Biosci. Biotechnol. Biochem., **74** (12), 2418–2425, 2010

Verification of the Antidiabetic Effects of Cinnamon (*Cinnamomum zeylanicum*) Using Insulin-Uncontrolled Type 1 Diabetic Rats and Cultured Adipocytes

Yan SHEN,[1] Misato FUKUSHIMA,[1] Yoshimasa ITO,[1] Etsuko MURAKI,[2] Takashi HOSONO,[1] Taiichiro SEKI,[1,†] and Toyohiko ARIGA[1]

[1]*Laboratory of Nutrition and Physiology, Department of Chemistry and Life Science, Nihon University College of Bioresource Sciences, Nihon University Graduate School of Bioresource Sciences, Kanagawa 252-0880, Japan*
[2]*Department of Clinical Dietetics and Human Nutrition, Faculty of Pharmaceutical Sciences, Josai University, Saitama 350-0295, Japan*

Received June 17, 2010; Accepted September 6, 2010; Online Publication, December 7, 2010
[doi:10.1271/bbb.100453]

It has long been believed that an intake of cinnamon (*Cinnamomum zeylanicum*) alleviates diabetic pathological conditions. However, it is still controversial whether the beneficial effect is insulin-dependent or insulin-mimetic. This study was aimed at determining the insulin-independent effect of cinnamon. Streptozotocin-induced diabetic rats were divided into four groups and orally administered with an aqueous cinnamon extract (CE) for 22 d. The diabetic rats that had taken CE at a dose of more than 30 mg/kg/d were rescued from their hyperglycemia and nephropathy, and these rats were found to have upregulation of uncoupling protein-1 (UCP-1) and glucose transporter 4 (GLUT4) in their brown adipose tissues as well as in their muscles. This was verified by using 3T3-L1 adipocytes in which CE upregulates GLUT4 translocation and increases the glucose uptake. CE exhibited its anti-diabetic effect independently from insulin by at least two mechanisms: i) upregulation of mitochondrial UCP-1, and ii) enhanced translocation of GLUT4 in the muscle and adipose tissues.

Key words: cinnamon; type-1 diabetes; uncoupling protein-1 (UCP-1); glucose transporter 4 (GLUT4); adipocyte

Type 1 diabetes mellitus is one of the serious endocrine diseases of childhood and adolescence.[1,2] The incidence of this type of disease varies between countries and different ethnic groups with a range from 0.1 to 37.4/100,000 among children from 0 to 14 years old.[1] The administration of insulin is the only and best therapeutic way of achieving metabolic balance and ensuring the well being of patients. Evidence shows that an improved blood glucose level reduces the risk of such chronic complications as microvascular, neurological, and macrovascular abnormalities.[2,3] The daily management of type 1 diabetes mellitus with the antidiabetic drug is, however, burdensome, and there are incidences of metabolic decompensation and long-term complications, including retinopathy, nephropathy, and cardiovascular diseases, leading to their increased morbidity and mortality.[4,5] Preventing or ameliorating these complications through daily eating habits is the major objective of this research.

Cinnamon is one of the oldest herbal treatments that has been mentioned in Chinese medicine since as long as 4000 years ago.[6] There have been several recent studies on cinnamon, focusing on improving the serum glucose and lipid levels in type 2 diabetic subjects.[7,8] It is known from *in vitro* studies that a cinnamon extract potentiated the action of insulin in isolated adipocytes and an adipocyte cell line.[9–11] Cinnamon has also increased endogenous insulin production, and ameliorated the nephropathy occurring sometimes at the early stage of diabetes.[12–14] Most of the studies on cinnamon have therefore confirmed its diabetic effect to be insulin-potentiating, although the effects independent from insulin have not yet been elucidated. Altschuler *et al.* have performed a prospective, double-blind, placebo-controlled study on cinnamon using adolescent type 1 diabetic subjects, and no significant effect on suppressing hemoglobin A1c was found.[15] Even their study itself could not prove the antidiabetic effect of cinnamon, since they studied subjects under insulin control.

It is thus necessary to demonstrate the antidiabetic effect of cinnamon on the insulin-uncontrolled type 1 diabetic model. The objective of this present study was to determine the effect of a hot water extract of cinnamon on streptozotocin (STZ)-induced type 1 diabetic model rats, and to elucidate the detailed mechanism by which cinnamon exhibits its antidiabetic activity independently from insulin.

Materials and Methods

Preparation of the cinnamon extract. Cinnamon (*Cinnamomum zeylanicum*) was provided in stick form by House Foods Corporation (Tokyo, Japan). The sticks (250 g) were soaked into 2,500 ml of water for 24 h at room temperature and then heated for 30 min at 100 °C. The cinnamon extract was lyophilized and stored at −20 °C until needed. The dried cinnamon extract was reconstituted with water and diluted to meet the needs of being given every 2–3 d. We termed this solution as

† To whom correspondence should be addressed. Fax: +81-466-84-3949; E-mail: tseki@brs.nihon-u.ac.jp

Abbreviations: AMPK, AMP kinase; BAT, brown adipose tissue; CE, cinnamon aqueous extract; GLUT4, glucose transporter 4; HDL, high-density lipoprotein; HPLC, high-performance liquid chromatography; NEFA, non-esterified fatty acid; STZ, streptozotocin; TRPA1, transient receptor potential A1; UCP-1, uncoupling protein-1

the aqueous cinnamon extract (CE). CE was analyzed by high-performance liquid chromatography (HPLC) in an MG-II C18 column (5 μm, φ 4.6 × 250 mm; Shiseido, Tokyo, Japan), using an LC-10A pump (Shimadzu, Kyoto, Japan) and a Chromato-Pro integrator (Run Time, Kanagawa, Japan). The solvent was a combined solution of water and acetonitrile (2:3), and CE applied to the column was eluted isocratically at a flow rate of 0.5 ml/min, the substances being optically detected at 254 nm.

