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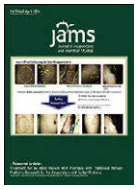
Journal of Acupuncture and Meridian Studies

journal homepage: www.jams-kpi.com



RESEARCH ARTICLE

Electroacupuncture Improves Insulin Resistance by Reducing Neuroprotein Y/Agouti-Related Protein Levels and Inhibiting Expression of Protein Tyrosine Phosphatase 1B in Diet-induced Obese Rats

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Available online 27 November 2015

Received: Sep 9, 2015
Revised: Nov 13, 2015
Accepted: Nov 17, 2015

KEYWORDS
adipocytes;
diet-induced obese rats;
homeostasis model assessment–insulin resistance index;
neuroprotein Y/agouti-related protein;
protein tyrosine

Abstract
Electroacupuncture (EA) has been shown to exert beneficial effects on obesity, but the mechanism is unclear. This study investigated the effects of EA on diet-induced obese (DIO) rats. Fifty male Sprague–Dawley rats were randomly divided into low-fat diet (LFD, 10 rats) and high-fat diet (HFD, 40 rats) groups. After the DIO models had been established, successful model rats were randomly divided into HFD, EA, and orlistat (OLST) groups. The EA group received EA at *Zusanli* (ST36) and *Quchi* (LI11) for 20 minutes once per day for 28 days. The OLST group was treated with orlistat by gavage. The body weight, homeostasis model assessment-insulin resistance index, adipocyte diameters, and neuroprotein Y/agouti-related protein and protein tyrosine phosphatase 1B levels were significantly lower in the EA group than in the HFD group. The rats of the OLST group showed watery stools and yellow hairs whereas those of the EA group had regular stools and sleek coats. The effect of EA on weight loss may be related to improved insulin

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pISSN 2005-2901 eISSN 2093-8152
http://dx.doi.org/10.1016/j.jams.2015.11.037

phosphatase 1B

resistance caused by changes in the adipocyte size and by reductions in the expressions of neuroprotein Y/agouti-related protein and protein tyrosine phosphatase 1B. This study indicates that EA may be a better method of alternative therapy for treating obesity and other metabolic diseases.

1. Introduction

Obesity is a worldwide public health problem and raises the risk of morbidity from hypertension, dyslipidemia, type 2 diabetes mellitus, coronary heart disease, stroke, gallbladder disease, and some cancers [1,2]. Acupuncture originated in China more than 2000 years ago and is one of the oldest medical procedures in the world [3]. Both experimental and clinical studies have shown that acupuncture has beneficial effects in the treatment of obese patients [4,5]. A study showed that electroacupuncture (EA) treatment could inhibit weight gain in rats with diet-induced obesity (DIO) that had been fed a high-fat diet [1]. However, the mechanism of acupuncture in rats with DIO is unclear.

Current data indicate that neuronal insulin signaling is required for both body weight control and glucose homeostasis [6]. A low dose of insulin delivery by intracerebroventricular infusion decreased both food intake and hepatic glucose production [7]. However, many obesity patients present insulin resistance. Insulin resistance arises from the inability of insulin to act normally in regulating nutrient metabolism in peripheral tissues [8].

Adipose tissue is an endocrine organ secreting cytokines and hormones, including leptin, adiponectin, and many other factors, influencing food intake, systemic insulin sensitivity, and nutrient homeostasis [6]. However, obesity from fat expansion disrupts the proper balance of cytokine and hormone generation, promoting insulin resistance [6].

The central nervous system has long been recognized as key in controlling energy homeostasis, and the hypothalamus has been identified as the pivotal structure regulating food intake and energy balance [9]. The arcuate nucleus contains neurons that respond to hormonal and nutrient-related afferent signals that are influenced by the size and the state of adipose tissue stores and by recent food ingestion. Among them are neurons that co-express neuropeptide Y (*NPY*) and agouti-related peptide (*AGRP*), two peptides that potently stimulate food intake, reduce energy expenditure, and, thus, promote weight gain [10]. Receptors for insulin are concentrated in the arcuate nucleus, and available evidence suggests that insulin inhibits *NPY*/*AGRP* neurons [11].

