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# **RESEARCH ARTICLE**

# Differential Effect of Electroacupuncture on Inflammatory Adipokines in Two Rat Models of Obesity



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#### KEYWORDS

adipokines; blood glucose; electroacupuncture; obesity; rat models

#### Abstract

Chronic inflammation is known to be associated with visceral obesity and insulin resistance which are characterized by altered levels of production of pro- and antiinflammatory adipokines. The dysregulation of the production of inflammatory adipokines and their functions in obese individuals leads to a state of chronic low-grade inflammation and may promote obesity-linked metabolic disorders and cardiovascular diseases such as insulin resistance, metabolic syndrome, and atherosclerosis. Electroacupuncture (EA) was tested to see if there was a difference in its effect on pro- and anti-inflammatory adipokine levels in the blood serum and the white adipose tissue of obese Zucker fatty rats and high-fat diet-induced obese Long Evans rats. In the two rat models of obesity, on Day 12 of treatment, repeated applications of EA were seen to have had a significant differential effect for serum tumor necrosis factor- $\alpha$ , adiponectin, the adiponectin:leptin ratio, and blood glucose. For the adipose tissue, there was a differential effect for adiponectin that was on the borderline of significance. To explore these changes further and how they might affect insulin resistance would require a modification to the research design to use larger group sizes for the two models or to give a greater number of EA treatments.

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### 1. Introduction

Obesity is a major public health concern and is associated with an increased risk of chronic diseases including type 2 diabetes, cardiovascular disease, cancer, decreased health-related quality of life and overall life expectancy, and increased health care costs [1]. It results from a complex interplay of many genetic and environmental factors.

Several studies have shown that the detrimental influence of abdominal obesity on metabolic processes is mediated by the intra-abdominal fat depot [2,3]. The visceral fat depot correlated with glucose intolerance in the presence of hyperinsulinemia during an oral glucose tolerance test, suggesting an insulin-resistant state in human obesity [4]. Insulin resistance and hyperinsulinemia are often associated with obesity, and the ability of obesity to engender insulin resistance is the major basis for the association of obesity with type 2 diabetes mellitus.

The accumulation of fat mass during the development of obesity is characterized by adipocyte hyperplasia and hypertrophy. The prevalence of hypertrophied adipocytes in adipose tissue results in a reduction in blood flow with subsequent hypoxia and macrophage infiltration, and increased production of proinflammatory adipokines [3-7]. Many of the proinflammatory cytokines are secreted by the adipocytes, whereas others are predominantly derived from adipose tissue-infiltrated macrophages. The dysregulation of pro- and anti-inflammatory adipokine production and their functions in obese individuals leads to a state of chronic low-grade inflammation and may promote obesity-linked metabolic disorders and cardiovascular diseases such as insulin resistance, metabolic syndrome, and atherosclerosis [8]. Obesityassociated inflammation may impair insulin action systemically through increasing free fatty acids (FFA) or proinflammatory adipokines [e.g., leptin, tumor necrosis factor- $\alpha$  $(TNF-\alpha)$ ] in circulation as both are able to target insulin receptor substrate proteins and modify the metabolic and mitotic effects of insulin leading to insulin resistance [9-13].

This paper examines whether the effect of electroacupuncture (EA) is different in two rat models of obesity by comparing the levels of pro- and anti-inflammatory adipokines in the two models with and without EA treatment. The two models used were the obese Zucker fatty rats and diet-induced obese Long Evans rats. Obesity develops in the former as a result of a genetic mutation in the leptin receptor, while in the latter it develops as a result of high dietary fat intake. The obese Zucker fatty rat has been used as a model of metabolic syndrome and develops hyperphagia, dyslipidemia, hyperleptinemia, hyperinsulinemia, central adiposity, hypertension, insulin resistance, and diabetes [14,15], all being risk factors involved in metabolic syndrome [16]. Homeostasis model assessment of insulin resistance was significantly greater for obese Zucker fatty rats than lean Zucker fatty rats [17]. Obesity induced in Long Evans rats by a high-fat diet (HFD) was reported to be associated with hyperleptinemia and insulin resistance [18,19]. Li et al [20] have shown that HFDinduced obese Long Evans rats at 25 weeks of age had significantly higher fasting plasma total cholesterol, triglyceride, insulin, leptin, and adiponectin levels when compared with lean animals of that age. Fasting plasma glucose was not significantly different to the lean animals. In oral glucose tolerance test at 21 weeks of age, both glucose and insulin levels were significantly higher after overnight fasting and at every time point after the oral glucose challenge in the diet-induced obese rats indicating impaired insulin sensitivity. There is increasing clinical evidence for the effectiveness of acupuncture as a treatment of insulin resistance [21], and possible changes in the circulating levels of pro- and anti-inflammatory adipokines by EA intervention could lead to a decrease in insulin resistance by affecting insulin sensitivity.