Animals and diets. Six-week-old male Wistar rats weighing 120–140 g were purchased from Japan SLC (Hamamatsu, Shizuoka, Japan) and housed individually in stainless steel wire-bottomed cages in a temperature-controlled room at 22–23 °C with a 12-h photoperiod. The rats were acclimatized for 1 week with a CE-2 diet (Clea Japan, Tokyo, Japan) and then treated with streptozotocin (STZ, Wako Pure Chemicals, Osaka, Japan) by a single intraperitoneal injection of 55 mg/kg of body weight. These rats formed the STZ-diabetic group (n = 20). Control rats were injected with the same volume of a sodium citrate buffer, the solvent for STZ, and formed the normal healthy animal group (n = 10). The diet was then changed to AIN-93G (Oriental Yeast, Tokyo, Japan), and water was supplied *ad libitum* throughout the experimental period for 10 d before giving CE. The rats were identified to be diabetic 10 d after the STZ injection based on a fasting blood glucose level higher than 220 mg/dl. The rats were then assigned to six groups: i) the normal rat group without cinnamon (N); ii) the normal, but with cinnamon group (NCE), cinnamon being administered *via* a catheter at a dose of 30 mg/kg, since they did not take enough water to provide the required daily dose; iii) the STZ-diabetic rat group without cinnamon (CE0); and iv)–vi) the STZ-diabetic rat groups dosed with varying amounts of cinnamon: 3 mg/kg (CE3), 30 mg/kg (CE30), and 100 mg/kg (CE100). To these CE-administered groups, cinnamon was given through water bottles containing the required daily dose, these being replaced with new water bottles to provide sufficient water. The CE administration was continued for 22 d, and every group of rats was sacrificed under anesthesia with sodium pentobarbital (Tokyo Kasei, Tokyo, Japan). Blood was withdrawn by cardiac puncture, and the gastrocnemius muscle from the hind legs and interscapular brown adipose tissue (BAT) were harvested and stored at −80 °C until needed. All animal experiments were performed in accordance with the Guidelines for Animal Experiments of the College of Bioresource Sciences at Nihon University.

Measurement of glucose and insulin. Blood glucose was measured by a Dexter-Z II (Bayer Medical, Leverkusen, Germany), using tail blood. Insulin was assayed by an ELISA kit (Shibayagi, Gunma, Japan) which enabled rat insulin to be measured in a concentration range of 0.156–10 ng/ml. The plasma total cholesterol (TC), triglyceride (TG), non-esterified fatty acid (NEFA), HDL-cholesterol and creatinine concentrations were determined by using commercially available assay kits (Wako Pure Chemicals).

Fractionation of BAT into the mitochondrial plasma membrane and cytosol fractions. The adipose tissue was fractionated essentially according to the methods of Chappell and Hansford[16] and Cunningham *et al.*[17] Interscapular BAT was homogenized with a Dounce homogenizer in an ice-cold sucrose-Tris-ethylene glycol (STE) buffer (pH 7.4) containing 250 mM sucrose, 5 mM Tris–HCl, 2 mM ethylene glycol tetraacetic acid (EGTA), and a protease inhibitor mixture (Sigma). The homogenate was centrifuged at 800 × *g* for 3 min at 4 °C to remove the tissue debris, and the resulting supernatant was recentrifuged at 12,000 × *g* for 10 min at 4 °C to produce a mitochondrial pellet. This pellet was resuspended in the STE buffer and subjected to western blotting to determine the UCP-1 and cytochrome *c* proteins. The supernatant above the pellet was then centrifuged at 35,000 × *g* for 60 min at 4 °C, and the resulting precipitate and supernatant were respectively obtained as the adipose tissue plasma membrane and cytosol for assaying the GLUT4 protein contents.

Isolation of the plasma membrane from muscle. The gastrocnemius muscle was homogenized by the Dounce homogenizer in an ice-cold HES buffer at pH 7.4, which had been prepared from 0.02 M HEPES, 0.25 M sucrose and 2 mM EGTA, and then centrifuged at 700 × *g* for 10 min. The resulting pellet was resuspended in an HES buffer containing a protease inhibitor mixture (Sigma), before being sonicated for 5 min at 3 kHz/130 W (UCD-130TM, Cosmo Bio, Tokyo, Japan), and centrifuged at 760 × *g* for 5 min. The supernatant was recentrifuged at 35,000 × *g* for 60 min, and the resulting pellet was used as the plasma membrane from the muscle. The supernatant was used as the muscle cytosol fraction.[18] These membrane and cytosol fractions were subjected to western blotting for GLUT4. The amounts of protein in the cytosol fraction and membrane pellet were quantified by a Protein Assay Dye reagent (Bio-Rad Laboratories, PA, USA).

Western blot analysis. The mitochondrial plasma membrane and cytosol fractions obtained from both BAT and muscle were subjected to SDS–PAGE, and the proteins that migrated were electrically transferred to a cellulose nitrate membrane (Advantec Toyo Kaisha, Tokyo, Japan) for western blotting. The membrane was incubated with anti-UCP-1 (1:1000; Abnova, Taiwan), mouse anti-cytochrome *c* (1:1000; Cell Signaling Technology, MA, USA), and the anti-GLUT4 antibody (1:1000; EMD Chemicals, NJ, USA) at 4 °C for 18 h. After this incubation, an anti-mouse IgG horseradish peroxidase conjugate (HRP, 1:2000 for UCP-1) or anti-rabbit IgG HRP (1:2000 for cytochrome *c* and GLUT4; DakoCytomation, Glostrup, Denmark) was added, and the culture allowed to stand for 30 min at room temperature. The antigenic proteins on the membrane were visualized by chemiluminescence, using a Lumi-LightPLUS (Roche Diagnostics, Basel, Switzerland), and the images were analyzed by an LAS-4000 image analyzer (Fujifilm, Tokyo, Japan).

Histochemical analysis. Tissue samples obtained from the kidney were immediately fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) at pH 7.4. Kidney sections (2 μm thick) were stained with haematoxylin and eosin (H-E), and digital images of the glomeruli and interstitial areas microscopically obtained (×400 magnification) were analyzed. The glomerular cross-sectional area (A_G) was measured in six glomerular profiles per rat kidney by using the Java image processing program (National Institutes of Health, MD, USA). The glomerular volume (V_G) was then calculated from the A_G value by the equation, $V_G = \beta/K[A_G]^{3/2}$, where $\beta = 1.38$, the size distribution coefficient, and $K = 1.1$, the shape coefficient for glomeruli idealized as spheres.[19]