As a negative regulator of the insulin signal transduction cascade, protein tyrosine phosphatase 1B (PTP1B) has been shown to function as a key insulin receptor phosphatase [12]. PTP1B-deficient mice are more sensitive to insulin, have improved glycemic control, and are more resistant to diet-induced obesity than wild mice [13]. These findings indicate that inhibition or downregulation of PTP1B is an effective strategy for improving insulin sensitivity [14].

The purpose of this study was to determine if the mechanism of EA in controlling weight was related to improving insulin sensitivity. This study also investigated whether or not other mechanisms were closely linked to changes in the sizes of adipocytes, reductions in the expressions of *NPY*/*AGRP*, and inhibition of the expression of PTP1B.

2. Materials and methods

2.1. Animals

Fifty rats (Sprague—Dawley, male, age 4 weeks, weighing 100 g) were purchased from Hunan SLAC Laboratory Animal Co., Ltd (Hunan, China) and were housed in the animal center of Hunan University of Chinese Medicine, Hunan, China. They were housed three to four per cage with *ad libitum* access to food and water under controlled conditions: temperature at 20—22°C and a 12-/12-hour light/dark cycle. The rats were kept in the laboratory for a week for habituation. The protocol was approved by the Care and Use of Laboratory Animals Committee of the University. Food for the low-fat diet (D12450B Rodent Diet with 10 kcal % Fat) and for the high-fat diet (D12492 Rodent Diet with 60 kcal% fat; Research Diets Inc., New Brunswick, NJ, USA).

2.2. Experimental protocols

Fifty Sprague—Dawley male rats were randomly divided into a low-fat-diet group (10 rats, LFD) fed with D12450B and a high-fat-diet group (40 rats, HFD) fed with D12492. After the rats had been fed for 5 weeks, 30 rats in the HFD group with body weights that were 20% higher than the average weight of the rats in the LFD were identified as rats with DIO (hereafter referred to as DIO rats). Those 30 rats were then randomly divided into an HFD group, an EA group, and an orlistat (OLST) group, with 10 rats in each group. In order to exclude the influence of diet change, we continued to feed the DIO rats a high-fat diet during the experiment. In the EA group, EA was applied at *Zusanli* (ST36) and *Quchi* (LI11) for 20 minutes (10 Hz, 1.5 mA), with the rats in the conscious state. The treatment was done once per day for 4 weeks. In the OLST group, orlistat was administered via gavage. The rats in the LFD group and the rats remaining in the original HFD group received no treatment. The body weights of all rats were recorded daily.

After 4 weeks, rats that had been deprived of food for 24 hours were intraperitoneally anaesthetized with 10% chloral hydrate (0.35 mL/100-g body weight). Blood samples were collected from the abdominal aorta. Perirenal fat samples were removed and placed into 10% buffered formalin. Each rat's hypothalamus was rapidly removed, immediately frozen using liquid nitrogen, and stored at −80°C in a refrigerator; these were then used to determine the expression of *NPY*/*AGRP* mRNA using real-time

quantitative polymerase chain reaction (RT-qPCR) and the expression of PTP1B by Western blotting.

2.3. Electroacupuncture and gavage

ST-36 is located 5 mm lateral to and below the anterior tubercle of the tibia in rats, and LI11 at the end of the lateral transverse elbow crease. Needles (0.25 mm in diameter; Suzhou Medical Appliance Factory, Jiangsu, China) were inserted to depths of 7 mm at ST36 and 4 mm at LI11 and were then stimulated by using EA with a constant-current square-wave output at 10 Hz and 1.5 mA from an electrical stimulator (SDZ-V; Suzhou Medical Appliance Factory, Jiangsu, China). Orlistat (120 mg/tablet, Zein Pharmaceutical Firms, Chongqing, China) was dissolved in distilled water (3.24 mg/mL). Rats in the OSLT group were given this by gavage 1 mL/d/100-g body weight for 4 weeks.

