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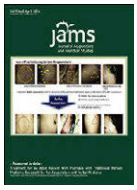

RESEARCH ARTICLE

Effects of Electroacupuncture on Pro-/Anti-inflammatory Adipokines in Serum and Adipose Tissue in Lean and Diet-induced Obese Rats

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Abstract
The effects of electroacupuncture (EA) on pro-/anti-inflammatory cytokines and blood glucose (BG) in lean and obese Long Evans rats were investigated. Group 1 and Group 3 had five lean and seven obese rats, respectively, and received EA at the Zhongwan/Guanyuan acupoints on Day 1, Day 3, Day 5, Day 8, Day 10, and Day 12. Group 2 and Group 4, with five lean and seven obese rats, respectively, did not undergo EA. After induction of anesthesia, BG was measured at 10 minutes and 20 minutes. EA was applied for 30 minutes, and BG was measured again. At the end of the study, blood and white adipose tissue were collected. Analyses showed that for all groups, the mean BG at 20 minutes (baseline) and 50 minutes were significantly greater on Day 1 than on any other day. Compared with Group 2, the baseline BG in Week 1 for Group 1 was significantly lower, but Groups 3 and 4 showed no difference. Group 1 had significantly higher serum interleukin-10 and tumor necrosis factor-α than Group 2, while Group 3's serum leptin was greater than Group 4's. White adipose tissue interleukin-10 and adiponectin:leptin ratio were higher for Group 1 than Group 2. EA affected no significant differences in any other components measured for lean and obese animals.

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1. Introduction

Obesity is a major global health problem and despite considerable efforts by scientists and health professionals, its incidence and prevalence continues to rise in adults and children. According to the World Health Organization global estimates, in 2014 39% of adults aged $\geq$ 18 years were overweight [body mass index (BMI) $\geq$ 25 kg/m^2] and 13% were obese (BMI $\geq$ 30 kg/m^2) [1]. Obesity-related health costs place a huge burden on the healthcare system as many diseases such as hypertension, cardiovascular disease, and type 2 diabetes may develop as a consequence of it [2]. Treatments that are commonly used include dietary restriction, exercise, and bariatric surgery [3]. Anti-obesity drugs have limited efficacy and many side effects including cardiovascular disease [4]. While genetic factors contribute to some individuals becoming obese, the increase in overweight individuals in developing countries as they modernize and become more Western-like, as well as the continued rise in the numbers of obese individuals in developed countries, indicate an important role of environmental factors. The consumption of a high-fat diet (HFD) is considered to be one of the main contributing factors. Obesity is a low-grade chronic inflammatory condition characterized by increased levels of circulating leptin [5], decreased levels of circulating adiponectin [6], and increased numbers of classically activated macrophages in the white adipose tissue (WAT) [7]. In obesity, macrophages and adipocytes in WAT produce many proinflammatory cytokines. Lumeng et al [8] reported that adipose tissue macrophages (ATM) undergo a shift in activation state from an anti-inflammatory M2 polarization state (alternatively activated) in lean animals which produce cytokines like interleukin-10 (IL-10) to a proinflammatory M1 polarization state (classically activated) in diet-induced obesity which produce cytokines like tumor necrosis factor-α (TNF-α).

Electroacupuncture (EA) has been suggested to be an effective therapy for obesity as shown in a study of obese women, where EA treatment two times/wk for 6 weeks at abdominal or lower leg acupoints was more effective at reducing body weight, weight circumference, and BMI than sit-up exercises for the same duration [9]. Cabioglu et al [10] also showed that EA applied to ear and body acupoints resulted in a 4.8% reduction in weight of obese women volunteers compared with a 2.5% reduction in a diet restriction group. HFD-induced obese male Sprague-Dawley rats showed a significant reduction in body weight and food intake with EA applied to hind leg acupoints for 30 minutes, three times/wk for 4 weeks. EA at 2 Hz decreased body weight by 3.3% while those not receiving EA had a 7.5% increase in body weight [11]. EA treatment in obese rats decreased leukocyte infiltration into WAT and decreased proinflammatory cytokines like TNF-α, IL-1, and IL-6 [12].