Cell culture. The 3T3-L1 fibroblasts were cultured and differentiation induced into adipocytes by the method described previously.[20] To determine the effect of CE on GLUT4 translocation, differentiated 3T3-L1 cells were serum-starved for 16 h, and washed three times with PBS at pH 7.4. The medium was replaced by DMEM alone, or by DMEM containing either insulin (10 or 100 nM) or CE (10 or 30 μg/ml) or by a combination of insulin and CE. After treating with these stimulants for 30 min, the cells were sonicated to isolate the plasma membranes by the method already described. Lipid droplets appearing in the matured adipocytes were colorimetrically quantified after being fixed with formaldehyde and stained with 0.5% Oil-Red O according to the method of Nagasawa *et al.*[21]

Measurement of glucose uptake. The glucose uptake was measured by the method described by Ragolia *et al.*[22] Briefly, 3T3-L1 adipocytes were serum-starved in DMEM for 16 h, before the cells were washed three times with PBS at pH 7.4 and then incubated for 30 min in DMEM alone, DMEM containing either 100 nM insulin or 30 μg/ml of CE, or both. A 0.5 mM amount of 2-deoxy-D-[2,6-^{3}H]glucose (1.5 μCi/well, GE Healthcare, Tokyo, Japan) was then added to the cells which were incubated for 15 min. The cells were then washed four times with PBS containing 0.3 mM phloretin and lysed with 1 ml of 1 N NaOH for scintillation counting.

Statistical analysis. Each result is expressed as the mean ± SE. The statistical comparison between groups was carried out by ANOVA with Dr. SPSS II 2002 software (SPSS Japan, Tokyo). Differences were considered significant at $p < 0.05$.

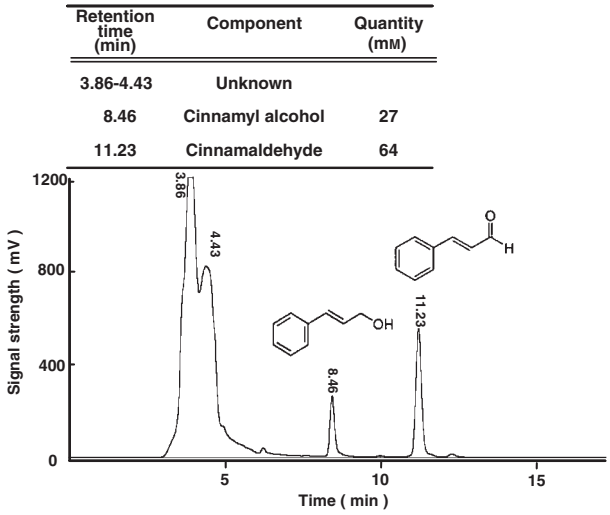

Fig. 1. Major Components of the Aqueous Cinnamon Extract (CE) Used in This Study.

The HPLC pattern shows two well-separated peaks coinciding with authentic cinnamaldehyde and cinnamyl alcohol in their retention times.

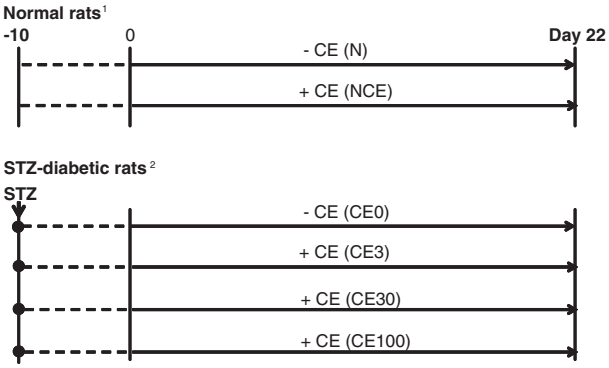

Fig. 2. STZ Treatment and CE Administration Schedule.

[1]Normal rat groups: N, normal rats not taking CE; and NCE, normal rats taking CE at a dose of 30 mg/kg of body weight/d. [2]STZ-treated diabetic rat groups: CE0, not taking CE; CE3, taking 3 mg of CE; CE30, taking 30 mg of CE; and CE100, taking 100 mg of CE/kg/d. The administration of CE lasted for 22 d either *via* drinking water for the diabetic group of rats or *via* a catheter for the normal rats.

Results

Effect of administering the aqueous cinnamon aqueous extract on the body weight and fasting blood glucose level of STZ-induced diabetic rats

The aqueous cinnamon extract prepared from cinnamon sticks was determined to contain 8.5 mg/ml (64 mM) of cinnamaldehyde and 3.6 mg/ml (27 mM) of cinnamyl alcohol by referring to the authentic compounds which have been reported as antidiabetic principles in cinnamon (Fig. 1).[23)]

The effect of CE administration on the body weight and fasting blood glucose level was studied. After being treated with STZ, the rats were raised for 10 d to complete their diabetic state. The CE administration was then started through their drinking water for 22 d (see Fig. 2 for the grouping and treatment of the rats). The body weight of the CE-administered normal rats (30 mg/kg, NCE in Table 1) increased at a rate similar to that of the CE-free normal rats in 22 d, *i.e.*, both groups of rats gained about 120% of the initial weight in this period. CE had no effect on the growth of the normal rats even after feeding for 22 d. In contrast, the STZ-diabetic rats lost from 153.2 ± 5.2 g to 134.8 ± 4.3 g of body weight during feeding for 22 d. However, this weight loss was attenuated by higher doses of CE of 30 mg and 100 mg/kg/d (the body weight at day 22 in Table 1). No significant difference was apparent in food consumption between the type 1 diabetic animal group CE0 and the CE3, CE30, or CE100 group (data not shown).

The fasting blood glucose level of the CE-administered normal rats (120.5 ± 7.9 mg/dl) was determined to be in the same range as that of the CE-free normal rats (105.8 ± 4.4 mg/dl). The blood glucose level of the STZ-diabetic rats was 3-fold more than that of the normal rats 10 d after STZ administration (the blood glucose at day 0 in Table 1). The high blood glucose level in these STZ-diabetic rats increased further in the succeeding experimental period for 22 d; however, the administration of CE to these rats for the same period

Table 1. Effect of CE Administration on Body Weight and Blood Glucose in STZ-Diabetic Rats

Group[2]	Body weight (g)[1]		Blood glucose (mg/dl)[1]	
	Day 0	Day 22	Day 0	Day 22
N	191.3 ± 5.0	243.6 ± 7.8	98.3 ± 4.6	105.8 ± 4.4
NCE	202.8 ± 3.1	249.0 ± 6.6	114.3 ± 2.5	120.5 ± 7.9
CE0	153.2 ± 5.2	134.8 ± 4.3	305.3 ± 27.3	580.5 ± 69.3
CE3	157.9 ± 5.2	141.1 ± 3.1	331.8 ± 27.1	433.0 ± 11.3
CE30	157.5 ± 2.1	153.4 ± 3.4*	338.3 ± 66.8	351.8 ± 31.5*
CE100	166.3 ± 3.9	164.9 ± 3.3*	307.3 ± 43.7	378.0 ± 12.8*

