructive sleep apnea [17] than general obesity. Currently, the treatments for AO mainly include lifestyle changes (dietary interventions, exercise), medication, and bariatric surgery [18]. Previous studies showed that lifestyle modification includes behavioral therapies, diet changes, and physical exercise, which requires at least 6 months or more to achieve bodyweight loss, and many patients lack high self-discipline and have difficulty adhering to it [19, 20]. Pharmaceutical drugs also have active effects but may rebound after stopping. Moreover, the side effects (gastrointestinal and menstrual disorders, possible liver damage) and instability limit its clinical application [21, 22]. Surgical intervention is also useful for obesity. A recent review reported that the most effective long-term treatments for severe obesity complicated by type 2 diabetes is bariatric procedures, but it is also associated with high costs, high surgical safety requirements, high risk of postoperative complications, and strict indications [23]. Since obesity is a disease that requires long-term treatment like hypertension, more and more people are seeking better alternative treatments. Acupuncture is favored because of its safety and effectiveness without toxic side effects.

As one of the treatment methods, catgut embedding has been proven to be used to treat many diseases, such as perimenopausal syndrome, chronic urticaria, depressive neurosis, refractory insomnia, obesity, etc. [24]. ACE is a treatment method of infixing surgical chromic catgut sutures into corresponding acupoints to form a continuous acupoint stimulation, which is generally considered to be more effective than ordinary acupuncture or electroacupuncture in the treatment of obesity [24, 25]. Recent research related that the beneficial effects of ACE in treating obesity, which is likely through improving leptin resistance in obese women [26], but the exact mechanism remains unclear. There are still many clinical

randomized controlled trials (RCTs) to further validate the efficacy and possible mechanisms of ACE for obesity [27].

The relationship between intestinal flora and metabolic disorder has been a hot research topic in recent years. Studies have shown that obesity is related to changes in intestinal flora and changes in serum metabolite levels corresponding to gut microbial patterns [28]. Studies in acupuncture and herb have shown that the mechanism of action anti-obesity is related to the modulation of intestinal flora [29, 30]. Based on this, we conjecture that the mechanism of efficacy of ACE for obesity may be related to altered intestinal flora, but no study has yet confirmed this. The purpose of this study is to observe the efficacy of ACE in the treatment of AO in women and to explore whether ACE can change the type and quantity of intestinal flora in AO patients, and study the mechanism of its efficacy.

Methods/design

Study design

This single-center, parallel, RCT will be conducted at The Second Affiliated Hospital of Yunnan University of Chinese Medicine and The Sports Trauma Specialist Hospital of Yunnan Province. Ninety participants will be recruited in this study. They will be blinded to group assignment and randomized to either ACE group, sham ACE group, or WL group, with 20 patients in each group. Each patient in the two ACE-based groups will receive one ACE treatment per week for 12 consecutive weeks. The primary outcomes are the change in intestinal flora and BMI. The secondary outcomes will include the changes in WC, BW, BP, HR, TC, TG, LDL, HDL, VAS of appetite, and GSRS. High-throughput 16S ribosomal ribonucleic acid (rRNA) gene sequencing will be used to detect changes in the intestinal flora composition. All outcomes will be evaluated at baseline and the end of treatment. This trial is reported following the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines [31] (Additional **file 1**). A flowchart of the research procedure is shown in **Fig. 1**

Patient recruitment

A public recruitment advertisement will be designed to recruit the patients online or offline. (e.g. WeChat public account, websites, outpatient clinics). Physicians will determine whether patients are eligible to participate in this study based strictly on inclusion and exclusion criteria. The included patients will sign an informed consent form before the trial. The schedule of patient enrolment, intervention, and assessment is illustrated in **Fig. 2**.

participant safety

The safety assessment of ACE will include 2 aspects. On one hand, each patient's blood pressure, heart rate, routine blood parameters, and hepatorenal function will be tested as a safety index before randomization and at the end of treatment. On the other hand, during the treatment, any adverse events such as pain, allergic reactions, local hematoma, infection, syncope, and other severe events will be

managed immediately and documented in the case report forms (CRFs) carefully and reported to the study director and the ethics committee of The Sports Specialist Hospital of Yunnan Province. If the adverse event is severe and associated with the trial, the participant will be withdrawn from the study and given appropriate treatment.

