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Improvement in glucose tolerance as a result of enhanced insulin sensitivity during electroacupuncture in spontaneously diabetic Goto-Kakizaki rats

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Abstract

We studied whether electroacupuncture (EA) applied on the abdomen improved glucose tolerance in the Goto-Kakizaki (GK) rat, a genetic model of type 2 diabetes mellitus. Male GK rats and nondiabetic Wistar rats were studied under pentobarbital anesthesia. Blood samples were drawn from the ventral tail artery during the fasting stage and after a glucose load (0.5 g/kg). Electroacupuncture (15 Hz, 10 mA) was performed for 90 minutes during both the fasting and intravenous glucose tolerance test (IVGTT) periods. A hyperinsulinemic euglycemic clamp was also carried out to assess glucose uptake during EA. A significant decrease in fasting blood glucose and an increase in plasma insulin levels were observed during the fasting period in GK rats treated with EA. Blood glucose levels after glucose load were also significantly lower in GK rats treated with EA compared with controls. The homeostasis model assessment index during IVGTT indicated an improvement in insulin sensitivity in GK rats treated with EA, whereas glucose infusion rate during hyperinsulinemic clamp was increased significantly during EA. The present study demonstrated that EA improved hyperglycemia in the fasting stage with a marked increase in plasma insulin levels. Electroacupuncture also restored impaired glucose tolerance during an IVGTT in GK rats by enhancing insulin sensitivity.

1. Introduction

There are several studies in both normal and diabetic rodents that reported that electroacupuncture (EA) applied on the abdomen or on the lower legs has marked hypoglycemic effects by increasing insulin secretion [1-4]. These studies indicate that electrical stimulation of these acupoints increases insulin secretion and decreases plasma glucose levels. Similarly, repeated applications of EA on the abdomen for a few days were shown to result in a prolonged improvement in hyperglycemia in an animal model of diabetes, probably by improving insulin resistance [5]. Based on these studies, we have demonstrated previously that electrical stimulation on the abdomen accelerated glucose consumption during both an intravenous glucose tolerance test (IVGTT) and a hyperinsulinemic euglycemic clamp in nondiabetic Sprague-Dawley rats. In these tests, an increase in both insulin secretion and sensitivity was observed [6]. As these data were obtained from a nondiabetic model, we consider it is necessary to investigate whether these beneficial effects are also observed in a model of type 2 diabetes mellitus. The spontaneously diabetic Goto-Kakizaki (GK) rat is a nonobese model of type 2 diabetes mellitus developed by selective breeding of glucose-intolerant Wistar rats over several generations [7,8]. This model is relevant for investigating the effects of EA on type 2 diabetes mellitus, as defects in both insulin secretion and resistance are seen at 2 to 4 weeks of age. Herein, we report an improvement in glucose tolerance and insulin sensitivity during EA on the abdomen in GK rats.

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2. Research design and methods

2.1. Animals

Male GK rats were purchased from Shimizu Laboratory Supplies (Kyoto, Japan) and studied at 8 to 9 weeks of age (weight, 170-250 g). Age-matched male Wistar rats served as controls. The rats were housed individually in an air-conditioned room with a 12:12-hour light/dark cycle (lights on at 7:30 AM) and allowed ad libitum access to standard rat chow and water. Food was removed from the cages between 4:00 PM and 8:00 PM the night before the experiment to ensure an overnight fast of 12- to 20-hour duration. All procedures in the present study were conducted with approval from the ethics committee of Meiji University of Integrative Medicine.

2.2. Anesthesia and surgical procedure

On the day of the experiment, the rats were anesthetized with an intraperitoneal administration of pentobarbital sodium (40 mg/kg body weight), with anesthesia being maintained by continuous intravenous infusion (0.15-0.30 mg/[kg min]). A dual-tail catheter method [9], modified for the conditions of the present study, was used to minimize the stress of the surgical procedure. Briefly, the tail artery was exposed by a longitudinal incision of approximately 1 cm at the tail root; and a catheter was inserted through a small incision made on the artery and tied with a silk suture. Blood samples were obtained through a Y-shaped connector connected to the catheter filled with saline containing heparin (500 U/mL). Anesthetic, glucose, or insulin was administered through a winged needle (27 × 1/2 G; Terumo, Tokyo, Japan) inserted in the tail vein. Body temperature was monitored at the rectum and was maintained at 37.5°C ± 0.5°C using a thermostatically controlled heating blanket system (Muromachi, Tokyo, Japan).

