RESEARCH ARTICLE

Diosgenin present in fenugreek improves glucose metabolism by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues

Taku Uemura[1], Shizuka Hirai[1], Noriko Mizoguchi[1], Tsuyoshi Goto[1], Joo-Yong Lee[1], Keiko Taketani[2], Yuki Nakano[2], Jinji Shono[2], Shohei Hoshino[2], Nobuaki Tsuge[2], Toshihiko Narukami[2], Nobuyuki Takahashi[1] and Teruo Kawada[1]

[1] Laboratory of Molecular Function of Food, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Uji, Kyoto, Japan
[2] Spice Research Laboratory, Somatech Center, House Foods Corporation, Yotsukaido, Chiba, Japan

In obesity, adipocyte hypertrophy and chronic inflammation in adipose tissues cause insulin resistance and type-2 diabetes. *Trigonella foenum-graecum* (fenugreek) can ameliorate hyperglycemia and diabetes. However, the effects of fenugreek on adipocyte size and inflammation in adipose tissues have not been demonstrated. In this study, we determined the effects of fenugreek on adipocyte size and inflammation in adipose tissues in diabetic obese KK-Ay mice, and identified the active substance in fenugreek. Treatment of KK-Ay mice with a high fat diet supplemented with 2% fenugreek ameliorated diabetes. Moreover, fenugreek miniaturized the adipocytes and increased the mRNA expression levels of differentiation-related genes in adipose tissues. Fenugreek also inhibited macrophage infiltration into adipose tissues and decreased the mRNA expression levels of inflammatory genes. In addition, we identified diosgenin, a major aglycone of saponins in fenugreek to promote adipocyte differentiation and to inhibit expressions of several molecular candidates associated with inflammation in 3T3-L1 cells. These results suggest that fenugreek ameliorated diabetes by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues, and its effects are mediated by diosgenin. Fenugreek containing diosgenin may be useful for ameliorating the glucose metabolic disorder associated with obesity.

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1 Introduction

Obesity correlates closely with insulin resistance [1, 2]. Insulin resistance is characterized by reduced actions of insulin in target tissues. It has been demonstrated that the size of adipocytes is inversely related to insulin sensitivity [3–5]. Excess energy results in adipocyte hypertrophy, which in turn exerts deleterious effects on insulin sensitivity. Large adipocytes are less insulin sensitive as shown by impaired insulin-stimulated glucose uptake [6]. The insulin-resistant state is a common cause of hypertension and coronary artery disease, as well as glucose metabolic disorders such as hyperglycemia, which is a typical symptom of type-2 diabetes [7]. Therefore, it is important for the improvement of glucose metabolic disorder associated with insulin resistance to suppress adipocyte hypertrophy, for instance, by promoting adipocyte differentiation [3].

Recent studies have shown that chronic inflammation characterized by the infiltration of macrophages in obese white adipose tissue (WAT) may cause whole-body insulin resistance in obese animals [8–10]. Chronic inflammation promotes the expression of proinflammatory cytokines such

Correspondence: Dr. Teruo Kawada, Laboratory of Molecular Function of Food, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan
E-mail: fat@kais.kyoto-u.ac.jp
Fax: +81-774-38-3752

Abbreviations: C/EBP, CCAAT/enhancer-binding protein; **HFD,** high fat diet; **MCP-1,** monocyte chemoattractant protein-1; **OGTT,** oral glucose tolerance test; **PPAR,** peroxisome proliferators-activated receptor; **TNF-α,** tumor necrosis factor-α; **WAT,** white adipose tissue

as monocyte-chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α), which induce the infiltration of macrophages into WAT and systemic insulin resistance, respectively [6, 11, 12]. On the other hand, expression of adiponectin, which inhibits inflammatory processes, is suppressed by adipocyte hypertrophy [13–15]. Thus, it is also important for the improvement of glucose metabolic disorder to suppress chronic inflammation in WAT.

Trigonella foenum-graecum (fenugreek) is native to the Eastern Mediterranean to Central Asia and Ethiopia, and is extensively cultivated in India, Pakistan, and China [16]. It has a long history of medicinal uses in Ayurvedic and Chinese medicine, and has been used for numerous indications, including labor induction, indigestion and as a general tonic to improve metabolism and health [17]. Over the past few years, fenugreek has been shown to display antidiabetic effects in some animals [18–20], as well as in humans [21]. These antidiabetic effects may be due to the intestinal effects of fiber [22, 23] or insulin secretagogue activity of 4-hydroxyisoleucine [24] in fenugreek seeds. However, there is no report that shows the effects of fenugreek on adipocyte size and inflammation in WAT.

Here, we examined the effects of fenugreek on adipocyte size and inflammation in WAT in obese diabetic KK-Ay mice. We showed that fenugreek improved high fat diet (HFD)-induced glucose metabolic disorders *via* miniaturization of adipocytes by promoting adipocyte differentiation and inhibiting inflammation in WAT. To elucidate the detailed mechanism, we identified diosgenin, a major aglycone of saponins in fenugreek, to promote adipocyte differentiation and to inhibit expressions of several molecular candidates associated with inflammation in 3T3-L1 cells. These results indicate that fenugreek directly affects adipose tissues and improves insulin sensitivity in obese diabetic mice, suggesting that the promotional effect of diosgenin on adipocyte differentiation and the inhibitory effect on inflammation play important roles in the therapeutic effects of fenugreek on glucose metabolism.

2 Materials and methods

2.1 Animals and chemicals

We purchased 4-wk-old male KK-Ay/Ta Jcl mice from Nippon CLEA (Tokyo, Japan). KK-Ay mouse is an obese diabetic model. All the mice were kept in individual cages in a temperature-controlled room at 24 ± 1°C and maintained on a 12 h light/dark cycle. All the animals were fed a commercial diet (CRF-1, Charles River Japan, Kanagawa, Japan) during 1 wk of adaptation, and were given a control HFD (D12492; Research Diets, NJ, USA) supplemented with fenugreek (0.5 or 2% w/w) for 4 wk of feeding in accordance with previous studies [17]. The control HFD is 60% kcal% fat, 20% carbohydrate, and 20% protein in kcal% (5.2 kcal/g diet) containing (in mg/kg diet): casein, 258.4; L-cystine, 3.9; maltodextrin-10, 161.5; sucrose, 88.9; cellulose, 64.6; soybean oil, 32.3; lard, 316.6; mineral mix (S10026, Research Diets), 12.9; dicalcium phosphate, 16.8; calcium carbonate, 7.1; potassium citrate, 21.3; vitamin mix (V10001, Research Diets), 12.9; choline bitartate, 2.6; FD&C Blue Dye♯1, 0.065. A control group fed the control HFD for the same period. Six mice were used in each group. The energy intake of all the mice was adjusted by pair feeding. Thus, the levels of food intake of each group were almost the same. The animal care procedures and methods were approved by the Animal Care Committee of Kyoto University.

Fenugreek with reduced bitterness was provided by House Foods (Chiba, Japan). Fenugreek powder was prepared as follows. We purchased fenugreek seeds cultivated in India from local market. Fenugreek seeds (1 kg) were boiled for 5 min, and then cooled to 40°C. The total weight was adjusted to 6.75 kg by adding water. An enzyme (SPEZYME CP, 95 g; Genencor, CA, USA) was added to the boiled seeds, which were then mashed to obtain fenugreek paste. Subsequently, the pH of the paste was adjusted to 5.0 using 1 N HCl, followed by incubated at 55°C for 6–10 h with stirring until the bitterness of the paste was sufficiently reduced. The enzyme was deactivated by heating at 90°C for 15 min. The paste was freeze dried and crushed into powder. We used the powder that was passed through a 500-μm sieve as the fenugreek powder. Diosgenin was from Wako (the structure is shown in Fig. 4B; Osaka, Japan). All the other chemicals were from Sigma (MO, USA) or Nacalai Tesque (Kyoto, Japan) and were guaranteed to be of reagent- or tissue-culture-grade.

2.2 Blood sample assays and oral glucose tolerance test

At the end of the treatment period, anesthetized animals were killed by a cervical dislocation after an overnight fast and blood sample was obtained. The plasma glucose level was determined using the glucose CII-test Wako (Wako). The plasma insulin level was measured using an ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan). Insulin sensitivity was assessed by the oral glucose tolerance test (OGTT). OGTT was carried out 4 wk after the start of feeding. Mice were fasted for 24 h, and then glucose (1.5 g/kg body weight) was administered orally. Blood samples were collected from the tail tip at 15, 30, 60, and 120 min, and the glucose and insulin concentrations were determined.

