

## ORIGINAL RESEARCH

# Observation on Short-Term Therapeutic Effect of Electroacupuncture on Compensatory Hypertrophy Of Gastrocnemius Muscle: A Retrospective Cohort Study

Jiamin Hu, MM; Feifei Lu, BM; Yanli You, PhD

### ABSTRACT

**Objective** • In Oriental women, having thick and short legs is a common posture problem, and having an imperfect lower leg shape is one of the factors that can cause feelings of inferiority. To address this issue, a retrospective cohort study was conducted to investigate the effectiveness of electroacupuncture in improving compensatory hypertrophy of the gastrocnemius muscle while also reducing side effects. The goal of this study is to improve the clinical treatment plan for this type of problem.

**Methods** • This retrospective cohort study was conducted between December 2020 and March 2022 at the Changhai Hospital of Shanghai, Shanghai, China. This study included 80 patients who were divided into two equal groups - the infrared (IR) group and the electroacupuncture (EA) group, based on the type of treatment they received during the research. The EA group received electroacupuncture and infrared treatment, while the IR group used an infrared therapeutic instrument to irradiate their lower legs on both sides. The main outcome measures were 3 calf circumference levels and the cross-sectional area of the gastrocnemius

muscle in two-dimensional ultrasound. The secondary outcome measure was the incidence of adverse events.

**Results** • According to the data, before the treatment there were no statistically significant differences between the two groups in calf circumference and ultrasound gastrocnemius cross-sectional area. After the treatment, the value of each calf circumference level and ultrasound gastrocnemius cross-sectional area were significantly lower than the value collected before the treatment in the EA Group. However, there is no significant change in the data of the infrared therapeutic group before and after treatment. By comparing the data between the 2 groups we collected after the treatment, the value of each calf circumference level and ultrasound gastrocnemius cross-sectional area of the EA group is significantly lower than that of the IR group. Only 8 patients suffered from lower limb pain and other discomfort after treatment, and these symptoms did not cause dissatisfaction.

**Conclusion** • Electroacupuncture is an effective treatment for compensatory hypertrophy of the gastrocnemius muscle. (*Altern Ther Health Med.* 2024;30(1):296-301).

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### INTRODUCTION

With the development of the times and aesthetics, modern women are more and more strict in the management of their body shape. The thick short legs are the common posture problems of oriental women.<sup>1,2</sup> In 2012, Benslim elaborated on the concept of the perfect calf shape. He defined the axis of the lower limb as a straight line from the midpoint of the femoral head to the midpoint of the knee joint and finally to the midpoint of the ankle joint. The maximum circumference of the ideal leg contour should be three-quarters the length of the

leg, and its height is usually at or slightly lower than the upper quarter of the leg.<sup>3</sup> In particular, in the Asian aesthetic concept, the convex rear leg is detrimental to the beauty of the leg. The physiological causes include obesity, edema, incorrect walking exertion, and poor leg posture.<sup>4,5</sup> Hayao Ozaki et al. observed the muscle morphology of lower limbs after long-term exercise in different ways, and found that jogging training would lead to muscle hypertrophy;<sup>6</sup> Henning Wackerhage found that the metabolic product of resistance exercise regulation may be a hypertrophic stimulus by comparing the effects of exercise injury and resistance exercise on the “hypertrophic receptor” in skeletal muscle;<sup>7</sup> Most of pathological short and thick leg can be rapidly improved by reducing fat and dehydration, while the compensatory hypertrophy of leg muscles caused by exertion and poor posture is difficult to improve, as we have known, the excessive use of the gastrocnemius muscle leads to compensatory hypertrophy, which causes the prominent back of the lower leg and affects the beauty. Improving the shape of the gastrocnemius muscle is also the key to improving the