2.3. Intravenous glucose tolerance test

At least 30 minutes after the surgical operation, a baseline blood sample (250 μL) was drawn. A further blood sample (250 μL) was collected 30 minutes after the initial sampling; and then within 1 minute, 0.5 g/kg glucose (20 wt/vol %; Otsuka Pharmaceutical, Tokyo, Japan) was administered as a bolus through the tail vein catheter. Two minutes later, a 30-μL blood sample was collected for measurement of blood glucose. Blood samples (250 μL) were then collected into Eppendorf tubes containing heparin (10 μg/mL plasma) and aprotinin (100 KIU/mL plasma) at 5, 10, 20, 30, 45, and 60 minutes for the measurement of blood glucose and plasma insulin. The blood samples were centrifuged immediately for 10 minutes at 12 000 rpm at 4°C using a centrifuge system (5417R; Eppendorf, Hamburg, Germany), and the plasma was stored at −40°C until assay. The areas under the curve (AUCs) for blood glucose and plasma insulin, which represented the total glucose level and total insulin secretion during the IVGTT, were determined according to the trapezoidal rule [10]. The homeostasis model assessment (HOMA) index (plasma glucose in millimoles per liter × insulin in microunits per milliliter/22.5) [11] was calculated to evaluate insulin sensitivity at each time point during the IVGTT studies.

2.4. Determination of blood glucose and plasma insulin

Blood glucose levels were measured by the glucose oxidase method using a glucose monitor (GR101, Terumo, Tokyo, Japan). Plasma insulin concentration was determined using a commercially available enzyme-linked immunosorbent assay kit (Shibayagi, Shibukawa, Gunma, Japan).

2.5. Hyperinsulinemic euglycemic clamp

Insulin sensitivity was assessed by the hyperinsulinemic euglycemic clamp [12]. After a blood sample (250 μL) was drawn for determination of fasting blood glucose and plasma insulin levels, an insulin solution (Humulin R; Eli Lilly Japan, Kobe, Japan) diluted in saline containing 0.25 wt/vol % bovine serum albumin (BSA RI, A7888; Sigma, St. Louis, MO) was infused through the venous catheter using a syringe pump (Harvard Apparatus, Holliston, MA) at 15 mU/(kg min) for 0 to 3 minutes, 10 mU/(kg min) for 3 to 6 minutes, and 7 mU/(kg min) for 6 to 10 minutes. This was followed by infusion of the insulin solution at 5 mU/(kg min). Blood samples (30 μL) were drawn at 5-minute intervals throughout the experiment for determination of blood glucose. An infusion of glucose (20 wt/vol %) was started 10 to 15 minutes after the insulin infusion using a peristaltic pump (Minipuls 3; GILSON, Middleton, WI), with the glucose infusion rate (GIR) being adjusted every 5 minutes according to the blood glucose concentration with the objective of maintaining the blood glucose level at 100 mg/dL. A stable GIR was obtained within 60 minutes after insulin infusion and maintained thereafter (ie, steady-state period).

2.6. Electroacupuncture

According to the details of a previous report, EA was performed at 2 points located between the top of the xiphoid process and the upper border of the pubis bond [1]. Acupuncture needles 30 mm in length and 0.32 mm in diameter (Seirin, Shizuoka, Japan) were inserted into the muscle layer to a depth of approximately 4 mm and fixed with glue. Electrical stimulation was carried out at a frequency of 15 Hz and intensity of 10 mA using EA apparatus (Han's Healthronics, Likon, Taipei). The intensity and frequency of the electrical stimulation were monitored with an oscilloscope located at both ends of a resistor (20 Ω) inserted into the circuit. Electroacupuncture was performed for 90 minutes through the IVGTT study, including the fasting period before administration of the glucose load. The needles were retained at the points after termination of the electrical stimulation to avoid unnecessary stimulation caused by removal of the fixed needles. In the hyperinsulinemic euglycemic clamp study, EA was performed for 60 minutes after the baseline data had been obtained; and the

Table 1
Changes in blood glucose and plasma insulin levels during the fasting period

