The FASEB Journal article fj.201601381R. Published online April 12, 2017.



# Autonomic nervous system activation mediates the increase in whole-body glucose uptake in response to electroacupuncture

**Anna Benrick,[*,†] Milana Kokosar,[*] Min Hu,[*] Martin Larsson,[*] Manuel Maliqueo,[‡] Rodrigo Rodrigues Marcondes,[§,¶] Marzia Soligo,[‖] Virginia Protto,[‖] Elisabet Jerlhag,[#] Antonina Sazonova,[**] Carl Johan Behre,[††] Kurt Højlund,[‡‡] Peter Thorén,[*] and Elisabet Stener-Victorin[§,1]**

[*]Department of Physiology and [#]Department of Pharmacology, Institute of Neuroscience and Physiology, [**]Department of Obstetrics and Gynecology, Institute of Clinical Sciences, and [††]Department of Cardiology, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [†]School of Health and Education, University of Skövde, Skövde, Sweden; [‡]Endocrinology and Metabolism Laboratory, West Division, School of Medicine, University of Chile, Santiago, Chile; [§]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; [¶]Disciplina de Ginecologia, Laboratório de Ginecologia Estrutural e Molecular (LIM 58), Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; [‖]Institute of Translational Pharmacology, Consiglio Nazionale delle Ricerche, Rome, Italy; and [‡‡]Department of Endocrinology, Odense University Hospital, Odense, Denmark

**ABSTRACT: A single bout of low-frequency electroacupuncture (EA) causing muscle contractions increases whole-body glucose uptake in insulin-resistant rats. We explored the underlying mechanism of this finding and whether it can be translated into clinical settings. Changes in glucose infusion rate (GIR) were measured by euglycemic–hyperinsulinemic clamp during and after 45 min of low-frequency EA in 21 overweight/obese women with polycystic ovary syndrome (PCOS) and 21 controls matched for age, weight, and body mass index (experiment 1) and in rats receiving autonomic receptor blockers (experiment 2). GIR was higher after EA in controls and women with PCOS. Plasma serotonin levels and homovanillic acid, markers of vagal activity, decreased in both controls and patients with PCOS. Adipose tissue expression of pro-nerve growth factor (NGF) decreased, and the mature NGF/proNGF ratio increased after EA in PCOS, but not in controls, suggesting increased sympathetic-driven adipose tissue metabolism. Administration of α-/β-adrenergic receptor blockers in rats blocked the increase in GIR in response to EA. Muscarinic and dopamine receptor antagonist also blocked the response but with slower onset. In conclusion, a single bout of EA increases whole-body glucose uptake by activation of the sympathetic and partly the parasympathetic nervous systems, which could have important clinical implications for the treatment of insulin resistance.—Benrick, A., Kokosar, M., Hu, M., Larsson, M., Maliqueo, M., Marcondes, R. R., Soligo, M., Protto, V., Jerlhag, E., Sazonova, A., Behre, C. J., Højlund, K., Thorén, P., Stener-Victorin, E. Autonomic nervous system activation mediates the increase in whole-body glucose uptake in response to electroacupuncture. FASEB J. 31, 000–000 (2017). www.fasebj.org**

**KEY WORDS: glucose homeostasis · polycystic ovary syndrome · insulin resistance · muscle contraction**

Physical exercise has beneficial effects on metabolic dysfunctions, including improved energy balance, reduced adiposity, and regulation of key signaling molecules in skeletal muscle and adipose tissue (1). A single bout of exercise induces rapid changes in skeletal muscle and adipose tissue, and these changes have been directly related to muscle contractions. Muscle contractions cause an immediate increase in glucose uptake in skeletal muscle and adipose tissue by activating glucose transporter (GLUT)-4 translocation to the cell membrane in an insulin-independent manner (2). This process is considered to be the main mechanism for the acute effect of physical exercise on glucose transport. Accumulating evidence suggests that the sympathetic nervous system also plays an important role in the regulation of glucose homeostasis (3). For example, stimulation of hepatic sympathetic nerves induces rapid and marked release of glucose from the liver, stimulation of pancreatic sympathetic nerves

**ABBREVIATIONS:** 5HIAA, 5-hydroxyindoleacetic acid; BMI, body mass index; CV, coefficient of variability; DOPAC, 3,4-dihydroxyphenylacetic acid; EA, electroacupuncture; GIR, glucose infusion rate; GLUT, glucose transporter; HOMA-IR, homeostatic model assessment; HVA, homovanillic acid; NGF, nerve growth factor; PCOS, polycystic ovary syndrome; $S/I_{clamp}$, insulin sensitivity

[1] Correspondence: Department of Physiology and Pharmacology, Karolinska Institutet, 171-77 Stockholm, Sweden. E-mail: elisabet.stener-victorin@ki.se

doi: 10.1096/fj.201601381R

This article includes supplemental data. Please visit *http://www.fasebj.org* to obtain this information.



 

causes a decrease in insulin secretion and stimulation of sympathetic nerves innervating adipose tissue causes lipolysis. These regulatory changes are important for handling the increased energy requirement during exercise.

Acupuncture with low-frequency electrical stimulation causing muscle contraction [electroacupuncure (EA)], activates afferent nerve fibers (Aδ fibers and C fibers) (4) and efferent sympathetic nerve fibers in the same segmental innervation during stimulation (5–8). Further, low-frequency EA has been shown to stimulate dopamine secretion that modulates vagal activity and controls inflammation (9), and it has been shown to modulate the release of endogenous opioids, which in turn might participate in the regulation of glucose metabolism (10, 11). Thus, EA induces changes in skeletal muscle and adipose tissue signaling pathways similar to exercise-induced muscle contractions (12, 13), and it has the potential to ameliorate various metabolic disorders associated with altered autonomic activity and insulin resistance (14).

A single bout of low-frequency EA increases whole-body glucose uptake during and after stimulation in both controls and insulin-resistant rats (15). However, there is only limited evidence in support of the effectiveness of EA for improving glucose homeostasis or insulin sensitivity during and after stimulation in humans (16), and there is no study evaluating the effect in insulin-resistant subjects. Clinically, EA can be a useful way of assisting muscle contraction for those who have difficulties performing voluntary exercise. However, it remains to be determined whether the rapid increase in glucose uptake by a single bout of EA in an insulin-resistant rodent model can be extrapolated to overweight and obese women with polycystic ovary syndrome (PCOS). PCOS is the most common endocrine and metabolic disorder among women and is associated with obesity, insulin resistance, and impaired fertility (17). Further, it remains to be determined *via* which pathways the effect of EA is mediated.

We aimed to test the hypothesis that a single bout of low-frequency EA increases whole-body glucose uptake in overweight and obese women with and without PCOS. We hypothesized that adipose tissue expression of pronerve growth factor (NGF) and mature (m)NGF, which are regulators of sympathetic nerve activity (18, 19), are modified and that plasma catecholamines and their metabolites are decreased by low-frequency electroacupuncture.

Because it has recently been demonstrated that the autonomic nervous system is activated to counteract decreased blood glucose (20, 21), we tested the hypothesis that increased glucose uptake by EA is mediated *via* activation of the autonomic nervous system and the opioid system.

## MATERIALS AND METHODS

### Experiment I

The human experiment was conducted in accordance with the Declaration of Helsinki and was approved by the regional ethics board at the Sahlgrenska Academy at the University of Gothenburg, Sweden (Dnr 520-11) (**Fig.1 *A***). The study was registered October 6, 2011, at ClinicalTrials.gov (NCT01457209) [U.S. National Institutes of Health (NIH), Bethesda, MD, USA] before the start of recruitment. All participants gave oral and written informed consent and were recruited through advertisements in local newspapers and in frequently visited places in the community between October 2011 and December 2013 in the Västra Götaland Region, Sweden.