A recent study has shown a molecular relationship between the HFD-induced obesity in Long Evans (LE) rats and human obesity, and that HFD-induced obesity in this rat strain represents an appropriate obesity model albeit with some limitations [13]. In the present study, the effect of repeated EA at abdominal acupoints on blood glucose, insulin, and various adipokines has been investigated as a way of determining whether the inflammatory state, hyperinsulinemia, and insulin resistance associated with obesity can be alleviated by such treatment.

2. Materials and methods

2.1. Animals

Male LE rats at 3 weeks and 11 weeks of age were obtained from a breeding colony maintained at the Taieri Animal Station and delivered to the Hercus Taieri Research Unit, University of Otago, Dunedin, New Zealand. The rats at 11 weeks of age were fed standard rat chow (Specialty Feeds irradiated rat and mouse diet, 4.8% fat, 20% protein, amino acids, vitamins, and minerals, 3.34 kcal/g; Specialty Feeds, Glen Forest, Western Australia, Australia), housed individually and acclimatized to the new environment for 1 week. These rats were designated to be the lean LE rats. The rats at 3 weeks of age were fed with a HFD (Specialty Feeds SF 03-020, 23% fat, 20% protein, 42% sucrose, amino acids, vitamins, and minerals, 4.78 kcal/g; Specialty Feeds) for 9 weeks immediately following delivery and group housed (5 rats/cage). All the rats were given free access to food and water in a room with 12-hour/12-hour light/dark cycle at a constant temperature. Rats fed with a HFD were weighed on the day immediately prior to the start of the study and those with a weight equal to or greater than the mean weight of the standard rat chow-fed rats plus two standard deviations were chosen for the study and placed in individual cages. These rats were designated to be the obese LE rats. Animals were deprived of food at 3:00 PM on the day prior to the experiment to ensure an overnight fast of at least 17 hours. This study was approved by the University of Otago Animal Ethics Committee.

2.2. Treatment of animals

2.2.1. Anesthesia and EA

At the start of the study, the mean weights (standard deviation, SD) of the lean LE rats and obese LE rats were 399 (27) g and 480 (32) g respectively. Twelve of the 14 obese LE rats had a weight equal or greater than 453 g. The lean and obese LE rats were each divided into two groups. Rats in all four groups were anesthetized with halothane (1%) in a 3:1 mixture of nitrous oxide:oxygen 1.2 L/min. This involved placing the nose of each animal in the nose cone of the anesthetic apparatus. BG was measured with a hand-held glucometer (Accu-Chek Advantage, Roche Diagnostics NZ Ltd., Mt Wellington, Auckland, New Zealand) after needle pricking the lateral saphenous vein of one of the hind limbs at 10 minutes and 20 minutes following insertion of the animal's nose into the nose cone of the anesthetic apparatus. The lean LE rats in Group 1 [n = 5; mean weight (SD) 390 (10) g] and obese LE rats in Group 3 [n = 7; mean weight 472 (4) g] were treated with EA applied at the Zhongwan (CV12) and Guanyuan (CV4) acupoints. The lean LE rats in Group 2 [n = 4; mean weight 410 (20) g] and obese LE rats in Group 4 [n = 7; mean weight 488 (16) g] were not treated with EA and served as controls. EA treatments were given on alternate weekdays giving a total of six applications of EA over 2 weeks. The acupoints were located using the acupoint detector of the EA unit. The

Zhongwan acupoint is 9/14 above the pubic crest of the distance measured between the top of the xiphoid process and the pubic crest, whereas the Guanyuan acupoint is 2/14 of this distance above the pubic crest [14]. The hair at the acupoint sites was removed with electric clippers. Sterile acupuncture needles (Seirin Japan, 0.25 mm, 15 mm) were inserted into the muscle layer at the chosen acupoints to a depth of 4 mm. EA was carried out for 30 minutes at a frequency 10 Hz, pulse width 200 μS, and intensity 10–15 mA (in most cases 10 mA) adjusted to induce weak muscle contractions using the Hans E600 EA unit (Han's Healthronics, Likon, Taipei, Taiwan). The positive and negative charges were connected to the Zhongwan and Guanyuan acupoints respectively. The frequency and intensity of the electrical stimulation were monitored in a previous study [15] with an oscilloscope located at both ends of a resistor (20 Ω) inserted into the circuit. On each day at the completion of treatment, BG was measured again (i.e., at 50 minutes) and the weights of the animals measured.