	Group	n	Time (min)				Difference[a] mean (SE)		*P* value[b]
			0		30				
			Mean	(SD)	Mean	(SD)			
Blood glucose (mmol/L)	GK control	14	7.09	(1.08)	6.96	(1.00)	−0.12	(0.14)	.395
	GK + EA	13	6.91	(0.90)	6.27	(0.99)	−0.65	(0.20)	.007
	Nondiabetic Wistar	10	5.35	(0.31)	5.51	(0.37)	+0.16	(0.14)	.272
Plasma insulin (pmol/L)	GK control	13	278.1	(117.6)	308.4	(106.4)	+30.4	(15.8)	.079
	GK + EA	13	235.0	(66.8)	418.5	(150.5)	+183.5	(34.7)	<.001
	Nondiabetic Wistar	10	71.3	(49.4)	94.3	(50.5)	+23.1	(11.6)	.078

Mean (SD) blood glucose and plasma insulin at baseline (0 minute) and 30 minutes later.
[a] Mean (SEM) difference in each value between baseline and 30 minutes.
[b] *P* values calculated by paired *t* tests.

measurements were continued for a further 60 minutes after termination of the electrical stimulation.

2.7. Statistical analysis

Data are expressed as means and standard errors (SEM) unless otherwise stated. Differences in blood glucose and plasma insulin concentrations between baseline (0 minute) and 30 minutes in the fasting period were analyzed using paired *t* tests. One-way analysis of variance (ANOVA) was used to determine differences between the 3 groups in the AUCs of glucose tolerance, insulin concentrations, and HOMA indices at each period. Scheffé multiple comparison was performed to detect significantly different pairs when the F test was significant in the 1-way ANOVA. In the hyperinsulinemic euglycemic clamp study, repeated-measures ANOVA was applied to detect differences in mean GIR before, during, and after EA. Post hoc pairwise comparisons using Bonferroni correction were performed to detect differences between the mean values at each period. Differences were considered statistically significant when the *P* value was less than .05. All the computations were performed with SYSTAT version 11 (SYSTAT Software Inc, San Jose, CA).

3. Results

3.1. Effect of EA during the fasting stage

Changes in blood glucose and plasma insulin levels in each group during the fasting stage are shown in Table 1. Both fasting blood glucose and insulin levels were significantly higher in the GK rats than in the nondiabetic Wistar rats (both *P*s < .001). Mean blood glucose level in the GK rats treated with EA for 30 minutes was decreased significantly, whereas the levels in the GK control and nondiabetic Wistar rats that had not received EA remained

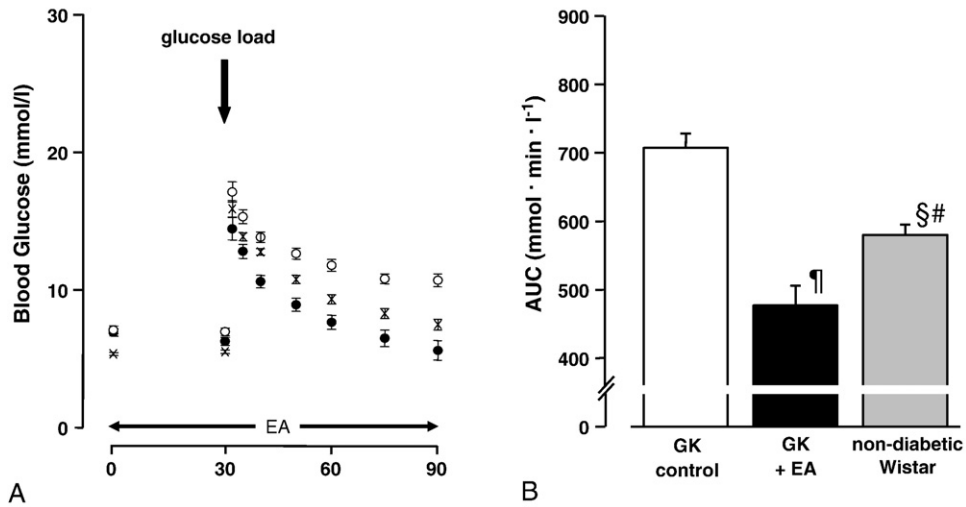

Fig. 1. Changes in blood glucose levels in the IVGTT study. (A) Changes in mean blood glucose level in the IVGTT study. Error bars indicate the SE of the mean (symbols). Glucose (0.5 g/kg) was infused as a bolus within 1 minute into the tail vein. Blood glucose levels after the glucose load were significantly higher in the GK controls (○) than in either the nondiabetic Wistar rats (×) or GK rats treated with EA (●). (B) The AUC after glucose load was significantly lower in the GK rats treated with EA and nondiabetic Wistar rats compared with GK controls. ¶*P* < .001 vs GK control; §*P* = .002 vs GK control; #*P* = .016 vs GK + EA.