2.4. Blood parameters

Serum glucose was determined using routine laboratory procedures. Serum insulin was measured using a radioimmunoassay kit (Beijing North Institute of Biological Technology, Beijing, China).

2.5. Adipose tissue

Perirenal fat samples were fixed in 10% buffered formalin, embedded in paraffin, cut into thick sections, and stained with hematoxylin and eosin. The images were captured by using a microscope (BA410; Motic, Xiamen, China). The diameters (μm) of the adipocytes were measured by using Motic Images Advanced 3.2 software at magnification of 400×.

2.6. RT-qPCR

NPY, *AGRP*, and β-actin primers were designed according to Genebank sequences. Primers were as follows: *NPY* forward 5′-GTGTTTGGGCATTCTGGCTG-3′ and *NPY* reverse 5′-GTG GGACAGGCAGACTGGTT-3′, 320 bp; *AGRP* forward 5′-TAGG CAAGGATCAACAAGCAA-3′ and *AGRP* reverse 5′-TAGACCT GAGAACTCTGGGAA-3′, 161 bp; β-actin forward 5′-CCCAT CTATGAGGGTTACGC-3′ and β-actin reverse 5′- TTTAATGT CACGCACGATTTC-3′, 118 bp.

Total RNA was extracted from the whole hypothalamus using TRIZOL (Invitrogen) according to the manufacturer's protocol. Total RNA (5.5 μL), 0.5 μL of oligo dT (0.5 μg/μL) and 6 μL of DEPC water were added into a PCR tube, mixed, centrifuged, and incubated for 5 minutes at 65°C. The reaction contained 2 μL of 5× reaction buffer, 1 μL of dNTPS (10 mM), 0.5 μl of RNase inhibitor, and 0.5 μL of reverse transcriptase. Reaction mixtures were incubated at 42°C for 1 hour and incubated in microtubes at 70°C for 10 minutes. cDNA was stored at −20°C until use.

The real-time PCR reaction system contained 10 μL of 2 × SYBR Green qPCR mix, 1 μL of forward primer (10 μM), 1 μL of reverse primer (10 μM), 1 μL of cDNA, and 7 μL of water. Reaction mixtures were amplified for 35 cycles (predenaturation at 95°C for 3 minutes, denaturation at 95°C for 10 seconds, and annealing at 58°C for 30 seconds).

2.7. Western blotting

Hypothalamus tissues were lysed in 7M urea, 2M thiourea, 60 mM dithiothreitol, 4% CHAPS, 2% pharmalyte 3-10, and 1.4 mg/mL phenylmethanesulfonyl fluoride. The protein samples were separated using sodium dodecyl sulfate—polyacrylamide gel electrophoresis and electrophoretically transferred to polyvinylidene difluoride membranes (380 mA, 1.5 hour), which were blocked with 5% milk in phosphate buffered saline with Tween. Primary rabbit-anti-mouse antibodies (Santa Cruz Biotechnology) were added and incubated overnight at 4°C. The next day, after four washes with phosphate buffered saline with Tween, goat anti-rabbit second antibodies were added and incubated at 37°C for 1 hour. The blots were then developed using the ECL detection kit and produced a chemiluminescence signal that was captured on X-ray film. The protein value presented by using the integrated optical density and glyceraldehyde-3-phosphate dehydrogenase was chosen as an internal control.

2.8. Statistics analysis

All results were expressed as means ± standard deviations and were analyzed by using the SPSS statistical software (version 17.0; SPSS, Chicago, IL, USA). Comparisons between multiple groups were assessed by using the one-way analysis of variance, with the least significant difference test (equal variances assumed) or Dunnett T3 test (equal variances not assumed) being used for *post hoc* tests between groups. Differences were considered statistically significant if $p < 0.05$.

3. Results

Before treatment, the average body weight in the LFD group was significantly lower than the average body weights in the HFD, OLST, and EA groups ($p < 0.05$). However, the differences among the average body weights in the HFD, OLST, and EA groups were not statistically significant ($p > 0.05$). At the end of trial, the average body weight of the HFD group was significantly higher than the average body weights of the OLST and the EA groups ($p < 0.05$). However, the average body weights of the OLST and the EA groups showed no statistically significant difference ($p > 0.05$; Table 1 and Fig. 1).

