The Therapeutic Effect of Acupuncture Treatment on Chronic Inflammation of High Fat Diet Induced Rats

Abstract

Objective: Obesity may be viewed as a chronic low-grade inflammatory disease, and there are increasing evidences proved that the acupuncture has the effect of anti-obesity; here we explored the therapeutic effect of acupuncture treatment on chronic inflammation of high fat diet induced rats.

Methods: Use the high fat diet to induced obesity rats, and treat the rats with specific acupoints acupuncture and non-acupoints acupuncture. After the interference, all the rats were exposure to CO₂ atmosphere and were killed by decapitation, the epididymis fat and the perirenal fat were isolated and weighted, and measured the serum level of FFA, TG, TC, IL-6 and TNFα. Results. The high-fat-diet induced rats had heavier body weight, epididymal and perirenal fat pad weight, and higher level of FFA, TC, TG, IL-6 and TNFα level in serum than the normal control group fed with standard chow. The specific acupoints and non-acupoints acupuncture treatment has the effect of significantly reducing body weight, epididymal and perirenal fat pad weight and serum FFA, TC, TG, IL-6 and TNFα. Compare the specific acupoints and non-acupoints acupuncture treatment, the body weight, and epididymal and perirenal fat pad weight in specific acupoints treatment group lower than the non-acupoints treatment group, however there is no significant difference of serum FFA, TC, TG, IL-6 and TNFα.

Conclusion: The specific acupoints (St 25, St 36, Sp6, P6 and St 40) acupuncture treatment have remarkable effect of anti-obesity and its mechanism may related to down-regulating the concentration of serum FFAs, IL-6 and TNFα.

Keywords: Obesity; Acupuncture; Chronic inflammation

Background

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health [1-3], leading to reduced life expectancy and/or increased health problems, the major adverse effects include diabetes, heart disease, sleep apnea and other problems [4,5]. In fact, some researchers believe that obesity is second only to smoking as a preventable cause of death [6]. The use of western anti-obesity drugs has been limited by side effects including mood changes, suicidal thoughts, and gastrointestinal or cardiovascular complications [7-9]. The effectiveness and safety of acupuncture provide an alternative established therapy for this medical challenge. As early as in the Han dynasty (BC 202-220), Chinese medicine practitioners have reported symptoms, mechanisms and risk factors of obesity and used acupuncture to control body weight. Acupuncture is the stimulation of specific acupuncture points along the skin of the body by thin needles, is one of the most popular types of traditional therapy. The multiple mechanisms of the efficacy of acupuncture have been attributed to the analgesic and anti-inflammatory effects [10,11]. Acupuncture is rapidly gaining recognition as an effective way to manage pain and discomfort [12,13]. There are more studies appearing that acupuncture is an effective way for treating obesity and this therapy is worldwide growing [14-16].

Base from the original theory of Chinese medicine, the key mechanism of obesity are “deficiency in origin and enrichment
in symptoms” and/or “deficiency of spleen Qi and over-strong of stomach Qi”. The patients with obesity gaining weight is because the deficiency of origin leads to the incompletely dispersing the nourishment and the un-thoroughly consuming of the energy which store in the adipocyte, the over much unburden fat brings about the enrichment complication including fatigue, hypertension, hyperglycemia, steatohepatitis. And what’s more, in the traditional Chinese medicine theory, spleen and stomach are not what we usually named in western medicine. Spleen and stomach is the center of the whole body, spleen and stomach Qi play vital important roles in the movement of Qi. The orientation of the spleen qi movement is up and the orientation of stomach qi movement is down. The opposite direction of movement of spleen and stomach Qi balance the whole body function. The spleen qi is to help disperse the nourishment; however the stomach qi is to help receiving food or digesting food. Thus, deficiency of spleen Qi and over-strong of stomach Qi leads to eat too much but cannot expend the energy properly.

Under the direction of this theory, we proposed that restoring the origin and strengthening spleen Qi and harmonizing the stomach Qi will rebalance the movement of Qi and push the body back to health. In previous studies [17-20], we use acupoints zusanli, sanyinjiao, taishu, fenglong and zhongwan. To balance spleen Qi and stomach Qi, which showed a significant reduced body index for rats of obesity or over weight. And considering the obesity is a kind of status of chronic inflammation [3,7,21] we speculate on the role of cytokines in our understanding of the links between anti-inflammatory activity and the effectiveness of acupuncture for obesity treatment and may uncover the acupuncture mechanism for the prevention and treatment of obesity.