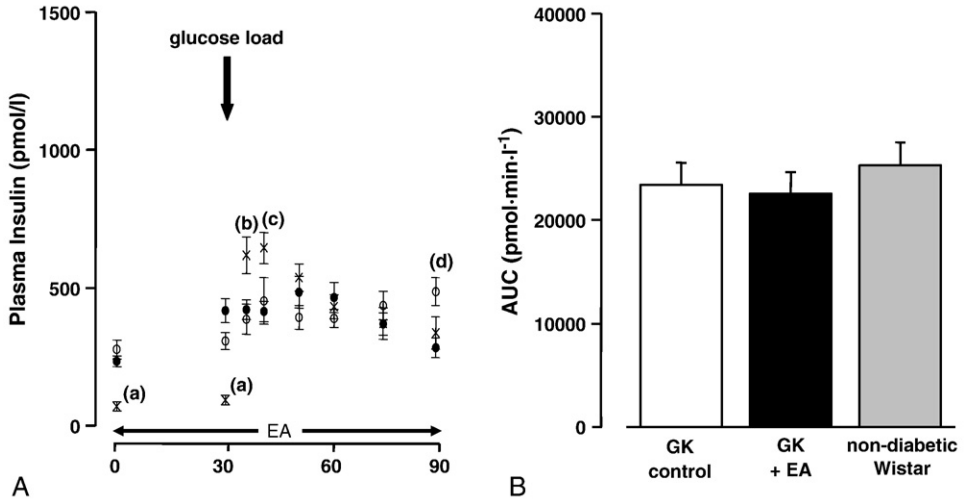

Fig. 2. Changes in plasma insulin levels in the IVGTT study. (A) Changes in mean plasma insulin level. Error bars indicate the SE of the mean (symbols). The first-phase insulin response to the administration of glucose (0.5 g/kg IV) was observed clearly in the nondiabetic Wistar rats (×), whereas this response was blunted in GK rats with (●) and without (○) EA. Plasma insulin levels recovered earlier in GK rats treated with EA than in the GK controls. [(a)] $P < .001$ vs GK controls and GK with EA; [(b)] $P = .011$ vs GK controls and $P = .034$ vs GK with EA; [(c)] $P = .048$ vs GK with EA; [(d)] $P = .009$ vs GK with EA. (B) The AUC after a glucose load was not significantly different between the groups ($P = .656$).

unchanged. Plasma insulin levels also showed a significant increase after EA.

3.2. Effect of EA on blood glucose and plasma insulin levels during the IVGTT study

Changes in blood glucose levels before and after the glucose load and the corresponding AUCs in each group are summarized in Fig. 1. As shown in this figure, it was apparent that recovery of blood glucose levels occurred faster in GK rats treated with EA and nondiabetic Wistar rats compared with recovery in the GK controls (Fig. 1A). This resulted in statistically significant differences in the corresponding AUCs between the 3 groups (Fig. 1B). Similar changes in plasma insulin levels before and after the glucose load are shown in Fig. 2. Mean plasma insulin level in nondiabetic Wistar rats increased rapidly within 10 minutes after the glucose load, whereas levels in the GK rats showed a delayed response to the glucose load ($P = .011$ [Wistar vs GK controls] and $P = .034$ [Wistar vs GK with EA] at 5 minutes after glucose load; $P = .048$ [Wistar vs GK with EA] at 10 minutes after glucose load). This delayed response was not improved by EA stimulation. On the other hand, mean plasma insulin levels in the GK rats treated with EA decreased earlier than levels in the GK controls ($P = .009$ at 60 minutes after the glucose load) (Fig. 2A). However, total insulin secretion after the glucose load, determined as AUC, was not significantly different between the groups (Fig. 2B).

