# Hypoglycemic Effects of Turmeric (*Curcuma longa* L. Rhizomes) on Genetically Diabetic KK-A$^y$ Mice

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**The turmeric (*Curcuma longa* L. rhizomes) EtOH extract significantly suppressed an increase in blood glucose level in type 2 diabetic KK-A$^y$ mice. In an *in vitro* evaluation, the extract stimulated human adipocyte differentiation in a dose-dependent manner and showed human peroxisome proliferator-activated receptor (PPAR)-$\gamma$ ligand-binding activity in a GAL4-PPAR-$\gamma$ chimera assay. The main constituents of the extract were identified as curcumin, demethoxycurcumin, bisdemethoxycurcumin, and ar-turmerone, which had also PPAR-$\gamma$ ligand-binding activity. These results indicate that turmeric is a promising ingredient of functional food for the prevention and/or amelioration of type 2 diabetes and that curcumin, demethoxycurcumin, bisdemethoxycurcumin, and ar-turmerone mainly contribute to the effects *via* PPAR-$\gamma$ activation.**

**Key words** *Curcuma longa* L.; adipocyte differentiation; curcuminoid; peroxisome proliferator-activated receptor (PPAR)-$\gamma$ ligand-binding activity; type 2 diabetes mellitus; sesquiterpenoid

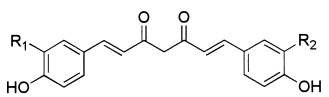
A crucial role in the development of the metabolic syndrome is played by adipocytes, which are highly specialized cells involved in energy regulation and homeostasis. Adipocyte differentiation is a tightly controlled process, in which determinant genes such as those of peroxisome proliferator-activated receptor-$\gamma$ (PPAR-$\gamma$) and CCAAT/enhancer binding protein-$\alpha$ lead to programmed adipocyte differentiation.[1,2] PPARs belong to the superfamily of nuclear receptors and modulate gene expression in the process of lipid metabolism.[3] Among PPARs, PPAR-$\gamma$ is the predominant molecular target for insulin-sensitizing thiazolidinedione drugs such as troglitazone, pioglitazone, and rosiglitazone, which have been approved for use in type 2 diabetic patients.[4,5] The thiazolidinedione derivatives activate PPAR-$\gamma$ and improve insulin resistance by increasing the number of small adipocytes with normal function differentiated from preadipocytes and inducing apoptosis in large adipocytes, which hyperproduce and hypersecrete adipocytokines such as leptin, tumor necrosis factor (TNF)-$\alpha$, and free fatty acids.[6] As part of our systematic search for new functional foods with preventive effects against type 2 diabetes mellitus, we found that the turmeric (*Curcuma longa* L. rhizomes) EtOH extract suppressed an increase in blood glucose levels in type 2 diabetic KK-A$^y$ mice and stimulated human adipocyte differentiation *in vitro*. To determine the mechanism of action, the extract and its components were evaluated for their PPAR-$\gamma$ ligand-binding activity.

## MATERIALS AND METHODS

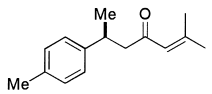
**Preparation of Turmeric Extract** Powdered turmeric (*C. longa* L. rhizomes; 100 g) was twice extracted with five volumes of EtOH, and the extract was concentrated under reduced pressure to give 12.2 g of EtOH extract.

**Isolation of Curcuminoids and Sesquiterpenoid** Powdered *C. longa* L. (1.0 kg) was extracted with EtOH (8.0 l) at room temperature for 2 d. The extract was filtered off and concentrated (118 g) and then subjected to porous polymer resin Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan) column chromatography, eluted with 30%, 50%, and 80% MeOH, MeOH, EtOH, and EtOAc. From the MeOH eluate portion (63.5 g), curcumin (**1**, 6.4 g), demethoxycurcumin (**2**, 1.3 g), and bisdemethoxycurcumin (**3**, 1.2 g) were purified on repeated silica gel column chromatography and eluted with hexane–$Me_2CO$ (2 : 1, 3 : 2, and 4 : 3) and $CHCl_3$–$Me_2CO$ (99 : 1 and 19 : 1). An essential oil fraction was obtained from the EtOH extract (100 g) on silica gel chromatography and eluted with hexane–EtOAc (9 : 1), and then ar-turmerone (**4**, 6.7 g) was purified on repeated ODS silica gel chromatography and eluted with $CH_3CN$–$H_2O$ (13 : 7). The structures of **1**—**4** were identified by comparison of their physical and spectral data with published values.[7—9]

***In Vivo* Animal Experiments** Female genetically diabetic KK-A$^y$/Ta mice were purchased from Clea Japan (Tokyo, Japan). The mice were housed in an animal laboratory with a controlled environment of 20—24 °C, 45—65% humidity, and a 12 h (07:30—19:30) light/dark cycle. The mice at 6 weeks of age divided into three groups of five mice each by body weight and blood glucose level. The control group was fed a basal diet (Oriental Yeast, Tokyo, Japan), and the treated groups were fed a diet containing the EtOH extract (0.2 or 1.0 g/100 g diet). Mice consumed these diets and water *ad libitum* for 4 weeks. Blood samples were obtained from the tail vein at fixed times (08:30—19:00) and blood glucose levels were determined using a Glutest Ace blood glucose level monitor (Sanwa Kagaku Kenkyusha,




| | $R_1$ | $R_2$ |
|---|---|---|
| curcumin (**1**) | OMe | OMe |
| demethoxycurcumin (**2**) | OMe | H |
| bisdemethoxycurcumin (**3**) | H | H |




ar-turmerone (**4**)

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Nagoya, Japan) before and after 4-week feeding.