#### *Participants*

Twenty-one overweight or obese women with PCOS and 21 controls matched for age, weight, and body mass index (BMI) were included. Inclusion criteria for the controls were 18–38 yr old, BMI >25 to <35, regular menstrual cycles of 28 ± 2 d, and normal ovarian morphology. Controls were excluded if they showed signs of hyperandrogenism (Ferriman-Gallwey score >4). Women with PCOS were diagnosed according to the Rotterdam criteria (22) fulfilling 2 of 3 criteria: ultrasound-verified polycystic ovaries (≥12 follicles of 2–9 mm and/or ovarian volume ≥10 ml in 1 or both ovaries), oligomenorrhea (>35 d between, or <6 menstruations in the past year), amenorrhea (absence of menstrual bleedings in the past 90 d), or clinical signs of hyperandrogenism (acne or hirsutism, Ferriman-Gallwey score ≥8) (23). The International Physical Activity Questionnaire estimated physical activity.

Participants reported to the laboratory in the morning after an overnight fast, anthropometrics were measured and BMI and waist–hip ratio (the ratio of waist and hip circumferences) were calculated. Basal blood samples were taken and homeostatic model assessment (HOMA-IR) [(fasting insulin (μU/ml) × fasting glucose (mM)/22.5)] and C-peptide index were calculated at baseline.

#### *Euglycemic–hyperinsulinemic clamp*

The euglycemic–hyperinsulinemic clamp was performed as described previously (24) at menstrual cycle d 1–10. In ameno-/oligomenorrheic women, the experiment was performed irrespective of cycle day. Body surface area was calculated using the Mosteller formula. Insulin was continuously infused (40 mU/m$^2$/min) for 120 min to reach steady state, and plasma glucose concentrations were measured every 10 min during the first 90 min of infusion, and thereafter every 5 min throughout the clamp. Steady state was defined as the last 30 min of the clamp. Euglycemia (5 mM) was achieved by infusing 20% glucose (1.1 M), and it was kept at 5 mM by adjusting the glucose infusion rate (GIR). At steady state a needle biopsy of subcutaneous abdominal adipose tissue was obtained under local anesthesia (Xylocain, AstraZeneca AB, Sweden) and snap frozen.

Blood samples for assessing plasma insulin were taken at baseline and at 60, 90, 100, 110, and 120 min during steady state; at 25, 35, and 45 min during acupuncture; and at 30, 40, 50, and 60 min after stimulation. Blood samples for assessing C-peptide were taken at baseline and the final 10 min of steady state, at 25 and 35 min during electroacupuncture, and at 40 min after stimulation. The clamp continued during and for 60 min after the end of electroacupuncture.

The GIR (mg/kg/min) during steady state, during the last 20 min of stimulation, and during the last 30 min after EA (post-stimulation) was used to measure whole-body glucose uptake (*M* value). Insulin sensitivity ($S/I_{clamp}$) was calculated according to Bergman *et al.* (25) at the same time points.

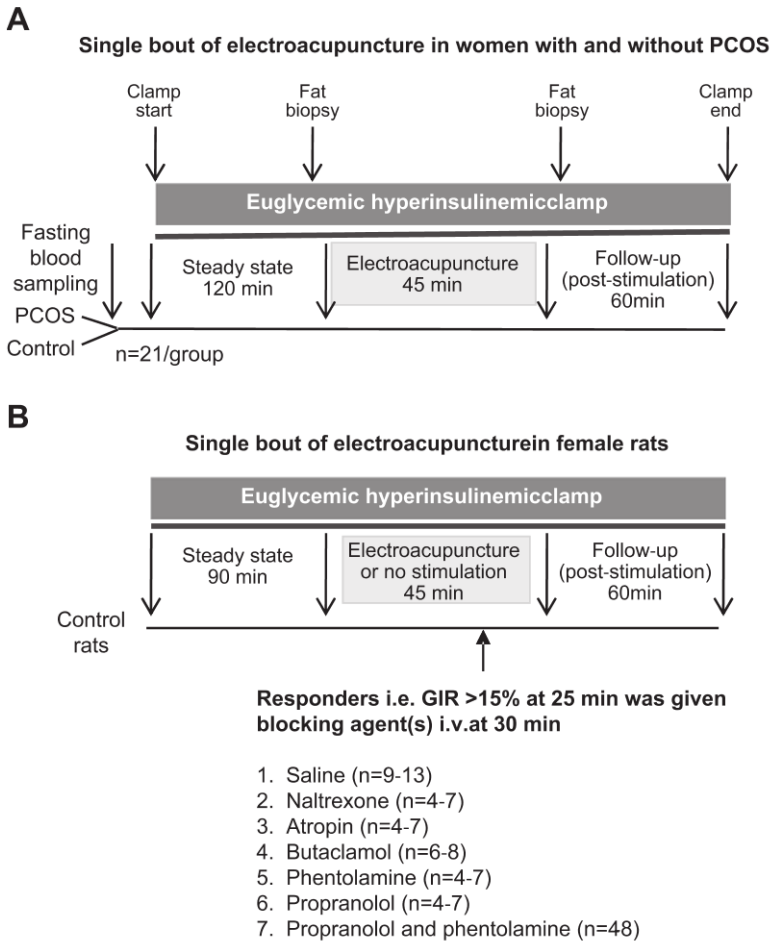


**Figure 1.** Overview of the study design and procedures.

### *Electroacupuncture*

We used a Western style of acupuncture (26). In brief, acupuncture needles were placed bilaterally in the abdominal muscle, in the quadriceps muscles, and in the muscles below the medial side of the knee, all in somatic segments corresponding to the innervation of the ovaries and pancreas. Needles were also placed in the hand. The needles were inserted to a depth of 15–40 mm and stimulated by manual rotation when inserted to evoke needle sensation (*de qi*). Needle placement and stimulation are presented in Table 1. Immediately after 45 min of electroacupuncture, a second needle biopsy of subcutaneous abdominal adipose tissue was collected.

## Experiment 2

The animal experiment was approved by the Animal Ethics Committee of the University of Gothenburg, Sweden, and followed the *Guide for the Care and Use of Laboratory Animals* (NIH). Female Wistar rats (Charles River, Sulzfeld, Germany) arrived at 13 wk of age, and were fed *ad libitum* with standard rat chow (Teklad Global Diet; Harlan, Rossdorf, Germany) (Fig. 1*B*). Rats were tested for the presence of an estrous cycle as determined by vaginal smears and randomly divided into a no-stimulation group and an EA group. Because the purpose of this study was to investigate the pathways through which acupuncture increases whole-body glucose uptake, the groups were further divided into rats receiving nonselective receptor blocking agents or saline as a control.

A subset of PCOS rats received EA in combination with phentolamine and propranolol (Supplemental Data).

### *Euglycemic–hyperinsulinemic clamp*

The clamp was performed in animals under anesthesia (15). Insulin (Actrapid, Novo Nordisk, Denmark) was continuously infused at 8 mU/kg/min, and glucose was infused to maintain a blood glucose level of 6 mM. Blood glucose levels were measured every 5 min. Insulin sensitivity was quantified as the GIR (mg/kg/min). The final GIR value during steady state was set to 1, and the fold change during EA and the poststimulation period was calculated.