2.3 Histochemical analysis of adipose tissues

Epididymal WAT was fixed in 10% formaldehyde/PBS. The fixed samples were embedded in paraffin wax. They were cut into 8 μm-thick sections and mounted on silanized slides. After deparaffinization and blocking with 2% normal goat serum/PBS, the sections were incubated with anti-mouse F4/80 rat antibody (1:50 dilution; Serotec, NC, USA) overnight at 4°C.

Immune complexes were detected with biotinylated anti-rat IgG antibody (1:100 dilution; Cappel research reagents, OH, USA), HRP-conjugated streptavidin (1:50 dilution; Amersham, Little Chalfont, UK), and the peroxidase substrate diaminobenzidine. Slides were observed with a light microscope. Adipocyte size was measured using Meta Morph software (Molecular Devices, Downingtown, PA, USA).

2.4 RNA preparation and RT-PCR

Total RNA was prepared from epididymal WAT using Qiazol lysis reagent (Qiagen, CA, USA) and from 3T3-L1 cells using Sepasol (Nacalai Tesque), a commercial solution of acid guanidium-phenol-chloroform in accordance with the manufacturer's protocol. Using M-MLV reverse transcriptase (Invitrogen, CA, USA), we reverse-transcribed 1 μg total RNA in accordance with the manufacturer's instructions using a thermal cycler (TaKaRa PCR Thermal Cycler SP: TaKaRa Shuzo, Shiga, Japan). To quantify mRNA expression, quantitative RT-PCR was performed using a LightCycler System (Roche Diagnostics, Mannheim, Germany) measuring SYBR green fluorescence signals intensity, as described previously [25, 26]. All the primers for the different genes are described in Table 1.

2.5 Preparation of saponin from fenugreek

Fenugreek powder was defatted using ether. The saponin fraction was extracted using 70% ethanol, concentrated using an evaporator, and separated using a polymer C18 (ODP-400) intermediate column (20 × 300 mm; SHOWA DENKO, Tokyo, Japan) with a solvent system of 50% aqueous ethanol after using a solvent system of 30% aqueous ethanol. Its products were designated as the crude fraction. The saponin fraction was obtained by separating the crude fraction by ODS HPLC (20 × 250 mm; Senshu Science, Tokyo, Japan) using a solvent system of 35% aqueous ethanol. The saponin fraction was dissolved in a solution containing 1 M sulfuric acid and 70% 2-propanol, and heated at 100°C for 2 h. After adding water, the extraction using *t*-butyl methyl ether was carried out three times. The extracted liquid was washed with 1 M NaOH twice and then with distilled water, and dehydrated with sodium sulfate. After cotton filtration, the extracted liquid was evaporated, and hydrolyzed saponin was obtained. Hydrolyzed saponin was fractionated by RP-HPLC on an Inertsil ODS-3 column (20 × 250 mm; GL Science, CA, USA) using a mobile phase of ACN–water (95:5, v/v). The flow rate was set at 7.5 mL/min and the detection wavelength was set at 210 nm. Six fractions were separated, as shown in Fig. 3C.

2.6 Identification and purification of active substance

The active substance was analyzed on an Inertsil ODS-3 column (2.1 × 150 mm, 3 μm; GL Science) using a mobile phase of ACN–5 mM ammonium acetate in water (95:5, v/v) and the flow rate was set at 0.2 mL/min. LC/MS and MS/

Table 1. Oligonucleotide sequences for primer used RNA analysis

Gene	Accession number	Sequence
m aP2	K02109	Fw: AAGACAGCTCCTCCTCGAAGGTT Re: TGACCAAATCCCCATTTACGC
m LPL	J03302	Fw: ATCCATGGATGGACGGTAACG Re: CTGGATCCCAATACTTCGACCA
m Glut4	AB008453	Fw: TAGGAGCTGGTGTGGTCAATACG Re: TAAAAGGGAAGGTGTCCGTCG
m PPARγ	AB008453	Fw: GGAGATCTCCAGTGATATCGACCA Re: ACGGCTTCTACGGATCGAAACT
m MCP-1	NM_011333	Fw: ATGCAGGTCCCTGTCATGCTTC Re: GGCATCACAGTCCGAGTCACAC
m TNF-α	NM_013693	Fw: ACACTCAGATCATCTTCTCAAAATTCG Re: GTGTGGGTGAGGAGCACGTAGT
m Adiponectin	NM_009605	Fw: ACAACCAACAGAATCATTATGACGG Re: GAAAGCCAGTAAATGTAGAGTCGTTGA
m F4/80	NM_010130	Fw: TTTCCTCGCCTGCTTCTTC Re: CCCCGTCTCTGTATTCAACC
m C/EBPβ	NM_009883	Fw: GCAAGAGCCGCGACCCG Re: GGCTCGGGCAGCTGCTT
m C/EBPδ	NM_007679	Fw: ACGACGAGAGCGCCATC Re: TCGCCGTCGCCCCAGTC
m 36B4	BC011291	Fw: TGTGTGTCTGCAGATCGGGTAC Re: CTTTGGCGGGATTAGTCGAAG

MS analyses were performed using a Quattro Premier XE (Waters, MA, USA) with an ESI interface and an ACQUITY UPLC (Waters). The data obtained were processed using Masslynx version 4.1 software. The mass spectrometer source was maintained at 130°C and a desolvation temperature of 400°C with a drying gas (nitrogen) at a flow rate of 800 L/h and a cone gas at a flow rate of 50 L/h. The cone energy was set at 40 V. The MS scan mode was used for LC/MS. The daughter scan mode was used for MS/MS, and the product ion spectra at 415.6 were collected. The collision energy was set at 20 V with the collision gas argon flow rate at 0.15 mL/min.

2.7 Cell-based assays

Differentiation of 3T3-L1 preadipocytes (American Type Culture Collection, Manassas, VA, USA) was performed as previously described [27, 28]. These cells were treated with diosgenin throughout the differentiation period. Triglyceride accumulation was measured by Oil Red O staining. Eight days after differentiation induction, the cells were rinsed with PBS and fixed with 10% formalin in PBS for 1 h. After washing twice with PBS, the cells were stained with Oil Red O for 1 h. Then, the cells were washed twice with water, and photographed. Stained oil droplets were extracted with isopropyl alcohol and the absorbance was measured at 490 nm. Activities of glycerol-3-phosphate dehydrogenase were measured as previously described [27]. The culture supernatants from the 3T3-L1 cells were obtained at day 6 or day 8, as previously described [29, 30]. Adiponectin and MCP-1 levels were determined using a mouse adiponectin ELISA development kit (R&D systems, MN, USA) and a READY-SET-GO! Mouse MCP-1 ELISA kit (eBioscience, CA, USA), respectively, in accordance with the manufacturer's instructions. To examine mRNA expression of CCAAT/enhancer-binding protein (C/EBP), total RNA was prepared using 3T3-L1 cells treated with/without diosgenin at 1, 3, 6, 12, and 24 h after the differentiation induction. We performed cell viability assay using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, WC, USA).

2.8 Glucose uptake using 3T3-L1

Measurement of glucose uptake into 3T3-L1 cells was performed as previously described [31]. Briefly, 6 days after differentiation induction, 3T3-L1 cells were incubated in serum-free DMEM for 5 h. After washing with HKR buffer (containing 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 2.5 mM $MgSO_4$, and 20 mM HEPES; pH 7.4), the cells were incubated with 0.5-mL HKR buffer containing 1% BSA at 37°C for 20 min in the presence or absence of 5 μg/mL insulin. [^{3}H]-2-Deoxyglucose (American Radiolabeled Chemicals, MO, USA) was added at a final concentration of 0.1 mM, followed by incubation for 10 min. The cells were washed with cold PBS and solubilized in 0.1 N NaOH. The radioactivity of a 200 μL aliquot was determined in a scintillation counter (LS6500, Beckman Coulter, CA, USA). Glucose uptake was expressed as the degree of increase compared with control (0 μM diosgenin in the presence of insulin), normalized to protein concentration.

2.9 Statistical analysis

The data are presented as mean ± SEM. Differences between two groups were assessed using unpaired *t*-test. Data involving more than two groups were assessed by one-way ANOVA and Dunnet's multiple comparison tests. Differences were considered significant at $p < 0.05$.