**Human Adipocyte Differentiation** Effects on human adipocyte differentiation were examined at Zen-Bio (Research Triangle Park, NC, U.S.A.). The test measured the accumulation of lipid droplets in the cells, which occurred during the differentiation from preadipocytes to adipocytes. Human preadipocytes (Zen-Bio) were isolated from subcutaneous adipose tissue healthy donors undergoing elective surgery after ethical approval had been obtained. The primary human subcutaneous preadipocytes were plated at $1.35\times10^4$ cells/well on 96-well plates and cultured with DMEM/Ham's F-12 nutrient medium (1 : 1, v/v) containing HEPES buffer 15 mM, fetal bovine serum (FBS) 30 $\mu$l/ml, biotin 33 $\mu$M, pantothenate 17 $\mu$M, human insulin 100 nM, 3-isobutyl-1-methylxanthine 0.25 mM, and dexamethasone 1.0 $\mu$M alone or supplemented with a sample for 3 d. The cells received three additional feedings with the above medium not containing 3-isobutyl-1-methylxanthine, alone or supplemented with a sample for 11 d. Medium alone was used as a control, and rosiglitazone maleate 1.0 $\mu$M as a positive control. The cells were washed with PBS and lysed. Accumulated triglycerides were then measured as glycerol using Infinity reagent (Sigma, St. Louis, MO, U.S.A.), which contained a microbial lipase. The degree of adipocyte differentiation was determined based on the amount of glycerol liberated from accumulated triglyceride.

**PPAR-$\gamma$ Ligand-Binding Assay** PPAR-$\gamma$ ligand-binding activity was assayed using the method described in our previous report.[10] Briefly, a plasmid expressing a fusion protein of the GAL4 DNA-binding domain and human PPAR-$\gamma$ ligand-binding domain and a reporter plasmid including the luciferase gene were transfected into CV-1 cells. After 24 h of transfection, the medium was changed to DMEM containing 10% charcoal-treated FBS and sample. Sample dissolved in DMSO was added to the medium at a final concentration of DMSO 1.0 $\mu$l/ml. The cells were washed with PBS containing $Ca^{2+}$ and $Mg^{2+}$, and luciferase activities were measured. PPAR-$\gamma$ ligand-binding activity was expressed as the relative luminescence intensity of the test sample to that of the solvent control.

**Statistical Analysis** Statistical significance was determined using the SAS/STAT computerized statistical analysis program software (SAS Institute, Cary, NC, U.S.A.). When significant differences were detected by one-way ANOVA, Turkey's multiple comparison test was applied.

## RESULTS AND DISCUSSION

***In Vivo* Animal Experiments** Hypoglycemic effects of the turmeric EtOH extract in genetically diabetic KK-A$^y$/Ta mice were investigated. No difference was observed in food intake or body weights of mice between the treated and control groups. The extract doses totaled almost 260 and 1500 mg/kg body weight/d in the 0.2 and 1.0 g EtOH extract/100 g diet groups, respectively. Compared with before feeding, the mean blood glucose level in the control group was significantly ($p<0.05$) increased (Fig. 1), indicating hyperglycemia after 4 weeks of feeding. However, the blood glucose levels in the EtOH extract groups remained the same as before feeding. These results indicate that turmeric is a promising ingredient of functional food for the prevention and/or amelioration of type 2 diabetes mellitus.

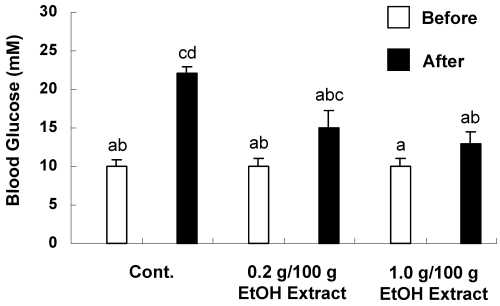


Fig. 1. Blood Glucose Levels in KK-A$^y$ Mice Fed Diet Alone (Cont.) or Diet Containing the Turmeric EtOH Extract 0.2 or 1.0 g/100 g Diet before and after 4 Weeks of Feeding

Values are expressed as mean±S.E.M., $n=5$. Means without a common letter differ, $p<0.05$

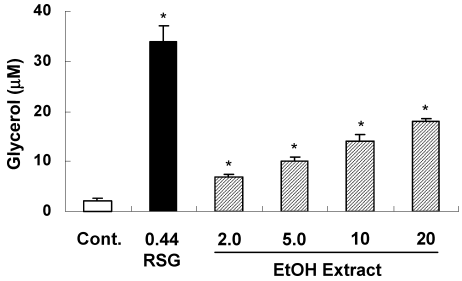


Fig. 2. Effecst of the Turmeric EtOH Extract 2.0, 5.0, 10, and 20 $\mu$g/ml on Human Adipocyte Differentiation