[1]Values are the mean $\pm$ SE, n = 4, *significantly different from CE0 value on day 22, $p < 0.05$

[2]The STZ-treated rats were grouped into 4 on the groups at 10th day to give similar levels of blood glucose, and the administration was started of different amounts of CE, 0–100 mg/kg b.w/day, for 22 d (see Fig. 2).

dose-dependently attenuated their hyperglycemia. There was a marked difference between the CE-free rats (CE0) and CE-administered rats (CE3–CE100). In particular, the blood glucose level in the CE30 and CE100 rat groups was significantly lower than that in the CE0 rats.

The aqueous cinnamon extract attenuated the organ weight loss in STZ-diabetic rats

The STZ-treated rats had mostly smaller organs than those of the untreated normal rats. Among the organs in the STZ-treated rats, the kidneys were unchanged in weight, but BAT was decreased by approximately 80% of that in the normal rats. The administration of CE to these diabetic rats increased not only their body weight but also their organ weights (Table 2). The weights of the liver, spleen, BAT and gastrocnemial muscles of the CE-administered rats (CE100) were significantly more than those of the CE-free animals (CE0). In particular, the respective weights of BAT and the spleen of the CE100 rats were as much as 200% and 170% higher than those the CE0 rats.

The aqueous cinnamon extract ameliorated the diabetic parameters that were deteriorated by the STZ treatment

The plasma insulin concentration of the normal rats was 7.5 ± 2.7 ng/ml, and this level was unchanged by

Table 2. Effect of CE Administration on the Organ Weights of STZ-Diabetic Rats

Group[2]	Organ weight (g)[1]					
	Liver	Pancreas	Spleen	Kidney	BAT	Muscle[3]
N	7.28 ± 0.42	0.86 ± 0.05	0.53 ± 0.03	1.63 ± 0.06	0.45 ± 0.03	2.88 ± 0.06
NCE	7.07 ± 0.22	0.93 ± 0.02	0.58 ± 0.02	1.67 ± 0.05	0.43 ± 0.03	2.92 ± 0.07
CE0	5.05 ± 0.58	0.62 ± 0.07	0.23 ± 0.07	1.79 ± 0.24	0.075 ± 0.02	1.20 ± 0.41
CE3	5.12 ± 0.09	0.72 ± 0.04	0.30 ± 0.02	1.68 ± 0.06	0.102 ± 0.01	1.45 ± 0.05
CE30	5.64 ± 0.22*	0.64 ± 0.04	0.31 ± 0.03	1.69 ± 0.07	0.139 ± 0.02*	1.61 ± 0.08
CE100	6.16 ± 0.20*	0.70 ± 0.05	0.38 ± 0.00*	1.86 ± 0.04	0.146 ± 0.01*	1.82 ± 0.06*

[1]Values are the mean ± SE, n = 4, *significantly different from the value for CE0, $p < 0.05$
[2]The grouping of rats and CE administration were performed as described under Fig. 2.
[3]The muscle sample was the gastrocnemius collected from the bilateral hind legs.

Table 3. Effect of CE Administration on the Diabetic Parameters of STZ-Treated Rat Plasma

Group[1]	NEFA (mEq/l)	HDL (mg/dl)	TC (mg/dl)	TG (mg/dl)	Creatinine (mg/dl)	Protein (g/dl)	Insulin (ng/ml)
N	1.71 ± 0.13	67.88 ± 9.12	79.91 ± 15.68	105.95 ± 46.61	0.856 ± 0.34	7.85 ± 0.47	7.51 ± 2.68
CE0	0.52 ± 0.12	51.06 ± 7.20	77.90 ± 11.46	92.08 ± 38.09	1.31 ± 0.10	5.79 ± 0.47	ND
CE3	0.70 ± 0.10	56.44 ± 7.94	95.20 ± 9.89	83.33 ± 17.46	0.949 ± 0.06	6.18 ± 0.82	ND
CE30	0.83 ± 0.02*	60.38 ± 4.23	95.27 ± 9.86	102.88 ± 14.81	0.937 ± 0.06*	6.50 ± 0.46	ND
CE100	0.84 ± 0.16*	62.96 ± 7.57	87.07 ± 13.64	97.78 ± 29.76	0.977 ± 0.23	7.42 ± 0.81*	ND

[1]The rat groups are termed as shown in Fig. 2. The NCE group was not analyzed except for the insulin assay. NEFA, non-esterified fatty acid; HDL, high-density lipoprotein; TC, total cholesterol; TG, total glyceride; Protein, plasma protein. Each value is the mean ± SE, n = 4, *$p < 0.05$.

the CE administration (6.3 ± 1.0 ng/ml; not shown in the figure). On the other hand, plasma insulin in the STZ-treated rats was undetectable. Since the minimum detection limit for insulin by the method was 0.156 ng/ml, these rats lost 98% or more insulin in 32 d from the first day of toxification, and thus, all the STZ-treated rats were likely to be exhausted in most of the diabetic parameters tested. However, the administration of CE had a beneficial effect on these rats (Table 3). The concentrations of non-esterified fatty acids (NEFA) and protein were significantly increased by a high dose of CE (CE30 and/or CE100). The other lipid parameters, HDL, TC and TG, tended to be improved. The decrease in creatinine level by CE administration may reflect the improvement of diabetes in these rats.

The aqueous cinnamon extract ameliorated the histological abnormality of the glomerulus in the kidneys of STZ-diabetic rats

Histological observation of the kidney sections showed that the STZ-diabetic rats had hypertrophic glomeruli with increased tubular and interstitial volumes as shown in Fig. 3A and B. CE administration to the diabetic rats clearly ameliorated such hypertrophy in the glomerulus and tubular surroundings (Fig. 3C–E). Since this amelioration seemed likely to be dose dependent, we performed a morphometric analysis on the kidney specimens to evaluate the change in glomerular volume, V_G, as described in the Materials and Methods section. V_G in the STZ-treated diabetic rats was about 3-times more than that in the normal rats. However, the administration of CE of 30 and 100 mg/kg/d significantly reduced V_G to a level that was nearly normal (Fig. 3F).