3 Results

3.1 Fenugreek improves insulin resistance in KK-Ay mice

To examine the effects of fenugreek on the development of insulin resistance, we fed obese diabetic model mice, KK-Ay, with HFD supplemented with 0.5 or 2.0% fenugreek for 4 wk. Energy intake during the feeding period in each group was almost the same (control, 20.5 ± 0.26; 0.5% fenugreek, 20.6 ± 0.23; 2% fenugreek, 20.2 ± 0.23 kcal/day). Weekly weights of the three experimental groups showed no difference (Supporting Information Fig. 1). At the end of this experiment, fenugreek affected neither the body weight (control, 39.6 ± 0.56; 0.5% fenugreek, 39.9 ± 0.50; 2% fenugreek, 38.8 ± 1.13 g) nor total WAT weight (epididymal, mesenteric, retroperitoneal, and renal adipose tissues) of KK-Ay mice fed HFD (control, 7.92 ± 0.43; 0.5% fenugreek, 8.64 ± 0.32; 2% fenugreek 7.77 ± 0.35 g/100 g BW). Fasting plasma glucose and insulin levels were lower in the 2% fenugreek-fed mice than in the control mice (Table 2). OGTT showed significant decrease in plasma glucose levels in the 2% fenugreek-fed mice compared with that in the control mice at 15 and 60 min after oral glucose injection (Fig. 1A). Plasma insulin levels during OGTT were lower in the 2% fenugreek-fed mice compared with that in the control mice (Fig. 1B). These data suggest that fenugreek improves glucose metabolic disorder by enhancing insulin sensitivity in KK-Ay mice fed HFD.

3.2 Fenugreek promotes adipocyte differentiation and inhibits obesity-related inflammation in WAT in KK-Ay mice

We next investigated the morphological change in epididymal WAT by histochemical analysis. The increase in adipocyte size induced by HFD was less pronounced in the

Table 2. Effect of fenugreek on plasma parameters in KK-Ay mice

	Control	0.5% Fenugreek	2% Fenugreek
Plasma glucose (mg/dL)	386.5±49.1	310.4±80.7	226.9±27.6*
Plasma insulin (ng/dL)	7.22±1.48	6.59±0.78	2.92±1.03*

Each value represents the mean±SEM ($n=5$). *$p<0.05$ compared with control, as analyzed by one-way ANOVA and Dunnet's multiple comparison tests.

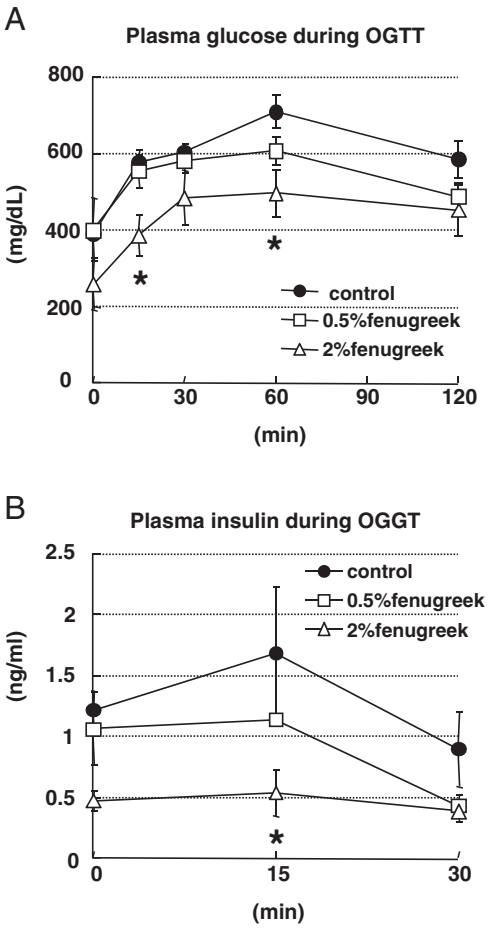

Figure 1. Effects of fenugreek on glucose tolerance in KK-Ay mice-fed HFD. We measured plasma glucose (A) and plasma insulin (B) levels during an OGTT in KK-Ay mice fed 0.5 or 2% fenugreek. Blood samples were collected from the tail tip at 15, 30, 60, and 120 min. ●, control; □, 0.5% fenugreek; Δ, 2% fenugreek. Each bar represents the mean±SEM ($n=5$). *$p<0.05$ compared with control, as analyzed by unpaired *t*-test.

2% fenugreek-fed mice than in the control mice (Figs. 2A and B). To clarify the mechanism by which fenugreek miniaturized WAT, we examined the effect of fenugreek on the mRNA expression levels of adipocyte-differentiation-related genes including *peroxisome-proliferator-activated receptor-γ* (*PPARγ*) and its target genes in WAT. The mRNA expression levels of *PPARγ* and its target genes (*adipocyte fatty acid-binding protein*, *aP2*; *lipoprotein lipase*, *LPL*; and *glucose transporter 4*, *Glut4*) in WAT were significantly higher in the 2% fenugreek-fed mice than in the control mice (Figs. 2C and D).

We next examined the effect of fenugreek on obesity-related inflammation in WAT. Fenugreek reduced the macrophage infiltration into WAT (Fig. 2E), and the mRNA expression level of *F4/80* in WAT was also significantly lower in the 2% fenugreek-fed mice than in the control mice (Fig. 2F). The mRNA expression levels of *MCP-1* and *TNF-α* in the epididymal WAT of the 2% fenugreek-fed mice tended to be lower than those in the epididymal WAT of the control mice, whereas that of *adiponectin* was significantly higher in the 2% fenugreek-fed mice than in the control mice (Fig. 2G). These results suggest that fenugreek miniaturizes adipocytes by promoting adipocyte differentiation and inhibits chronic inflammation in WAT in KK-Ay mice under HFD-fed conditions.

3.3 Effect of saponin fraction from fenugreek on adipocyte differentiation in 3T3-L1 cells

To elucidate the mechanism by which fenugreek promotes adipocyte differentiation, we determined the effect of fenugreek on adipocyte differentiation in 3T3-L1 cells. Fenugreek contains various alkaloids, flavonoids, and saponins. Of these, the saponin content is higher than those of the other two substances. It has been demonstrated that some saponin components have hypoglycemic effects [32, 33]. Therefore, we investigated the effect of the saponin fraction from fenugreek on adipocyte differentiation in 3T3-L1 cells. 3T3-L1 cells were treated with the saponin fraction for 48 h after differentiation induction, followed by incubation for 8 days. The saponin fraction had no effect on the adipocyte differentiation in 3T3-L1 cells (data not shown). Next, the saponin fraction was hydrolyzed with acid, and 3T3-L1 cells were treated with the hydrolyzed saponin for 48 h after differentiation induction, followed by incubation for 8 days. The hydrolyzed saponin promoted lipid accumulation in 3T3-L1 cells, as shown by Oil Red O staining (Fig. 3A). To identify the active compound, the hydrolyzed saponin was fractionated into six fractions by RP-HPLC (Fig. 3B). 3T3-L1 cells were treated with the fractionated hydrolyzed saponin for 48 h after the induction of differentiation. Fraction nos. 4 and 5 promoted lipid accumulation in 3T3-L1 cells (Fig. 3B).