### 2. Materials and methods

#### 2.1. Animals

Male Zucker fa/fa rats 12–14 weeks of age and male Long Evans rats at 3 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 obese Zucker rats were fed standard rat chow (Specialty Feeds irradiated rat and mouse cubes, 4.8% fat, 20% protein, amino acids, vitamins and minerals, 3.34 kcal/g; Specialty Feeds, Glen Forest, Western Australia), housed in individual cages and acclimatized to the new environment for 1 week. The justweaned Long Evans rats 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. The HFD-fed Long Evans rats 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 plus two standard deviations of normal rat chow-fed male Long Evans rats of the same age were selected for the study and placed in individual cages. These rats were designated as obese Long Evans rats. All 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

This involved lightly anesthetizing the animals with 1% halothane in a 3:1 mixture of nitrous oxide:oxygen 1.2 L/ min. Blood glucose (BG) was measured with a hand-held glucometer (Accu-Chek Advantage, Roche, 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 anesthesia. The obese Zucker rats in Group 1 (n = 5) were treated with EA applied at the Zhongwan (conception vessel; CV12) and Guanyuan (CV4) acupoints on alternate weekdays (Monday/Wednesday/Friday) over 2 weeks giving a total of six applications of EA; those in Group 2 (n = 7) were not treated with EA and served as controls [22]. Similarly the obese Long Evans rats in Group 3 (n = 7) were

treated with EA applied at the *Zhongwan* (CV12) and *Guanyuan* (CV4) acupoints on alternate weekdays (Monday/Wednesday/Friday) over 2 weeks, while those in Group 4 (n = 7) served as controls [23]. Controls were handled in the same way as EA-treated animals.

Sterile acupuncture needles (Seirin Corporation, Shizuoka, Japan; 0.25 mm  $\times$  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  $\mu$ S, and intensity 10–15 mA using Hans E600 EA unit (Han's Healthronics, Likon, Taipei, Taiwan). The positive and negative charges were connected to the *Zhongwan* and *Guanyuan* acupoints, respectively. On each day at the completion of treatment, BG was measured again (i.e., at 50 minutes) and the weights of the animals measured.

**2.2.2. Collection of blood serum and white adipose tissue** At the completion of the study on Day 12, the rats were given sodium pentobarbitone (60 mg/mL in sterile saline, 0.4 mL intraperitoneal injection). Blood was collected by cardiac puncture into tubes and white adipose tissue harvested from the pelvic region and frozen immediately in liquid nitrogen. The blood samples were centrifuged (1,500g for 10 minutes) and the sera stored at  $-20^{\circ}$ C and adipose tissue samples stored at  $-80^{\circ}$ C until assays for adiponectin, leptin, TNF- $\alpha$ , interleukin-10 (IL-10), and insulin were performed.

#### 2.3. Homogenization of white adipose tissue

Adipose tissue (0.200 g) was homogenized in radioimmunoprecipitation assay (RIPA) buffer (0.60 mL) using a Qiagen tissue lyser II (Venlo, Netherlands; Program P5, 20 Hz, 2 minutes). The radioimmunoprecipitation assay buffer (0.625% Igepal CA-630, 0.625% sodium deoxycholate, 6.25mM sodium phosphate, and 1mM EDTA at pH 7.4) contained 10  $\mu$ g/mL of protease inhibitor cocktail (Product P8340, Sigma Aldrich, St. Louis, MO, USA). Homogenates were centrifuged at 4°C (12,000g for 5 minutes). An opening was made in the fat layer and the infranatants were collected and stored at  $-20^{\circ}$ C [24].