Participants

Diagnostic criteria

Owing to this study will be conducted in China, participants must meet the diagnostic criteria in the guideline of “the Prevention and Control of Overweight and Obesity in Chinese Adults (Trial)”, proposed by the Chinese Working Group on Obesity in 2003, published by the Chinese Center for Disease Control (CDC). At the same time, in order to obtain more reliable results, according to the characteristics of AO, the diagnostic criteria include the following two points, ①BMI≥28kg/m^2; ②female waistline≥80cm.

Inclusion criteria

Inclusion criteria are as follows:

1. Meet the diagnostic criteria for abdominal obesity;
2. Females, aged between 18-40 (including 18 and 40 years old);
3. Have a certain writing and reading ability to understand the entire process of research;
4. Signed informed consent forms;
5. No other trials were taken within three months.

Exclusion criteria

Patients with any one of the following criteria are excluded from this study:

1. Extremely obese patients (BMI≥40kg/m^2);
2. Secondary obesity, such as obesity caused by endocrine disease (Cushing syndrome, thyroid disease, hypothalamic disease, pituitary disease, gonadal disease, etc.) and medication (glucocorticoid or antipsychotics);
3. Pregnancy, lactation, or who have fertility requirements or are unable to provide adequate contraception within the next 6 months;
4. Mental disorder, cardiovascular disease, liver and kidney impairment, immunodeficiency, diabetes, blood disease;
5. There has been a history of surgery for obesity.
6. Patients with symptoms of the gastrointestinal system, such as active peptic ulcers/bleeding, or previous recurrent ulcers/bleeding;
7. Previously undergone major intestinal excision or gastrointestinal major surgery;

8. People who take antibiotics for 3 days or more in the last 3 months;
9. Patients with long-term dysmenorrhea;
10. People who tend to bleed, or who are allergic to alcohol or animal protein.

Dropout criteria

1. Patients who do not meet the inclusion criteria but are mistakenly enrolled;
2. Decide to withdraw from the study;
3. Occurrence of severe AEs or complications which result in stopping the trial;
4. violate the protocol or refuses to give feedback on treatment information.

Sample size

Intestinal flora

According to the previous literature [32], the average BMI of the obese patients after treatment decreased 1.43 Kg/m^2 after being treated with acupuncture, while increased 0.54 Kg/m^2 after being treated with sham acupuncture. The adjusted mean difference between the two groups was 1.97 Kg/m^2. In this study, we anticipated a 1.5Kg/m^2 improvement by the ACE group; 0.3Kg/m^2 by the sham ACE group; and 0 Kg/m^2 in the waiting-list group. The standard deviation is set as 1.3. Assuming that $\alpha = 0.05$ and $1- \beta = 0.9$, 18 patients will be required for each group, as calculated using G*Power (version 3.1.2, Franz Faul, Universität Kiel, Germany). There is no consensus on sample size for 16S rRNA gene sequencing. Based on similar studies and the research data analysis requirements of 16S rRNA gene sequencing, the sample size range of 15 to 20 cases shows enough statistical power for intestinal flora analysis [33, 34]. Finally, allowing for a 10% withdrawal rate, a total of 60 participants will be recruited with 20 participants in each group.

Randomization and allocation

All participants will be randomly assigned to three groups of group A (ACE group), group B (sham ACE group), and group C (WT group) at a ratio of 1:1:1. Random numbers will be generated by computer and sealed in opaque envelopes by an independent research assistant. After participants accept the principle of random allocation, they will be randomly conducted to selects an opaque envelope and obtains an allocation sequence number, which will be recorded in a case report form (CRF) by the research assistant. The result of a participant's allocation will be given to the operator in charge of ACE treatment.

Blinding

Considering the particularity of ACE manipulation, the operators can't be blinded for the entire process. At the time of ACE operation, two group patients will be treated in separate rooms to avoid interaction. The acupuncturist and assistants will receive specialized training before participating in the study and will not disclose the allocation of the participants at any moment. Furthermore, to eliminate subjective bias,

outcome assessors and statisticians are also blinded to group assignment. The allocation will only be revealed under some adverse events, such as severe allergy, serious infection, uncontrolled pain, etc.

Interventions

Standard operating procedures (SOPs) will be formulated to ensure the standards and quality of the study. ACE will be performed by qualified TCM doctors with at least 3 years of clinical experience in acupuncture.