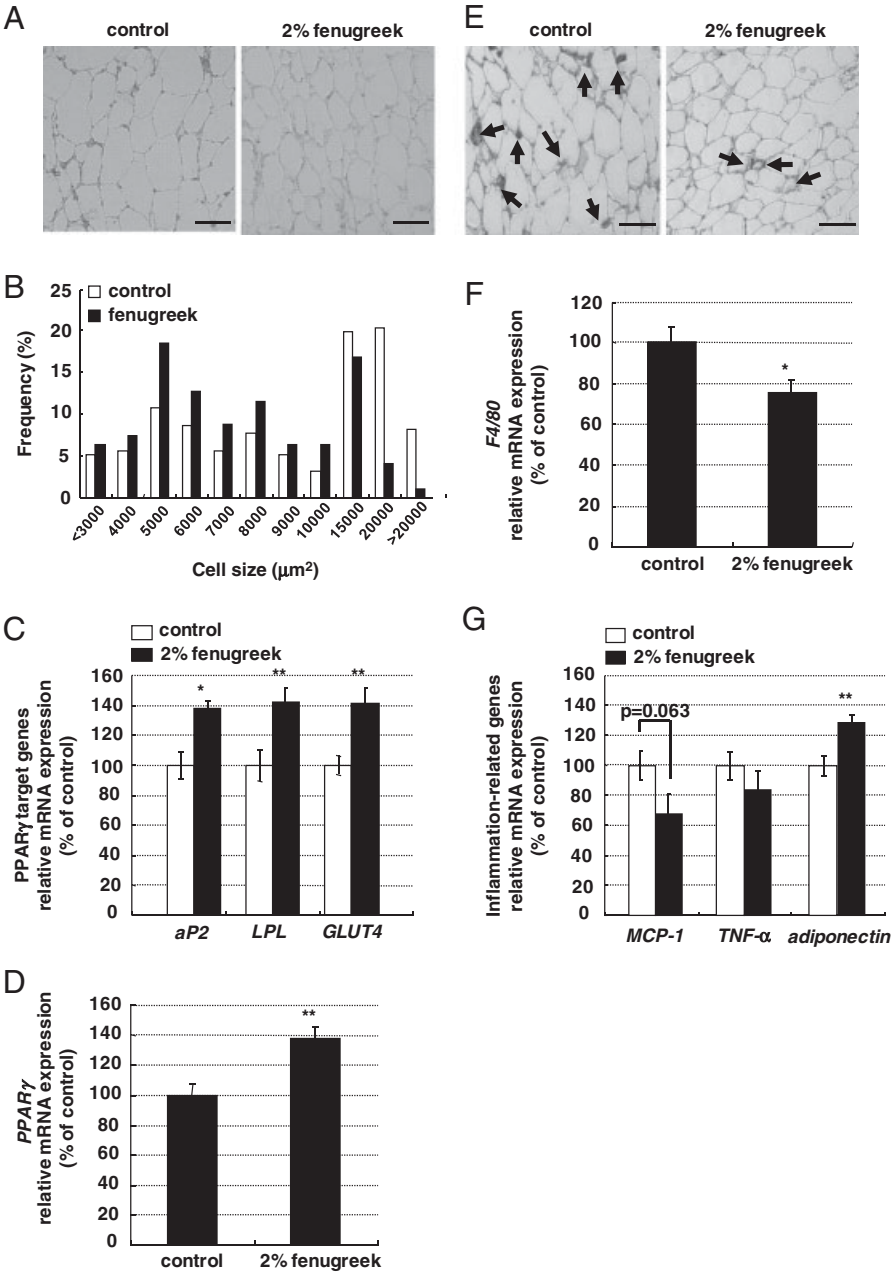

Figure 2. Effect of fenugreek on adipocyte differentiation and inflammation in KK-Ay mice-fed HFD. (A) and (E) histological analyses of epididymal WAT of KK-Ay mice-fed fenugreek. Scale bars: 100 μm. (E) The sections were stained with anti-F4/80 antibody. Arrows indicate F4/80-expressing cells. (B) Size distribution of adipocytes in epididymal fat tissue of KK-Ay mice. Data are mean values from analyses of five sections from each five mice. (C), (D), (F), and (G): amounts of mRNAs of *PPARγ* target genes (C), *PPARγ* (D), *F4/80* (F), *MCP-1*, *TNF-α*, and *adiponectin* (G) in epididymal WAT of mice fed 2% fenugreek. The amounts of mRNAs of these molecules were quantified by a RT-PCR method. The relative amount of each transcript was normalized to the amount of the *36B4* transcript. Each bar represents the mean ± SEM ($n = 6$). $^{*}p < 0.05$; $^{**}p < 0.01$ *versus* control group, as analyzed by unpaired *t*-test.

These results suggest that fraction nos. 4 and 5 of the fractionated hydrolyzed saponin stimulate the adipocyte differentiation of 3T3-L1 cells.

3.4 Identification of active substance in fenugreek

We identified the active substance in fraction no. 4, because its amount in fenugreek is more than that of fraction no. 5. To identify the active substance in fraction no. 4, we purified the fraction further to obtain a single peak by RP-HPLC and analyzed it by LC/MS. In the positive ESI mode, a protonated ion at *m/z* 415$[M+H]^+$ was detected. This finding suggests that the active substance was diosgenin, which is a major aglycon of fenugreek saponin [34], and whose molecular weight is 414. When analyzed by LC/MS/MS, the active substance yielded exactly the same product ions as those of authentic diosgenin (Fig. 4A). The identity of the active substance was further confirmed by ^{1}H-NMR and ^{13}C-NMR analyses (data not shown). The chemical structure of diosgenin is shown in Fig. 4B. 3T3-L1 cells were treated with diosgenin for 48 h after differentiation induction. Diosgenin promoted lipid accumulation in 3T3-L1 cells, as well as fraction no. 4 (Fig. 4C). These results suggest that

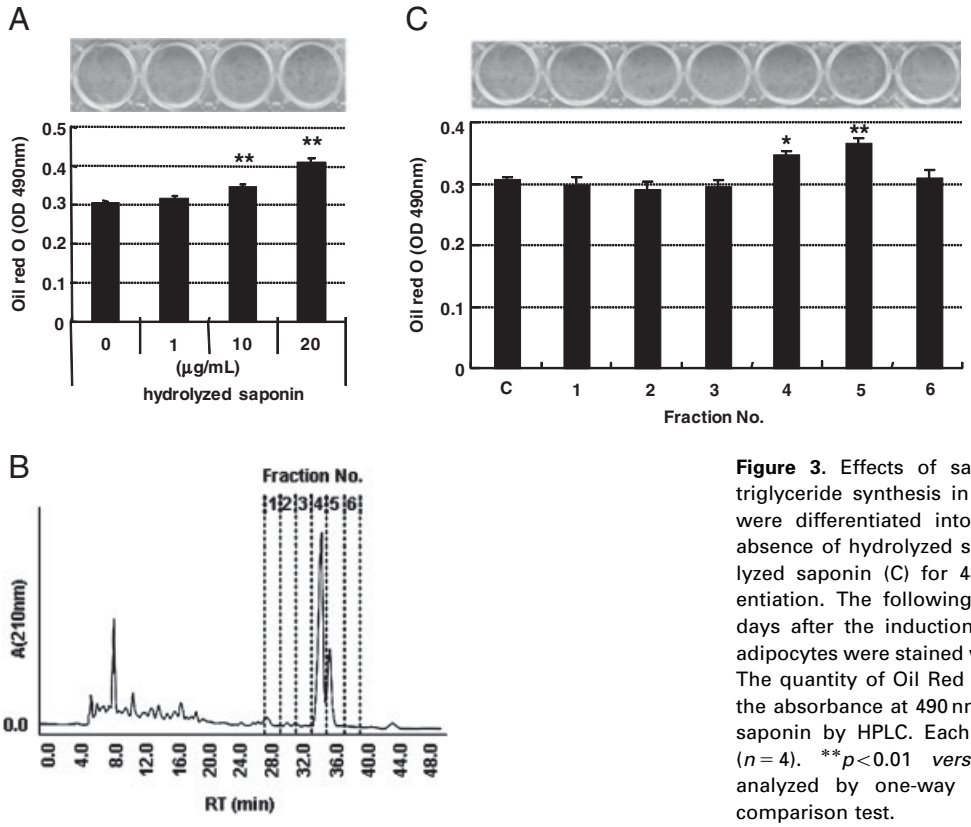

Figure 3. Effects of saponin fraction of fenugreek on triglyceride synthesis in 3T3-L1 cells. (A, C) 3T3-L1 cells were differentiated into adipocytes in the presence or absence of hydrolyzed saponin (A) or fractionated hydrolyzed saponin (C) for 48 h after the induction of differentiation. The following experiments were performed 8 days after the induction of differentiation. Differentiated adipocytes were stained with Oil Red O and photographed. The quantity of Oil Red O was determined by measuring the absorbance at 490 nm. (B) Fractionation of hydrolyzed saponin by HPLC. Each bar represents the mean ± SEM ($n = 4$). $^{**}p < 0.01$ *versus* control (0 μM fraction), as analyzed by one-way ANOVA and Dunnet's multiple comparison test.

diosgenin is the active compound in fenugreek, which causes adipocyte differentiation.

3.5 Diosgenin enhances glucose uptake by promoting adipocyte differentiation and affecting the expression of inflammation-related factors in 3T3-L1 cells

Next, we investigated the details of the effect on adipocyte differentiation. 3T3-L1 cells were treated with diosgenin throughout the differentiation period. Oil Red O staining showed that diosgenin promotes lipid accumulation in 3T3-L1 cells in a dose-dependent manner (Fig. 5A) and glycerol-3-phosphate dehydrogenase activity (Supporting Information Fig. 2). None of the tested concentrations of diosgenin affected the viability of 3T3-L1 cells (relative cell viability: 0 μM diosgenin, 100.0 ± 3.08; 1 μM, 103.1 ± 2.42; 5 μM, 99.0 ± 2.73; 10 μM, 99.1 ± 2.49%). Under the same conditions, diosgenin significantly increased the mRNA expression levels of *PPARγ* and its target genes (*aP2*, *LPL*, and *Glut4*) in a dose-dependent manner (Figs. 5B–E). Moreover, the diosgenin treatment throughout the differentiation period decreased both the mRNA expression level and protein secretion of MCP-1 (Figs. 6A–C), and increased those of adiponectin (Figs. 6B–D) in a dose-dependent manner. These data suggest that diosgenin promotes adipocyte differentiation in 3T3-L1 cells.

Finally, we investigated the effect of diosgenin on the insulin-dependent enhancement of glucose uptake into differentiated 3T3-L1 cells. The glucose uptake into differentiated 3T3-L1 cells (day 10) tended to be higher in diosgenin-treated groups in the absence of insulin than in no diosgenin-treated groups in the absence of insulin, but the difference was not significant. The glucose uptake into control cells (no diosgenin treatment) was increased to 1.25-fold by insulin stimulation, whereas in the 5 and 10 μM diosgenin-treated cells, the glucose uptake was increased to approximately two- and fourfold (*versus* basal 0 μM diosgenin), respectively (Fig. 7). These results indicate that diosgenin promotes the insulin-dependent glucose uptake into differentiated 3T3-L1 cells.