# 2.4. Outcomes measured in blood sera and adipose tissue homogenates

The rat blood sera collected at the end of the study were assayed for insulin, adiponectin, leptin, TNF- $\alpha$ , and IL-10. The white adipose tissue homogenates were assayed for adiponectin, leptin, TNF- $\alpha$ , and IL-10. Insulin and the four mentioned adipokines were measured using rat enzyme-linked immunosorbent assay kits (Millipore, Abacus ALS, Auckland, New Zealand). The product numbers for the Millipore enzyme-linked immunosorbent assay kits were EZRADP-62K (rat adiponectin), EZRL-83K (rat leptin), IBBE45311 (IBL rIL-10), EZRTNFA (rat TNF- $\alpha$ ), and EZRMI-13K (rat/mouse insulin). The assays were performed by New Zealand Veterinary Pathology, Palmerston North, New Zealand.

### 2.5. Statistical analysis

Means and standard deviations of BG at 20 minutes and change in BG over a 30 minute interval for Groups 1-4 on

Day 1, Day 3, Day 5, Day 8, Day 10, and Day 12 were compared using analysis of variance and when a significant p value was found, Duncan's *post hoc* test was performed. Significance was taken to be at the level of p < 0.05.

Descriptive statistics (means and standard deviations) were presented for each outcome measured in the blood serum and adipose tissue by animal type and EA intervention combination. For each outcome, a regression model was used to allow modeling a two-way factorial design with interaction. Where there was evidence that model assumptions were not satisfied (including evidence for nonnormality from inspecting residual histograms and conducting formal tests of skew and kurtosis and evidence for heteroscedasticity from Levene's test for equality of variance), robust standard errors (from Huber-White sandwich estimators) were used. This was the case for all outcomes aside from IL-10 and TNF- $\alpha$  for the blood sera. Overall differences between all four groups were assessed, with pairwise differences presented where the overall test was statistically significant, along with a test for an interaction between the intervention and animal type. Stata 13.1 (StataCorp LP, College Station, TX, USA) was used for all analyses with two-sided p < 0.05 considered statistically significant in each case.

The "overall difference" tests the four values for a measured outcome and if a p value was < 0.05 then there was a significant difference between at least two of the values. With the exception of one outcome all the p values were significant for the sera, and also for the adipose tissue, and justified testing for a differential effect of EA.

The "*p* value for interaction" tests the difference between the differences of EA and control values for the two models. If a *p* value was < 0.05 then there was a differential effect of EA between the two models. Even with the small group sizes used, the statistical testing indicated that the effect of EA was different between the two models for three of the measured adipokine outcomes in the sera.

#### 3. Results

#### 3.1. For control (i.e., untreated) animals

Mean values of BG at 20 minutes for Group 2 of obese Zucker rats were significantly greater than for Group 4 of obese Long Evans rats on Day 1, Day 3, Day 5, Day 8, Day 10, and Day 12 (Table 1). There was no significant difference in mean change in BG over a 30-minute interval between Groups 2 and 4 on Day 1, Day 3, Day 5, Day 8, Day 10, and Day 12 (Table 2). The BG values at 20 minutes for obese Long Evans rats in Group 4 did not differ significantly from those of lean Long Evans rats except on Day 3 and Day 5 when a lower value was found for the obese Long Evans rats [18], thus confirming that the obese Long Evans rats were not hyperglycemic. The highest BG value of 7.96 mmol/L for obese Zucker rats in Group 2 occurred on Day 1 and indicated that these rats were also not hyperglycemic.

For the assays of blood sera on Day 12, the mean levels of leptin, TNF- $\alpha$ , and insulin were significantly greater for Group 2 than Group 4. There were no significant differences in mean levels of adiponectin and IL-10 between Groups 2 and 4. The mean adiponectin:leptin ratio and IL-10:TNF- $\alpha$ 

Table 1	Baseline blood glucose	(mmol/L) at 20 minutes.