ACE group

Based on clinical experiences, previous researches, and TCM theory of invigorating the spleen and resolving dampness, the back-shu points and front-mu points of the spleen, stomach, and large intestine will be selected as the prescription. It includes BL 20 (Pi-shu), BL 21 (Wei-shu), BL 25 (Dachang-shu), RN 12 (Zhong-wan), LR 13 (Zhang-men), and ST 25 (Tian-shu). All acupoints except Zhong-wan are selected bilaterally, for a total of 11 points. All acupoints will be positioned according to the national standard developed by the People's Republic of China in 2006 (GB/T 12346-2006). The locations of the acupoints are shown in **Table 1** and **Fig. 3**.

Appliance selection

The instruments that will be used in this trial include disposable embedding needles (9#, Jiangsu Huahong Medical Instruments Co., Ltd., Jiangsu, China), absorbable catgut sutures (Shanghai Pudong Jinhuan Medical Products Co., Ltd., Shanghai, China), and disposable embedding aids (Yangzhou City Dragon Tiger Medical Instrument Factory, Jiangsu, China).

Operation

The operation of ACE will refer to the relevant provisions of the Operation Standard for Acupuncture, Part 10: Catgut Implantation at Acupoints (GB/T 21709.10–2008) [35]. Firstly, Patients take a supine position and routinely disinfect the skin around the points. Secondly, the assistant will place an absorbable catgut suture of an appropriate length at the front end of the trocar before the stylet is connected, and handed to the acupuncturist. Thirdly, the skin where the acupoint is located will be tensed or lifted by the acupuncturist with the thumb and index finger of the left hand, and the needle will be inserted into the acupoint with the appropriate force by the right hand to the required depth. When a needling sensation (Deqi) occurs, the stylet will be pushed while the tube is withdrawn, which will embed the absorbable catgut suture into the muscular layer or subcutaneous tissue of the acupoint. Finally, a dry cotton ball can be used to press the acupoint for a few moments after the needle is withdrawn to avoid bleeding, and the patient will be told to keep the skin at the acupoint dry for 3 days.

Sham ACE group

The prescription and procedure of the operation for the sham ACE group will be the same as the ACE group. The only difference between the two groups is the sham ACE group will not be placed in the absorbable catgut suture.

WL group

The WT group will not receive any intervention. The patients will be asked to receive relevant tests, fecal samples collecting, and scale evaluations at the same time as the other two groups and delayed ACE therapy for free after a waiting period of 12 weeks.

Intestinal flora data acquisition and analysis

A total of 120 fecal samples will be obtained from 60 patients (before and after treatment) for high-throughput 16S rRNA gene sequencing. Participants will be given instructions by oral, video, or written teaching about collecting fecal samples, including usage of fecal kit, weight and volume of fecal sample, storage, and transport. The main tests are the species and the abundance of the intestinal flora.

Collection of fecal samples

Collection method

1. Select a clean squat toilet or flush toilet, rinse it well, and then pad two layers of clean paper towels in the commode to prevent the fecal sample from falling directly into the commode water;
2. Defecate on prepared tissues;
3. Open the sampling bag, put on disposable gloves, take out the sampling protection kit, and prepare for sampling.
4. Unscrew the collection tube (Guangdong Longsee Biomedical Co., Ltd. Guangzhou, China), hold the liquid-holding tube at one end, and use the sampling rod to take the stool. Collecting from the medial part of the feces (approximately 3 g).
5. Insert the sampling rod with the feces taken back into the tube and tighten the sealing screw cap.
6. Hold the collection tube in your hand and push the middle of the liquid-holding tube with your thumb near the side of the easy-to-fold rod, so that the rod in the liquid-holding tube breaks along the root, to reveal a small hole, and gently squeeze the liquid-holding tube to make the protective liquid flow through the small hole into the tube containing feces.
7. Squeezing all the liquid in the liquid-holding tube into the lower tube, gently shaking the collection tube so that the stool sample in the groove of the sampling head separates from the sampling head and mixes fully with the protective liquid so that the liquid in the tube is in suspension.
8. Return the fecal collection tube to its original bag and give it to the researchers as soon as possible.

Collection time

The fecal samples should be collected within 1 week before treatment and within one week after 12 weeks of treatment, 2 times in total. Fasting is required the night before the collection, and the collection should be done on an empty stomach in the early morning of the next day. Each collection tube will be labeled with the subjects' names, dates, and times of the sample was collected. All samples will be returned to the laboratory as soon as possible and stored in a -80°C refrigerator until analysis. It can also be stored in a household refrigerator at -4°C, but that must be handed over to the researcher for subsequent analysis within 24 hours.