3.6 Diosgenin promotes adipocyte differentiation by increasing *C/EBP* mRNA expression

To elucidate the mechanism, by which diosgenin promotes adipocyte differentiation, we determined the effect of diosgenin on mRNA expression levels of *C/EBP*s at the early phase of adipocyte differentiation, which play a crucial role in the adipocyte differentiation. Diosgenin significantly increased mRNA expression level of *C/EBPδ* (Fig. 8A) at 1 h after the induction of adipocyte differentiation, but not that of *C/EBPβ* (Fig. 8B). These results indicate that diosgenin increases the mRNA expression of *C/EBPδ* at the early

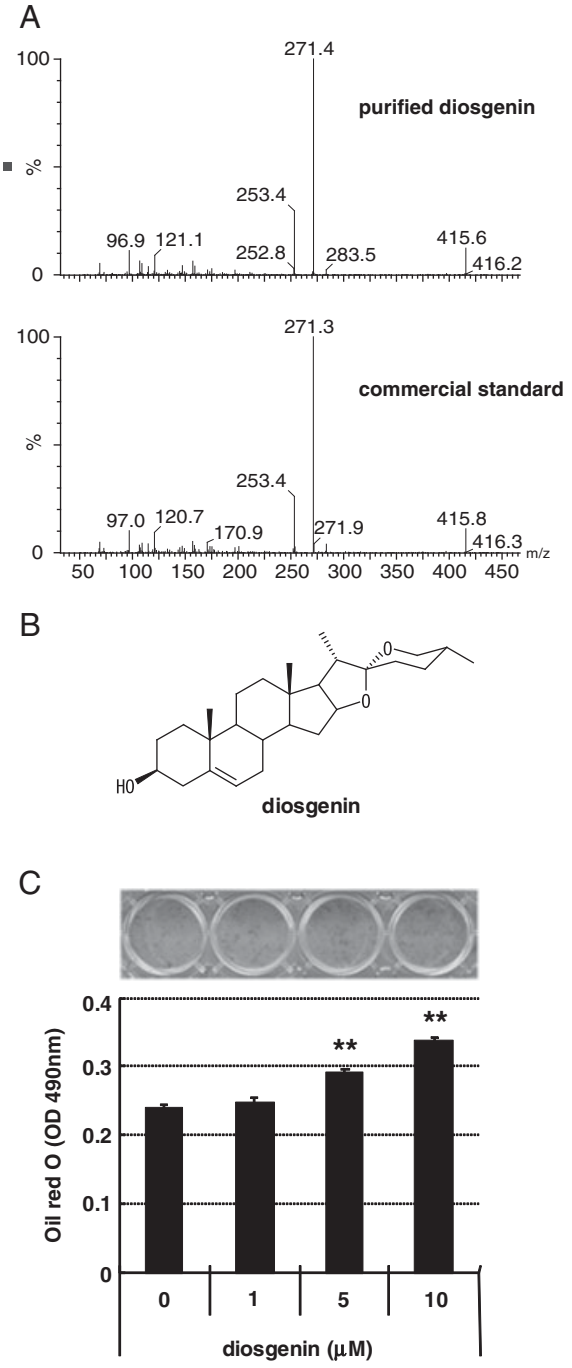

Figure 4. Identification of active substance in saponin fraction. (A) LC-MS/MS trace obtained by direct infusion of (A) purified fraction no. 4 (purified diosgenin) and authentic diosgenin (commercial standard). (B) Structure of diosgenin. (C) 3T3-L1 cells were differentiated into adipocytes in the presence or absence of diosgenin for 48 h after the induction of differentiation. Differentiated adipocytes were stained with Oil Red O and photographed. The quantity of Oil Red O was determined by measuring the absorbance at 490 nm. Each bar represents the mean ± SEM ($n = 4$). $^{**}p < 0.01$ *versus* control, as analyzed by one-way ANOVA and Dunnet's multiple comparison test.

phase of adipocyte differentiation, suggesting that the increase of the *C/EBPδ* expression level results in the promoting effect of diosgenin on adipocyte differentiation.

4 Discussion

We showed here that fenugreek ameliorated hyperglycemia and hyperinsulinemia accompanied by HFD-induced obesity in KK-Ay mice. Moreover, OGTT showed that fenugreek improved glucose intolerance. This is consistent with previous reports, in which fenugreek or its extracts have similar effects under different experimental conditions [18–20]. However, the detailed mechanism was unclear. In this study, we showed that fenugreek miniaturized the size of adipocytes under HFD-fed conditions. In addition, fenugreek enhanced the mRNA expression levels of *PPARγ* and its target genes, which are involved in adipocyte differentiation, in WAT. It has been reported that an increase in the number of small adipocytes can improve insulin resistance, whereas adipocyte hypertrophy causes insulin resistance [3, 35] and that the mRNA expression levels of such adipocyte-differentiation-related genes are decreased in large adipocytes of obese individuals [36]. Thiazolidinediones show antidiabetic effects by promoting adipocyte differentiation through the increase in the number of small adipocytes [3, 35]. Thus, these results suggest that fenugreek improves glucose intolerance by increasing the number of small adipocytes in WAT and that the increase in small adipocyte number is due to the promotion of adipocyte differentiation, similar to the effect of thiazolidinediones. Several reports have also demonstrated the effect of fenugreek seeds on adipocytes [37–39]. In the reports, aqueous extract of fenugreek seeds is used, in which no saponin is contained. In addition, the extract is administrated by intraperitoneal injections. Thus, our findings indicate another aspect of fenugreek as dietary components. Moreover, we found that fenugreek enhanced the mRNA expression of *Glut4* in WAT. The enhancement may partly contribute to the improvement of insulin resistance, because both the translocation of GLUT4 to the plasma membrane and the *Glut4* mRNA expression decrease in obese WAT [40–42]. Therefore, the enhancement of *Glut4* expression in WAT may contribute to the improvement of insulin resistance and diabetic conditions by the fenugreek treatment.

To determine the compound responsible for the effects of fenugreek on the *in vivo* promotion of adipocyte differentiation, we fractionized the fenugreek used in this study. It has been demonstrated that some saponin components have a hypoglycemic effect [32, 33]. Therefore, first, we investigated the effect of the saponin fraction in fenugreek on adipocyte differentiation in 3T3-L1 cells, but no effect was observed. It was assumed that saponin is hydrolyzed into aglycon, and that the hydrolyzed aglycon is absorbed in the intestine. Therefore, aglycon, not intact saponin