contour of the lower leg, therefore, there are several clinically targeted treatment methods, such as Musclectomy, and selective neurectomy which cause “disuse atrophy” of muscles by cutting off nerves. However, the treatments mentioned above will cause large trauma to the human body and the side effects are obvious, which is not easy to be accepted by the public.<sup>8</sup> In 2004, Lee et al. studied the effect of botulinum toxin injection on patients with leg protrusion. He injected botulinum toxin into the medial head of the gastrocnemius muscle, where the calf muscle function is redundant and is the most prominent part of the leg. The results show that this method has a significant effect on the correction of leg shape, and the maintenance time is more than 6 months.<sup>9</sup> Since then, botulinum toxin injection into the gastrocnemius muscle has become the main treatment method for leg plasticity. However, this method may still have some side effects, such as abnormal gait and fatigue after walking or running caused by muscle disorders in the leg. Patients with gastrocnemius muscle hypertrophy and allergies to botulinum toxin cannot use it.

Acupuncture is a traditional Chinese medicine treatment method, which has more than 2000 years of history. It is guided by the basic theory of traditional Chinese medicine and stimulates the meridians and acupoints of the human body, which plays the role of dispersing blood, Qi-benefiting, and relieving pain.<sup>10</sup> Relevant mechanism studies show that electroacupuncture can improve the degree of denervated skeletal muscle atrophy, and down-regulate the expression of autophagy-related factors: Beclin-1, Vps34, LC3II/LC3I.<sup>11</sup> However, there are few studies on the use of electroacupuncture to improve the compensatory hypertrophy of the gastrocnemius muscle. The clinical application is rare. Therefore, to improve the clinical treatment plan for the treatment of gastrocnemius compensatory hypertrophy and reduce the side effects. This study evaluated the effect of electroacupuncture on the compensatory hypertrophy of gastrocnemius muscle by comparing the changes in leg and muscle dimensions of subjects after electroacupuncture and infrared therapeutic.

## MATERIAL AND METHODS

### Study design

This retrospective cohort study was conducted between December 2020 and March 2022 at the outpatient department of Changhai Hospital of Shanghai, Shanghai, China.

### Patient

Inclusion criteria: Outpatients included calf muscular hypertrophy and visible leg contour in high heels, with a strong desire for leg appearance improvement. Exclusion criteria would be subcutaneous fat hypertrophy of the lower leg; patients with congenital lower leg muscle disease; contradiction to acupuncture; and pregnant and lactating women.

The patient data included in this study were divided into the infrared (IR) group and the electroacupuncture (EA) group according to the difference in the treatment conducted during the research. The EA group received electroacupuncture and infrared treatment, while the IR group used an infrared

therapeutic instrument to irradiate their lower legs on both sides.

### Intervention

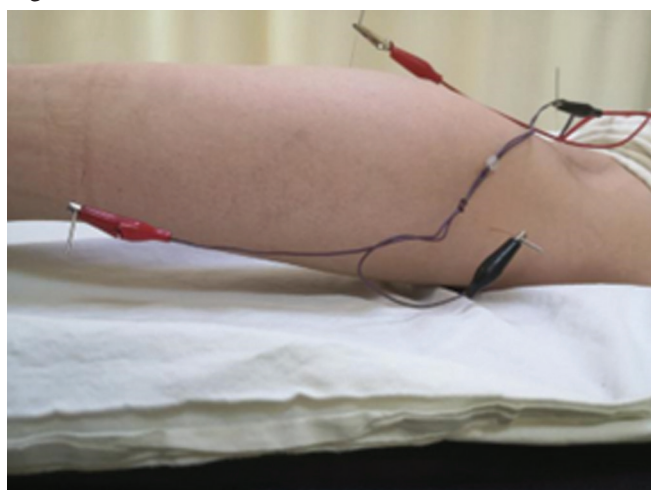
During the treatment, after the subjects arrived at the outpatient department, a routine examination would be adopted, the doctor would observe the muscle-fat ratio of both legs while the patient lay prostrate on the examination table, by using an MyLab™ Alpha Twice ultrasonic diagnostic instrument (probe model LA52, Italy), two-dimensional ultrasound imaging was performed, the patient's feet should hang on the edge of the table so that the ultrasound examination can be performed on medial head of the gastrocnemius muscle and the ventral side of the lateral head muscle at the relaxed ankle position. During the examination, the ultrasonic probe should be placed horizontally on the skin and gently pressed on it to obtain an ultrasonic image according to the method of Bénar et al.<sup>12</sup> The ultrasonic probe would be centered on the middle longitudinal axis, and collect the image of the distance from the popliteal fossa is 25%, 55%, and 75% of the length of the gastrocnemius muscle. The ultrasonic probe takes ultrasonic images with a series of tilt and rotation angles in 5-degree increments. On the obtained two-dimensional ultrasound image, the muscle cross-section is marked, and the ultrasound image processing software automatically calculates the cross-sectional area of the gastrocnemius muscle at 25%, 55%, and 75% from the popliteal fossa. In addition, the doctor would measure the circumference of the first leg at three levels (level 1: fibula capitulum, level 2: tibial midpoint, level 3: gastrocnemius insertion).