Precautions for collection

1. Subjects were instructed to maintain their former diet, eating habits, and physical activity as usual, but to avoid foods rich in probiotics and prebiotics, such as yogurt.
2. Subjects should not take any food containing probiotics and prebiotics, such as yogurt, for 7 days before sample collection, and should also avoid spicy, raw, cold, and other stimulating foods.
3. If the patient suddenly develops constipation or diarrhea before sampling, or the patient is in a menstrual period, it is advisable to delay sampling until the patient returns to normal bowel movements.
4. If the patient has taken antibiotics for more than 3 days before sampling, it should be postponed for 1 month according to the time of taking the drug.
5. Try to avoid urine contamination when collecting feces.

Intestinal flora sequencing

Intestinal flora will be quantified by Knorigene Technologies Co., Ltd.

The main steps from extraction to sequencing include the following:

1. The CATB method will be applied to extract intestinal floras' DNA, and the DNA will be quantified using Nanodrop.
2. Polymerase chain reaction (PCR) amplification of target fragments. The sequence of the variable region V3 to V4 of the 16S rRNA gene, which can reflect the composition and diversity of the intestinal flora, will be targeted, and the corresponding primers will be designed according to the conserved regions in the series.
3. The PCR products are purified and recovered by magnetic beads, and then they will be quantified by fluorescence, (fluorescence reagent is Quant-iT PicoGreen dsDNA Assay Kit, quantification instrument is Microplate reader (BioTek, FLx800).
4. Sequencing library preparation. Based on the characteristics of the amplified 16S, a small fragment library was constructed, and the library was sequenced by double-end sequencing (Paired_End) based on Illumina's TruSeq Nano DNA LT Library Prep kit sequencing platform, and the corresponding reagent was MiSeq Reagent kit V3 (600 cycles).

5. The Illumina MiSeq platform (Illumina, San Diego, USA) will be used for high-throughput sequencing to obtain the base sequence information of the corresponding microbiomes.

Analysis processing

QIIME 2 (QIIME 2, RRID: SCR_008249, URL: http://qiime.org/) and Greengenes database (Release 13.8, http://greengenes.secondgenome.com/)(DeSantis et al., 2006) will be applied. The classify-sklearn algorithm (Bokulich et al., 2018) (https://github.com/QIIME2/q2-feature-classifier) of QIIME2 will be used for analysis. According to each feature sequence of ASVs or representative sequences of each OTU, the pre-trained Naive Bayes classifier in the QIIME2 software with default parameters will be used for species annotation, to identify the corresponding taxonomic and its abundance information. Alpha diversity analysis, including species in richness, diversity, and evenness will be used to assess the diversity of the intestinal flora; The number of high abundances and rare ASV/OTU in the intestinal flora can be visualized by using the Rank abundance curve. (https://en.wikipedia.org/wiki/Rank_abundance curve). In addition, principal component analysis, principal coordinate analysis, Nonmetric Multidimensional scaling, and other methods will be used to characterize the bacteria in each experimental group. Then, the interrelationships between groups of intestinal flora will be performed network analysis and the functional profiles of intestinal floras will be predicted by the software PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Gavin M. Douglas, et al., preprint).

Outcome measurement

The clinical outcomes will be used to assess the effectiveness of ACE to AO, the results of the intestinal flora will be linked to clinical outcomes to further elucidate the mechanism of ACE for AO. All measurements including, weight, BMI, VAS of appetite, and GSRS will be undertaken before and after the intervention. All subjects will be tested in School of Acupuncture and Massage-Rehabilitation/The Second Affiliated Hospital of Yunnan University of Chinese Medicine.

The primary outcomes are intestinal flora and BMI. The intestinal flora will be detected by high-throughput 16S rRNA gene sequencing, which is considered an intuitive and reliable assay to study the composition and dynamics of the gut microbial community at present [36]. Through the relevant results of intestinal flora, the number and species of intestinal flora in patients with AO before and after the intervention will be obtained, and the results will be analyzed and compared. The BMI will be used to assess patients' obesity levels and the effectiveness of ACE to AO. The BMI is calculated as BMI = weight (kg)/ $(\text{height(m)})^2$. Weight will be measured after the overnight fast, using an electronic balance scale (QX-QB2015). Before being weighed, the subjects will be asked to wear lightweight clothing and take off their shoes. Height will be measured using a wall-mounted stadiometer.