Materials and Methods

Ethics Statement

All protocols were approved by the institutional animal care and use committee of Guangxi University of Chinese Medicine.

Animals and diets

50 Adult male (50-60 days) Sprague-Dawley rats (160-200 g) were purchased from Hunan Slack Scene of Laboratory Animal Co. Ltd. (Hunan, China). All animals were housed 5 animals per plastic cages with soft bedding on a 12 h light-dark cycle (light cycle: 08:00 ~ 20:00) and at a constant temperature (22 ± 2°C) and humidity (50 ± 10%). The high fat diet were fed to 40 rats containing COO. Body weights were recorded once a week. After 8 weeks, determined the successfully obesity rats as their body weight excessed the average weight of the rats fed with regular diet. And then, select 30 successful obesity rats from the 40 rats fed with high fat diets. And then, all the 30 obesity rats and 10 control rats were separately administrated with different intervention. After 5 weeks of intervention, all the rats were feed-deprived overnight, sedated by exposure to CO₂ atmosphere, and killed by decapitation. Blood was collected and processed for serum and the pigidymisadipose and perirenal fats were collected.

Experimental groups and the administration

The experiment was designed for four groups: a) control with regular diet and situation (CTL), totally 10 rats. b) Nutritional obesity only (Nu.), totally 10 rats. c) Nutritional obesity following manual acupuncture on the specific acupoints (Nu.A.), totally 10 rats. d) Nutritional obesity following manual acupuncture on non-acupoints (NA-For), totally 10 rats. In the control group, rats were bonded and tied with the same duration as the other groups under the same conditions.

Acupuncture needle and manual acupuncture

All experimental needles were provided by Ruikang Hospital affiliated to Guangxi University of Chinese Medicine (Nanning, China). We used stainless steel needles (0.3 mm in diameter and 30 mm in length, Huatuo Sterile Needles, Suhou, China). The acupoints of zusanli (ST36), Tianshu (St-25), Fenglong (S40), Zhongwan (CV12), Yinlingquan (SP9), and Guanyuan (CV4) were selected to use in the experiments. The Zusanli (ST36) acupoint is located between the tibia and fibula at approximately 5 mm lateral and 5 mm lower to the anterior tubercle of the tibia [23]. Tianshu (St-25), the only bilateral point of this series, is located on a horizontal line 5 Tsun above the symphysis pubis and 2 Tsun lateral to the midline (right and left) [24]. All the acupoints location and the needles applying to these acupoints were shown in Figure 1. A lipid tissue found along the border of the body’s trunk at the thigh or at the abdomen on the ipsilateral side was selected as our non-acupoints. A stainless-steel needle was inserted into the appropriate acpoint by twirling two times clockwise and counter-clockwise at each second. Such twirling continued for 1 min and paused for 5 min for 5 times which took a total of 30 min. St-25, CV12, CV4 acupoints and their related non-acupoint insertion was at a depth of 4 ~ 5 mm, while the ST36, S40, SP9 acupoints and the related non-acupoint depth insertion was 2 ~ 3 mm (Figure 1B). Rats received acupuncture every other day and continuous for constant 35 days, whereas the control rats received only pressures without a needle. All the interference begins at 7 weeks’ diet.

Measurements of serum triglyceride, total cholesterol, IL-6 and TNFα levels

Serum samples from the rats were maintained at -80°C until analysis. Levels of triglyceride, total cholesterol, IL-6 and TNFα were measured using an enzyme-linked immunosorbent assay kit according to the manufacturer’s method (Boster, Wuhan, China).