Firstly, the acupoints were fixed in the electroacupuncture group: Yanglingquan GB34 (a influential point of sinews, particularly sinew spasm and motor disturbance), Xuanzhong GB39 (benefits the sinews, bones, neck, and activates the channel, alleviates pain), Chengshan BL57 (main point to relieve cramp of the venter of gastrocnemius muscle.), Weizhong BL40 (Main point for pains of the back and lumbar, spasm of the popliteal tendons, weakness or paralysis in the lower limb), Yanglingquan is located on the outside of the lower leg, anterior lower hollow of the fibular head. Xuanzhong is located on the outside of the lower leg, at 4 transverse fingers on the tip of the lateral ankle, anterior edge of the fibula. Chengshan is located at the posterior of the lower leg, with sharp angle depression under the gastrocnemius muscle. Weizhong is located on the back of the knee joint, right at the midpoint of popliteal striation. A routine sterilization was performed after positioning confirmation. Needles were vertically inserted in these acupoints, and the depth of needling was 0.5~0.8 inch, Paired electrodes from the electroacupuncture apparatus were attached transversely to the needle handles at two groups of acupoints: Yanglingquan, Xuanzhong, and Weizhong, Chengshan. The electroacupuncture stimulation lasted for 30 minutes with alternating dilatational waves of 2 Hz / 100 Hz. It is considered to be effective for acupuncture when the frequency of electrical stimulation is adjusted to cause pain, numbness, swelling, or radiation sensation. This process was continued for 20 minutes, during which the infrared therapeutic instrument was used to

**Figure 1.**



**Figure 2.**



**Figure 3.**



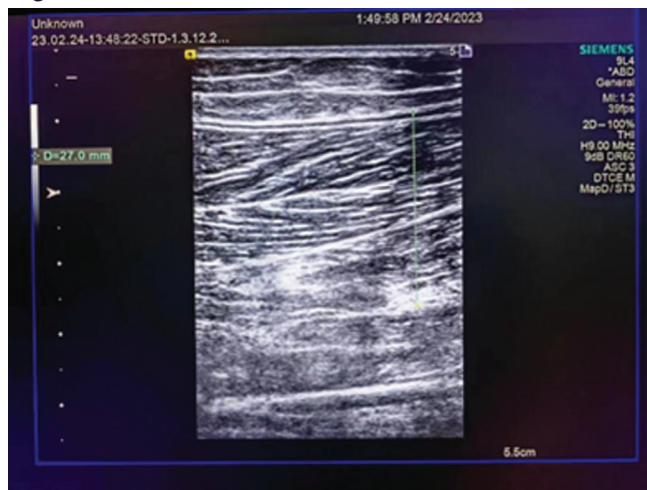
irradiate the lower legs of both sides. After the treatment, the patients were instructed to continue the treatment on the next day for 4 weeks (Figures 1, 2 and 3).

