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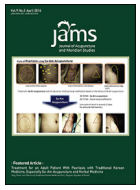

RESEARCH ARTICLE

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

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