Statistical analysis

The results presented reflect means ± SD per group. A one way analysis of variance (ANOVA) was used to compare the body weight, BMI, the serum triglyceride, total cholesterol after acupuncture intervene, serum IL-6 and TNFα levels. Less than 0.05 was considered statistically significant (SPSS 14.0, SPSS Inc., Chicago, Illinois, USA).
Serum free fatty acids (FFA), triglyceride (TG), total cholesterol (TC) were measured by enzymatic colorimetric methods using commercial kits from Nanjing JianCheng bioengineering institute (Nanjing, China). Interleukin-6 (IL-6) and TNFα concentration were estimated by ELISA with a commercially available kit from Boster (Wuhan, China). The effect of acupuncture are shown in Figures 4 and 5. The Nutritional obesity group had significantly (p<0.05) high level of FFA, TC and TG relative to the normal control rats. The serum FFA, TC and TG level were affected by the 35 days manual acupuncture on specific acupoints, while the nutritional obesity rats were followed with manual acupuncture on the specific acupoints more significantly reduced serum FFA, TC and TG level compared with nutritional obesity rats followed with manual acupuncture on the non-acupoints.

The serum IL-6 and TNFα showed the same tendency with the changes of FFA, TC and TG. The serum IL-6 and TNFα level of nutritional obesity group had a significant difference compare with normal control group. And the acupuncture on the specific acupoints significantly reduced them. And there is statistical significantly different between Nu.A group and NA-For group (Figure 5).

**Results**

**Body weight were measured every week throughout the experiments**

Body weights were measured every week throughout the experiment. At week 6, the beginning of body weight of group Nu, Nu.A, NA-FOR weighted significantly more (n=8, p<0.01 one way ANOVA) than control rats. At week 7, the differences were further increase and it showed the MAX difference. And during the first 7 weeks, body weight increased steadily in all the four groups (CTL, Nu, Nu.A, NA-FOR). At week 8, rats of group Nu.A and NA-FOR received acupuncture every other day and continuous for constant 35 days, whereas the control rats received only presses without a needle. The body weight of Nu still steadily increased weekly but not as fast as the first 7 weeks (the duration when the rats were feed with diet). The body weight of group Nu.A, NA-FOR begun to decrease and the rats of group Nu.A decrease faster than them in NA-FOR group. And at week 12, the body weight of group Nu.A significantly lower than Nu, NA-FOR and control group (Figure 2).

After the interference, all the rats were exposure to CO₂ atmosphere and were killed by decapitation, the epididymis fat and the perirenal fat were isolated and weighted. The piddidymisadipose mass and the perirenal fat of Nu group significantly higher than control group (P<0.05). The treatment of acupuncture decreased the piddidymisadipose mass and the perirenal fat, and the piddidymisadipose mass and the perirenal fat of Nu.A group were statically significantly lower than them in Nu group (P<0.05). Even the NA-For group, the piddidymisadipose mass and the perirenal fat also significantly lower than them in Nu group (P<0.05) but significantly higher than control group (P<0.05). There were no significantly difference of piddidymisadipose mass and the perirenal fat between Nu.A group and NA-For group (P>0.05) (Figure 3).

Blood was collected by cardiac puncture after the rats were killed under CO₂ anesthesia at the end of the experiments. Serum was obtained by blood centrifugation at 4000 rpm for 10 min at 4°C and immediately stored at -80°C for further analyses. Serum free fatty acids (FFA), triglyceride (TG), total cholesterol (TC) were measured by enzymatic colorimetric methods using commercial kits from Nanjing JianCheng bioengineering institute (Nanjing, China). Interleukin-6 (IL-6) and TNFα concentration were estimated by ELISA with a commercially available kit from Boster (Wuhan, China). The effect of acupuncture are shown in Figures 4 and 5. The Nutritional obesity group had significantly (p<0.05) high level of FFA, TC and TG relative to the normal control rats. The serum FFA, TC and TG level were affected by the 35 days manual acupuncture on specific acupoints, while the nutritional obesity rats were followed with manual acupuncture on the specific acupoints more significantly reduced serum FFA, TC and TG level compared with nutritional obesity rats followed with manual acupuncture on the non-acupoints.