The patients of the infrared therapeutic group were instructed to put themselves in the right position, the

**Figure 4.**



**Figure 5.**



**Figure 6.**



infrared therapeutic instrument was used to irradiate the lower legs of both sides, this process was continued for 20 minutes. After the treatment, the patients were instructed to continue the treatment on the next day for 4 weeks.



## Outcomes and data collection

During the study period, Major patients were evaluated right before the operation. Then the patients were assigned to the electro-acupuncture group (1:1 ratio) and infrared therapeutic group. The data of patients were retrospectively abstracted from computerized medical records by clinical staff.

The primary outcome included three Calf circumference levels (level 1: fibula capitulum, level 2: tibial midpoint, level 3: gastrocnemius insertion), and cross-sectional area of the gastrocnemius muscle in two-dimensional ultrasound. The secondary outcome was AE (adverse event) which was used to observe the occurrence of adverse reactions after the operation. (Figures 4, 5, and 6).

## Statistical analysis

This study determined the sample size by comparing the mean leg circumference of the two groups. According to reference,<sup>13</sup> the effect of different anesthesia depths on postoperative delirium scores was substituted into two sets of data: the treatment group was  $35.42 \pm 1.35$  cm, and the control group was  $36.49 \pm 2.98$  cm. The sample size was determined by comparing the mean leg circumference of the two groups. The study also had the following abilities for type 1 errors and unilateral delirium  $\alpha$  90% of 0.025 ( $\beta = 0.10$ ), using PASS software for sample size calculation ( $z \alpha = 1.96$ ,  $z \beta = 1.28$ ,  $SD = 1.645$ ), with a minimum number of participants in each group of 40. Analyses were carried out using SAS software V.9.4. Of the eligible participants, 50% were assigned to the EA group, while the remaining 50% were assigned to the IR group and using PASS software to calculate the sample size,

the number of participants in each group is 40. Statistical Product and Service Solutions (SPSS) software (SPSS 17.0, IBM Corp., Armonk, NY, USA) was used for data analysis. For continuous data, the Student's *t* test or Mann–Whitney U test was applied based on data with normal or non-normal distribution. We set a value of  $P < .05$  (2-side) as having statistically significant.

## RESULTS

### Baseline monitor

Baseline assessment was conducted by comparing the three Calf circumference levels of the two groups and ultrasound gastrocnemius cross-sectional area before the test. There were no statistically significant differences between the two groups (Tables 1, 2).

### Calf circumference outcomes

As illustrated in Table 3, compared with the pre-treatment, there were significant differences in the decrease in each level's circumference of the lower leg in the EA group ( $P < .001$ ), however, in the IR group, there was no statistically significant change in the lower legs circumference of patients before and after treatment.

### Gastrocnemius cross-sectional area outcomes

By collecting the patients' cross-sectional area of gastrocnemius under two-dimensional ultrasound, and analyzing the data difference between the two groups before and after treatment. the results showed that the measurements of gastrocnemius cross-sectional area in the EA group after the treatment were significantly smaller than that IR group ( $P < .001$ ). Meanwhile, there was no significant difference between the data collected from the sham acupuncture group before and after treatment (Table 4).

## DISCUSSION

Short and thick legs are a common problem for Asian women, and imperfect leg shape is one of many factors that cause women's inferiority. As we have known, The muscle at the back of the lower leg includes the gastrocnemius muscle and soleus muscle. The medial and lateral heads of the gastrocnemius muscle are located in the shallow layer, and the soleus muscle is located in the deep layer. The muscle that determines the shape of the lower leg is the superficial gastrocnemius muscle at the back of the lower leg. Many patients have deep atrophy and weakness of the soleus muscle due to incorrect force posture, while the excessive use of the gastrocnemius muscle leads to compensatory hypertrophy, which leads to a prominent back of the lower leg and affects the beauty. Therefore, enhancing the strength of soleus muscle and reducing the compensatory force of gastrocnemius muscle are also the key to improve the lower leg contour. It is believed that the gastrocnemius muscle is the