Medium alone was used as a control (Cont.), and rosiglitazone (RSG) 0.44 $\mu$g/ml (1.0 $\mu$M) as a positive control. Accumulated triglyceride was determined as glycerol concentration. Values are expressed as mean±S.D., $n=3$. $*p<0.05$ indicates a significant difference from control

***In Vitro* Experiments** When human preadipocytes were cultured with the turmeric EtOH extract, glycerol liberated from accumulated triglyceride increased ($p<0.05$) in a dose-dependent manner (Fig. 2), indicating that the EtOH extract stimulated adipocyte differentiation. The EtOH extract showed human PPAR-$\gamma$ ligand-binding activity in a GAL4-PPAR-$\gamma$ chimera assay (Fig. 3A). The activity of 5.0 $\mu$g/ml and 10 $\mu$g/ml of the EtOH extract was more potent than that of 0.22 $\mu$g/ml and 0.44 $\mu$g/ml of troglitazone used as a positive control, respectively. The main constituents of the EtOH extract, curcumin (**1**), demethoxycurcumin (**2**), bisdemethoxycurcumin (**3**), and ar-turmerone (**4**), also exhibited significant PPAR-$\gamma$ ligand-binding activity (Fig. 3B). Adipocyte differentiation was considered to be associated with the PPAR-$\gamma$ ligand-binding activity of the turmeric EtOH extract, which resulted in the suppression of an increase in blood glucose levels in type 2 diabetic KK-A$^y$ mice.

## CONCLUSION

Since the turmeric EtOH extract stimulated human adipocyte differentiation in a dose-dependent manner and reduced the blood glucose levels of genetically diabetic KK-A$^y$ mice in association with PPAR-$\gamma$ ligand-binding activity, turmeric is concluded to be a promising ingredient of functional food for the prevention and/or amelioration of type 2 diabetes mellitus. The PPAR-$\gamma$ agonistic thiazolidinedione derivatives such as troglitazone, pioglitazone, and rosiglita-

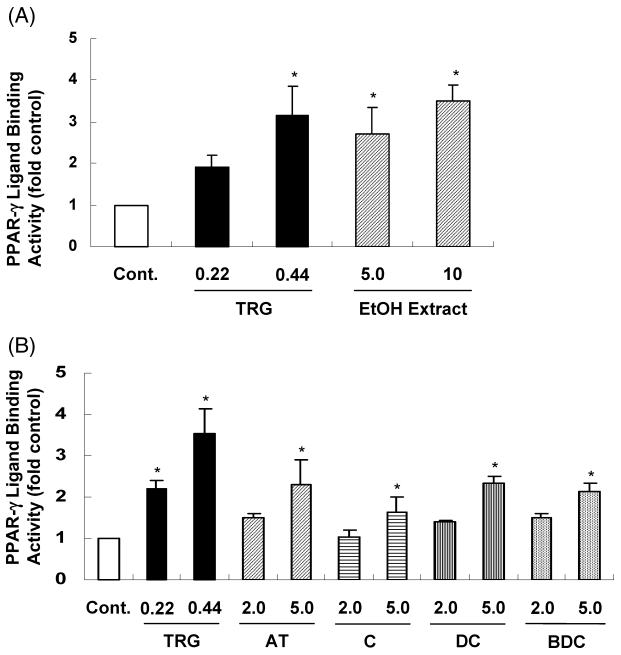


Fig. 3. PPAR-$\gamma$ Ligand-Binding Activity of the Turmeric EtOH Extracts 5.0 and 10 $\mu$g/ml (A), and Ar-turmerone (AT), Curcumin (C), Demethoxycurcumin (DC), and Bisdemethoxycurcumin (BDC) 2.0 and 5.0 $\mu$g/ml (B) in a GAL4-PPAR-$\gamma$ Chimera Assay System

DMSO 1.0 $\mu$l/ml was used as a solvent control (Cont.), and troglitazone (TRG) 0.22 $\mu$g/ml (0.5 $\mu$M) and 0.44 $\mu$g/ml (1.0 $\mu$M) as a positive control. Values are expressed as mean±S.D., $n=3$ experiments. $*p<0.05$ indicates a significant difference from control

zone stimulate adipocyte differentiation, improve insulin resistance, and exert hypoglycemic effects. The PPAR-$\gamma$ active compounds of turmeric, curcumin (**1**), demethoxycurcumin (**2**), bisdemethoxycurcumin (**3**), and ar-turmerone (**4**), are assumed to improve insulin resistance and ameliorate type 2 diabetes mellitus by the same biological mechanism as the thiazolidinedione derivatives. When the four compounds were mixed at the same concentrations as in the turmeric EtOH extract, the PPAR-$\gamma$ ligand-binding activity of the mixture accouted for more than 90% of that of the EtOH extract (data not shown). *In vivo* evaluation of **1**—**4** is in progress.

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