On completion of the study on Day 12, the rats were given sodium pentobarbitone (60 mg/mL in sterile saline, 0.2 mL intraperitoneal for lean LE and 0.4 mL intraperitoneal for obese LE rats). Blood was collected by cardiac puncture into tubes and WAT was harvested from the pelvic region and frozen immediately in liquid nitrogen. The blood samples were centrifuged (1,500 *g*, 10 minutes) and the sera stored at −20°C and adipose tissue samples stored at −80°C until assays for adiponectin, leptin, TNF-α, IL-10, and insulin were performed.

2.2.2. Measurement of rat BMI

At the completion of treatment on Day 1 and Day 12, the anesthetized animals were photographed lying prone with a ruler placed alongside at the same level as the dorsum of the rat. The length from the base of the tail to the tip of the nose was measured from the photographs using ImageJ processing programme (U.S. National Institutes of Health, Bethesda, MD, USA). The BMI was calculated as body weight/(length of rat)^2 (kg/m^2).

2.3. Homogenization of WAT

There was a large depot of WAT in the pelvic region of the obese LE rats but a much smaller depot in the lean LE rats. Adipose tissue (0.200 g) was homogenized in a radioimmunoprecipitation assay (RIPA) buffer (0.60 mL) using a Qiagen tissue lyser (Program P5, 20 Hz, 2 minutes). The RIPA buffer (0.625% Igepal CA-630, 0.625% sodium deoxycholate, 6.25mM sodium phosphate, and 1mM ethylenediaminetetraacetic acid at pH 7.4) contained 10 μg/mL of protease inhibitor cocktail (Product P8340, Sigma-Aldrich, St. Louis, MO, USA). Homogenates were centrifuged at 4°C (12,000 *g*, 5 minutes). A small opening was made in the fat layer and the infranatants collected and stored at −20°C [16].

2.4. Assay of blood sera and WAT homogenates

The rat blood sera collected at the end of the study were assayed for glucose, insulin, adiponectin, leptin, TNF-α, and IL-10. The WAT homogenates were assayed for adiponectin, leptin, TNF-α, and IL-10. Glucose was measured spectrophotometrically using the hexokinase-glucose-6-phosphate dehydrogenase method (Modular analyzer, Roche Diagnostics NZ Ltd.). Insulin and the four mentioned adipokines were measured using rat enzyme-linked immunosorbent assay kits (Millipore, Abacus ALS, Auckland, New Zealand).

2.5. Statistical analysis

Mean values of BG for Groups 1–4 on Day 1, Day 3, Day 5, Day 8, Day 10, and Day 12 were compared with analysis of variance (ANOVA) and when a significant *p*-value was found, Duncan's post hoc test was performed. BG values were also compared over Week 1 (Day 1, Day 3, and Day 5 combined) and Week 2 (Day 8, Day 10, and Day 12 combined) with unpaired *t*-test. Body weights of rats in each group on Day 12 were compared with those on Day 1 with paired *t* tests. The levels of insulin, glucose, and adipokines in the serum and adipose tissue for the four groups were compared with ANOVA. Significance was taken to be at the level of $p < 0.05$.

3. Results

3.1. Effect of repeated EA on BG

As in a previous study [15], the baseline BG level was taken to be at 20 minutes and the change in BG was measured over a 30-minute period from 20 minutes to 50 minutes following anesthesia. Table 1 shows the mean BG levels at 20 minutes and 50 minutes and the change in BG on the various days of treatment for Groups 1–4. Combined BG measurements at 20 minutes and the change in BG over 30 minutes for Week 1 and Week 2 for Groups 1–4 are presented in Table 2.

3.1.1. For lean LE rats

By ANOVA, for Groups 1 and 2 on Day 1, the mean BG at 20 minutes ($p = 0.004$ and $p = 0.009$, respectively) and at 50 minutes ($p < 0.0005$ and $p < 0.0005$, respectively) were significantly higher than those on all other days. For Group 2, the mean BG at 50 minutes on Day 3 was significantly greater than on Day 8, Day 10, and Day 12. There were no significant differences in the mean change in BG for Groups 1 and 2 ($p = 0.086$ and $p = 0.911$, respectively). For Group 1, there was an increase in the mean BG over 30 minutes on Day 1 and Day 3 but a decrease on all the other days, while for Group 2 there was a decrease in the mean BG over 30 minutes on all the treatment days except Day 3 when no change occurred.