### *Electroacupuncture*

At steady state, 2 acupuncture needles (HEGU Svenska AB, Landsbro, Sweden; 0.20 × 15 mm) were placed in the *rectus abdominis* muscle, which corresponds to the acupuncture points ST27, -28, and -29, and 2 needles were placed in the triceps surae muscle bilaterally, which corresponds to SP6 and -9. All acupuncture points were in somatic segments corresponding to the innervation of the pancreas and ovaries. The needles were attached to an electrical stimulator (CEFAR ACU II; Cefar-Compex Scandinavia, Malmö, Sweden) and stimulated with low frequency (2 Hz) at an intensity high enough to evoke muscle twitches, which varied from 0.8 to 2.2 mA, due to receptor adaptation. Responders to EA were identified as those with >15% increase in GIR at 25 min of stimulation, and blocking agents were administered to these rats at 30 min. A bolus of naltrexone (10 mg/kg), phentolamine (5 mg/kg), propranolol (1 mg/kg), phentolamine and propranolol, atropine (1 mg/kg), butaclamol (2 mg/kg), or saline was given. After the bolus, a continuous infusion of


 

TABLE 1. *The acupuncture protocol was fixeda ndb asedo np reviouss tudiesi nvestigatingt hee ffecto fa cupunctureo nr eproductivef unctioni n women with PCOS and on experimental studies*

| Acupuncture point | Stimulation | Localization | Muscle | Muscle innervation |
|---|---|---|---|---|
| CV3, *Guan Yuan* | EA | 3 cm caudal to the umbilicus | Fibrous tissue, linea alba | L1 |
| CV12, *Zhongwan* | EA | On the midline, 4 cm superior to the umbilicus | Fibrous tissue, linea alba | Th7–8 |
| ST29, *Guilai* | EA, bilateral | 1 cm cranial to the pubic bone and 2 cm lateral of the midline | Musculus rectus abdominis | Th6–12 |
| ST34, *Futu* | EA, bilateral | 2 cm above the superior lateral border of the patella on the line connecting the anterior superior iliac spine found | Musculus quadriceps femoris | Femoral nerve |
| ST32, *Liangqiu* | EA, bilateral | 6 cm above the superior lateral border of the patella on the line connecting the anterior superior iliac spine found | Musculus quadriceps femoris | Femoral nerve |
| SP6, *Sanyinjiao* | Manual stim. every 10 min | 3 cm proximal to the medial malleolus | Musculusi flexor digitorum longus, tibialis posterior | L4–5, S1–2 |
| ST36, *Zusanli* | Manual stim. every 10 min | On the anterior lateral side of the leg, 3 cm below *Dubi* (ST35), 1 finger width (middle finger) from the anterior crest of the tibia | Musculi tibialis anterior | L4–5, S1 |
| LI4, *Hegu* | Manual stim. every 10 min | On the highest point at the musculus interosseus dorsalis | Musculusi interosseus dorsalis I, lumbricalis II, adductor pollicis | C8, Th1 |

Acupuncture needles (0.25 × 30 or 0.30 × 50 mm) were placed in the acupuncture points listed, in somatic segments corresponding to the innervation of the ovaries and pancreas. All needles were stimulated manually when inserted, and thereafter every 10th minute or electrically with low-frequency (2 Hz) for 45 min. The intensity was adjusted to produce local muscle contractions without pain or discomfort. Acupuncture was delivered by a therapist educated in Western medical acupuncture and with over 25 yr of experience. C, cervical vertebra; CV, conception vessel; L, lumbar vertebra; LI, large intestine; LR, liver; PC, pericardium; S, sacral vertebra; SP, spleen; ST, stomach; Th, thoracic vertebra.

atropine (0.5 mg/kg), butaclamol (1 mg/kg), phentolamine (7 mg/kg/h), propranolol (0.5 mg/kg/h), both phentolamine and propranolol or saline was administered.

After the 45 min of electroacupuncture, the clamp and blocking continued for another 60 min. Animals in the no-stimulation group underwent the same procedure as those in the EA group but with a wooden toothpick that touched the skin.

*Laboratory analyses* Plasma glucose was measured by One Touch Ultra2 (LifeScan, West Chester, PA, USA). Insulin was analyzed at an accredited laboratory. Serum insulin was measured with immunometric two-step sandwich method (Advia Centaur Insulin ReadyP ack;B ayerH ealthCare, Leverkusen, Germany) where 2 antibodies are used: anti-insulin (Lite Reagent, Siemens, Upplands Väsby, Germany) and a monoclonal mouse anti-insulin antibody (Solid Phase; Siemens) to initiate a chemiluminescence reaction, the intra-assay coefficient of variability (CV%) was 10%. Serum C-peptide was measured with a human diabetes C-peptide magnetic bead set (Bio-Rad, Hercules, CA, USA), the intra- and interassay CV% was 5 and 4%, respectively. Plasma noradrenaline, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin, and 5-hydroxyindoleacetic acid (5HIAA) were measured on a split-fraction HPLC-ED system (27). Serum testosterone, estradiol, and progesterone were measured by gas chromatography-tandem mass spectrometry (28).

*Adipose tissue extraction and ELISA* Adipose tissue was homogenized in ice-cold lysis buffer [20 mM Tris (pH 7.4), 150 mM NaCl, 10% glycerol, 1 mM EDTA (pH 8.0), 20 mM NaF, 30 mM sodium pyrophosphate ($Na_4P_7O_2$)], and protease and phosphatase inhibitor cocktails (P8340, P5276, and P0044; Sigma-Aldrich, Stockholm, Sweden). The homogenized tissue samples were centrifuged at 3000 *g* for 6 min at 4°C, and the lower phase was collected. Five volumes of ice-cold detergent mix (6% NP40, 0.6% SDS, and 1.5% NaDOC) were added, followed by shaking on ice for 45 min and centrifugation at 3000 *g* for 20 min at 4°C. The protein concentration was quantified by the Bradford assay. mNGF and proNGF were detected and quantified using MAB5260Z clone 27/21 and EP1318Y antibodies (Merck Millipore, Billerica, MA, USA) in a homemade sandwich ELISA (29).

## Statistical analyses

Data are presented as means ± SEM, and Fisher's permutation test was used to assess differences between women with PCOS and controls at steady state, during electroacupuncture, and after stimulation. Fisher's test for paired comparisons was used to analyze changes between steady state and during EA and between steady state and post-stimulation in women with PCOS and controls. Fisher's permutation test and Fisher's test for paired comparisons are nonparametric tests and are not to be confused with Fisher's exact test (30). In rats, changes in GIR during steady state, electroacupuncture, and after stimulation within each group and between the groups were compared with repeated-measures 2-way ANOVA followed by the Bonferroni *post hoc* test. Statistical analyses were performed with SPSS software (v.21.0; SPSS, Chicago, IL, USA), and a value of $P \leq 0.05$ was considered significant.

## RESULTS

### Baseline characteristics

Baseline characteristics are presented in Table 2. There was no difference in age, weight, and BMI between women with and without PCOS. Women with PCOS had higher serum testosterone, number of antral follicles, and FG score ($P < 0.001$ for all parameters) *vs.* controls. Oligo-/amenorrhea was recorded in 18 women with PCOS, whereas all controls had regular cycles. Fasting insulin and HOMA-IR was nonsignificantly higher ($P = 0.068$ and $P = 0.061$, respectively), fasting C-peptide and C-peptide index were higher ($P = 0.028$ and $P = 0.008$), and fasting glucose was lower ($P = 0.01$). Fourteen women with PCOS and 10 controls had a HOMA-IR >2.0, which is indicative of insulin resistance. There was no difference in the overall total physical activity between women with PCOS and controls [2219 ± 1820 *vs.* 1887 ± 2272 MET-min/wk ($P = 0.675$)].

### Changes in whole-body glucose uptake in women

The GIR was higher during the poststimulation period compared with steady state in patients with PCOS ($P = 0.019$) and controls ($P = 0.003$) (Fig. 2*A*), whereas calculated insulin sensitivity $S/I_{clamp}$, taking insulin levels before and during the clamp into account, was higher during the poststimulation period, only in patients with PCOS ($P = 0.045$; Fig. 2*B*). There were no significant within-group changes in the GIR and $SI_{clamp}$ during electroacupuncture, which might be explained by decreased insulin clearance or increased insulin secretion as evidenced by increased insulin concentration in controls and increased C-peptide concentrations in patients with PCOS during EA (Fig. 2*C*, *D*). There were no significant differences in GIR or $SI_{clamp}$ between cases and controls during or after electroacupuncture.