**Table 1.** The three calf circumference levels

|          | Level 1             | Level 2             | Level 3             |
|----------|---------------------|---------------------|---------------------|
| Group EA | $37.3 \pm 1.06$ cm  | $38.79 \pm 1.07$ cm | $36.45 \pm 0.86$ cm |
| Group IR | $38.58 \pm 0.98$ cm | cm                  | $36.64 \pm 0.84$ cm |
| P-value  | .430                | .350                | .340                |

**Table 2.** The three ultrasound gastrocnemius cross-sectional area levels

|          | 25%                              | 55%                               | 75%                              |
|----------|----------------------------------|-----------------------------------|----------------------------------|
| Group EA | $13.40 \pm 1.00$ cm <sup>2</sup> | $20.11 \pm 0.616$ cm <sup>2</sup> | $12.24 \pm 0.79$ cm <sup>2</sup> |
| Group IR | $13.19 \pm 0.99$ cm <sup>2</sup> | $19.91 \pm 0.89$ cm <sup>2</sup>  | $12.13 \pm 0.97$ cm <sup>2</sup> |
| P value  | .317                             | .14                               | .678                             |

**Table 3.** The three circumference levels of the lower leg before and after the trial

|          | Level1(pre)         | Level1(post)        | Level2(pre)         | Level2(post)        | Level3(pre)         | Level3(post)        |
|----------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Group EA | $37.3 \pm 1.06$ cm  | $35.97 \pm 0.93$ cm | $38.79 \pm 1.07$ cm | $36.64 \pm 0.85$ cm | $36.45 \pm 0.86$ cm | $35.30 \pm 0.82$ cm |
| P value  | <.001               |                     | <.001               |                     | <.001               |                     |
| Group IR | $37.15 \pm 0.97$ cm | $37.06 \pm 0.90$ cm | $38.58 \pm 0.98$ cm | $38.30 \pm 1.06$ cm | $36.64 \pm 0.84$ cm | $36.67 \pm 0.80$ cm |
| P value  | .647                |                     | .246                |                     | .791                |                     |

**Table 4.** The 3 levels' cross-sectional area of gastrocnemius under two-dimensional ultrasound before and after the trial

|          | 25%(pre)                         | 25%(post)                        | 55%(pre)                         | 55%(post)                        | 75%(pre)                         | 75%(post)                        |
|----------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Group EA | $13.40 \pm 1.00$ cm <sup>2</sup> | $12.09 \pm 0.88$ cm <sup>2</sup> | $20.20 \pm 0.73$ cm <sup>2</sup> | $18.91 \pm 0.60$ cm <sup>2</sup> | $12.24 \pm 0.29$ cm <sup>2</sup> | $10.99 \pm 0.71$ cm <sup>2</sup> |
| P value  | <.001                            |                                  | <.001                            |                                  | <.001                            |                                  |
| Group IR | $13.19 \pm 0.99$ cm <sup>2</sup> | $13.16 \pm 1.02$ cm <sup>2</sup> | $19.91 \pm 0.89$ cm <sup>2</sup> | $19.85 \pm 0.81$ cm <sup>2</sup> | $12.13 \pm 0.97$ cm <sup>2</sup> | $12.03 \pm 0.93$ cm <sup>2</sup> |
| P value  | .817                             |                                  | .750                             |                                  | .537                             |                                  |

center of human movement, and the changes in the muscle neurons of the gastrocnemius muscle will seriously affect the efficiency of movement.<sup>13</sup> So, clinicians should be cautious when it comes to more radical treatment methods in modern medicine, such as muscle resection, nerve damage, and even intramuscular injection of botulinum toxin.<sup>14</sup> So now come to the question: Why not choose the mild and effective therapy on the way to beauty?