The aqueous cinnamon extract increased UCP-1 expression and GLUT4 translocation in BAT of STZ-diabetic rats

The UCP-1 protein level in BAT of normal rats was in the low range and not increased by taking CE (N and NCE in Fig. 4A and B). However, UCP-1 protein was above the normal level in the STZ-diabetic rats (N *vs.* CE0 in Fig. 4), and the administration of CE to these rats elevated its level far above normal. There was significant elevation of UCP-1 (Fig. 4), especially at the highest dose of CE (CE100).

The GLUT4 protein level in BAT of normal rats was highly expressed in both the plasma membrane and cytosolic fractions of this tissue (N in Fig. 5). The STZ treatment markedly suppressed the GLUT4 level, and its level in the plasma membrane became around 1/2 of that in the normal rats (CE0 *vs.* N). The administration of CE to normal rats did not affect the GLUT4 level (NCE); however, administration to the STZ-diabetic rats significantly increased the GLUT4 level which was accompanied by a decrease in GLUT4 in the cytosolic fraction, demonstrating that CE could stimulate GLUT4 translocation (see Fig. 5B and C, CE100). It has been reported that the stimulation by insulin of GLUT4 translocation to the plasma membrane did not affect the amount of total cellular GLUT4.[24] Although we were not able to determine the total cellular GLUT4 protein content due to the limitation of BAT samples, it was clearly demonstrated that CE significantly stimulated the translocation of GLUT4 to the plasma membrane of BAT.

The aqueous cinnamon extract stimulated GLUT4 translocation in the muscle

We next measured the amount of GLUT4 protein in the muscle of CE-administered rats. Since muscle is the largest organ in the animal body, it incorporates more than 80% of the glucose taken.[25] If CE stimulated the GLUT4 translocation, it would be extremely beneficial to ameliorate diabetic hyperglycemia. To verify the translocation of GLUT4 from the cytosol to membrane, we also fractionated the gastrocnemial muscle into its plasma membrane and cytosolic fractions, and determined the amounts of GLUT4 protein in both these

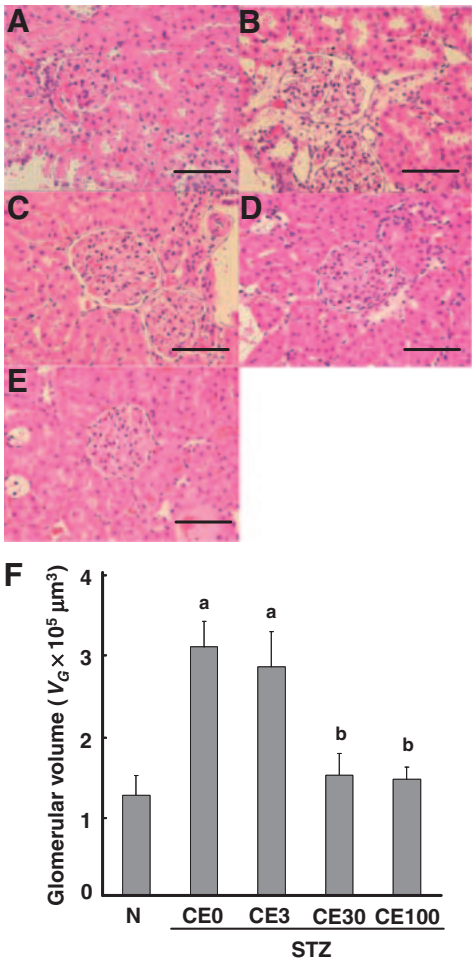

Fig. 3. Representative Micrographs of Kidney Tissues Stained with Haematoxylin and Eosin, and Glomerular Volume (V_G) Measured Morphometrically.

Panels: A, normal rat kidney; B, STZ-treated CE-free rat kidney; C–E, STZ-treated and CE-administered rat kidney specimens: C, CE3; D, CE30 and E, CE100 (see Fig. 1 for the group names). All the specimens were prepared from the kidneys on day 22 after administering CE. The scale bar in each panel is 8.5 μm; F, V_G value measured by the morphometric analysis of 10 glomeruli in each specimen. Error bars represent the mean ± SE. The significant difference between the bars with a and b is $p < 0.01$.

fractions (Fig. 6A). It was apparent that CE stimulated GLUT4 translocation to the membrane (Fig. 6B). On the other hand, the GLUT4 content in the cytosol was also significantly increased in the diabetic animals took the highest amount of CE, suggesting CE also enhanced GLUT4 production in the muscle (Fig. 6C). In fact, the total GLUT4 protein content in the lysate of the gastrocnemial muscle was significantly higher in the CE100 group than in the CE0 group (data not shown). We obtained here striking results that the GLUT4-elevating effect of cinnamon was demonstrated only on the STZ-diabetic rats, and had almost no effect on normal rats.

The aqueous cinnamon extract up-regulated GLUT4 expression and stimulated glucose uptake by differentiated 3T3-L1 adipocytes

An adipocyte phenotype differentiated from the mouse 3T3-L1 fibroblastic cell line (see Fig. 7A) was serum-starved for 16 h in DMEM, and then challenged for 30 min by either insulin or CE or both to assess the

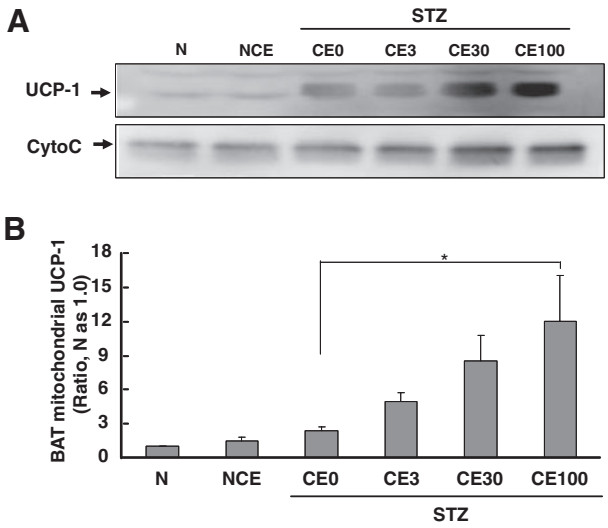

Fig. 4. The Aqueous Cinnamon Extract Elevated Mitochondrial UCP-1.