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**Discussion**

In the present study, we observed that the high-fat-diet induced rats had heavier body weight, epididymal and perirenal fat pad weight, and higher level of FFA, TC, IL-6 and TNFα level in serum than the normal control group fed with standard chow. The administration of acupuncture on specific acupoints significantly reduced body weight, epididymal and perirenal fat pad, and serum FFA, TC, IL-6 and TNFα level compared with the Nutritional obesity group fed with high fat diet. The results implicated that acupuncture on specific acupoints could...
significantly reduced body weight, epididymal and perirenal fat pads. In this project, we found that the specific acupoints acupuncture regulating Hypothalamus ob-R mRNA expression [17-20]. In contrast with non-treatment high fat diet induced rats, and these specific acupoints significantly reduce the body weight, Lee's index of high fat diet induced rats compared with ST36, St-25, S40, CV12, SP9, and CV4 significantly reduce the body weight, epididymal and perirenal fat pads. However, the non-acupoints treatment, the specific acupoints base on the traditional Chinese medicine has a better effect on reducing body weight, epididymal and perirenal fat pad weight, serum TG, TC, IL-6 and TNFα level significantly effect on reducing body weight, epididymal and perirenal fat pad weight of obesity rats and the results were consistent with previous results.

One of the greatest factors contributing to the obesity is high fat diet. Fat consumption has been found to be a key player in the obesity epidemic [28-30]. Long-term consumption of the high fat food can lead to obesity and consequently damaging effects on general health. Adipose tissue is an active endocrine organ; adipocytes control whole body energy homeostasis through the storage of triglycerides and release of fatty acids. Long-term high fat food consumption increase the size of the adipocytes and the number of the adipocytes, and then high level of triglyceride (TG) and total cholesterol (TC) will be found in the serum. In the previous experiments [17-20] we examined the serum TG, TC, high density lipoprotein (HDL) and low density lipoprotein (LDL), and found that the high fat diet significantly increase serum TG, TC, LDL and decrease the serum HDL, however the specific acupoints acupuncture treatment reversed the effect and it significantly decrease the serum level of TG, TC, LDL and increased the serum level of HDL. The present results similar with these results. It implicated that the high fat diet successfully induce obesity fat rat model and the specific acupoints acupuncture have the effect of anti-obesity.

In obesity high levels of free fatty acids (FFA) contribute to inflammation. Numerous studies have reported that elevated adiposity is associated with increased plasma pro-inflammatory cytokines [31-33]. The excess storage of TAG from dietary intake results in an excessive influx of free fatty acids into blood circulation. And then, the free fatty acids leads to low-grade inflammation characterized by over production of pro-inflammatory adipocytokines [34,35]. Adipocytes mediate inflammatory response by regulating the release of free fatty acids and adipocytokines, in particular tumor necrosis factor-α (TNFα), interleukin-6 (IL-6). A recent study reported that the consumption of high fat mixed meal increased plasma IL-6 concentration compared with control rats [36]. Previous works conducted a 6-week crossover dietary intervention with reduced energy diets in 15 overweight men and found that Plasma TNFα, IL-6 concentrations did not differ between diets but appreciably reduced compared with baseline. In this study, the similar results were found: the concentration of FFAs, IL-6 and TNFα significantly were up-regulated by high fat diet induced, and the specific acupuncture treatments have the effect of down-regulating the serum FFAs, IL-6 and TNFα concentration.

In the present project, we also compared the effect of specific acupoints and non-acupoint acupuncture treatment. The results showed that the non-acupoints acupuncture treat have significantly effect on reducing body weight, epididymal and perirenal fat pad weight, serum TC, TG, IL-6 and TNFα level compared with Nutritional obesity rats. But compared with non-acupoints treatment, the specific acupoints base on the traditional Chinese medicine has a better effect on reducing body weight, epididymal and perirenal fat pad weight. However, the two different acupoints acupuncture treatment have a similar effect on reducing obesity’s rats’ serum FFA, FTG, TC, IL-6 and TNFα. It looks like that the specific acupoints and non-specific acupoints acupuncture have different mechanism on treatment...
and maybe that’s why the specific acupoints acupuncture have a better effect on treating obesity. It still needs more evidences to proof that.

**Conclusion**

High fat diet provoke inflammatory response in obesity rats however the effect of types of dietary fats remained uncertain. The specific acupoints (St 25, St 36, Sp6, P6 and St 40) acupuncture treatment have remarkable effect of anti-obesity and its mechanism may related to down-regulating the concentration of serum FFAs, IL-6 and TNFα. The specific acupoints and non-acupoints acupuncture which base on the theory of Chinese medicine may have different mechanism on treating obesity. A complete understanding on the role of acupuncture treatment on low grade inflammation merits further investigation. Information is needed based on rigorously well designed clinical studies.
References