The second outcomes include WC, VAS of appetite, and GSRS. The WC will be measured twice by a standard measuring tape at the midpoint between the lowest rib and the iliac crest, the average of the two measurements will be taken. The VAS of appetite of Canadian E Doucet scholars [37] will be used,

consisting of 4 components, including the intensity of desire to eat VAS, the intensity of hunger VAS, the intensity of satiety VAS, and the intensity of willingness to eat quantity VAS, all on a 10-point scale, with 0 no appetite, minimal intake, 1-3 light appetite, a small amount of intake, 4-6 moderate appetite, moderate intake, and 7-10 strong appetite, huge intake. Subjects will be asked to mark their level of appetite on the VAS. Subjects will not be allowed to access their previous VAS records on subsequent sessions. The scale of the visual analogue score (VAS) of appetite is shown in **Fig. 4**. The GSRS is a widely used questionnaire for patients' gastrointestinal symptoms in the past 1 week, which is a 15-item specific scale covering 5 GI symptoms: reflux, abdominal pain, dyspepsia, diarrhea, and constipation. The GSRS is rated on a 7-point Likert scale from “asymptomatic” to “very severe”; The higher the score, the more severe the symptoms [38, 39].

Other outcomes, including basal metabolic rate, blood pressure (BP), heart rate (HR), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels, will be tested before and after treatment.

Statistical analysis

Before data analysis, the research group will draw up a statistical plan, including the required data and method of data processing.

The demographics, baseline characteristics, and efficacy of the subjects will be analyzed with different methods by SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA). Qualitative data will be described as percentages or proportions and will be compared using chi-square (x^2) tests. Quantitative data will be expressed as mean ± standard deviation. For continuous variables, if the data are normally distributed, a one-way analysis of variance (ANOVA) will be used to detect differences among the three groups. If not, the Kruskal-Wallis (K-W) test may be considered. For longitudinal and repeated measures data, repeated measures ANOVA will be used to determine differences in the same group at 2-time points (baseline and the end of 12 weeks intervention). Two-sided tests will be used during the analysis and *P*-value < 0.05 will be considered as the threshold of statistical significance.

Quality control

To ensure the objectivity of the study results, the study will be conducted in strict accordance with the basic requirements of a clinical randomized controlled trial, with randomized grouping and enrollment, strict control of subject recruitment criteria, and evaluate the trial results by evaluators who are unaware of the characteristics of the groups, and perform a blind statistical analysis by a third party. All researchers, data collectors, and statisticians are involved in the study should strictly abide by the rules of the study.

To ensure a smooth study, specialized clinical training will be provided to all the clinical researchers who will receive specialized training before the initiation of the trial to familiarize each clinical researcher with the study’s process and implementation.

Among them, the operator of ACE should have at least 3 years of clinical work experience and be able to cope with any possible AEs during the treatment; The data collectors are responsible for keeping and managing various data and performing rigorous proofreading of the data. Moreover, keeping detailed records of subjects' withdrawals and AEs during the study period, and those who have already passed 1/2 course of treatment should enter the efficacy statistics; The statisticians will be fully responsible for data management and statistical analysis. Regular team meetings will also be held and fully documented during the conduct of the study.

Ethics approval

The protocol of this study has been approved by the ethics committee of the Sports Specialist Hospital of Yunnan Province (2021-02) and registered in the Chinese Clinical Trial Registry (NO: CHiCTR2100048853). Before the start of the study, patients will be fully informed of the project information including the study procedures, benefits, and potential risks, and they will be informed that they are free to withdraw from the study without penalty at any time subject to careful consideration of participation. If the protocol modifications are required, all trial information will be reported to the Ethics Committee, and the modified protocol will be implemented only after consent has been obtained.

Data management and confidentiality

The researchers will record subjects' information on the CRFs and verify that data are collected in timely, accurately, and fully. Personal information such as the patient's name, phone number, ID number, and medical records will be kept anonymously to prevent information leakage. All participants' paper data will be kept by the researchers in a special cabinet and preserved for at least 5 years after publication.