3.3. Effect of EA on HOMA index during IVGTT study

The HOMA indices in each group during the IVGTT study are shown in Table 2. Significantly lower indices were observed in the nondiabetic Wistar rats compared with the GK rats during the fasting period. In the nondiabetic Wistar

Table 2
Changes in the HOMA index during the fasting period and the IVGTT study

Group	n	Fasting period		Time after glucose load (min)					
		0 min	30 min	5	10	20	30	45	60
GK control	13	12.3 (5.8)	13.5 (5.4)	37.4 (19.7)	39.4 (29.6)	31.5 (15.0)	28.4 (10.4)	29.0 (11.2)	33.1 (15.2)
GK + EA	13	10.1 (3.2)	16.1 (5.6)	33.5 (10.8)	27.4 (10.2)	27.2 (13.7)	22.7 (12.1)	15.8[§] (8.6)	11.3[‖] (7.6)
Nondiabetic Wistar	10	2.4* (1.7)	3.2* (1.7)	53.2[†] (18.0)	50.6[‡] (12.3)	35.6 (8.6)	25.1 (8.5)	19.5 (10.1)	16.0[¶] (9.0)

Data are expressed as mean (SD).
* $P < .001$ vs GK controls and GK + EA.
[†] $P = .027$ vs GK + EA.
[‡] $P = .032$ vs GK + EA.
[§] $P = .008$ vs GK control.
[‖] $P = .001$ vs GK control.
[¶] $P = .004$ vs GK control.

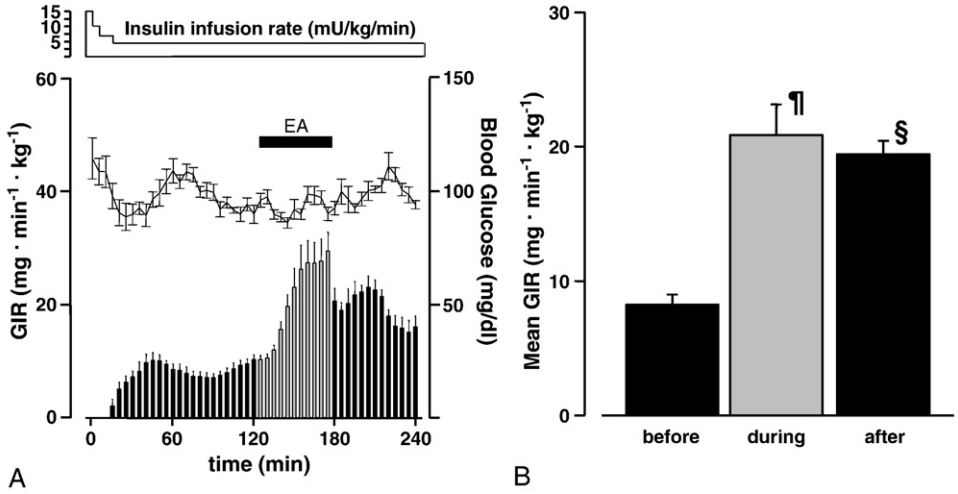

Fig. 3. Changes in GIR in the hyperinsulinemic euglycemic clamp study. (A) Glucose infusion rates are indicated as bars (error bars indicate SE). The corresponding blood glucose levels are shown as lines and error bars (SE). The GIR showed a marked increase during EA stimulation, with the elevated level being maintained after termination of the stimulation. (B) Mean GIR before (0-60 minutes), during (65-120 minutes), and after EA (125-180 minutes). $^{¶}P = .001$ vs before; $^{§}P < .001$ vs before.

rats, HOMA indices were significantly higher during the first 10 minutes after the glucose load, but decreased rapidly after that. In GK rats treated with EA, HOMA indices were significantly lower than those in the GK controls at 45 and 60 minutes after the glucose load.

3.4. Effect of EA on glucose uptake during hyperinsulinemic euglycemic clamp study

Fig. 3 shows changes in GIR during continuous infusion of insulin in the hyperinsulinemic euglycemic clamp study in the GK rats. A steady state was obtained approximately 60 minutes after commencement of insulin infusion. After attainment of the steady state, EA was applied for a further 60 minutes. As shown in the left panel of the figure (Fig. 3A), GIR increased significantly during EA stimulation, with this higher level being maintained for at least 60 minutes after termination of EA. Mean GIRs during and after EA stimulation were significantly higher than the rate measured during the baseline period (Fig. 3B).