A, western blotting of UCP-1 and cytochrome *c* in the protein extracts from BAT obtained from CE-administered normal and STZ-diabetic rats. A representative western blot is shown. The rat groups are termed as already described. B, densitometric analysis of the blots on three separate gel samples. The values obtained were normalized to those of cytochrome *c*, and are presented as the mean ± SE, n = 3, $^*p < 0.05$.

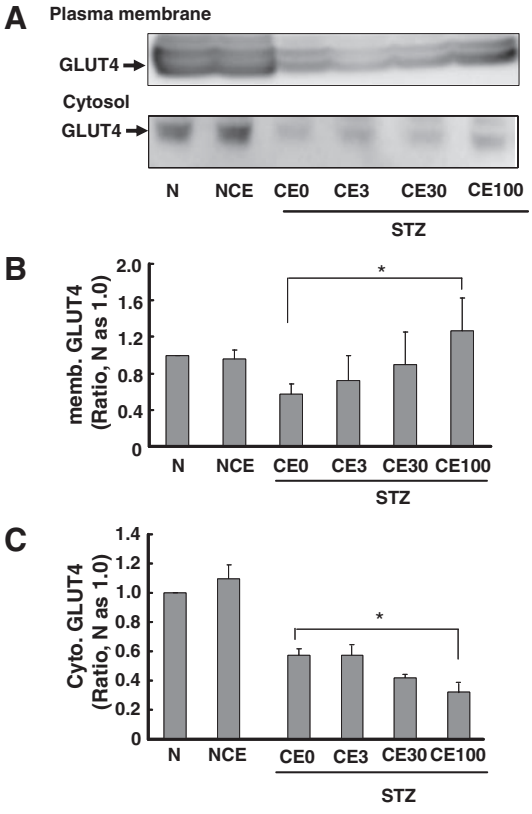

Fig. 5. The Aqueous Cinnamon Extract Stimulated GLUT4 Translocation in BAT of the STZ-Diabetic Rats.

A, the amounts of GLUT4 protein in the plasma membrane and cytosolic fractions of BAT were analyzed by western blotting. B and C, relative blot intensity analyzed as above for GLUT4 protein from the plasma membrane (B) and that from the cytosolic fraction (C). Each value is the mean ± SE, n = 3, $^*p < 0.05$.

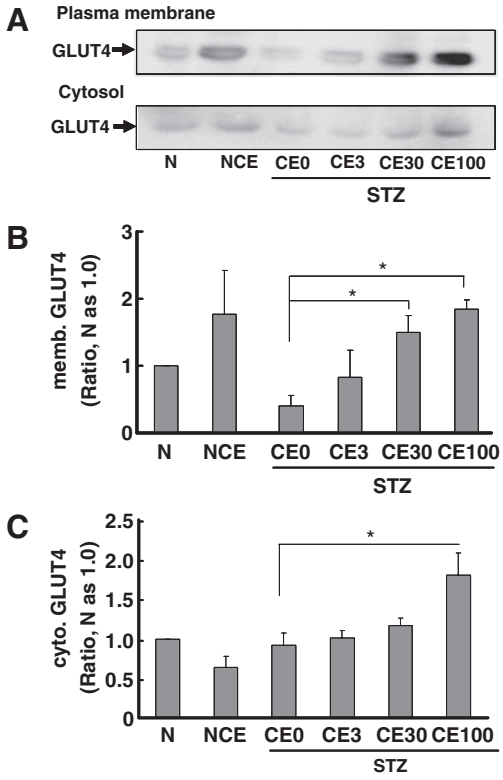

Fig. 6. CE Administration Increased the Expression of GLUT4, and Stimulated Its Translocation to the Plasma Membrane in Gastrocnemius Muscle.

The gastrocnemius muscle of the CE-administered rats was analyzed after fractionation into the cytosolic and plasma membrane fractions as described in the Materials and Methods section. A, western blotting of GLUT4 in the plasma membrane fraction (the upper gel samples), and that of GLUT4 in the cytosolic fraction (the lower gel). B and C, calculated values for GLUT4 expression from the gel samples analyzed for the plasma membrane fraction (B), and the cytosolic fraction (C) from three rats in each group. Each value is expressed as the mean ± SE, n = 3. There is significant difference between the bars tied with a line, $^{*}p < 0.05$.

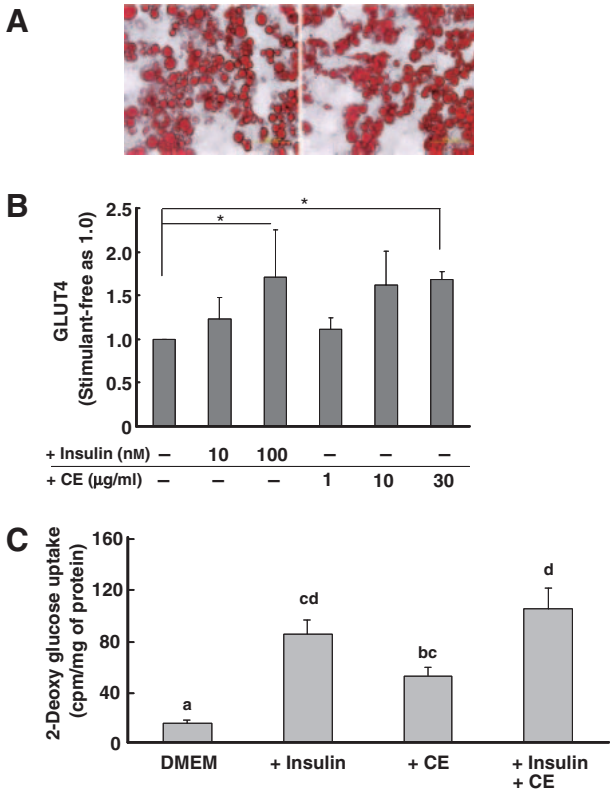

Fig. 7. Lipid-Accumulated 3T3-L1 Adipocytes and Their Glucose Uptake in the Presence of CE or Insulin.