The glucose (GLU) and the fasting insulin (FINS) levels and the homeostasis model assessment—insulin resistance index (HOMA-IR) were assessed in the four groups. The GLU and the FINS, as well as the HOMA-IR, values were increased in the HFD group compared to the LFD group, but there were no statistically significant difference ($p > 0.05$). The OLST and the EA groups were observed to have significantly lower GLU, FINS, and HOMA-IR values compared to the HFD group ($p < 0.05$), but no significant differences were observed between the OLST and the EA groups ($p > 0.05$; Table 2).

Table 1 Changes in daily body weight in four different groups.

Group	*n*	Body weight premodel establishment (g)	Body weight postmodel establishment (g)	Body weight posttreatment (g)	Change in body weight during treatment (g)
LFD	10	117.8 ± 8.5	344.6 ± 15.5	470.7 ± 19.3†	126.0 ± 8.2†
HFD	10	121.6 ± 7.3	440.5 ± 25.8*	655.4 ± 14.3*	214.9 ± 21.9*
OLST	10	122.0 ± 6.6	413.2 ± 28.3*	540.0 ± 35.0*,†	126.7 ± 21.4†
EA	10	126.7 ± 3.4	411.8 ± 13.7*	514.4 ± 12.0*,†	96.1 ± 11.4*,†

Data are presented as mean ± standard deviation.
EA = electroacupuncture; HFD = high-fat group; LFD = low-fat diet group; OLST = orlistat.
* $p < 0.01$ versus LFD.
† $p < 0.01$ versus HFD.

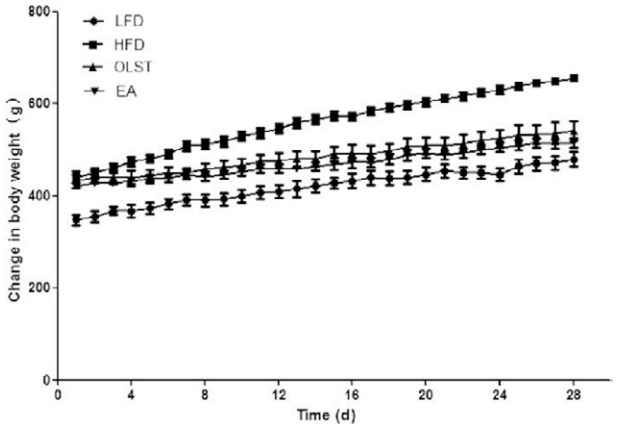

Figure 1 Changes in daily body weight in four different groups. EA = electroacupuncture; HFD = high-fat group; LFD = low-fat diet group; OLST = orlistat.

Table 2 Glucose (GLU) and fasting insulin (FINS) levels and homeostasis model assessment—insulin resistance index (HOMA-IR) in four different groups.

Group	*n*	FINS (μIU/mL)	GLU (mM)	HOMA-IR*
LFD	10	14.68 ± 8.60	11.5 ± 3.11	7.16 ± 5.00
HFD	10	20.14 ± 4.79	13.8 ± 1.25	12.41 ± 3.14
OLST	10	10.26 ± 6.78	11.62 ± 3.81	5.16 ± 3.98‡
EA	10	9.24 ± 8.15‡	9.63 ± 1.31‡	3.49 ± 2.51‡

Data are presented as mean ± standard deviation.
EA = electroacupuncture; HFD = high-fat group; LFD = low-fat diet group; OLST = orlistat.
* HOMA-IR = fasting GLU × FINS/22.5.
‡ $p < 0.05$ versus HFD.

The adipocytes in the HFD group (290.62 ± 48.38 μm) were significantly larger than those in the LFD group (193.98 ± 11.13 μm; $p < 0.05$). After the treatment, the adipocytes in the OLST group (246.82 ± 23.78 μm) and in the EA group (213.62 ± 20.85 μm) were significantly smaller than those in the HFD group ($p < 0.05$). No statistically significantly difference in adipocyte size was observed between the OLST and the EA groups ($p > 0.05$; Fig. 2).