In addition, the ethics committee of the Sports Specialist Hospital of Yunnan Province will periodically review the progress of the trial and monitor the collection, allocation, and concealment of data. The modification or termination of the trial can be implemented by the committee. The data monitoring committee is independent of the sponsor and has no conflict of interest.

Discussion

Obesity, as a pandemic public health issue in the world today, not only brings a heavy medical burden to society but also seriously affects people's quality of life. In recent years, with the development of complementary medicine, acupuncture has been widely applied as an alternative therapy in the clinic, among which, ACE is more popular among obese patients because of its economical, time-saving, less frequent needling, and long-lasting efficacy. In the context of new evidence for the efficacy of ACE interventions in obesity, it is necessary to elucidate the mechanism of ACE in the treatment of obesity.

There is growing evidence that changes in the intestinal flora are associated with metabolism-related diseases such as obesity [40], and the intestinal flora consists of many bacteria that contribute to nutrient and energy regulation and they are closely related to the energy metabolism of the host [41]. It was found

that there are significant changes in the intestinal micro-ecosystem in obese people compared to normal people. Compared with the intestinal flora of lean people, the level of thick-walled bacteria in the intestinal tract of obese people is increased and accompanied by a decrease in the number of mimics [42]. Study has shown that obesity can be effectively suppressed by intervening in the intestinal flora related to obesity [43]. It was also found that acupuncture in the treatment of simple obesity can reduce the BMI by increasing the number of their intestinal Lactobacillus and Bifidobacterium,and decreasing the number of Bacteroides and Clostridium perfringens [29]. However, the current researches on the mechanism of ACE to regulate intestinal flora to treat obesity is still limited.

This is a randomized, single-blind, parallel-group study that aims to further evaluate the efficacy of ACE in treating AO and to investigate the relationship between changes in the composition of the intestinal flora and treatment outcomes. According to the theory of TCM, obesity is closely related to the digestive function of the spleen, stomach, and intestines. In the theory of acupuncture, the back points and the collection points are the gathering points of the qi of the internal organs on the back, chest, and abdomen, respectively. The back-shu points and front-mu points are the points where the qi of the internal organs converges in the back and chest and abdomen respectively. The two are often used together to bring out their synergistic effect in the clinic, which is also one of the classic combinations acupoints in Chinese medicine, known as Shu-mu points. An experimental study showed that electrical stimulation of the back-shu points “Dachangshu” (BL 25) and front-mu points “Tianshu”(ST 25) of the large intestine can improve diarrhea in IBS model rats by regulating the expressions of colon c-kit and TRPV1 [44]. Therefore, the back-shu points and front-mu points of the spleen, stomach, and large intestine are chosen as the prescription for ACE.

There are clinical trials with non-meridian non-acupuncture points as a control group in the clinic [45]. Due to the special nature of ACE, the acupoints prescription for the control group in this trial will be the same as the observation group, with the difference that the absorbable catgut suture will not leave in the body after the needle is withdrawn. At the same time, considering that the sham ACE may produce clinical effectiveness, including some stimulation of acupoints and unavoidable psychological factors, we added awaiting treatment group to exclude this confounding factor.

In addition, since the prevalence of obesity among women is higher than that of men, women are recruited as the subjects of this experiment [2, 46], and abdominal obesity is relatively more significant in women [46, 47]. What’s more, in clinical practice, the majority of patients on diet pills are women, and diet pills can have serious effects on female fertility in addition to the conventional side effects such as nausea, headache, and constipation [48].

In this trial, in addition to BMI as the classical clinical evaluation index, GSRS, which is currently more widely used, will be used to assess changes in the gastrointestinal tract, as well as several aspects such as WC, body weight, appetite changes, and blood lipids to assess the clinical efficacy. More importantly, the main purpose of the study is to observe the changes of intestinal flora, and 16S rRNA gene sequencing, which is currently more widely used, will be used to detect changes in the intestinal flora

composition before and after treatment. We do not restrict the diet of the subjects during the treatment period, they can maintain their diet, eating habits, and physical activity as usual during the study except for some that could interfere with the analysis of intestinal flora, such as taking probiotic foods or using antibiotics, which ensure the participant's diet components, and levels of physical activity did not differ compared to before the treatment and also exclude the effects of dietary interventions to facilitate a more objective evaluation of the trial results.