In addition to those radical therapies mentioned above, there are many relatively mild therapies in clinical practice such as exercise therapy strengthening the deep muscle group and posterior thigh muscle group, infrared therapy, cryotherapy, and other treatment methods to reduce the gastrocnemius muscle compensation and improve the calf contour. One study observed the forearm muscle pain and blood circulation status with cryotherapy and infrared therapy and found that both of them relieved muscle pain and improved local blood circulation.<sup>15</sup> These exercise therapy and physical therapy methods have certain curative effects on reducing muscle compensation and alleviating muscle fatigue, but they have the disadvantages of a long treatment cycle and insignificant clinical effect; The theory of “myofascial chain” was first proposed by Ida Rolf, a famous American physiotherapist.<sup>16</sup> Thomas Myers confirmed this in his book “Anatomy Train” through human anatomy, which further promoted the improvement of myofascial theory.<sup>17</sup> In recent years, there have been more and more physical therapies for skeletal muscle and even medical diseases based on the fascia theory. Many studies have also confirmed that stimulating the trigger point of the fascia chain can effectively alleviate many stubborn soft tissue diseases.<sup>18</sup> At the same time, the corresponding mechanism research also confirmed that the good distribution of nerves through the combing of fascia is conducive to better management of muscles and surrounding soft tissues.<sup>19</sup> As we have known, there are many ways to stimulate these trigger points in the clinic, including, but not limited to, acupuncture, compression, and shock waves. In this study, acupuncture therapy was chosen because it effectively treats deep fascia problems more than non-invasive treatments such as compression and shock waves.

Nowadays, there is more and more clinical research confirming that the recovery effect of electroacupuncture for muscle function is significant. Stefano Schiafino treated a 62-year-old female Facioscapulohumeral muscular dystrophy (FSHD) patient with acupuncture. After about 10 months of long-term treatment, the facial muscle function of the patient recovered significantly.<sup>20</sup> At the same time, the efficacy of electroacupuncture in alleviating muscle fatigue is also recognized by the public. Ricardo Cardosod observed 45 volunteers with exercise-induced muscle injury, treated with electroacupuncture and sham acupuncture, and evaluated the threshold of muscle pain and pressure pain at different time points. It was found that acupuncture and moxibustion can reduce the occurrence of acute muscle soreness by half, and the occurrence of delayed muscle soreness by one-third.<sup>21</sup> These seemingly contradictory studies manifest the

effects of the two-way regulation of acupuncture. This function of acupuncture can excite the depressed and inhibited organism, and also make the hyperactive and excited organism converge, but it has no obvious effect on the normal physiological state. The occurrence of simple gastrocnemius hypertrophy is related to the atrophy and weakness of deep soleus muscle, and the compensation and fatigue of shallow gastrocnemius muscle.

In this research, the investigator chose GB34, and GB39 on Gallbladder Meridian which patrols the outside of the lower leg, and based on the theory that “where the meridians patrols, the main governing point is” mentioned by Lingshu of Inner Canon of the Yellow Emperor. We conclude that by stimulating these two acupoints, the “Qi” and “blood” along the meridian path will be enchanted,<sup>22</sup> and not only the strength of the lateral head of the deep soleus muscle be enhanced, but also the fatigue of the lateral head of the shallow, gastrocnemius muscle is reduced. GB34 also known as “jinhui” (influential point of tendons) in acupuncture theory, it’s an important point for the clinical treatment of soft tissue diseases, which can treat the diseases of lower limb meridians and tendons, the position of this acupoint coincides with the outlet of the superficial peroneal nerve, which innervates the deep peroneal long and short muscles.<sup>23</sup> By stimulating this acupoint and the surrounding blood vessels and nerves, the local meridian “Qi” and “Blood” will also be stimulated, and when the deep muscles are nourished, the goal to reduce the compensation of the gastrocnemius muscle is then achieved. The ancient classic medical book “Compendium of Acupuncture and Moxibustion” mentioned that when it comes to lower limb problems, GB39 in combination with GB34 is often stimulated to relieve the pain, and it also mentioned that GB39 alone is effective in treating muscle spasms after stroke. Su C et al. explored the mechanism of electroacupuncture at GB39 and GB36 in treating adjuvant arthritis and found that it can improve joint injury by regulating the p53 signal pathway and inducing apoptosis.<sup>24</sup> As we have known, apoptosis is immediately related to skeletal muscle atrophy. Therefore, this study hopes to induce apoptosis by electroacupuncture at GB39 and improve the morphology of the gastrocnemius muscle. Another two acupoints-BL40, BL57 belong to Bladder Meridian which patrols the back of the lower leg. by stimulating these acupoints, the “Qi” and “blood” along the back of the lower leg will be enchanted, achieving the plasticity of the rear leg. The BL40 is located in the middle of the popliteal fossa, and the popliteal artery and the tibial nerve pass through the deep layer of this position. The tibial nerve is the main structure that controls the movement of the soleus and gastrocnemius muscles.<sup>25</sup> Meanwhile, the deep level of BL40 is the gastrocnemius muscle abdomen, and it is also the pathway of the tibial nerve, In traditional Chinese medicine, these two acupoints are often used together to treat leg spasms and fatigue after exercise. Jun Wang et al. stimulated the explosive force of muscles around the knee joint by electroacupuncture at BL40 to improve the joint