RT-qPCR studies revealed that *NPY*/*AGRP* mRNA expression in the hypothalamus of the HFD group was the highest among the four groups ($p < 0.05$). After the treatment, *NPY* and *AGRP* mRNA expressions of the OLST and the EA groups were significantly lower compared to those in the HFD group ($p < 0.05$) (Table 3). In addition, the PTP1B protein levels in the HFD group (0.72 ± 0.11) were higher than that in the LFD group (0.17 ± 0.06; $p < 0.05$), and the PTP1B protein levels were decreased significantly in the OLST (0.33 ± 0.09) and the EA (0.51 ± 0.09) groups compared to the level in the HFD group ($p < 0.05$; Fig. 3).

During the treatment, the rats in the OLST group showed watery stools and yellow hairs. However, the rats in the EA and the LFD groups had regular stools and sleek coats.

4. Discussion

EA is an acupuncture procedure that uses electrical current stimulation instead of manual manipulations of needles [15]. Because of its reproducibility, EA has been used more frequently in clinical and research settings in recent years [15]. *Yangming* meridians are closely related to *qi* and blood circulation throughout the body and regulate energy metabolism [16]. Acupuncture and EA stimulation of the *Yangming* acupoints *Zusanli* (ST36) and *Quchi* (LI11) have been shown to have beneficial effects in the treatment of obesity [17,18]. Therefore, in our study, ST 36 and LI11 were chosen as acupoints for the treatment of DIO rats. Our findings were consistent with those of earlier studies; i.e., the rate of increase in the body weights of the rats in the EA group was significantly slower than that of the rats in the HFD group.

Insulin resistance, defined as failure of ordinary levels of insulin to trigger its downstream metabolic actions, is closely associated with obesity, type 2 diabetes mellitus (T2DM), metabolic syndrome, and hypertension. Insulin resistance results in impaired insulin action in insulin-sensitive tissues, leading to abnormalities in glucose metabolism and dysfunction in insulin secretion [19]. Insulin resistance-related diseases have become epidemic worldwide, and the number of such cases is predicted to increase in the future, which will threaten human health. Urgent action is needed now to prevent insulin resistance so as to halt the progression of the comorbidities of insulin resistance-related diseases [19]. Several studies have concluded that acupuncture can improve insulin sensitivity [20], as it is effective against metabolic disturbances such as hyperglycemia [21], being overweight [22], and insulin signal defects [23], which are closely associated with the pathogenesis of insulin resistance [19]. Acupuncture at