A, oil red O-stained 3T3-L1 adipocytes. The two pictures show different fields of the differentiated cells. B, GLUT4 expression on the adipocytes stimulated with either insulin or CE. The values were obtained by western blots of the plasma membrane proteins. C, uptake of 2-deoxy-D-[2,6-^{3}H] glucose by the adipocytes. The cultured cell groups are as follows: DMEM, cells starved in the medium without containing FBS; + Insulin, cells in the medium containing 100 nM insulin; + CE, cells in the medium containing 30 μg/ml of CE; + Insulin and CE, cells in the medium containing both insulin and CE at identical concentrations to each alone. Bars show the mean ± SE of five different experiments. There is significant difference between the bars tied with a line (B) and the bars with different characters (C), $^{*}p < 0.05$.

stimulative activity of these compounds for GLUT4 expression and glucose uptake. As shown in Fig. 7B, the addition of CE to the cells caused dose-dependent elevation of GLUT4 in the plasma membrane, and this mode of elevation was comparable to the addition of insulin to the same cells. When comparing the stimulating power between CE and insulin on a weight basis, 30 μg/ml of CE was roughly equivalent to 100 nM (or 0.58 μg/ml) of insulin; *i.e.*, CE had approximately 1/50 the power of insulin in the translocation of this protein. As expected, the uptake of 2-deoxy-D-[2,6-^{3}H] glucose by the cells was stimulated by 100 nM insulin, and by 30 μg/ml of CE. The effect of combining the use of insulin and CE was nearly additive, although no statistical significance was apparent between the absorption in combination and that with insulin alone (Fig. 7C).

Discussion

Cinnamon is a commercially available form obtained as the inner bark of a tree (*Cinnamomum zeylanicum*) grown in India, China and Ceylon. The utilization of this aromatic material is diverse, from a flavor for candy or cakes to a physiologically functional spice. Its antimicrobial function has been well accepted world wide as one of the latter uses.[26,27] Although there have been several studies on cinnamon or its ingredients against diabetes mellitus,[7,28] the mechanism by which cinnamon could prevent the hyperglycemic state has remained to be clarified. The fact that cinnamon is effective against STZ-induced type-1 diabetes prompted us to investigate its activities other than insulin-related activity. We prepared in our present investigation an aqueous cinnamon extract (CE for short) at first which is known to contain effective principles of cinnamaldehyde and cinnamic alcohol.[23,29] We were able to confirm that hot water efficiently extracted both compounds from cinnamon powder. We employed STZ-induced type-1 diabetic rats as an animal model, since these rats would demonstrate the effects of cinnamon itself independently from those of insulin.

The effect of CE administration was precisely confirmed by alterations in the body weight, fasting blood glucose level and renal histology, which is known as nephropathy, a characteristic complication with diabetes.[30] We also measured the UCP-1 expression and GLUT4 translocation in the adipose tissue and

muscle to verify any function of cinnamon as a catabolic stimulant.

Treatment with STZ resulted in the rats falling into type 1 diabetes with a marked body weight loss by as much as 35% of the healthy animal, a 3-fold elevation of the fasting blood glucose level, and characteristic nephropathy with glomerular hypertrophy. The administration of CE composed mainly of cinnamaldehyde surprisingly attenuated any further increases in these disease conditions. Although the body weight of the diabetic rats did not increase over the initial weight by CE administration, its further loss was significantly impeded. The increases in organ weights of the CE-fed rats were above our expectations; *i.e.*, as compared with the initial weight in the diabetic rats, the average weight of the liver became 120%, spleen 165% and muscle 150%. The most prominent organ was intrascapular BAT which nearly doubled (195%) in its weight. Restoration of the hypertrophic renal histology in diabetic rats to that of normal healthy rats was apparent as a comprehensive result of the effect of cinnamon against this disease. The approximately 130% increase in plasma protein concentration of the diabetic rats as a result of CE administration for 22 d seems to have been due to the amelioration of renal malfunction, although we did not perform a urinalysis in this study.

We and other investigators have found that, by using a pancreatic islet cell line, cinnamon or its extract played the role of a stimulant for insulin secretion from the cells.[14,31] Although we could not prove this effect on the insulin-producing β-cells that had deteriorated due to STZ-induced type 1 diabetes, we are able to provide evidence that cinnamon prevented diabetes by enhancing the catabolism of glucose through up-regulation of the UCP-1 protein in BAT and, at the same time, translocation of GLUT4 to the plasma membranes in both BAT and muscle.

Interestingly, the upregulation of UCP-1 by CE was confined to BAT in the STZ-diabetic rats, and it had almost no effect on BAT in the normal healthy rats. We cannot yet explain why CE exhibited such different modes of action between rats with and without diabetes. We do however suggest that these two animal groups had quite different sensitivity to the spicy substance, cinnamon, and that the diabetic animals were hypersensitive to the spice, which is known to evoke the neuroterminal nociceptor, TRPA1, leading to thermogenesis involving the mitochondrial activity in BAT.[32]

The adrenergic agent stimulates the uptake of 2-deoxy-D-glucose by BAT; however, this stimulation has not been apparent in UCP-1-deficient mice,[33] suggesting that UCP-1 would play an important role in the adrenergic-stimulated glucose uptake by BAT. We observed in this study the increase by CE of GLUT4 translocation to the plasma membrane of BAT. This might have been due to the stimulation of adrenaline secretion by CE[34] as well as to the stimulation of GLUT4 translocation by CE.

We found in the STZ-diabetic rats that the abundance of GLUT4 in the plasma membrane was decreased by about 50% in BAT, whereas there was no significant change in muscle GLUT4 in comparison with that in the normal rats. However, it was noted that the administration of CE to the diabetic rats elevated the GLUT4 level in both tissues in a dose-dependent fashion. Since this elevation in the plasma membrane of BAT was accompanied by its depression in the cytosolic fraction, it is most likely that CE stimulated the translocation of GLUT4 from the cytosol to cell membrane. The fact that CE administration elevated GLUT4 in the muscle membrane of the diabetic rats by 4.5-fold or more than that of the control rats (see Fig. 6B, bars CE100 and CE0) demonstrates the possible use of cinnamon to treat this type of diabetes. We also found for the first time the up-regulation by CE of GLUT4 production in the gastrocnemial muscle. We also verified the function of CE *in vitro* by using 3T3-L1 adipocytes that CE increased glucose absorption by stimulating GLUT4 translocation with a comparable mode of action to that of insulin. We have not yet determined the detailed mechanism by which CE up-regulated GLUT4 production in the muscle, although Cao *et al.* have reported that cinnamon polyphenol increased the production of GLUT4 in 3T3-L1 adipocytes; however, the mechanism for this still remains to be clarified.[35] Of the events connecting the administration of CE and the observed phenomena, we are about to verify details of the mechanism related to excitation of the central nervous system and AMPK activity.[36,37]

In conclusion, CE had two major functions: i) it up-regulated mitochondrial UCP-1, and ii) enhanced the production and translocation of GLUT4 in the muscle and, at least, the translocation of GLUT4 in adipose tissues. These activities may allow cinnamon to be used in the daily care of diabetes mellitus by its dietary or supplementary use, and in alleviating the long-term complications, including cardiovascular disease, chronic renal failure and retinal damage.