One limitation of this study is the relatively small sample size, partly due to the relatively high cost of intestinal flora testing, but even so, our analysis of the gut flora results will not be affected. Also, our study will only observe changes before and after treatment and no follow-up will be done, also in consideration of the study funding. Secondly, the treatment period of this trial is relatively long, and the sham buried group may have some risk in blinding control because the absorbable catgut suture is not left in the patients, which requires good communication between the operator and the subjects.

In conclusion, the results of this study are expected to demonstrate that Shu-mu points ACE is effective in relieving AO and altering intestinal flora. We expect the results will further support the use of ACE to treat AO, thus providing reliable evidence for future research and practical recommendations for clinical practice.

Trial Status

This trial is recruiting patients at present. The trial began recruitment on September 1, 2021, and is anticipated to be completed on December 31, 2023.

Abbreviations

AO: abdominal obesity; ACE: Acupoint catgut embedding; AE: Adverse event;

BMI: Body mass index; WC: Waist circumference; BW: body weight; HR: Heart rate; BP: Blood pressure; TC: Total cholesterol; TG: Triglyceride; HDL: High-density lipoprotein; LDL: low-density lipoprotein; T2DM: Type 2 diabetes mellitus; RCT: Randomized controlled trial; WT: Waiting list; SD: Standard deviation; CDC: Chinese Center for Disease Control; SOPs: Standard operating procedures; WHO: World Health Organization; K-W: Kruskal-Wallis; GSRS: the Gastrointestinal Symptom Rating Scale; VAS: visual analogue score CRFs: the case report forms

Declarations

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Authors' contributions

QFL and JLF contributed equally to this paper. TPG and CHX are the corresponding authors. QFL participated in the conception and design of the trial. QFL and JLF drafted the manuscript. SWZ and XXZ are in charge of the recruitment and data collection. MK and YQZ are in charge of the treatment of patients, XHZ participated in data analysis. CHX and TPG provided the funding, ethical approval, total design, and proofread the final manuscript. All authors approved the submitted version of the manuscript.

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Availability of data and materials

The full data from this study will be available upon reasonable request after completion of the study.

Ethics approval and consent to participate

This study was approved by the ethics committee of the Sports Specialist Hospital of Yunnan Province (2021-02). All participants will sign written consent to participate in the study after being fully informed of the project information including the study procedures, benefits, and potential risks.

Consent for publication

Not applicable. Results of the study will be published in papers in a peer-reviewed academic journal or presented at relevant national and international conferences.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Location of acupoints in the two ACE groups

Acupoints	Location
BL 20 (Pishu)	On the back, on the same level of the 11th subspinous of thoracic spine, and 1.5 cun lateral to the posterior median line.
BL21 (Weishu)	On the back, on the same level of the 12th subspinous of thoracic spine, and 1.5 cun lateral to the posterior median line.
BL25 (Dachangshu)	On the back, on the same level of the 4th subspinous of lumbar vertebra, and 1.5 cun lateral to the posterior median line.
RN12 (Zhongwan)	on the anterior median line, 4 cun above the umbilicus
LR13 (Zhangmen)	On the lateral abdomen, inferior to the free extremity of the 11th rib at the liver meridian,or 1.8 inch up GB 26 (Daimai) at the intersection of the vertical line of the free end of the 11th rib and horizontal line on the same level of umbilicus
ST 25 (Tianshu)	Level with the umbilicus, and 2 cun lateral to the anterior median line

Figures

Figure 1

Flowchart of the study design.

	STUDY PERIOD			
	Enrolment	Allocation	Treatment	
TIMEPOINT	-1 week	0 week	1-12th week of treatment	Post of treatment (12th week)
ENROLMENT:				
Eligibility screen	×			
Informed consent	×			
Physical examination	×			
Randomization		×		
INTERVENTIONS:				
ACE group (n=30)			×	×
Sham ACE group (n=30)			×	×
Waiting-list group (n=30)			•	•
INTESTINAL FLORA TESTING:				
ACE group (n=30)		×		×
Sham ACE group (n=30)		×		×
Waiting-list group (n=30)		×		×
ASSESSMENTS				
BMI, WC		×		×
Basal metabolic rate, BP, HR		×		×
TC, TG, HDL		×		×
VAS of appetite		×		×
GSRS		×		×
PARTICIPANTS SAFETY				
Adverse events		×	×	×

Figure 2

Study schedule foe data collection.

Figure 3

Location of acupoints.

Figure 4

The scale of the VAS of appetite.

Supplementary Files

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