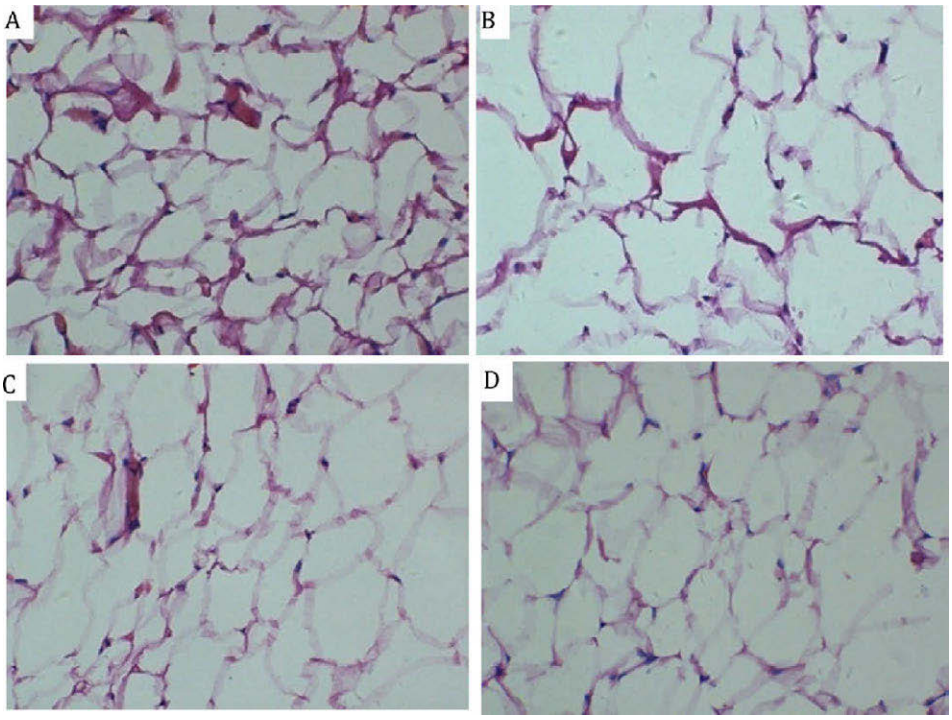

Figure 2 Adipocytes in four different groups (400× magnification). (A) Low-fat diet group, (B) high-fat group, (C) orlistat group, and (D) electroacupuncture group.

combined acupoints has been shown to decrease insulin levels in T2DM and hypertension with hyperinsulinemia [24]. In our experiment, the GLU and the FINS levels, as well as the HOMA-IR, of the HFD group were higher than those of the other three groups, which indicates that rats in the HFD group present insulin resistance. Using EA at the bilateral ST36 and LI11 acupoints, our study also showed that EA could improve insulin resistance by lowering the GLU and the FINS levels in DIO rats. The improvement in the insulin sensitivity seems to be related to changes in fat tissues [6], reductions in the orexigenic peptides in the hypothalamic arcuate nuclei [25], and changes in the insulin signal [12].

Large adipocytes are thought to cause insulin resistance in skeletal muscle and the liver in part by secreting large amounts of tumor necrosis factor-α and free fatty acid [26]. A study showed that an increased population of small white adipocytes might account for improved insulin sensitivity [27]. Consistent with that, in our experiment, the diameters of the adipocytes in the HFD group were significantly bigger than those in the LFD group, which indicated insulin resistance in the HFD group. With EA treatment, the adipocytes in the EA group were significantly smaller than those in the HFD group, and the HOMA-IR in the EA group was significantly smaller than that in the HFD group. Therefore, we suggest that the amelioration of insulin resistance is correlated with the promotion of adipocytes.

NPY and *AGRP*, two peptides that potently stimulate food intake and reduce energy expenditure, and thereby promote weight gain [10], are inhibited by insulin. Under conditions of reduced hypothalamic signaling by insulin, increased *NPY*/*AGRP* signaling may contribute not only to the resultant hyperphagia and weight gain but also to systemic insulin resistance and glucose intolerance as well [28]. Our experiment revealed that *NPY*/*AGRP* mRNA

Table 3 Neuropeptide Y (*NPY*) and agouti-related peptide (*AGRP*) expression in the hypothalamus of rats in four different groups.

Group	*n*	*AGRP*	*NPY*
LFD	10	0.93 ± 0.15	1.05 ± 0.17
HFD	10	2.37 ± 0.56*	2.06 ± 0.21*
OLST	10	0.42 ± 0.11*,†	1.08 ± 0.14†
EA	10	0.96 ± 0.12†	1.08 ± 0.19†

Data are presented as mean ± standard deviation.
EA = electroacupuncture; HFD = high-fat group; LFD = low-fat diet group; OLST = orlistat.
* $p < 0.05$ versus LFD.
† $p < 0.05$ versus HFD.

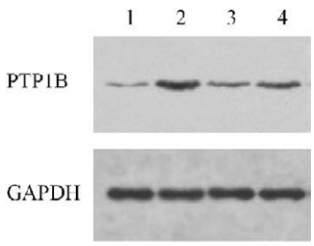

Figure 3 Expression of protein-tyrosine phosphatase 1B (PTP1B) protein and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in four different groups. 1, low-fat diet group; 2, high-fat group, 3 orlistat group; and 4, electroacupuncture group.

expression in the hypothalamus of the HFD group was the highest among the four groups and was accompanied by weight gain and insulin resistance. After the treatment, *NPY/AGRP* mRNA expression in the EA group was decreased significantly compared to that in the HFD group. Thus, EA may improve insulin resistance by inhibiting the expression of *NPY/AGRP*.