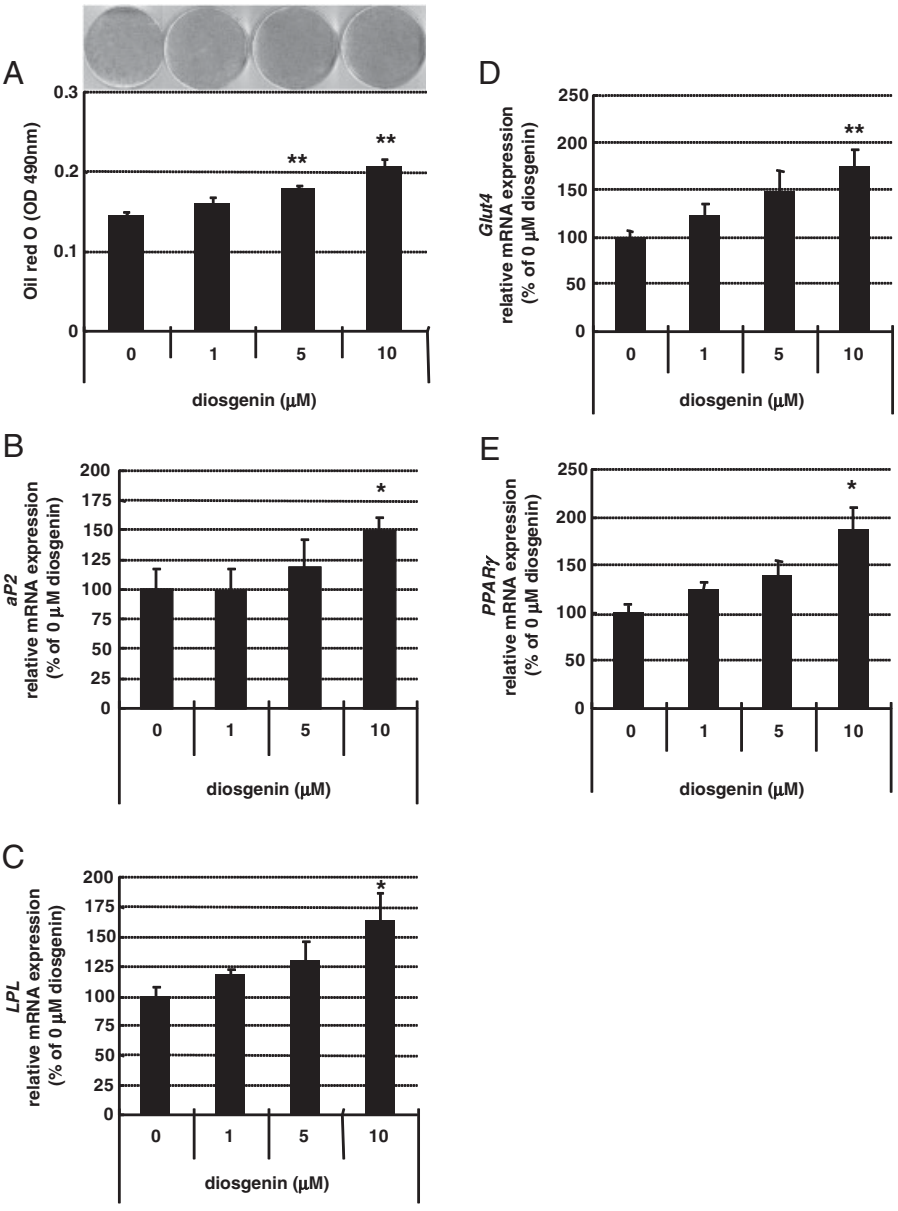

Figure 5. Effects of diosgenin on adipocyte differentiation. (A) 3T3-L1 cells were treated with diosgenin throughout the differentiation. The following experiments were performed at 6–8 days after the induction of differentiation. Differentiated adipocytes were stained with Oil Red O and photographed. The quantity of Oil Red O was determined by measuring the absorbance at 490 nm. Amounts of mRNAs of *aP2* (B), *LPL* (C), *Glut4* (D), and *PPARγ* (E) in differentiated 3T3-L1 cells treated with diosgenin throughout the differentiation period. The amounts of mRNAs were quantified as described in the caption of Fig. 2. Each bar represents the mean ± SEM ($n = 4$). $^{*}p < 0.05$; $^{**}p < 0.01$ *versus* 0 μM diosgenin, as analyzed by one-way ANOVA and Dunnet's multiple comparison test.

(glycosides), must be the active component that induces various effects *in vivo*. Given this assumption, we hydrolyzed the saponin fraction and added the hydrolyzed fraction to the medium of 3T3-L1 cells. Indeed, the hydrolyzed saponin fraction promoted adipocyte differentiation, and experiments using additional fractionized samples revealed that the most effective sample was found to contain diosgenin. This bioactive compound is a major aglycon of saponin in fenugreek [34, 43] and has been found to have various effects [44, 45]. We found no other fraction of our saponin extract showing the promoting effects on adipocyte differentiation. Therefore, we concluded that diosgenin is the compound in fenugreek responsible for the antidiabetic effects observed in this study, although we cannot completely exclude the possibility that other saponin(s) and/or compounds other than saponins are also responsible.

We showed that diosgenin enhanced the mRNA expression levels of *PPARγ* and its target genes in 3T3-L1 cells. The treatment with diosgenin induced the mRNA expressions of *aP2*, *LPL*, and *Glut4*, which are both adipocyte differentiation markers and PPARγ target genes. These results indicate that diosgenin promotes adipocyte differentiation. This is confirmed by the fact that diosgenin enhanced insulin-dependent glucose uptake and intracellular lipid accumulation in 3T3-L1 cells. A similar induction of differentiation marker genes was observed in the fenugreek-treated mice, as described earlier. Moreover, a stimulatory effect on adipocyte differentiation causes the

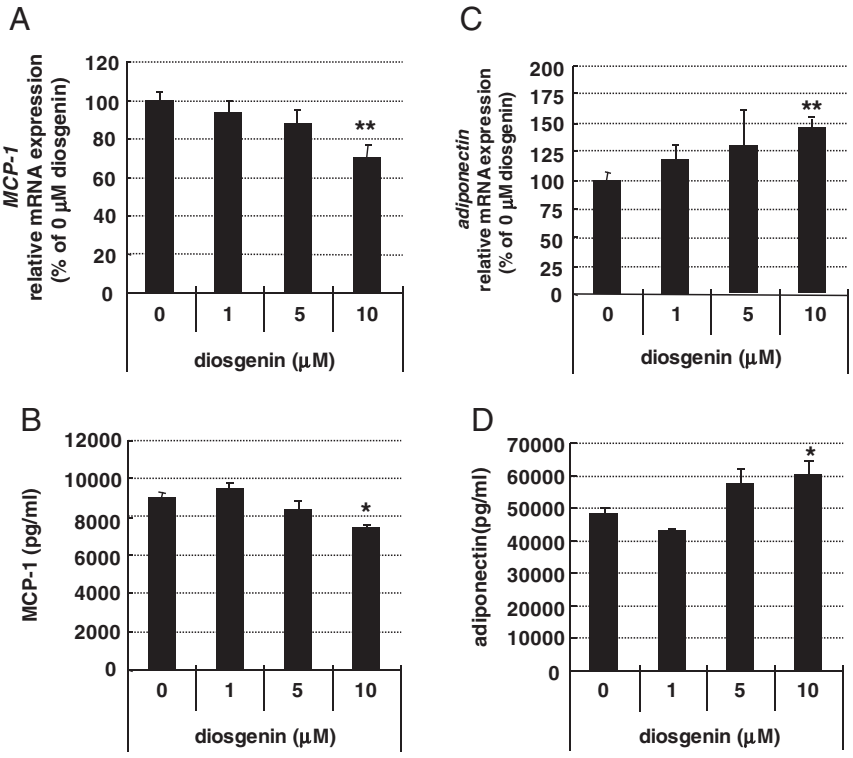

Figure 6. Effects of diosgenin on inflammation-related factors in 3T3-L1 cells. Cell culture was performed as described in the caption of Fig. 3. Protein secretion was measured using aliquots collected from differentiated 3T3-L1 cell culture media 6–8 days after the induction of differentiation. Amounts of mRNAs of *MCP-1* (A) and *adiponectin* (B) in 3T3-L1 cells. The amounts of mRNAs were quantified as described in Fig. 2 legend. MCP-1 (C) and adiponectin (D) secretion from 3T3-L1 cells. Each bar represents the mean ± SEM ($n = 4$). $^{*}p < 0.05$; $^{**}p < 0.01$ *versus* 0 μM diosgenin, as analyzed by one-way ANOVA and Dunnet's multiple comparison test.

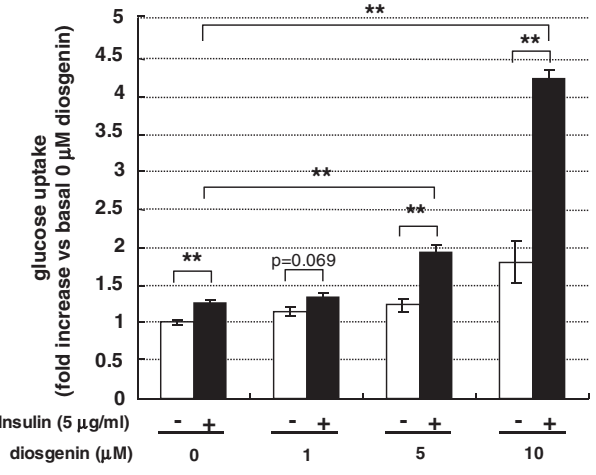

Figure 7. Effects of diosgenin on glucose uptake in 3T3-L1 cells. Cell culture was performed as described in the caption of Fig. 3. [^{3}H]-2-Deoxyglucose uptake was assessed 10 days after the induction of differentiation in the absence (A) or presence (B) of insulin. Glucose uptake was expressed as the degree of increase compared with basal control (basal 0 μM diosgenin). Each bar represents the mean ± SEM ($n = 4$). $^{*}p < 0.05$; $^{**}p < 0.01$, as analyzed by unpaired *t*-test.

enhancement of insulin-dependent glucose uptake. Thus, it is suggested that diosgenin (and fenugreek containing diosgenin) could contribute to the improvement of systemic insulin resistance by promoting adipocyte differentiation. Kato *et al.* have demonstrated that the diosgenin treatment shows no hypoglycemic effect [46]. However, they examined only the short-term effect of diosgenin (4 h after the diosgenin administration). On the other hand, we anticipated the long-term effect (4 wk with the diosgenin treatment). Therefore, our findings demonstrated that the long-term treatment shows the hypoglycemic effect. In general, the PPARγ ligand enhances the mRNA expression levels of PPARγ target genes and promotes the differentiation of adipocytes [47–49]. However, diosgenin did not enhance the transactivation of PPARγ in a luciferase reporter assay using CV-1 cells (data not shown). On the other hand, diosgenin increased the mRNA expression of *C/EBPδ* at the early phase of adipocyte differentiation. The increase in the *C/EBPδ* expression at the early phase promotes subsequent adipocyte differentiation processes [50, 51]. Therefore, it is suggested that diosgenin promotes adipocyte differentiation by enhancing the mRNA expression of *C/EBPδ* at the early phase of adipocyte differentiation. However, further studies are required to verify the complete mechanism of the stimulatory effect of diosgenin on adipocyte differentiation.