Acknowledgment

This study was performed under grant-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan to Y.S. (no. 074510) and by a grant from Nihon University to T.S. The authors thank House Foods Co. of Japan for providing the sticks of cinnamon (*C. Zeylanicum*). We also thank Dr. Nobuyo Tsunoda and Professor Keizo Kasono at Josai University, Mr. Liu Nan Jia, Dr. Hiroyuki Fujisawa and Dr. Nobuaki Okumura at Nihon University for helpful discussion, criticism and technical advice.

References

1) Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, and Tuomilehto J, *Diabetes Care*, **23**, 1516–1526 (2000).
2) Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, and Zinman B, *N. Engl. J. Med.*, **353**, 2643–2653 (2005).
3) Genuth S, *Endocr. Pract.*, **1**, 34–41 (2006).
4) National Diabetes Data Group, Diabetes in America: diabetes data compiled 1984, Bethesda, Md, National Institutes of Health, NIH publication no. 85–1468 (1985).
5) Deckert T, Poulsen JE, and Larsen M, *Diabetologia*, **14**, 363–377 (1978).
6) Toriizuka K, *Kampo Med.*, **11**, 431–436 (1998).
7) Khan A, Safdar M, Ali Khan MM, Khattak KN, and Anderson RA, *Diabetes Care*, **26**, 3215–3218 (2003).
8) Mang B, Wolters M, Schmitt B, Kelb K, Lichtinghagen R,

Stichtenoth DO, and Hahn A, *Eur. J. Clin. Invest.*, **36**, 340–344 (2006).
9) Broadhurst CL, Polansky MM, and Anderson RA, *J. Agric. Food Chem.*, **48**, 849–852 (2000).
10) Imparl-Radosevich J, Deas S, Polansky MM, Baedke DA, Ingebritsen TS, Anderson RA, and Graves DJ, *Horm. Res.*, **50**, 177–182 (1998).
11) Jarvill-Taylor KJ, Anderson RA, and Graves DJ, *J. Am. Coll. Nutr.*, **20**, 327–336 (2001).
12) Kim HS, Hyun SH, and Choung SY, *J. Ethnopharmacol.*, **104**, 119–123 (2006).
13) Mishra A, Bhatti R, Singh A, and Singh Ishar MP, *Planta Med.*, **76**, 412–417 (2010).
14) Anand P, Murali KY, Tandon V, Murthy PS, and Chandra R, *Chem. Biol. Interact.*, **186**, 72–81 (2010).
15) Altschuler JA, Casella SJ, MacKenzie TA, and Curtis KM, *Diabetes Care*, **30**, 813–816 (2007).
16) Chappell JB and Hansford RG, "Subcellular Components: Preparation and Fractionation," ed. Birnie GD, Butterworths, pp. 77–91 (1972).
17) Cunningham O, McElligott AM, Carroll AM, Breen E, Reguenga C, Oliveira ME, Azevedo JE, and Porter RK, *Biochim. Biophys. Acta*, **1604**, 170–179 (2003).
18) Nishiumi S and Ashida H, *Biosci. Biotechnol. Biochem.*, **71**, 2343–2346 (2007).
19) Su J, Zhang P, Zhang JJ, Qi XM, Wu YG, and Shen JJ, *Phytomedicine*, **17**, 254–260 (2009).
20) Seki T, Miyasu T, and Ariga T, *J. Cell. Physiol.*, **189**, 72–78 (2001).
21) Nagasawa K, Yamamoto N, Hiroi N, and Yoshino G, *J. Med. Soc. Toho Univ.*, **53**, 21–30 (2006).
22) Ragolia L and Begum N, *Endocrinology*, **138**, 2398–2404 (1997).
23) Subash Babu P, Prabuseenivasan S, and Ignacimuthu S, *Phytomedicine*, **14**, 15–22 (2007).
24) Ragolia L, Hall CE, and Palaia T, *Prostaglandins Other Lipid Mediat.*, **87**, 34–41 (2008).
25) Richter EA, "Handbook of Physiology," ed. Rowell LB, American Physiological Society, Bethesda, pp. 912–951 (1996).
26) Inouye S, Yamaguchi H, and Takizawa T, *J. Infect. Chemother.*, **7**, 251–254 (2001).
27) Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V, and Parker M, *Int. J. Food Microbiol.*, **107**, 180–185 (2006).
28) Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, Schonene NW, and Graves DJ, *J. Agric. Food Chem.*, **52**, 65–70 (2004).
29) Lee HS, *J. Pharm. Pharm. Sci.*, **5**, 226–230 (2002).
30) Gavin JR 3rd and Bohannon NJ, *Postgrad. Med.*, **122**, 43–51 (2010).
31) Shen Y, Muraki E, Ebata M, Tsunoda N, and Kasono K, Abstracts of Papers, 10th Asian Congress of Nutrition, Taiwan, 2007, p. H-51.
32) Tominaga M and Caterina MJ, *J. Neurobiol.*, **61**, 3–12 (2004).
33) Inokuma K, Ogura-Okamatsu Y, Toda C, Kimura K, Yamashita H, and Saito M, *Diabetes*, **54**, 1385–1391 (2005).
34) Iwasaki Y, Tanabe M, Kobata K, and Watanabe T, *Biosci. Biotechnol. Biochem.*, **72**, 2608–2614 (2008).
35) Cao H, Polansky MM, and Anderson RA, *Arch. Biochem. Biophys.*, **459**, 214–222 (2007).
36) Chernogubova E, Cannon B, and Bengtsson T, *Endocrinology*, **145**, 269–280 (2004).
37) Mulder AH, Tack CJ, Olthaar AJ, Smits P, Sweep FC, and Bosch RR, *Am. J. Physiol. Endocrinol. Metab.*, **289**, 627–633 (2005).