The ability of PTP1B to regulate insulin receptor kinase negatively has been established at the molecular level [29]. PTP1B inhibits insulin signaling and, when over-expressed, plays a role in insulin resistance [30]. PTP1B-deficient mice have been shown to be resistant to weight gain and to remain insulin sensitive when subjected to a high-fat diet, even though the amount of food consumed was not different [31]. Ablation of the PTP1B gene yields mice displaying characteristics that suggest that inhibition of the PTP1B function may be an effective strategy for the treatment of diabetes and obesity [13]. In accordance with this, our results showed decreased expression of PTP1B in the EA group. Furthermore, the reduction of PTP1B activity in the rats was accompanied by decreased HOMA-IR, which suggested that EA might increase insulin sensitivity by inhibiting the expression of PTP1B.

In conclusion, EA affects weight loss. Based on the data presented in this study, the mechanism of that weight loss may be related to improved insulin resistance due to changes in the sizes of adipocytes, reduction in the expression of *NPY/AGRP*, and inhibition of the expression of PTP1B. In addition, although the rats administered orlistat also showed weight loss, as did the rats in the EA group, the adverse effects of orlistat, such as watery stools and yellow hairs, cannot be ignored. Thus, compared to orlistat, EA may be considered to be a better method of alternative therapy for obesity and other metabolic diseases.

Disclosure statement

The authors declare that they have no conflicts of interest and no financial interests related to the material of this manuscript.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81373717), the Fund of Open Innovation Platform in Hunan College (No. 12K084), the Fund of National Key Subjects of TCM Diagnostics of Hunan University of Chinese Medicine (2013ZYZD13) and the *225* Training Project of High-level Medical Talents of Hunan Province.

References

[1] Xu J, Chen L, Tang L, Chang L, Liu S, Tan J, et al. Electroacupuncture inhibits weight gain in diet-induced obese rats by activating hypothalamic LKB1-AMPK signaling. *BMC Complement Altern Med*. 2015;15:147.

[2] Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *J Am Coll Cardiol*. 2014;129(25 Suppl 2):S102—S138.

[3] Abou Ismail LA, Ibrahim AA, Abdel-Latif GA, Abd El-Haleem DA, Helmy G, Labib LM, et al. Effect of acupuncture on body weight reduction and inflammatory mediators in Egyptian obese patients. *OA Maced J Med Sci*. 2015;3:85—90.

[4] Lee YC, Li TM, Tzeng CY, Chen YI, Ho WJ, Lin JG, et al. Electroacupuncture at the ST-36 acupoint induces a hypoglycemic effect by stimulating the cholinergic nerve in a rat model of streptozotocine-induced insulin-dependent diabetes mellitus. *Evid-Based Complement Altern Med*. 2011;2011:1—6.

[5] Lee MS, Kim JH, Lim HJ, Shin BC. Effects of abdominal electroacupuncture on parameters related to obesity in obese women: a pilot study. *Complement Ther Clinic Pract*. 2006;12:97—100.

[6] Guo S. Insulin signaling, resistance, and metabolic syndrome: insights from mouse models into disease mechanisms. *J Endocrinol*. 2014;220:T1—23.

[7] Woods SC, Lotter EC, McKay LD, Porte DJ. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature*. 1979;282:503—505.

[8] Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*. 2003;112:1821—1830.

[9] Andreazzi AE, Grassiolli S, Marangon PB, Martins AG, de Oliveira JC, Torrezan R, et al. Impaired sympathoadrenal axis function contributes to enhanced insulin secretion in prediabetic obese rats. *Exp Diabetes Res*. 2011;2011:947917.

[10] Porte D, Baskin DG, Schwartz MW. Insulin signaling in the central nervous system a critical role in metabolic homeostasis and disease from *C. elegans* to humans. *Diabetes*. 2005;54:1264—1276.

[11] Schwartz MW, Woods SC, Porte DJ, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature*. 2000;404:661—671.