In this study, in addition to promotion of adipocyte differentiation, it is shown that fenugreek treatment suppressed the mRNA expression of an activated macrophage-derived proinflammatory factor, *TNF-α*, which promotes inflammatory processes and induces insulin resistance. Moreover, the fenugreek treatment decreased the number of F4/80-expressing macrophages and the mRNA expression levels of *F4/80* in WAT. We previously reported that diosgenin suppresses the inflammatory changes associated with the interaction of adipocytes and macrophages,

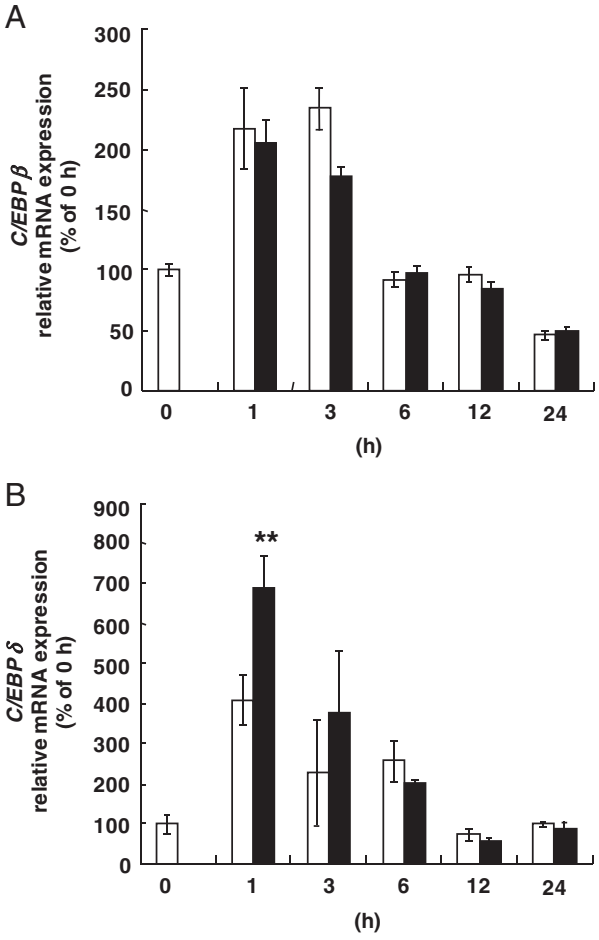

Figure 8. Effects of diosgenin on expression of *C/EBPβ* and *δ* at earlier adiopcyte differentiation in 3T3-L1 cells. 3T3-L1 cells were differentiated into adipocytes in the presence or absence of 10 μM diosgenin for 1, 3, 6, 12, or 24 h after the differentiation induction. Amounts of mRNAs of *C/EBPβ* (A) and *C/EBPδ* (B) in 3T3-L1 cells. The amounts of mRNAs were quantified as described in the caption of Fig. 2. Each bar represents the mean ± SEM ($n = 4$). $^{**}p < 0.01$ *versus* 0 μM diosgenin, as analyzed by unpaired *t*-test.

using a coculture system of macrophages and adipocytes [52]. Therefore, these results indicate that the fenugreek treatment suppresses chronic inflammation of WAT by the antiinflammatory effect of diosgenin, suggesting that the hypoglycemic effects of fenugreek are partly caused by the inhibition of chronic inflammation by suppressing the infiltration and activation of macrophages in WAT. In addition to the effects on macrophages, we showed that the fenugreek treatment significantly decreased the mRNA expression level of *MCP-1* and increased that of adiponectin in adipocytes. The inhibition of *MCP-1* expression by fenugreek results in the suppression of macrophage infiltration. The stimulatory effect on adiponectin expression suppresses macrophage activation that induces chronic inflammation in WAT. Therefore, fenugreek and diosgenin affect inflammation in not only macrophages but also adipocytes. It is suggested that the antiinflammatory effects of fenugreek and diosgenin contribute to the improvement of insulin resistance, in addition to promoting adipocyte differentiation.

In conclusion, we found that fenugreek ameliorated hyperglycemia and insulin resistance by promoting adipocyte differentiation and inhibiting chronic inflammation in WAT in diabetic obese mice. In addition, *in vitro* experiments using 3T3-L1 adipocytes showed that diosgenin, which is a major aglycon of saponin in fenugreek, promoted 3T3-L1 adipocyte differentiation to enhance insulin-dependent glucose uptake, as fenugreek does *in vivo*, indicating that diosgenin is the active compound in fenugreek. These findings indicate that fenugreek directly affects WAT and improves abnormalities of glucose metabolism in WAT in diabetic obese mice. In addition to the effects on the improvement of insulin resistance, we and others have reported that fenugreek and/or diosgenin also improve dyslipidemia in obesity-related diabetes [44, 53, 54]. Therefore, fenugreek containing the diosgenin is useful as a food factor for not only the control of obesity-related insulin resistance, but also the treatment of metabolic syndrome.

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The authors have declared no conflict of interest.

5 References

[1] Flier, J. S., Obesity wars: molecular progress confronts an expanding epidemic. *Cell* 2004, *116*, 337–350.

[2] Wellen, K. E., Hotamisligil, G. S., Inflammation, stress, and diabetes. *J. Clin. Invest.* 2005, *115*, 1111–1119.

[3] Okuno, A., Tamemoto, H., Tobe, K., Ueki, K. *et al.*, Troglitazone increases the number of small adipocytes without the change of white adipose tissues mass in obese Zucker rats. *J. Clin. Invest.* 1998, *101*, 1354–1362.

[4] Kubota, N., Terauchi, Y., Miki, H., Tamemoto, H. *et al.*, PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol. Cell* 1999, *4*, 597–609.

[5] Kadowaki, T., Insights into insulin resistance and type 2 diabetes from knockout mouse models. *J. Clin. Invest.* 2000, *106*, 459–465.

[6] Kamei, N., Tobe, K., Suzuki, R., Ohsugi, M. *et al.*, Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. *J. Biol. Chem.* 2006, *281*, 26602–26614.

[7] Nielsen, S., Jensen, M. D., Obesity and cardiovascular disease: is body structure a factor? *Curr. Opin. Lipidol.* 1997, *8*, 200–204.

[8] Dandona, P., Aljada, A., Bandyopadhyay, A., Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol.* 2004, *25*, 4–7.

[9] Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M. *et al.*, Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 2003, *112*, 1796–1808.

[10] Xu, H., Barnes, G. T., Yang, Q., Tan, G. *et al.*, Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* 2003, *112*, 1821–1830.

[11] Kanda, H., Tateya, S., Tamori, Y., Kotani, K. *et al.*, MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J. Clin. Invest.* 2006, *116*, 1494–1505.

[12] Hotamisligil, G. S., Peraldi, P., Budavari, A., Ellis, R. *et al.*, IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 1996, *271*, 665–668.

[13] Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y. *et al.*, The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat. Med.* 2001, *7*, 941–946.

[14] Díez, J. J., Iglesias, P., The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur. J. Endocrinol.* 2003, *148*, 293–300.

[15] Kadowaki, T., Yamauchi, T., Adiponectin and adiponectin receptors. *Endocr. Rev.* 2005, *26*, 439–451.

[16] Morton, J. F., Mucilaginous plants and their uses in medicine. *J. Ethnopharmacol.* 1990, *29*, 215–266.

[17] Basch, E., Ulbricht, C., Kuo, G., Szapary, P., Smith, M., Therapeutic applications of fenugreek. *Altern. Med. Rev.* 2003, *8*, 20–27.

[18] Ribes, Y., Sauvaire, C. D., Costa, J. C., Loubatieres-Mariani, M. M., Antidiabetic effects of subtractions from fenugreek seeds in diabetic dogs. *Proc. Soc. Exp. Biol. Med.* 1986, *182*, 159–166.

[19] Swanston-Flatt, S. K., Day, C., Flatt, P. R., Gould, B., Bailey, C. J., Glycemic effect of traditional European plants treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetes Res.* 1989, *10*, 69–73.

[20] Grover, J. K., Yadav, S., Vats, V., Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol.* 2002, *81*, 81–100.