Because the effect of electroacupuncture, at least in part, has been shown to be mediated *via* changes in the autonomic nervous system, dopamine and its metabolites were measured. Serotonin and HVA decreased in controls and patients with PCOS (**Fig. 3*A*, *B***) immediately after a single bout of electroacupuncture, indicating modulation of the vagal system. The plasma levels of dopamine, noradrenalin, and DOPAC were below the detection limit.

The expression of pro-NGF was decreased (Fig. 3*C*) and the mNGF/proNGF ratio was increased (Fig. 3*E*) in adipose tissue immediately after EA only in women with PCOS (Fig. 3*C*–*E*).

The decrease in serotonin and HVA by EA indicates modulation of the parasympathetic nervous system. Also, the increase in mNGF/proNGF ratio indicates that EA modulates the sympathetic nervous system. Therefore, we investigated whether the increased whole-body glucose uptake by EA could be inhibited by blocking agents for sympathetic, parasympathetic, dopamine, and opioid receptors in rats.

### Pathways by which EA increases glucose uptake in rats

The GIR in the no-stimulation and saline-stimulated rats did not change during the clamp (**Fig. 4*A***). In the EA and saline group, the mean GIR was increased during stimulation ($P < 0.001$) and poststimulation ($P < 0.001$) compared to steady state and was higher than in the no-stimulation and saline group.

TABLE 2. *Clinical characteristics of women with PCOS and controls pair-wise matched for age, weight, and BMI*

| Variable | Control ($n = 21$) | PCOS ($n = 21$) | $P$* |
|---|---|---|---|
| Age (yr) | 29.76 ± 6.36 | 31.19 ± 5.56 | 0.520 |
| Body composition | | | |
| Body mass index (kg/m$^2$) | 30.41 ± 3.62 | 31.16 ± 4.12 | 0.534 |
| Weight (kg) | 84.64 ± 11.35 | 85.11 ± 13.60 | 0.902 |
| Clinical reproductive | | | |
| Antral follicle <9 mm ($n$) | 8.00 ± 4.55 | 23.00 ± 7.21 | <0.000 |
| Oligo-/amenorrhea, $n$ (%) | 0 (0) | 18 (86) | N/A |
| FG score | 1.86 ± 2.35 | 10.90 ± 7.19 | <0.000 |
| Endocrine variables | | | |
| E2 (pg/ml) | 57.35 ± 41.76 | 87.12 ± 63.16 | 0.080 |
| Progesterone (pg/ml) | 822.2 ± 2622.5 | 1185.6 ± 2628.3 | 0.672 |
| Testosterone (pg/ml) | 247 ± 87 | 488 ± 303 | <0.001 |
| Metabolic measurements | | | |
| Glucose (mM) | 5.2 ± 0.5 | 4.8 ± 0.3 | 0.010 |
| Insulin (mU/L) | 9.6 ± 4.1 | 3.1 ± 7.5 | 0.068 |
| C-peptide (mg/ml) | 0.95 ± 0.37 | 1.25 ± 0.48 | 0.028 |
| C-peptide index | 6.12 ± 2.42 | 8.76 ± 3.65 | 0.008 |
| HOMA-IR | 2.20 ± 1.11 | 3.18 ± 2.05 | 0.061 |

Values are means ± SD. FG score, Ferriman Gallwey score; E2, estradiol. *Fisher permutation test. Statistical significance is underlined.


 

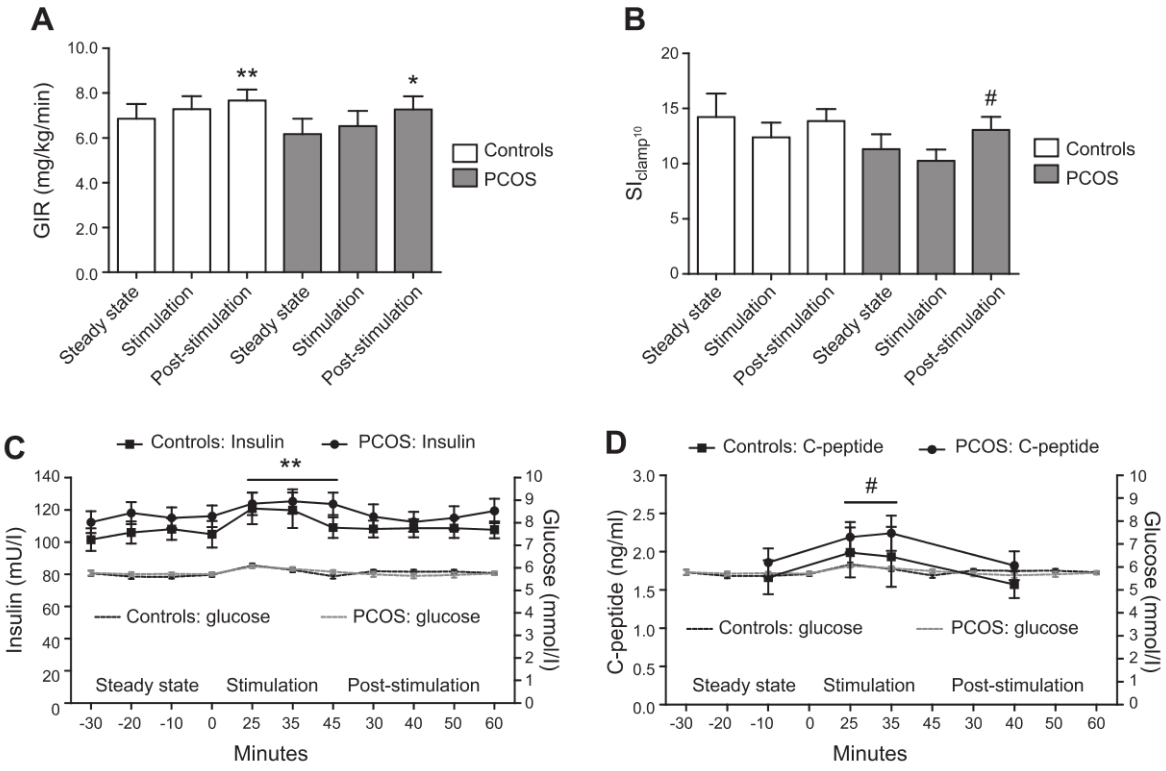


**Figure 2.** Regulation of whole-body glucose uptake by a single bout of EA in overweight and obese women with and without PCOS. GIR (*A*) and insulin sensitivity (*B*) calculated as $SI_{clamp}$ at steady state (last 30 min before stimulation), during EA, and during the poststimulation period. Glucose, insulin (*C*), and C-peptide (*D*) values during the study period. Values are mean ± SEM. $^{**}P \leq 0.01$ for within-control group differences, $^{\#}P \leq 0.05$ for within-PCOS group differences (as determined with Fisher's test for pair-wise comparisons).

### *Opioid receptor blocker*

Administration of naltrexone did not block the increased GIR induced by a single bout of EA ($P < 0.01$) and did not affect GIR in the no-stimulation group (Fig. 4*B*).

### *Dopamine receptor blocker*

Blocking with butaclamol returned the GIR values to steady-state levels in the poststimulation period in the low-frequency EA group ($P < 0.001$; Fig. 4*C*). Administration of butaclamol to the no-stimulation group decreased GIR in the poststimulation period compared with steady state ($P < 0.05$), but it did not differ from the EA and butaclamol or the no-stimulation and saline groups.

### *Muscarinic receptor blocker*

Blocking with atropine returned the GIR values to steady-state levels in the poststimulation period in the low-frequency EA group ($P < 0.001$; Fig. 4*D*). Administration of atropine to the no-stimulation group decreased GIR in the poststimulation period compared with steady state ($P < 0.001$), but did not differ from the EA and atropine or no-stimulation and saline groups.