	Obese Zucker rats Group 1 EA Group 2		Obese Long Evans rats		
			Group 3 EA	Group 4	
D 1	7.92 (1.19) <sup>a</sup>	7.96 (1.44) <sup>a</sup>	5.73 (0.81) <sup>b</sup>	5.83 (0.60) <sup>b</sup>	
D 3	5.42 (0.91) <sup>c</sup>	5.74 (1.36) <sup>c</sup>	3.90 (0.24) <sup>d</sup>	3.87 (0.37) <sup>d</sup>	
D 5	4.94 (0.47) <sup>e</sup>	4.84 (1.22) <sup>e</sup>	3.94 (0.29) <sup>f</sup>	3.76 (0.35) <sup>f</sup>	
D 8	6.08 (0.78) <sup>g</sup>	5.10 (0.84) <sup>h</sup>	4.01 (0.40) <sup>j</sup>	4.11 (0.44) <sup>j</sup>	
D 10	7.04 (1.16) <sup>k</sup>	5.84 (0.83) <sup>l</sup>	4.10 (0.22) <sup>m</sup>	4.13 (0.39) <sup>m</sup>	
D 12	5.54 (0.95) <sup>n</sup>	5.40 (0.42) <sup>n</sup>	4.41 (0.44) <sup>p</sup>	4.41 (0.49) <sup>p</sup>	

Data presented as mean (standard deviation).

Data tested for each group with analysis of variance and Duncan's test with significance taken to be at the level p < 0.05. For each day, values with different superscripts across individual rows are significantly different.

EA = electroacupuncture.

ratio were significantly lower for Group 2 than Group 4 (Table 3).

From the assays of white adipose tissue homogenates on Day 12, the mean level of leptin was significantly greater for Group 2 compared with Group 4, whereas the mean levels of adiponectin and IL-10 were significantly lower for Group 2 than Group 4. No significant difference was found in mean TNF- $\alpha$  levels between Groups 2 and 4. The mean adiponectin:leptin ratio and IL-10:TNF- $\alpha$  ratio were significantly lower for Group 2 than Group 4 (Table 4).

#### 3.2. For EA-treated animals

Mean values of BG at 20 minutes were significantly higher for Group 1 of obese Zucker rats compared with Group 3 of obese Long Evans rats on Day 1, Day 3, Day 5, Day 8, Day 10, and Day 12 (Table 1). No significant difference in mean values of change in BG over a 30-minute interval were found between Groups 1 and 3 on Day 1, Day 3, and Day 8, but significantly greater values were obtained for Group 1 compared with Group 3 on Day 5, Day 10, and Day 12. For Group 1 the mean BG values increased over 30 minutes on each study day, whereas for Group 3 the mean BG values decreased over 30 minutes on all of the days (Table 2).

Table 2 Change in blood glucose (mmol/L) measured at 50 minutes over a 30-minute interval.

	Obese Z	ucker rats	Obese Long Evans rats			
	Group 1 EA	Group 2	Group 3 EA	Group 4		
D 1	0.62 (1.34)	-0.32 (0.45)	-0.20 (0.51)	-0.31 (0.47)		
D 3	0.48 (1.71)	-0.60 (0.16)	-0.30 (0.24)	-0.10 (0.30)		
D 5	1.02 (1.09) <sup>a</sup>	-0.18 (0.26) <sup>b</sup>	-0.41 (0.12) <sup>b</sup>	-0.09 (0.17) <sup>b</sup>		
D 8	0.22 (0.41)	-0.04 (0.61)	-0.06 (0.23)	-0.16 (0.33)		
D 10	2.54 (4.00) <sup>c</sup>	0.22 (0.66) <sup>d</sup>	-0.19 (0.32) <sup>d</sup>	0.13 (0.46) <sup>d</sup>		
D 12	1.04 (0.35) <sup>e</sup>	$-0.04 (0.54)^{f}$	$-0.16 (0.17)^{f}$	0.03 (0.46) <sup>f</sup>		

Data presented as mean (standard deviation).

Data tested for each group with analysis of variance and Duncan's test with significance taken to be at the level p < 0.05. For each day, values with different superscripts across individual rows are significantly different.