hardness of the knee joint.<sup>26</sup> Therefore, these acupuncture points mentioned above had an effective therapeutic effect in treating calf muscle strain. They were also considered effective for reducing gastrocnemius compensatory hypertrophy by improving flatfish muscle weakness.

Due to the scarcity of clinical studies on the treatment of skeletal muscle hypertrophy with electroacupuncture, the clinical application of this disease is also limited. According to the results of this study, we found that electroacupuncture has a relatively obvious improvement effect on the compensated hypertrophic gastrocnemius muscle. Through the observation of the circumference of the three layers of the lower leg, we can see that electroacupuncture has a certain improvement effect on this kind of problem. Among the results, the most significant effect is shown in the second level of the lower leg, the midpoint of the flat tibia, which is also the most bulging part of the posterior calf muscle. The numerical variation of level two in Table 3 shows a decrease from  $38.79 \pm 1.07$  cm to  $36.64 \pm 0.85$  cm. Many patients are very satisfied with the plastic effect of the leg after acupuncture because of the reduction of the circumference here. In addition, by observing the cross-sectional area of the gastrocnemius muscle under ultrasound can find that the thickness of the gastrocnemius muscle is also significantly reduced at three levels, The most effective site is the cross-sectional area of the gastrocnemius muscle at 55%, which decreased from  $20.20 \pm 0.73$  cm<sup>2</sup> to  $18.91 \pm 0.60$  cm<sup>2</sup>. Accordingly, it can be ruled out that the reduction of lower leg circumference caused by acupuncture is due to the reduction of fat and other soft tissues, and it is powerful clinical evidence for electroacupuncture to improve gastrocnemius hypertrophy. After the end of the treatment, the clinical observation form of adverse reactions filled out by the patients, we found that only 8 patients suffered from lower limb pain and other discomfort after treatment, lasting for 1-2 days, 13 patients had ecchymosis greater than 2 cm, and the symptoms caused by the electroacupuncture were relieved after 7 days. These symptoms did not cause dissatisfaction, and no patients withdrew from the treatment.

This study has several limitations. First, it is a retrospective study with the inherent potential biases of that design. Second, the experience levels of investors who manage measurement indicators are varied. This could lead to subjective measurement errors. Third, this experiment has not been followed up, and it is impossible to confirm the long-term efficacy of electroacupuncture in improving the compensatory hypertrophy of the gastrocnemius muscle.

## CONCLUSION

The results of this study showed that electroacupuncture is an effective treatment for compensatory hypertrophy of the gastrocnemius muscle. Giving electroacupuncture does not cause serious side effects and adverse reactions in the treatment of this disease, this therapy should be extended to clinical practice.

## CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report relevant to this article.

## AUTHOR CONTRIBUTIONS

JH and YY designed the study and performed the experiments, JH and FL collected the data, YY and FL analyzed the data, and JH and YY prepared the manuscript. All authors read and approved the final manuscript.

## FUNDING

This study did not receive any funding in any form.

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