### *α-Adrenergic receptor blocker*

Phentolamine (a nonselective α-receptor antagonist) returned the increased GIR values to steady-state levels in the EA group ($P < 0.01$; Fig. 4*E*). GIR was below steady-state levels during the poststimulation period ($P < 0.05$) and differed from the no-stimulation phentolamine group. Phentolamine did not affect GIR in the no-stimulation group.

### *β-Adrenergic receptor blocker*

Propranolol (a nonselective β-receptor antagonist) returned the increased GIR values to steady-state levels in the EA group ($P < 0.01$), and these levels did not differ from the no-stimulation propranolol group during the poststimulation period (Fig. 4*F*). However, GIR values in the poststimulation period were higher than steady-state levels ($P < 0.05$). Propranolol did not affect GIR in the no-stimulation group.

Combined phentolamine and propranolol returned the increased GIR values to steady-state levels in the poststimulation period ($P < 0.01$; Fig. 4*G*) but did not significantly affect GIR in the no-stimulation group.

Administration of combined phentolamine and propranolol to PCOS rats in the EA group resulted in an immediate return of GIR values to steady-state

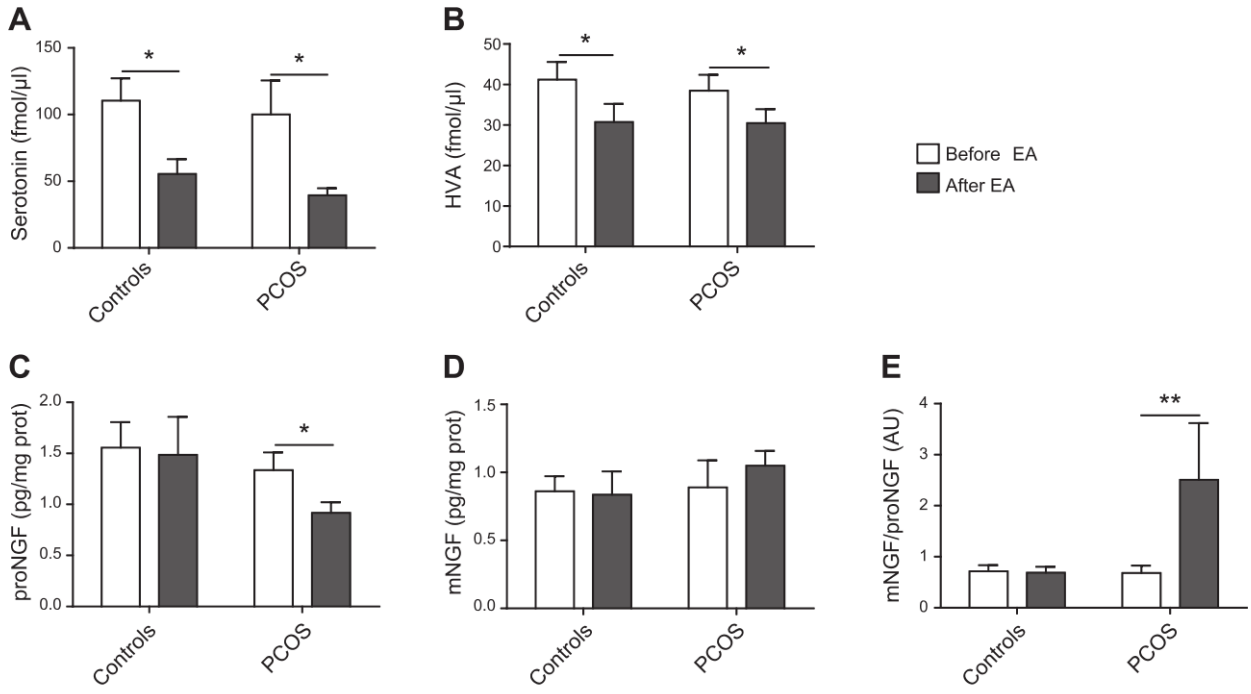


**Figure 3.** Changes in plasma serotonin and HVA and in adipose tissue protein expression of proNGF, mNGF, and proNGF/mNGF ratio by a single bout of EA. Changes in plasma serotonin (*A*) and HVA (*B*), adipose tissue expression of proNGF (*C*), mNGF (*D*), and the mNGF/proNGF ratio (*E*) from before the start of EA, at steady state, and immediately after 45 min of EA. Values are means ± SEM. $*P \leq 0.05$, $**P \leq 0.001$ for within-group differences as determined with Fisher'st estf orp air-wise comparisons.

levels in the poststimulation period (Supplemental Fig. 1).

## DISCUSSION

The effect of low-frequency EA on whole-body glucose uptake is largely unknown. In this study, a single bout of EA resulted in a significant increase in insulin-stimulated glucose uptake in overweight women with and without PCOS. Also, EA modulates circulating serotonin and HVA levels in cases and controls, whereas adipose tissue markers of sympathetic nerve activity were only increased in women with PCOS. In rats, the increased glucose uptake was completely blocked by administration of inhibitors of sympathetic nerve activity and partially blocked by administration of inhibitors of parasympathetic nerve activity.

Physical exercise with muscle contractions is the first line of therapy to improve insulin sensitivity in persons with impaired glucose tolerance and compensatory hyperinsulinemia, but the use of complementary and alternative medicine is increasing and may serve as an adjunct to conventional medical care (31). We hypothesized that low-frequency EA that causes muscle contractions would alter metabolic stress by activating the autonomic nervous system and that the effect would be stronger in women with PCOS than in healthy controls. The reason for this is that we have previously demonstrated that low-frequency EA modulates sympathetic nerve activity in a rat model of PCOS (32) and in control rats (7, 8), as well as in women with PCOS (33). The GIR increased after EA stimulation, but not significantly during stimulation despite slightly higher insulin concentrations in this period. A similar tendency with increased insulin levels was seen in a previous human study using transcutaneous low-frequency electrical stimulation with electrodes placed on the skin instead of needles inserted into the muscle (34). During the euglycemic–hyperinsulinemic clamp, insulin infusion is set to a fixed rate and the GIR is adjusted to keep blood glucose stable. The increased insulin levels seen during EA must therefore be due to increased endogenous production of insulin or decreased clearance of insulin. C-peptide levels were increased in patients with PCOS during stimulation, suggesting that EA triggers insulin release. Insulin levels increased in controls during stimulation, and we speculate that insulin clearance might be affected in controls whereas insulin production is more affected in patients with PCOS.

The effect of EA involves modulation of sympathetic nerve activity (7, 33) and vagal activity (9). Therefore, we analyzed adipose tissue protein expression of NGF, which is known to be a regulator of sympathetic innervation of adipose tissue. Both mNGF and proNGF activate the tyrosine kinase A receptor in supporting sympathetic activity (35), and mNGF regulates adipocyte metabolism *in vitro* (36). In our study, a single bout of EA decreased adipose tissue proNGF and increased the mNGF/proNGF ratio in patients with PCOS, indicating that mNGF activity is stimulated by EA in combination with hyperinsulinemia. It has been reported that proNGF acts as an inducer of sympathetic denervation in peripheral tissues (37, 38). Our data are


 