[21] Sharma, R. D., Sarkar, A., Hazra, D. K., Mishra, B. *et al.*, Use of fenugreek seeds powder in the management of non-insulin dependent diabetes mellitus. *Nutr. Res.* 1996, *16*, 1331–1339.

[22] Hannan, J. M. A., Ali, L., Rokeya, B., Khaleque, J. *et al.*, Soluble dietary fibre fraction of *Trigonella foenum-graecum* (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. *Br. J. Nutr.* 2007, *97*, 514–521.

[23] Raghuram, T. C., Sharma, R. D., Sivakumar, B., Sahay, B. K., Effect of fenugreek seeds on intravenous glucose disposition in non-insulin dependent diabetic patients. *Phytother. Res.* 1994, *8*, 83–86.

[24] Sauvaire, Y., Petit, P., Broca, C., Manteghetti, M. *et al.*, 4-Hydroxyisoleucine: a novel amino acid potentiator of insulin secretion. *Diabetes* 1998, *47*, 206–210.

[25] Goto, T., Takahashi, N., Kato, S., Egawa, K. *et al.*, Phytol directly activates peroxisome proliferator-activated receptor alpha (PPAR alpha) and regulates gene expression involved in lipid metabolism in PPAR alpha-expressing HepG2 hepatocytes. *Biochem. Biophys. Res. Commun.* 2005, *337*, 440–445.

[26] Takahashi, N., Kawada, T., Goto, T., Yamamoto, T. *et al.*, Dual action of isoprenols from herbal medicines on both PPAR gamma and PPAR alpha in 3T3-L1 adipocytes and HepG2 hepatocytes. *FEBS Lett.* 2002, *514*, 315–322.

[27] Takahashi, N., Kawada, T., Yamamoto, T., Goto, T. *et al.*, Overexpression and ribozyme-mediated targeting of transcriptional coactivators CREB-binding protein and p300 revealed their indispensable roles in adipocyte differentiation through the regulation of peroxisome proliferator-activated receptor gamma. *J. Biol. Chem.* 2002, *277*, 16906–16912.

[28] Takahashi, N., Kawada, T., Goto, T., Kim, C. S. *et al.*, Abietic acid activates peroxisome proliferator-activated receptor-gamma (PPAR gamma) in RAW264.7 macrophages and 3T3-L1 adipocytes to regulate gene expression involved in inflammation and lipid metabolism. *FEBS Lett.* 2003, *550*, 190–194.

[29] Hirai, S., Kim, Y. I., Goto, T., Kang, M. S. *et al.*, Inhibitory effect of naringenin chalcone on inflammatory changes in the interaction between adipocytes and macrophages. *Life Sci.* 2007, *81*, 1272–1279.

[30] Kuroyanagi, K., Kang, M. S., Goto, T., Hirai, S. *et al.*, Citrus auraptene acts as an agonist for PPARs and enhances adiponectin production and MCP-1 reduction in 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.* 2008, *366*, 219–225.

[31] Takahashi, N., Goto, T., Taimatsu, A., Egawa, K. *et al.*, Bixin regulates mRNA expression involved in adipogenesis and enhances insulin sensitivity in 3T3-L1 adipocytes through PPAR gamma activation. *Biochem. Biophys. Res. Commun.* 2009, *390*, 1372–1376.

[32] Omoruyi, F. O., Jamaican bitter yam sapogenin: potential mechanisms of action in diabetes. *Plant. Foods. Hum. Nutr.* 2008, *63*, 135–140.

[33] Trinh, H. T., Han, S. J., Kim, S. W., Lee, Y. C., Kim, D. H., Bifidus fermentation increases hypolipidemic and hypoglycemic effects of red ginseng. *J. Microbiol. Biotechnol.* 2007, *17*, 1127–1133.

[34] Taylor, W. G., Zamanr, M. S., Mir, Z., Mir, P. S. *et al.*, Analysis of steroidal sapogenins from amber fenugreek (*Trigonella foenum-graecum*) by capillary gas chromatography and combined gas chromatography/mass spectrometry. *J. Agric. Food. Chem.* 1997, *45*, 753–759.

[35] Tsuchida, A., Yamauchi, T., Takekawa, S., Hada, Y. *et al.*, Peroxisome proliferator-activated receptor (PPAR) alpha

activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPAR alpha, PPAR gamma, and their combination. *Diabetes* 2005, *54*, 3358–3370.

[36] Dubois, S. G., Heilbronn, L. K., Smith, S. R., Albu, J. B. *et al.*, Decreased expression of adipogenic genes in obese subjects with type 2 diabetes. *Obesity* 2006, *14*, 1543–1552.

[37] Vijayakumar, M. V., Singh, S., Chhipa, R. R., Bhat, M. K., The hypoglycaemic activity of fenugreek seed extract is mediated through the stimulation of an insulin signalling pathway. *Br. J. Pharmacol.* 2005, *146*, 41–48.

[38] Vijayakumar, M. V., Bhat, M. K., Hypoglycemic effect of a novel dialysed fenugreek seeds extract is sustainable and is mediated, in part, by the activation of hepatic enzymes. *Phytother. Res.* 2008, *22*, 500–505.

[39] Vijayakumar, M. V., Pandey, V., Mishra, G. C., Bhat, M. K., Hypolipidemic effect of fenugreek seeds is mediated through inhibition of fat accumulation and upregulation of LDL receptor. *Obesity* 2010, *18*, 667–674.

[40] Kahn, B. B., Charron, M. J., Lodish, H. F., Cushman, S. W., Flier, J. S., Differential regulation of two glucose transporters in adipose cells from diabetic and insulin-treated diabetic rats. *J. Clin. Invest.* 1989, *84*, 404–411.

[41] Furuta, M., Yano, Y., Gabazza, E. C., Araki-Sasaki, R. *et al.*, Troglitazone improves GLUT4 expression in adipose tissue in an animal model of obese type 2 diabetes mellitus. *Diabetes Res. Clin. Pract.* 2002, *56*, 159–171.

[42] Abel, E. D., Peroni, O., Kim, J. K., Kim, Y. B. *et al.*, Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 2001, *409*, 729–733.

[43] Trivedi, P. D., Pundarikakshudu, K., Rathnam, S., Shah, K. S., A validated quantitative thin-layer chromatographic method for estimation of diosgenin in various plant samples, extract, and market formulation. *J. AOAC Int.* 2007, *90*, 358–363.

[44] Son, I. S., Kim, J. H., Sohn, H. Y., Son, K. H. *et al.*, Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (*Dioscorea* spp.), on high-cholesterol fed rats. *Biosci. Biotechnol. Biochem.* 2007, *71*, 3063–3071.

[45] Shishodia, S., Aggarwal, B. B., Diosgenin inhibits osteoclastogenesis, invasion, and proliferation through the downregulation of Akt, I kappa B kinase activation and NF-kappa B-regulated gene expression. *Oncogene* 2006, *25*, 1463–1473.

[46] Kato, A., Miura, T., Fukunaga, T., Effects of steroidal glycosides on blood glucose in normal and diabetic mice. *Biol. Pharm. Bull.* 1995, *18*, 167–168.

[47] Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O. *et al.*, An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J. Biol. Chem.* 1995, *270*, 12953–12956.

[48] Berger, J., Leibowitz, M. D., Doebber, T. W., Elbrecht, A. *et al.*, Novel peroxisome proliferator-activated receptor (PPAR) gamma and PPAR delta ligands produce distinct biological effects. *J. Biol. Chem.* 1995, *274*, 6718–6725.

[49] Spiegelman, B. M., PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 1998, *47*, 507–514.

[50] Park, J. R., Jung, J. W., Seo, M. S., Kang, S. K. *et al.*, DNER modulates adipogenesis of human adipose tissue-derived mesenchymal stem cells *via* regulation of cell proliferation. *Cell Prolif.* 2010, *43*, 19–28.

[51] Yeh, W. C., Cao, Z., Classon, M., McKnight, S. L., Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes Dev.* 1995, *9*, 168–181.

[52] Hirai, S., Uemura, T., Mizoguchi, N., Lee, J. Y. *et al.*, Diosgenin attenuates inflammatory changes in the interaction between adipocytes and macrophages. *Mol. Nutr. Food Res.* 2010, DOI: 10.1002/mnfr.200900208.

[53] Annida, B., Stanely Mainzen Prince, P., Supplementation of fenugreek leaves lower lipid profile in streptozotocin-induced diabetic rats. *J. Med. Food* 2004, *7*, 153–156.

[54] Raju, J., Bird, R. P., Alleviation of hepatic steatosis accompanied by modulation of plasma and liver TNF-alpha levels by *Trigonella foenum graecum* (fenugreek) seeds in Zucker obese (fa/fa) rats. *Int. J. Obes.* 2006, *30*, 1298–1307.