By unpaired *t* test, for Group 1, there was no significant difference in baseline BG for Week 2 compared with Week 1 ($p = 0.09$); however, there was a significant difference in change in BG for Week 2 compared with Week 1 ($p = 0.034$). For Group 2, the baseline BG in Week 2 was significantly lower compared with Week 1 ($p = 0.005$) and there was no significant difference in change in BG for Week 2 compared to Week 1 ($p = 0.84$). For Group 1, the baseline BG in Week 1 was significantly lower than that for Group 2 ($p = 0.039$), but no difference was found in Week 2.

Table 1 Blood glucose measurements (mmol/L) on days of treatment.

Day of study	BG at 20 min	BG at 50 min	Change in BG
Group 1 (Lean Long Evans rats, EA treated)			
1	5.22 (0.21)[a]	5.34 (0.09)[c]	0.12 (0.19)
3	4.34 (0.18)[b]	4.56 (0.12)[d]	0.22 (0.22)
5	4.50 (0.13)[b]	4.30 (0.22)[d]	−0.20 (0.12)
8	4.56 (0.19)[b]	4.24 (0.17)[d]	−0.32 (0.10)
10	4.38 (0.10)[b]	4.12 (0.13)[d]	−0.26 (0.07)
12	4.26 (0.12)[b]	4.16 (0.09)[d]	−0.10 (0.10)
Group 2 (Lean Long Evans rats, control)			
1	5.58 (0.14)[e]	5.38 (0.11)[g]	−0.20 (0.23)
3	4.90 (0.21)[f]	4.90 (0.15)[h]	0 (0.35)
5	4.88 (0.15)[f]	4.65 (0.03)[hi]	−0.23 (0.15)
8	4.43 (0.12)[f]	4.30 (0.07)[i]	−0.13 (0.07)
10	4.38 (0.29)[f]	4.30 (0.09)[i]	−0.08 (0.24)
12	4.78 (0.26)[f]	4.45 (0.22)[i]	−0.33 (0.11)
Group 3 (Obese Long Evans rats, EA treated)			
1	5.73 (0.31)[a]	5.53 (0.36)[c]	−0.20 (0.19)
3	3.90 (0.09)[b]	3.60 (0.13)[d]	−0.30 (0.09)
5	3.94 (0.11)[b]	3.53 (0.09)[d]	−0.41 (0.05)
8	4.01 (0.15)[b]	3.96 (0.15)[de]	−0.06 (0.09)
10	4.10 (0.08)[b]	3.91 (0.14)[de]	−0.19 (0.12)
12	4.41 (0.17)[b]	4.26 (0.16)[e]	−0.16 (0.06)
Group 4 (Obese Long Evans rats, control)			
1	5.83 (0.23)[f]	5.51 (0.30)[i]	−0.31 (0.18)
3	3.87 (0.14)[g]	3.77 (0.05)[jk]	−0.10 (0.11)
5	3.76 (0.13)[g]	3.67 (0.12)[j]	−0.09 (0.06)
8	4.11 (0.17)[gh]	3.96 (0.14)[jkl]	−0.16 (0.12)
10	4.13 (0.15)[gh]	4.26 (0.22)[kl]	0.13 (0.18)
12	4.41 (0.19)[h]	4.44 (0.13)[l]	0.03 (0.18)

Data are presented as mean (SE).
* Data tested separately for EA treated and control groups with analysis of variance and Duncan's test with significance taken to be at the level $p < 0.05$. For each table, values with different superscripts within individual columns are significantly different.
BG = blood glucose; EA = electroacupuncture.