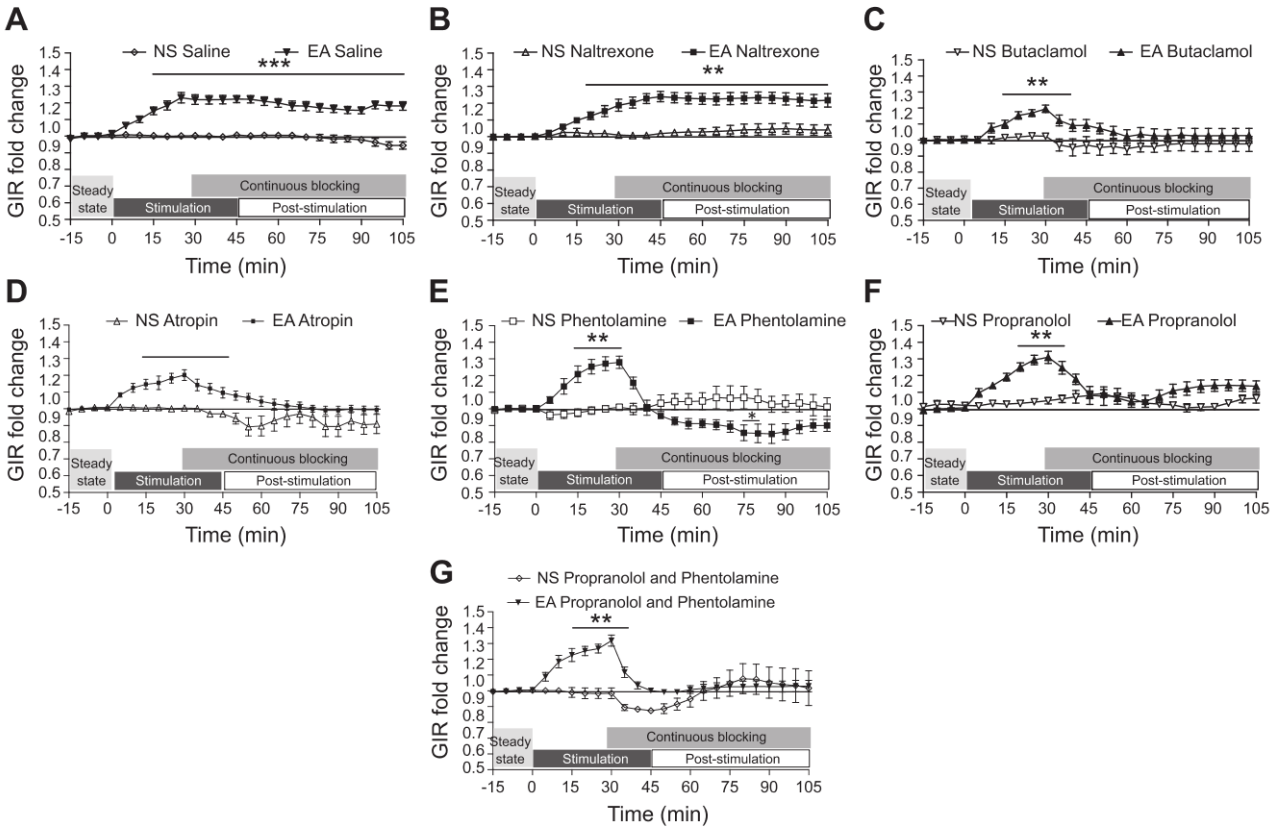


**Figure 4.** Changes in whole-body glucose uptake after a single bout of EA, with or without blockers. Fold change in GIR at steady state, during treatment, and after treatment in rats given no stimulation (NS) or EA together with saline (*A*), naltrexone (opioid receptor blocker) (*B*), butaclamol (dopamine receptor blocker) (*C*), atropine (muscarinic receptor blocker) (*D*) phentolamine (α-adrenergic receptor blocker) (*E*), propranolol (β-adrenergic receptor blocker) (*F*), or propranolol and phentolamine (*G*) i n female rats. The final GIR during steady state is set to 1, and the mean fold changes during treatment and the posttreatment period are presented. The 45–60 min before steady state was reached is excluded. Values are expressed as the mean ± SEM ($n$ = 6–8/EA group; $n$ = 4/NS group). The changes in GIR were determined with repeated-measures ANOVA with the Bonferroni *post hoc* test. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* NS.

consistent with a possible increased conversion of proNGF into mNGF (39), which would support sympathetic activity far beyond the stimulation period with possible long-lasting beneficial effects on adipose tissue metabolism (36, 40) and glucose uptake (26).

Serotonin affects glucose homeostasis and insulin resistance *via* vagal afferent serotonergic neurons and receptors in peripheral tissue. It is increased in type 2 diabetes (41), and HVA is responsive to changes in blood glucose (42). It is noteworthy, therefore, that serotonin and HVA decreased in response to EA in both controls and patients with PCOS, indicating that increased glucose uptake is mediated *via* modulation of the vagal system.

Exercise can increase the glucose uptake rate by either insulin-independent or -dependent mechanisms. Low-frequency EA lowers plasma glucose and increases plasma insulin in normal and type 2 diabetic rats, but not in type 1 diabetic rats, and it can therefore be considered insulin dependent (5, 10). However, the increased response to exogenous insulin after electrical stimulation seen in both types 1 and 2 diabetic rats indicates an insulin-sensitizing effect (43, 44), possibly mediated by increased GLUT4 concentrations in muscle and adipose tissue (10, 44).

We previously demonstrated that a single bout of EA in rats is superior to manual stimulation of the needles, but they are equally effective during the poststimulation period, indicating that it is the activation of sensory afferents rather than muscle contractions *per se* that causes the observed changes (15). Here, we show that the increased glucose uptake in rats is mediated by activation of sympathetic nerves because blocking adrenergic receptors reverses the increased glucose uptake. These results are supported by *in vitro* studies demonstrating that $\alpha_1$- and $\beta_2$-receptor agonists can stimulate GLUT4 translocation in skeletal muscle and white adipose tissue (45). Further, adrenergic stimulation of α- and β-adrenoceptors increases glucose uptake in rodents and antagonists can block this effect.

Electroacupuncture modulates the production of catecholamines in the adrenal glands, leading to increased serum levels of dopamine and noradrenaline in rodents (9), this effect is completely blocked by the dopamine receptor antagonist butaclamol. We show that butaclamol reverses the increased glucose uptake in rats, suggesting that all 3 catecholamines may mediate the enhanced glucose uptake during and after electroacupuncture.

When blood glucose levels fall below the normal basal values, parasympathetic activity stimulates glucagon

secretion as a first response to restore normoglycemia (46). However, parasympathetic innervation of the pancreatic islet is also involved during hyperglycemia in the potentiation of insulin secretion through the release of acetylcholine, which binds to muscarinic receptors on pancreatic β cells. In the current study, the increased glucose uptake in response to EA in rats is partly mediated by activation of parasympathetic nerves because blocking muscarinic receptors with atropine reverses the increased glucose uptake, but with a slower onset compared with adrenergic receptor blockers. These results support previous findings showing that EA can improve glucose tolerance through cholinergic nerve activation in rats, an effect that is modulated by atropine (43).

Phentolamine's primary action is vasodilatation, which lowers arterial blood pressure and induces tachycardia. Propranolol also lowers blood pressure, but its antihypertensive effect is less potent (47). We did not monitor blood pressure in this study, but an obvious question is if possible changes in blood pressure contributed to the decrease in GIR after administration of autonomic blockers in electroacupuncture-stimulated rats. We do not think that any such unspecific effect is important, because we did not see any effect on GIR when the α- or β-adrenergic blockers were given to rats not receiving any acupuncture, which indicates that α- or β-adrenergic blockers do not affect glucose uptake at steady state *via* unspecific effects on blood pressure.

Administration of naltrexone did not block the electroacupuncture-induced increase in GIR. This result does not support previous observations showing that the increased glucose uptake is mediated *via* the endogenous opioid system in rats (10), resulting in activation of specific opioid receptors (48), and that this effect is partly blocked by naloxone, a μ-opioid receptor antagonist (10, 11). This difference may be due to the rather short-term stimulatory effect β-endorphin seems to exert on the pancreas (49).