4. Discussion

The present study demonstrated, for the first time, that EA has a hypoglycemic effect in spontaneously diabetic GK rats. In the fasting state, mean blood glucose level was significantly higher in GK rats than in Wistar rats, the original parent strain. After application of EA for 30 minutes, baseline blood glucose levels were decreased significantly, accompanied by a significant increase in plasma insulin levels. This hypoglycemic activity, however, was lower than what we observed in earlier studies on normal Sprague-Dawley rats [6] and what Lin et al [3] reported on nondiabetic Wistar rats. Chang et al [1] reported that the hypoglycemic activity of EA during the fasting stage was due primarily to an elevation in plasma insulin levels. This raises the possibility that the existing insulin resistance in the spontaneously diabetic GK rats [13-15] diminished the effectiveness of the increase in insulin induced by EA stimulation. In fact, the increase in insulin secretion that we observed after EA in the present study was comparable with that seen in earlier studies.

Basal insulin secretion in the GK rats in the present study was significantly higher than that in the nondiabetic Wistar rats, a finding that is consistent with a previous report [16]. It is remarkable that EA accelerated insulin secretion even under hyperinsulinemic conditions in the spontaneously diabetic rats. The secretion of insulin is stimulated by vagal nerve fibers and inhibited by sympathetic nerve fibers [17-19]. Because EA has been reported to be capable of altering autonomic functions [20], we presume that activation of the vagal nerve or suppression of sympathetic nerve activity resulted in enhanced insulin secretion in the GK rats. In further investigations, it would therefore be interesting to study these possibilities by administering appropriate blocking agents such as atropine or, alternatively, after a vagotomy.

In the IVGTT study, recovery of blood glucose levels was delayed significantly in the GK rats compared with the nondiabetic Wistar rats. In addition, the first-phase insulin response to the elevation in blood glucose levels was blunted in the GK rats, indicating an impairment in glucose-stimulated β-cell response in this strain [16,21,22]. In contrast, the recovery of blood glucose levels after a glucose load in the GK rats treated with EA was significantly faster than that in GK rats not given EA and nondiabetic Wistar rats. However, the first-phase insulin response in the rats treated with EA remained blunted, resulting in total insulin secretion after the glucose load being similar in all 3 groups. Taken together, these results indicate that the improvement in

glucose tolerance associated with EA stimulation during the IVGTT study was dependent primarily on an increase in insulin sensitivity in peripheral tissues. Evidence of such an effect is our finding that HOMA indices calculated at each time point were lowest in the GK rats that received EA.

The acceleration in glucose uptake during EA was also confirmed by a hyperinsulinemic euglycemic clamp test. Abnormalities in insulin-mediated glucose uptake that are responsible, in part, for glucose intolerance in the GK rats have been reported previously [13,23]. However, whether abnormalities in the insulin-signaling pathway are restored by EA stimulation remains unknown; and the results of the present study do not provide any further information on this point. There is a possibility that contraction of the electrically stimulated skeletal muscle may increase glucose uptake by accelerating the translocation of glucose transporter 4 [24]. However, it remains unclear whether this type of stimulation affects whole-body glucose metabolism, as it has been demonstrated primarily in isolated skeletal muscles [24-26].

Shapira et al [5] demonstrated in diabetic *Psammomys obesus* that 3 applications of EA on the abdomen within 5 days of an intervention period resulted in a sustained improvement in insulin resistance for up to 3 weeks. Whether or not GK rats have a similar sustained effect of EA is an interesting topic for further study, as a sustained improvement in glucose tolerance has the potential to restore abnormalities in the insulin-signaling pathway in skeletal muscles of this strain [14].

There is a possibility that administration of pentobarbital suppresses plasma catecholamine level [27,28], which in turn reduces lipolysis [29] and/or increases insulin secretion through its inhibitory effect on K_{ATP} channels [30], although plasma glucose level and insulin signaling in the peripheral tissue may be less influenced [30,31]. It is therefore considered valuable to determine the effect of EA under conscious state in further studies.

In conclusion, our study demonstrated that EA on the abdomen reduced hyperglycemia with a simultaneous increase in insulin secretion during the fasting stage and also improved glucose tolerance during an IVGTT by enhancing insulin sensitivity in peripheral tissues.

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