EA = electroacupuncture.

From the assays of blood sera on Day 12, the mean levels of leptin and insulin were significantly higher for Group 1 compared with Group 3, whereas there was no significant difference in mean TNF- $\alpha$  levels between Groups 1 and 3. The mean levels of adiponectin and IL-10 were significantly lower for Group 1 than Group 3. The mean adiponectin:leptin ratio and IL-10:TNF- $\alpha$  ratio were also significantly lower for Group 1 than Group 3 (Table 3).

For the assays of the white adipose tissue homogenates on Day 12, the mean leptin level was significantly greater for Group 1 compared with Group 3, while the mean levels of adiponectin and IL-10 were significantly lower for Group 1 compared with Group 3. No significant difference was found in the mean TNF- $\alpha$  level of Groups 1 and 3. The mean adiponectin:leptin ratio and IL-10:TNF- $\alpha$  ratio were significantly lower for Group 1 than Group 3 (Table 4).

#### 3.3. EA-treated animals versus control animals

Mean values of BG at 20 minutes for EA-treated and control obese Zucker rats did not differ significantly on Day 1, Day 3, Day 5, and Day 12, but were significantly greater for EAtreated animals on Day 8 and Day 10. No significant difference in mean values of change in BG over a 30-minute interval were found between EA-treated and control obese Zucker rats on Day 1, Day 3, and Day 8, but a significantly greater (all positive) value was observed for EA-treated animals on Day 5, Day 10, and Day 12. For obese Long Evans rats, there were no significant differences in mean values of BG at 20 minutes or change in BG over a 30-minute interval for EA-treated and control animals.

For the assays of blood sera on Day 12, the mean levels of adiponectin, leptin, adiponectin:leptin ratio, IL-10, IL-10:TNF- $\alpha$  ratio, and insulin did not differ significantly between EA-treated and control obese Zucker rats, whereas that for TNF- $\alpha$  was significantly lower for EA-treated compared with control animals. For obese Long Evans rats, there was no difference in the mean values of adiponectin, IL-10, TNF- $\alpha$ , IL-10:TNF- $\alpha$  ratio, and insulin between EA-treated and control animals, but that for leptin was significantly increased and for adiponectin:leptin ratio significantly decreased for EA-treated compared with control animals.

From the assays of the white adipose tissue homogenates on Day 12, there were no significant differences in mean values between EA-treated and control obese Zucker rats. Also no significant differences in mean values were found between EA-treated and control obese Long Evans rats.

### 3.4. Interaction between EA intervention and animal model

The effect of EA was different in the two obese rat models with there being a significant interaction in serum adiponectin, adiponectin:leptin ratio, and TNF- $\alpha$  for obese Zucker rats compared with obese Long Evans rats. For example, in Zucker rats there was a nonsignificant difference in adiponectin in EA-treated rats which was less than the control, and for Long Evans rats there was a nonsignificant difference in EA-treated rats which was greater than the control, but the difference of these differences was significant (p = 0.024). For adiponectin:leptin ratio, for Zucker rats

Table 3	Measured	outcomes i	in blood	l sera	on Da	y 12. <sup>",</sup>
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Outcome	Obese Zucker		Obese L	ong Evans	p for overall	p for			
	Group 1 EA	Group 2	Group 3 EA	Group 4	difference	interaction			
Adiponectin (µg/mL)	15.28 (6.82) <sup>a</sup>	21.72 (2.55) <sup>ab</sup>	25.99 (3.68) <sup>c</sup>	22.74 (2.07) <sup>bc</sup>	0.035	0.024			
Leptin (ng/mL)	66.16 (8.72) <sup>a</sup>	63.23 (4.93) <sup>a</sup>	5.62 (1.71)	3.85 (0.72)	<0.001	0.814			
Adiponectin:leptin ( $\times 10^3$ )	0.24 (0.13) <sup>a</sup>	0.33 (0.03) <sup>a</sup>	4.72 (1.00)	6.01 (0.82)	<0.001	0.038			
IL-10 (pg/mL)	12.91 (5.10) <sup>a</sup>	20.15 (7.57) <sup>ab</sup>	27.86 (8.32) <sup>b</sup>	23.90 (10.96) <sup>ab</sup>	0.082	0.167			
TNF-α (pg/mL)	3.46 (1.78) <sup>a</sup>	18.89 (1.48)	3.43 (1.44) <sup>a</sup>	3.26 (1.15) <sup>a</sup>	<0.001	<0.001			
IL-10:TNF-α	2.66 (0.93)	1.00 (0.49)	9.01 (3.79) <sup>a</sup>	6.93 (2.18) <sup>a</sup>	<0.001	0.821			
Insulin (ng/mL)	13.63 (1.91) <sup>b</sup>	12.42 (3.24) <sup>b</sup>	3.13 (1.31) <sup>a</sup>	2.87 (1.02) <sup>a</sup>	<0.001	0.628			