In summary, we demonstrate here that a single bout of electroacupuncture causes an immediate increase in whole-body glucose uptake in overweight and obese women, both controls and women with PCOS, and this effect is in part mediated *via* activation of the sympathetic and parasympathetic nervous system but not by the opioid receptor system. These results could have important clinical implications for the nonpharmacological treatment of insulin resistance. FJ

## ACKNOWLEDGMENTS

The authors thank Carola Gustafsson (University of Gothenburg) for performing all euglycemic hyperinsulinemic clamp studies in the human experiment as well as tissue biopsies. Professor Elisabet Stener-Victorin (Karolinska Institutet) is the guarantor of this study and had full access to all the data and takes responsibility for the integrity of the data and the accuracy of the data analysis. This work was supported by the Swedish Medical Research Council (Project 2014-2775, to E.S.V.); the Jane and Dan Ohlsson Foundation (to E.S.V.); Wilhelm and Martina Lundgrens's Science Fund (to E.S.V. and A.B.); the Hjalmar Svensson Foundation (to E.S.V. and A.B.); the Adlerbert Research Foundation (to E.S.V. and A.B.); the Novo Nordisk Foundation (NNF16OC0020744) (to E.S.V.); Strategic Research Programme in Diabetes at Karolinska Institutet (to E.S.V.); The Royal Society of Arts and Sciences in Gothenburg (to A.B.), the Swedish federal government under the LUA/ALF agreement ALFGBG-429501 (to E.S.V.); and the regional agreement on medical training and clinical research (to A.L.F.) between Stockholm County Council and Karolinska Institutet (to E.S.V.). M.M. thanks the Becas Chile Programme and the University of Chile for financial support through a postdoctoral fellowship. M. S. and V. P. thank the Italian National Research Council (CNR) Short Mobility Program 2015 for mobility fellowships. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript. The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

E. Stener-Victorin and A. Benrick designed the study, acquired and analyzed the data, and wrote the manuscript; M. Kokosar, M. Hu, M. Larsson, M. Maliqueo, M. Soligo, and V. Protto, acquired the data contributed to the discussion, and reviewed/edited the manuscript; R. R. Marcondes, and E. Jerlhag acquired the data and reviewed and edited the manuscript; A. Zaxonova screened all of the study subjects and reviewed and edited the manuscript; and C. J. Behre, K. Højlund., and P. Thorén contributed to the study design, acquired the data, and reviewed/edited the manuscript.

## REFERENCES

1. Hawley, J. A., Hargreaves, M., Joyner, M. J., and Zierath, J. R. (2014) Integrative biology of exercise. *Cell* **159**, 738–749
2. Pedersen, S. B., Lund, S., Buhl, E. S., and Richelsen, B. (2001) Insulin and contraction directly stimulate UCP2 and UCP3 mRNA expression in rat skeletal muscle in vitro. *Biochem. Biophys. Res. Commun.* **283**, 19–25
3. Schlaich, M., Straznicky, N., Lambert, E., and Lambert, G. (2015) Metabolic syndrome: a sympathetic disease? *Lancet Diabetes Endocrinol.* **3**, 148–157
4. Kagitani, F., Uchida, S., and Hotta, H. (2010) Afferent nerve fibers and acupuncture. *Auton. Neurosci.* **157**, 2–8
5. Higashimura, Y., Shimoju, R., Maruyama, H., and Kurosawa, M. (2009) Electro-acupuncture improves responsiveness to insulin via excitation of somatic afferent fibers in diabetic rats. *Auton. Neurosci.* **150**, 100–103
6. Shimoju-Kobayashi, R., Maruyama, H., Yoneda, M., and Kurosawa, M. (2004) Responses of hepatic glucose output to electro-acupuncture stimulation of the hindlimb in anaesthetized rats. *Auton. Neurosci.* **115**, 7–14
7. Stener-Victorin, E., Fujisawa, S., and Kurosawa, M. (2006) Ovarian blood flow responses to electroacupuncture stimulation depend on estrous cycle and on site and frequency of stimulation in anesthetized rats. *J. Appl. Physiol.* **101**, 84–91
8. Stener-Victorin, E., Kobayashi, R., and Kurosawa, M. (2003) Ovarian blood flow responses to electro-acupuncture stimulation at different frequencies and intensities in anaesthetized rats. *Auton. Neurosci.* **108**, 50–56
9. Torres-Rosas, R., Yehia, G., Peña, G., Mishra, P., del Rocio Thompson-Bonilla, M., Moreno-Eutimio, M. A., Arriaga-Pizano, L. A., Isibasi, A., and Ulloa, L. (2014) Dopamine mediates vagal modulation of the immune system by electroacupuncture. *Nat. Med.* **20**, 291–295
10. Chang, S. L., Lin, J. G., Chi, T. C., Liu, I. M., and Cheng, J. T. (1999) An insulin-dependent hypoglycaemia induced by electroacupuncture at the Zhongwan (CV12) acupoint in diabetic rats. *Diabetologia* **42**, 250–255
11. Lin, J.-G., Chen, W.-C., Hsieh, C.-L., Tsai, C.-C., Cheng, Y. W., Cheng, J.-T., and Chang, S.-L. (2004) Multiple sources of endogenous opioid peptide involved in the hypoglycemic response to 15 Hz electroacupuncture at the Zhongwan acupoint in rats. *Neurosci. Lett.* **366**, 39–42
12. Kaufman, M. P., Waldrop, T. G., Rybicki, K. J., Ordway, G. A., and Mitchell, J. H. (1984) Effects of static and rhythmic twitch contractions


 