3.1.2. For obese LE rats

By ANOVA, for Groups 3 and 4 the mean BG at 20 minutes and 50 minutes on Day 1 were significantly higher than on all other days (all cases $p < 0.0005$). In Group 3 the mean BG at 50 minutes on Day 12 was significantly higher than on Day 3 and Day 5. In Group 4 the mean BG values at 20 minutes and 50 minutes on Day 12 were significantly higher than on Day 3 and Day 5. There were no significant differences in change in BG for Group 3 and Group 4 ($p = 0.313$ and $p = 0.369$, respectively). For Group 3, there was a decrease in the mean BG over 30 minutes on all the treatment days, whereas for Group 4 there was a decrease in the mean BG over 30 minutes on all the treatment days except Day 10 and Day 12 when an increase was observed. A statistically significant difference was found in mean change in BG for Group 3 compared with Group 4 on Day 5 (end of Week 1; unpaired t test, $p = 0.0012$).

By unpaired t test, for Groups 3 and 4 there were no significant differences in mean baseline BG or change in BG for Week 2 compared to Week 1, and no significant differences between these two groups ($p > 0.05$).

Table 3 Body weight measurements (g) for Groups 1−4.

Day of Study	Lean Long Evans rats		Obese Long Evans rats	
	Group 1	Group 2	Group 3	Group 4
1	390 (4.5)	410 (19.6)	472 (4.3)	488 (16.4)
12	382 (3.7)*	395 (15.5)	444 (5.5)*	453 (15.0)*

Data are presented as mean (SE). Data tested separately for each group with paired t test with significance taken to be at the level $p < 0.05$.
* Denotes significantly different from Day 1 with paired t test ($p < 0.05$).

3.2. Effect of repeated EA on body weight

Table 3 shows the mean body weight measurements on Day 1 and Day 12 for Groups 1−4.

3.2.1. For lean LE rats

For Group 1 the mean body weight on Day 12 was significantly lower than on Day 1, but no significant change occurred for Group 2. The mean BMI on Day 8 was 7.35 (0.21) kg/m^2 ($n = 2$, with 1 rat from Group 1 and 1 rat from Group 2).

Table 2 Blood glucose measurements (mmol/L) over Week 1 and Week 2.

	Wk 1		Wk 2	
	Baseline (BG at 20 min)	Change in BG over 30 min	Baseline (BG at 20 min)	Change in BG over 30 min
Lean Long Evans rats				
Group 1	4.69 (0.14)*	0.05 (0.11)	4.40 (0.08)	−0.23 (0.06)†
Group 2	5.12 (0.13)	−0.14 (0.14)	4.53 (0.13)†	−0.18 (0.09)
Obese Long Evans rats				
Group 3	4.52 (0.22)	−0.30 (0.07)	4.18 (0.09)	−0.13 (0.05)
Group 4	4.49 (0.23)	−0.17 (0.07)	4.22 (0.10)	0 (0.09)

Data are presented as mean (SE).
* Denotes significantly different from Group 2 by unpaired t test ($p < 0.05$).
† Denotes significantly different from Week 1 by unpaired t test ($p < 0.05$).

3.2.2. For obese LE rats

For both Groups 3 and 4 the mean body weight on Day 12 was significantly lower than on Day 1. The mean BMI on Day 1 for Groups 3 and 4 was 8.80 (0.21) kg/m^2 and 8.79 (0.10) kg/m^2, respectively, while on Day 12 for Groups 3 and 4 it was 8.00 (0.19) kg/m^2 and 7.70 (0.15) kg/m^2, respectively. By paired *t* test, there was a significant decrease in BMI for both these groups on Day 12 compared with Day 1 ($p < 0.05$).

3.3. Effect of repeated EA on adipokines, insulin, and glucose in blood sera

The mean values for adiponectin, leptin, IL-10, TNF-α, insulin, and glucose in the blood sera collected at the end of the study on Day 12 are presented in Table 4. The mean adiponectin levels were significantly higher for Groups 1 and 2 of lean LE rats than Groups 3 and 4 of obese LE rats. The opposite was found for mean leptin levels which were significantly lower for Groups 1 and 2 compared with Groups 3 and 4. As a consequence, the mean adiponectin:leptin ratio was significantly greater for Groups 1 and 2 compared with Groups 3 and 4.

In addition, the mean IL-10 levels were significantly higher for Groups 1 and 2 compared with Groups 3 and 4. The mean TNF-α level was significantly higher for Group 1 than the other three groups which did not differ from each other. The mean IL-10:TNF-α ratio was significantly greater for Groups 1 and 2 compared with Groups 3 and 4.