Data presented as mean (standard deviation).

<sup>\*</sup> A regression model was used to allow modeling a two-way factorial design with interaction. Where there was evidence that model assumptions were not satisfied, robust standard errors were used. Stata 13.1 was used for all analyses with two-sided p < 0.05 considered statistically significant in each case.

<sup>†</sup> Stata lettering system identifies which particular pairs of groups are different, so columns sharing a letter showed no evidence of being different and columns not sharing a letter (including columns where both are "blank") showed evidence of being different; "blanks" are different to all other values including other "blanks."

EA = electroacupuncture; IL = interleukin; TNF = tumor necrosis factor.

Та	ble	4	Measured	outcomes	in	adipose	tissue	on	Day	12.	,
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Outcome	Obese	Zucker	Obese Long Evans		p for overall	p for	
	Group 1 EA	Group 2	Group 3 EA	Group 4	difference	interaction	
Adiponectin (µg/mL)	13.35 (6.35) <sup>a</sup>	7.35 (3.38) <sup>a</sup>	20.19 (2.23) <sup>b</sup>	20.02 (2.06) <sup>b</sup>	<0.001	0.098	
Leptin (ng/mL)	48.16 (7.58) <sup>b</sup>	43.06 (5.23) <sup>b</sup>	7.65 (1.76) <sup>a</sup>	6.16 (1.44) <sup>a</sup>	<0.001	0.362	
Adiponectin:leptin ( $\times 10^3$ )	0.27 (0.11) <sup>a</sup>	0.17 (0.07) <sup>a</sup>	2.79 (0.83) <sup>b</sup>	3.39 (0.73) <sup>b</sup>	<0.001	0.119	
IL-10 (pg/mL)	469.38 (36.66) <sup>a</sup>	486.70 (63.19) <sup>a</sup>	597.88 (89.95) <sup>b</sup>	580.86 (89.80) <sup>b</sup>	0.005	0.562	
TNF-α (pg/mL)	21.13 (3.64) <sup>b</sup>	17.87 (3.81) <sup>ab</sup>	17.60 (2.23) <sup>ab</sup>	16.20 (2.69) <sup>a</sup>	0.078	0.469	
IL-10:TNF-α	22.70 (4.09) <sup>a</sup>	27.97 (5.73) <sup>ab</sup>	34.56 (7.47) <sup>bc</sup>	37.01 (9.67) <sup>c</sup>	0.015	0.650	

Data presented as mean (standard deviation).

A regression model was used to allow modeling a two-way factorial design with interaction. Where there was evidence that model assumptions were not satisfied, robust standard errors were used. Stata 13.1 was used for all analyses with two-sided p < 0.05 considered statistically significant in each case.

<sup>†</sup> Stata lettering system identifies which particular pairs of groups are different, so columns sharing a letter showed no evidence of being different and columns not sharing a letter (including columns where both are "blank") showed evidence of being different; "blanks" are different to all other values including other "blanks."

there was a nonsignificant difference in EA-treated rats which was less than the control, and for Long Evans rats a significant difference in EA-treated rats which was less than the control, with the difference of these differences being significant (p = 0.038). With regards to TNF- $\alpha$ , for Zucker rats there was a significant difference in EA-treated rats which was less than the control, and a nonsignificant difference EA-treated rats which was greater than the control for Long Evans rats, with the difference of these differences being significant (p < 0.001). A similar statistical analysis of mean BG values on Day 12 gave a significant difference between the two models with and without EA but not within the models for EA-treated versus control (p = 0.004).