on the discharge of group III and IV muscle afferents. *Cardiovasc. Res.* **18**, 663–668
13. Johansson, J., Feng, Y., Shao, R., Lönn, M., Billig, H., and Stener-Victorin, E. (2010) Intense electroacupuncture normalizes insulin sensitivity, increases muscle GLUT4 content, and improves lipid profile in a rat model of polycystic ovary syndrome. *Am. J. Physiol. Endocrinol. Metab.* **299**, E551–E559
14. Liang, F., and Koya, D. (2010) Acupuncture: is it effective for treatment of insulin resistance? *Diabetes Obes. Metab.* **12**, 555–569
15. Benrick, A., Maliqueo, M., Johansson, J., Sun, M., Wu, X., Mannerås-Holm, L., and Stener-Victorin, E. (2014) Enhanced insulin sensitivity and acute regulation of metabolic genes and signaling pathways after a single electrical or manual acupuncture session in female insulin-resistant rats (published correction in *Acta Diabetol.* 52, 219–220). *Acta Diabetol.* **51**, 963–972
16. Belivani, M., Lundeberg, T., Cummings, M., Dimitroula, C., Belivani, N., Vasilakos, D., and Hatzitolios, A. (2015) Immediate effect of three different electroacupuncture protocols on fasting blood glucose in obese patients: a pilot study. *Acupunct. Med.* **33**, 110–114
17. Diamanti-Kandarakis, E., and Dunaif, A. (2012) Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocr. Rev.* **33**, 981–1030
18. Al-Shawi, R., Hafner, A., Chun, S., Raza, S., Crutcher, K., Thrasivoulou, C., Simons, P., and Cowen, T. (2007) ProNGF, sortilin, and age-related neurodegeneration. *Ann. N. Y. Acad. Sci.* **1119**, 208–215
19. Howard, L., Wyatt, S., Nagappan, G., and Davies, A. M. (2013) ProNGF promotes neurite growth from a subset of NGF-dependent neurons by a p75NTR-dependent mechanism. *Development* **140**, 2108–2117
20. Wang, M., Wang, Q., and Whim, M. D. (2016) Fasting induces a form of autonomic synaptic plasticity that prevents hypoglycemia. *Proc. Natl. Acad. Sci. USA* **113**, E3029–E3038
21. Holz, R. W., and Anantharam, A. (2016) Food deprivation induces presynaptic plasticity in the autonomic nervous system. *Proc. Natl. Acad. Sci. USA* **113**, 5766–5767
22. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Fertil. Steril.* **81**, 19–25
23. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum. Reprod.* **19**, 41–47
24. DeFronzo RA, Tobin, J. D., and Andres R. (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol.* **237**, E214–E223
25. Bergman, R. N., Finegood, D. T., and Ader, M. (1985) Assessment of insulin sensitivity in vivo. *Endocr. Rev.* **6**, 45–86
26. Stener-Victorin, E., Maliqueo, M., Soligo, M., Protto, V., Manni, L., Jerlhag, E., Kokosar, M., Sazonova, A., Behre, C. J., Lind, M., Ohlsson, C., Højlund, K., and Benrick, A. (2016) Changes in HbA1c and circulating and adipose tissue androgen levels in overweight-obese women with polycystic ovary syndrome in response to electro-acupuncture. *Obes. Sci. Pract.* **2**, 426–435
27. Prieto-Garcia, L., Egecioglu, E., Studer, E., Westberg, L., and Jerlhag, E. (2015) Ghrelin and GHS-R1A signaling within the ventral and laterodorsal tegmental area regulate sexual behavior in sexually naïve male mice. *Psychoneuroendocrinology* **62**, 392–402
28. Nilsson, M. E., Vandenput, L., Tivesten, Å., Norlén, A. K., Lagerquist, M. K., Windahl, S. H., Börjesson, A. E., Farman, H. H., Poutanen, M., Benrick, A., Maliqueo, M., Stener-Victorin, E., Ryberg, H., and Ohlsson, C. (2015) Measurement of a comprehensive sex steroid profile in rodent serum by high-sensitive gas chromatography-tandem mass spectrometry. *Endocrinology* **156**, 2492–2502
29. Soligo, M., Protto, V., Florenzano, F., Bracci-Laudiero, L., De Benedetti, F., Chiaretti, A., and Manni, L. (2015) The mature/pro nerve growth factor ratio is decreased in the brain of diabetic rats: Analysis by ELISA methods. *Brain Res.* **1624**, 455–468
30. Odén, A., and Wedel, H. (1975) Arguments for Fisher's permutation test. *Ann. Stat.* **3**, 518–520
31. Nahin, R. L., Dahlhamer, J. M., Taylor, B. L., Barnes, P. M., Stussman, B. J., Simile, C. M., Blackman, M. R., Chesney, M. A., Jackson, M., Miller, H., and McFann, K. K. (2007) Health behaviors and risk factors in those who use complementary and alternative medicine. *BMC Public Health* **7**, 217
32. Stener-Victorin, E., Kobayashi, R., Watanabe, O., Lundeberg, T., and Kurosawa, M. (2004) Effect of electro-acupuncture stimulation of different frequencies and intensities on ovarian blood flow in anaesthetized rats with steroid-induced polycystic ovaries. *Reprod. Biol. Endocrinol.* **2**, 16
33. Stener-Victorin, E., Jedel, E., Janson, P. O., and Sverrisdottir, Y. B. (2009) Low-frequency electroacupuncture and physical exercise decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R387–R395
34. Hamada, T., Sasaki, H., Hayashi, T., Moritani, T., and Nakao, K. (2003) Enhancement of whole body glucose uptake during and after human skeletal muscle low-frequency electrical stimulation. *J. Appl. Physiol.* **94**, 2107–2112
35. Masoudi, R., Ioannou, M. S., Coughlin, M. D., Pagadala, P., Neet, K. E., Clewes, O., Allen, S. J., Dawbarn, D., and Fahnestock, M. (2009) Biological activity of nerve growth factor precursor is dependent upon relative levels of its receptors. *J. Biol. Chem.* **284**, 18424–18433
36. Ng, T. B., and Wong, C. M. (1985) Epidermal and nerve growth factors manifest antilipolytic and lipogenic activities in isolated rat adipocytes. *Comp. Biochem. Physiol. B* **81**, 687–689
37. Al-Shawi, R., Hafner, A., Olsen, J., Chun, S., Raza, S., Thrasivoulou, C., Lovestone, S., Killick, R., Simons, P., and Cowen, T. (2008) Neurotoxic and neurotrophic roles of proNGF and the receptor sortilin in the adult and ageing nervous system. *Eur. J. Neurosci.* **27**, 2103–2114
38. Lorentz, C. U., Parrish, D. C., Alston, E. N., Pellegrino, M. J., Woodward, W. R., Hempstead, B. L., and Habecker, B. A. (2013) Sympathetic denervation of peri-infarct myocardium requires the p75 neurotrophin receptor. *Exp. Neurol.* **249**, 111–119
39. Bruno, M. A., and Cuello, A. C. (2006) Activity-dependent release of precursor nerve growth factor, conversion to mature nerve growth factor, and its degradation by a protease cascade. *Proc. Natl. Acad. Sci. USA* **103**, 6735–6740
40. Mannerås, L., Cajander, S., Lönn, M., and Stener-Victorin, E. (2009) Acupuncture and exercise restore adipose tissue expression of sympathetic markers and improve ovarian morphology in rats with dihydrotestosterone-induced PCOS. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **296**, R1124–R1131
41. Hasegawa, Y., Suehiro, A., Higasa, S., Namba, M., and Kakishita, E. (2002) Enhancing effect of advanced glycation end products on serotonin-induced platelet aggregation in patients with diabetes mellitus. *Thromb. Res.* **107**, 319–323
42. Bello, N. T., and Hajnal, A. (2006) Alterations in blood glucose levels under hyperinsulinemia affect accumbens dopamine. *Physiol. Behav.* **88**, 138–145
43. Lee, Y. C., Li, T. M., Tzeng, C. Y., Cheng, Y. W., Chen, Y. I., Ho, W. J., Lin, J. G., and Chang, S. L. (2011) Electroacupuncture-induced cholinergic nerve activation enhances the hypoglycemic effect of exogenous insulin in a rat model of streptozotocin-induced diabetes. *Exp. Diabetes Res.* **2011**, 947138
44. Ishizaki, N., Okushi, N., Yano, T., and Yamamura, Y. (2009) Improvement in glucose tolerance as a result of enhanced insulin sensitivity during electroacupuncture in spontaneously diabetic Goto-Kakizaki rats. *Metabolism* **58**, 1372–1378
45. Dehvari, N., Hutchinson, D. S., Nevzorova, J., Dallner, O. S., Sato, M., Kocan, M., Merlin, J., Evans, B. A., Summers, R. J., and Bengtsson, T. (2012) β(2)-Adrenoceptors increase translocation of GLUT4 via GPCR kinase sites in the receptor C-terminal tail. *Br. J. Pharmacol.* **165**, 1442–1456
46. Thorens, B. (2014) Neural regulation of pancreatic islet cell mass and function. *Diabetes Obes. Metab.* **16**(Suppl 1), 87–95
47. Tabrizchi, R., and Pang, C. C. (1990) Adrenalectomy abolishes antagonism of alpha-adrenoceptor-mediated hypotension by a beta-blocker in conscious rats. *Br. J. Pharmacol.* **101**, 358–362
48. Han, J.-S. (2003) Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. *Trends Neurosci.* **26**, 17–22
49. Curry, D. L., Bennett, L. L., and Li, C. H. (1987) Stimulation of insulin secretion by beta-endorphins (1-27 & 1-31). *Life Sci.* **40**, 2053–2058

*Received for publication December 23, 2016.*
*Accepted for publication March 27, 2017.*

# Autonomic nervous system activation mediates the increase in whole-body glucose uptake in response to electroacupuncture

Anna Benrick, Milana Kokosar, Min Hu, et al.

*FASEB J* published online April 12, 2017
Access the most recent version at doi:10.1096/fj.201601381R