The mean insulin level for Group 1 was significantly lower than Groups 3 and 4. There were no significant differences in mean glucose levels between the four groups.

3.3.1. For lean LE rats

Group 1 had significantly higher mean IL-10 and TNF-α levels than Group 2, while the means of all the other biochemical components measured were not significantly different.

3.3.2. For obese LE rats

Group 3 had a significantly greater mean leptin level than Group 4, while the means of all the other biochemical components measured were not significantly different.

3.4. Effect of repeated EA on adipokines in WAT

The mean values for adiponectin, leptin, IL-10, and TNF-α in the WAT collected at the end of the study on Day 12 are given in Table 5. While there were no significant differences in mean adiponectin level between Groups 1–4, the mean leptin level was significantly lower for Group 1 of LE rats compared with Group 3 of obese LE rats. As a consequence, the mean adiponectin:leptin ratio was significantly greater for Group 1 compared with Group 3. There were no differences in mean leptin or adiponectin:leptin ratio between Group 2 of lean LE rats compared to Group 4 of obese LE rats.

The mean IL-10 and TNF-α levels for Groups 1 and 2 were significantly higher than for Groups 3 and 4, but there were no significant differences in mean IL-10:TNF-α ratio between the four groups.

3.4.1. For lean LE rats

The mean adiponectin:leptin ratio and IL-10 level were significantly increased for Group 1 compared with Group 2.

3.4.2. For obese LE rats

No significant differences were found between Groups 3 and 4 for all of the biochemical components measured.

4. Discussion

Obesity is a low-grade chronic inflammatory disease characterized by an excess accumulation of visceral fat especially WAT. Increased WAT mass is known to produce and secrete a wide range of proinflammatory mediators including TNF-α and IL-6, resulting in an imbalance in the production of anti-/pro-inflammatory cytokines [17,18]. Recent studies indicate that local proliferation of adipose tissue macrophages, in addition to macrophage infiltration from the bloodstream, occurs in the WAT [7,8,19]. The adipose tissue macrophages may thus be a major source of proinflammatory cytokines which may have local effects on the WAT physiology besides exerting systemic effects on other organs. Most of the obesity-related metabolic disturbances are reversible with weight loss [20] and this is associated with a reduction in the macrophage infiltration

Table 4 Measurements of adiponectin, leptin, interleukin-10, tumor necrosis factor-α, insulin, and glucose in blood sera.

	Lean Long Evans rats		Obese Long Evans rats	
	Group 1 EA	Group 2	Group 3 EA	Group 4
Adiponectin (μg/mL)	40.03 (3.80)^a	37.48 (8.17)^a	25.99 (1.39)^b	22.74 (0.78)^b
Leptin (ng/mL)	1.39 (0.34)^c	1.64 (0.19)^c	5.62 (0.70)^d	3.85 (0.27)^e
Adiponectin:leptin (×10^3)*	35.36 (6.76)^f	24.14 (6.49)^f	4.72 (0.41)^g	6.01 (0.31)^g
Interleukin-10 (pg/mL)	116.50 (20.91)^h	69.25 (10.45)^j	27.86 (3.15)^k	23.90 (4.14)^k
Tumor necrosis factor-α (pg/mL)	6.37 (0.73)^l	4.18 (1.05)^m	3.42 (0.54)^m	3.26 (0.47)^m
Interleukin-10:tumor necrosis factor-α†	19.70 (5.57)^n	20.93 (6.51)^n	9.01 (1.43)^p	6.93 (0.89)^p
Insulin (ng/mL)	0.94 (0.47)^q	2.12 (0.61)^{qr}	3.13 (0.50)^r	2.87 (0.42)^r
Glucose (mmol/L)	6.44 (0.33)	7.43 (0.35)	6.60 (0.46)	7.08 (0.27)

Data are presented as mean (SE); tested with analysis of variance.
Values with different superscripts across a row are significantly different ($p < 0.05$).
* Adiponectin:leptin ratio determined from levels expressed in ng/mL.
† Interleukin-10:Tumor necrosis factor-α ratio determined from levels expressed in pg/mL.

Table 5 Measurements of adiponectin, leptin, interleukin-10, and tumor necrosis factor-α in white adipose tissue.