For the adipose tissue, no significant interactions were found although for adiponectin there was a nonsignificant difference in EA-treated rats greater than the control for Zucker rats, and a small nonsignificant difference in EAtreated rats greater than the control for Long Evans rats, with the difference of these differences being on the borderline of significance (p = 0.098).

### 4. Discussion

Insulin resistance can be defined as decreased sensitivity or decreased responsiveness, or by combinations of the two, to the metabolic actions of normal concentrations of insulin such as insulin-mediated glucose disposal [25]. Obesity is the most common cause of insulin resistance and at least three candidate molecules have been implicated in the development of insulin resistance due to obesity [26]. Circulating lipids such as FFA, which are increased in obesity, inhibit glucose uptake and use in muscle [27]. TNF- $\alpha$  inhibits glucose uptake in muscle, and causes phosphorylation of insulin receptor substrate-1 which inhibits insulin signal transduction [28] or downregulation of glucose transporter type 4 [29]. Increased level of leptin which is associated with obesity may cause insulin resistance [30]. Adiponectin and IL-10 are both anti-inflammatory cytokines and act to increase insulin sensitivity.

The present study has examined whether the effect of EA is different in two animal models of obesity, the Zucker

fatty rat and the HFD-induced obese Long Evans rat. The study measured levels of BG and important adipokines that contribute to a pro-/anti-inflammatory cytokine imbalance in obesity and a possible insulin-resistant state. Insulin resistance is closely associated with obesity, type 2 diabetes mellitus, hypertension, and metabolic syndrome, and is a risk factor for serious diseases such as cardiovascular disease. EA has been shown to be effective in improving insulin sensitivity and lowering BG in diabetic rodents and obese humans [31–34].

The male obese Zucker rats were at 15 weeks of age, and the male Long Evans rats had been fed HFD from weaning at 3 weeks of age and those at 12 weeks of age with a body weight two standard deviations greater than that of male lean Long Evans rats were selected for the study. While both of these animal models were normoglycemic, the mean baseline BG for controls (no EA, measured at 20 minutes after inducing anesthesia) was greater for the obese Zucker rats than the obese Long Evans rats. In addition, the control obese Zucker rats had a much higher serum leptin, TNF- $\alpha$ , and insulin than the control obese Long Evans rats, but with no difference in the serum levels of adiponectin and IL-10. These findings are consistent with obese Zucker rats being leptin resistant and insulin resistant. Insulin resistance is by definition tethered to hyperinsulinemia [35] and insulin resistance induces hyperleptinemia [36]. As a consequence of the pro-/anti-inflammatory imbalance being much greater in the obese Zucker rats than in the obese Long Evans rats, the obese Zucker rat may be a better model to test EA as a treatment modality to reverse the pro-/anti-inflammatory imbalance that contributes to metabolic dysfunction and insulin resistance in obesity. The obese Long Evans rats were most likely of lower insulin resistance than the obese Zucker rats as evidenced by the low serum leptin and insulin levels and this may be due to the relatively short period of 9 weeks for feeding the HFD and the young age of the rats at which this was started. As previously reported, there was no significant difference in serum insulin levels between control lean and obese Long Evans rats [23]. In a study by Posey et al [19], Long Evans rats 8-10 weeks of age and fed HFD for 7 weeks, in 4 hourfasted animals the mean plasma leptin was 22 ng/mL and insulin 5.0 ng/mL. Also in the study by Woods et al [18], Long Evans rats 8-11 weeks of age and fed HFD for 10 weeks, the mean plasma leptin was 35 ng/mL and insulin 6.6 ng/mL. These levels are much higher than those for the obese Long Evans rats in the present study.