[12] Moller DE. New drug targets for type 2 diabetes and the metabolic syndrome. *Nature*. 2001;414:821—827.

[13] Elchebly M, Payett P, Michaliszyn E, Cromlish W, Collins S, Loy AL, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science*. 1999;283:1544—1548.

[14] Sun C, Zhang F, Ge X, Yan T, Chen X, Shi X, et al. SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. *Cell Metabol*. 2007;6:307—319.

[15] Luo D, Liu S, Xie X, Hou X. Electroacupuncture at acupoint ST-36 promotes contractility of distal colon via a cholinergic pathway in conscious rats. *Digest Dis Sci*. 2008;53:689—693.

[16] Zheng YH, Wang XH, Lai MH, Yao H, Liu H, Ma HX. Effectiveness of abdominal acupuncture for patients with obesity-type polycystic ovary syndrome: a randomized controlled trial. *J Altern Complement Med*. 2013;19:740—745.

[17] Ji B, Hu J, Ma S. Effects of electroacupuncture *Zusanli* (ST36) on food intake and expression of POMC and TRPV1 through afferents-medulla pathway in obese prone rats. *Peptides*. 2013;40:188—194.

[18] Cabıoğlu MT, Ergene N. Changes in levels of serum insulin, C-peptide and glucose after electroacupuncture and diet therapy in obese women. *Am J Chin Med*. 2006;34:367—376.

[19] Liang F, Koya D. Acupuncture: is it effective for treatment of insulin resistance? *Diabetes Obes Metab*. 2010;12:555—569.

[20] Chang SL, Lin KJ, Lin RT, Hung PH, Lin JG, Cheng JL. Enhanced insulin sensitivity using electroacupuncture on bilateral *Zusanli* acupoints (ST 36) in rats. *Life Sci*. 2006;79:967—971.

[21] Hu H. A review of treatment of diabetes by acupuncture during the past forty years. *J Tradit Chin Med*. 1995;15:145—154.

[22] Cho SH, Lee JS, Thabane L, Lee J. Acupuncture for obesity: a systematic review and meta-analysis. *Int J Obes*. 2009;33:183—196.
[23] Morino S, Kondo T, Sasaki K, Adachi H, Suico MA, Sekimoto E, et al. Mild electrical stimulation with heat shock ameliorates insulin resistance via enhanced insulin signaling. *PLoS One*. 2008;3:e4068.
[24] Shang W, Cheng H. Advances in TCM research of insulin resistance. *J Trad Chin Med*. 2001;21:141—146.
[25] Tian N, Wang F, Tian DR, Zou Y, Wang SW, Guan LL, et al. Electroacupuncture suppresses expression of gastric ghrelin and hypothalamic NPY in chronic food restricted rats. *Peptides*. 2006;27:2313—2320.
[26] Kubota N, Terauchi Y, Miki H, Tamenoto H, Yamauchi T, Komeda K, et al. PPARγ mediates high-fat diet—induced adipocyte hypertrophy and insulin resistance. *Mol Cell*. 1999;4:597—609.
[27] Okuno A, Tamemoto H, Tobe K, Ueki K, Mori Y, Iwamoto K, et al. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. *J Clin Invest*. 1998;101:1354—1361.
[28] Schwartz MW, Porte D. Diabetes, obesity, and the brain. *Science*. 2005;307:375—379.
[29] Myers MP, Andersen JN, Cheng A, Tremblay ML, Horvath CM, Parisien JP, et al. TYK2 and JAK2 are substrates of protein-tyrosine phosphatase 1B. *J Biol Chem*. 2001;276:47771—47774.
[30] Di Paola R, Frittitta L, Miscio G, Bozzali M, Baratta R, Centra M, et al. A variation in 3′ UTR of hPTP1B increases specific gene expression and associates with insulin resistance. *Am J Hum Genet*. 2002;70:806—812.
[31] Niswender KD, Morrison CD, Clegg DJ, Olson R, Baskin Jr DG, Myers MG, et al. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus a key mediator of insulin-induced anorexia. *Diabetes*. 2003;52:227—231.