	Lean Long Evans rats		Obese Long Evans rats	
	Group 1 EA	Group 2	Group 3 EA	Group 4
Adiponectin (μg/mL)	23.07 (1.45)	23.24 (1.42)	20.19 (0.84)	20.02 (0.78)
Leptin (ng/mL)	4.50 (0.85)[a]	5.65 (0.34)[ab]	7.65 (0.67)[b]	6.16 (0.55)[ab]
Adiponectin:leptin ($\times 10^3$)*	5.87 (1.07)[c]	4.15 (0.31)[d]	2.79 (0.31)[d]	3.39 (0.28)[d]
Interleukin-10 (pg/mL)	1,636.3 (211.0)[e]	1,244.0 (156.6)[f]	597.9 (34.0)[g]	580.9 (33.9)[g]
Tumor necrosis factor-α (pg/mL)	36.43 (5.69)[h]	28.93 (3.24)[h]	17.60 (0.85)[j]	16.20 (1.01)[j]
Interleukin-10:tumor necrosis factor-α†	46.55 (4.22)	43.13 (3.52)	34.56 (2.83)	37.01 (3.66)

Means (SE) tested with ANOVA.
Values with different superscripts across a row are significantly different ($p < 0.05$).
* Adiponectin:leptin ratio was determined from levels expressed in ng/mL.
† Interleukin-10:Tumor necrosis factor-α ratio determined from levels expressed in pg/mL. White adipose tissue, 0.2 g, homogenized in 0.6 mL radioimmunoprecipitation assay buffer with protease inhibitor, centrifuged, and infranatant assayed.

of WAT and an improvement in the inflammatory profile of gene expression. Factors derived from adipocytes and adipose tissue macrophages may also play a role in the pathogenesis of insulin resistance characteristic of obesity. Many proinflammatory adipokines such as leptin, TNF-α, and IL-6 are overproduced during obesity [21]. Expression and plasma levels of adiponectin, an anti-inflammatory and insulin-sensitizing cytokine derived from adipocytes, are also downregulated in obese states [6].

The present study was designed to test whether repeated EA application would lead to a change in the imbalance of pro- and anti-inflammatory cytokines produced by WAT and reduce the body weight in a HFD-induced rat model of obesity. As anti-obesity treatments or medications should ideally not increase BG levels, otherwise there would be a tendency towards diabetes which often coexists with obesity, the effect of repeated EA on BG was also examined. A previous study had shown that repeated EA in obese women was an effective treatment for obesity and can decrease serum BG by increasing insulin secretion [22].

In the present study, the effects of repeated EA on BG and biochemical components in blood serum and WAT of lean and HFD-induced obese LE rats have been examined. The EA treatment procedure in previous studies with obese Zucker Diabetic Fatty rats was used as it had been found to be effective in reducing BG. It was also successful in reducing serum leptin and increasing adiponectin in WAT of obese Zucker Diabetic Fatty rats 12—15 weeks [23]. EA treatment was applied 3 times/wk for 2 weeks using Zhongwan (CV12) and Guanyuan (CV4) acupoints [15,23,24].

For the lean rats, repeated EA treatment using the above mentioned abdominal acupoints decreased the baseline BG in Week 1 compared with the controls and a decrease in change in BG over 30 minutes occurred in Week 2 compared with Week 1. For the obese rats there were no significant alterations in baseline BG or change in BG over 30 minutes in Week 2 compared with Week 1. The assays of the blood sera of the lean and HFD-induced obese rats confirmed that leptin was increased while adiponectin and IL-10 were decreased in obesity. EA treatment for the lean rats increased IL-10 and TNF-α levels, while for the obese rats it increased the leptin level. The adiponectin:leptin ratio and IL-10:TNF-α ratio were greater for the lean rats than the obese rats, while EA treatment of the obese rats did not alter either of these ratios.