Repeated EA treatment at CV12/CV4 acupoints significantly increased serum leptin and decreased adiponectin:leptin ratio in obese Long Evans rats, consistent with these rats not being fully leptin resistant [23], and repeated EA in obese Zucker rats significantly decreased serum TNF- $\alpha$ and increased IL-10:TNF- $\alpha$  ratio [22]. The effect of EA differed for the obese Zucker and obese Long Evans rats with there being a significant interaction between EA intervention and animal type for serum adiponectin, adiponectin:leptin ratio, and TNF- $\alpha$  on Day 12. Serum adiponectin was nonsignificantly lower for Zucker EA-treated versus Zucker control, while it was nonsignificantly greater for Long Evans EA-treated versus Long Evans control (i.e., in opposite direction to Zucker rats). Serum adiponectin:leptin ratio was nonsignificantly lower for Zucker EA-treated versus Zucker control, while it was significantly lower for Long Evans EA treated versus Long Evans control (i.e., in the same direction). Serum TNF- $\alpha$  was significantly lower for Zucker EA-treated versus Zucker control, while it was nonsignificantly lower for Long Evans EA-treated versus Long Evans control (i.e., in the same direction).

Acupoints CV4 and CV12 are located on the middle line of the abdomen, and both belong to the *Ren Mai* meridian. The anatomical structure of these two acupoints predominantly consists of the connective tissues. However, these two acupoints have different nerve innervations (CV4 is innervated by T12 while CV12 by T7/8). According to classic acupuncture theories and clinical reports, CV4 has invigorating effects on the whole body and can regulate the function of the genitourinary system, whereas CV12 has regulatory effects on functions of the gastrointestinal system [37].

To explore the differential effect of EA further (e.g., on serum adiponectin, adiponectin:leptin ratio) would require a modification to the research design and this could be to use larger group sizes for the two models or to give a greater number of EA treatments. Also, Woods et al [18] found that feeding HFD for 10 weeks to Long Evans rats at 10 weeks of age resulted in decreased insulin sensitivity as shown by insulin tolerance test. This may provide a better diet-induced rat model of obesity with hyperinsulinemia and insulin resistance. Furthermore, Li et al [20] found for Long Evans rats fed HFD from weaning, in oral glucose tolerance test at 21 weeks of age, both glucose and insulin levels were significantly higher after overnight fasting and at every time point after the oral glucose challenge indicating impaired insulin sensitivity. These rats would have been fed HFD for 18 weeks.

The findings from the statistical comparison of data for the two rat models of obesity would suggest that the decrease in serum TNF- $\alpha$  for Zucker EA model may lead to a decrease in insulin resistance in this model. However, with there being no significant decrease in circulating insulin and BG compared with controls, the data would more likely indicate that EA has not lowered insulin resistance in this model when applied 3 times/wk over 2 weeks. To test for an alteration in insulin resistance an insulin tolerance test would need to be performed in which blood is sampled before and at timed intervals after an intraperitoneal injection of insulin and BG measured [18]. Previous studies had shown EA to decrease insulin resistance by lowering circulating FFA levels in steroid-background rats [38] and HFD mice [39], and by decreasing circulating TNF- $\alpha$  level and insulin in HFD rats [40]. In addition, in a study by Tominaga et al [41] using a hyperinsulinemic—euglycemic clamp with HFD-induced obese rats treated with EA at bilateral ST36, the glucose infusion rate and phospho-5' AMP-activated protein kinase- $\alpha$  in the EA group were significantly greater compared with the control group. It was suggested that insulin resistance was significantly improved by EA probably by activation of 5' AMP-activated protein kinase signaling in skeletal muscle.

In summary, repeated EA treatment at CV12/CV4 acupoints has been shown to cause alterations in the balance of pro-/anti-inflammatory adipokines in each of the two rat models of obesity examined. For obese Zucker rats there was an increase in the IL-10:TNF- $\alpha$  ratio, while for obese

Long Evans rats the adiponectin:leptin ratio was decreased by EA. This comparative study has indicated the possibility of other alterations in the levels of adipokines by EA in the two models and this could be examined in larger sized studies. It could also be used to compare the effects of EA in other models such as obese and lean animals or animals with different extents of metabolic disturbance.

# **Disclosure statement**

The author declares to have no conflicts of interest and no financial interests related to the material of this manuscript.

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