Lumeng et al [8] identified a population of macrophages in the adipose tissue of obese mice that was not present in lean mice. ATMs from lean mice expressed many genes characteristic of M2 or "alternatively activated" macrophages, including arginase-1 and IL-10. HFD-induced obesity decreased expression of these genes in ATMs, while increasing the expression of genes such as those encoding TNF-α and induced nitric oxide synthase that are characteristic of M1 or "classically activated" macrophages. The anti-inflammatory cytokine IL-10, which was overexpressed in ATMs from lean mice, protected adipocytes from TNF-α-induced insulin resistance. Thus, diet-induced obesity leads to a shift in the activation state of ATMs from a M2 polarization state in lean animals to a M1 polarization state that contributes to insulin resistance [8,19]. A study by Wen and Lee [12] showed that EA 10 Hz 20 min/d given 7 d/wk for 1 week at Zusanli (ST36) acupoints in HFD-induced obese rats decreased body weight and ATM infiltration. Adipose tissue inflammatory responses indicated by TNF-α, IL-6, monocyte chemotactic protein-1 were reduced by EA in obese rats, and serum levels of TNF-α, IL-6, and IL-1 were also lowered. The data from the present study using EA 10 Hz 30 min/d given 3 d/wk for 2 weeks at Zhongwan (CV12) and Guanyuan (CV4) acupoints would suggest that EA did not reduce adipose tissue inflammatory responses or ATM activation state in HFD-induced obese LE rats as TNF-α and leptin levels were not significantly changed compared with non-EA treated obese rats, and in addition adiponectin and IL-10 levels in adipose tissue were unaltered by EA. In fact there was a trend towards EA increasing leptin level in adipose tissue of obese rats and would need to be checked using a larger number of animals. This differed from the lean animals where EA treatment increased adiponectin:leptin ratio and IL-10 levels in adipose tissue.

The increase in serum leptin level by EA in HFD-induced obese rats differs from other studies in rodents and humans where EA caused a lowering in circulating leptin level [25—27]. It is possible that this difference may be due to these animals not fully developing resistance to leptin at this time. The animals had been fed the HFD at 3 weeks of age for a short period of 9 weeks, and the levels of

circulating leptin were much lower than found in other studies [28,29]. Research studies have shown that EA has a bidirectional adjustment of circulating leptin levels. Within a short period of time before rats develop resistance to leptin, EA can increase leptin and its receptor levels [30].

The present study showed that EA increased the serum IL-10 level as well as the adiponectin:leptin ratio and IL-10 level in adipose tissue of lean rats. The latter may be responsible for the raised serum IL-10 level of these animals. It also suggests that in the EA-treated lean rats the increased serum IL-10 level is correlated with a lower serum insulin level. For the EA-treated lean rats, none of the four values for serum insulin were $>$ 2.4 ng/mL, whereas for the control rats three of the four values for serum insulin were $>$ 2.4 ng/mL (2×2 Chi-square test, $p < 0.05$). There is evidence in literature of IL-10 having an insulin-sensitizing effect [31—33] and this could be responsible for the serum glucose level of the EA-treated lean rats not being significantly different from the controls. For the obese rats, EA did not alter the serum insulin level compared to controls. It is likely that the raised level of serum leptin in these animals indicates a tendency towards developing leptin resistance and therefore a decrease in leptin-mediated improvement of insulin sensitivity [34].

The body weights of the HFD-induced obese rats decreased during the study period and there was no difference between EA-treated and control obese rats. Using another group of HFD-obese LE rats ($n = 8$), it was found that after overnight fasting for at least 17 hours on alternate days over 2 weeks and a brief exposure of 5 minutes to 1% halothane in 3:1 nitrous oxide:oxygen, the body weights of these rats increased over the 2 week period. It therefore appears that exposure to the anesthetic gas for longer periods (50 minutes) may be responsible for the decrease in body weight of EA-treated and control animals. Inhaled anesthetic gases, particularly nitrous oxide, are reported to suppress appetite [35]. In future studies, to test the effect of repeated EA on body weight of HFD-induced obese rats it may be necessary to use conscious animals that have been trained to be restrained inside a plastic cylinder. A previous study used EA 10 Hz, 2 mA for 30 minutes at bilateral Zusanli and Neiting acupoints three times/wk for 4 weeks in conscious male HFD-induced obese Sprague-Dawley rats aged 16 weeks restrained in a plastic holder [25]. There was a significant decrease in body weight compared with the control group fed the HFD and restrained for 30 minutes without EA stimulation.

Disclosure statement

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

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