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Phosphorylated glucosamine inhibits adipogenesis in 3T3-L1 adipocytes

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Abstract

Phosphorylated glucosamine (glucosamine-6-phosphate, PGlc) was synthesized using methanesulfonic acid, phosphorus pentoxide (P_2O_5), NH_2NH_2 and DMF. Its inhibitory effect on lipid accumulation in cultured 3T3-L1 adipocytes was investigated by measuring triglyceride contents and Oil Red O staining. In order to understand the mechanism by which lipid accumulation in adipocytes is decreased by PGlc, we examined the expression levels of several genes and proteins associated with adipogenesis and lipolysis using reverse transcription polymerase chain reaction, real-time polymerase chain reaction and Western blot analysis. Treatment with PGlc significantly reduced lipid accumulation during adipocyte differentiation and induced down-regulation of peroxisome proliferator-activated receptor-γ, sterol regulatory element binding protein 1 and CCAAT/enhancer binding protein-α in a dose-dependent manner. Moreover, treatment with PGlc during adipocyte differentiation induced significant up-regulation of preadipocyte factor 1 mRNA and down-regulation of such adipocyte-specific gene promoters as adipocyte fatty acid binding protein, fatty acid synthase, lipoprotein lipase and leptin. According to the lipolytic response, PGlc up-regulated hormone-sensitive lipase mRNA expression and suppressed the expression levels of tumor necrosis factor-α mRNA compared with fully differentiated adipose tissue. These results suggest that the inhibitory effect of PGlc on adipocyte differentiation might be mediated through the down-regulation of adipogenic transcription factors, such as peroxisome proliferator-activated receptor-γ, sterol regulatory element binding protein 1 and CCAAT/enhancer binding protein-α, which are related to the downstream adipocyte-specific gene promoters.

Keywords: Phosphorylated glucosamine; Adipogenesis; 3T3-L1; Adipocytes

1. Introduction

The growth rate of the obese population has been increasing worldwide, and obesity has become one of the most serious public health problems. Obesity is defined as excessive body weight in the form of fat and is associated with numerous pathological disorders, such as diabetes, hypertension and cardiovascular disease, among others [1,2]. Adipocytes play a vital role in regulating adipose mass and obesity, related not only to lipid homeostasis and energy balance but also to secreting various transcription factors [3]. Research on the relationship between occurrence of obesity and adipocyte differentiation or fat accumulation has been recently conducted [4]. The cellular regulatory mechanisms of adipocyte differentiation have been facilitated using 3T3-L1 cells. 3T3-L1 cells can induce the differentiation of preadipocytes into mature adipocytes. The programmed differentiation of preadipocytes involves several stages related to obesity [5]. For these reasons, many research efforts have been conducted in 3T3-L1 cells to search for new health-beneficial food/agents for obesity or weight control.

Numerous clinical trials for glucosamine (Glc) supplements have been carried out widely. Three kinds of Glc, such as Glc hydrochloride, sulfated Glc and *N*-acetyl-Glc, are generally used as Glc nutritional supplements [6]. Among them, the positive effect of sulfated Glc on MMP and osteoblastic differentiation was reported [6,7]. These Glc's can be derived from chitin, which is a biopolymer found in the exoskeleton of marine invertebrate animals. Also, chitosan obtained by alkaline deacetylation of the chitin has received considerable attention for its commercial applications in the biomedical, food and chemical industries [8]. Among derivatives of chitosan, phosphorylated derivatives exhibited the highest inhibitory activity on the formation of calcium phosphate [9]. Therefore, it could be expected that phosphorylated Glc (Glc-6-phosphate, PGlc) contributes to some kind of biomedical activity.

In this study, we synthesized PGlc and investigated its effects on lipid accumulation in cultured 3T3-L1 adipocytes by measuring triglyceride (TG) contents and Oil Red O staining as indicators of lipid accumulation. To understand the mechanism by which lipid accumulation in adipocytes is decreased by PGlc, we examined the expression levels of several genes and proteins associated with

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adipogenesis and lipolysis using reverse transcription polymerase chain reaction (RT-PCR), real-time PCR and Western blot analysis.

2. Materials and methods

2.1. Synthesis of PGlc

Glc was phosphorylated by phosphorus pentoxide (P_2O_5) [10]. Briefly, *N*-phtalamide Glc (1 mM) was dissolved in 10 ml of methanesulfonic acid with the addition of phosphorus pentoxide, followed by stirring at 5°C for 4 h. The products were precipitated with ether and centrifuged. The precipitates were air-dried and dissolved in DMF (50 ml), hydrazine monohydrate (20 ml) and water (40 ml) in order to remove the residue phtalamide. The mixture was heated to 100°C under N_2 atmosphere with stirring. After reaction for 15 h, the suspension was filtrated, 50 ml of water was added into the filter layer and the filtrates were then dried in vacuum with a rotary evaporator. Fifty milliliters of water was added into the dried product. The resultant solution was cooled and then dialyzed exhaustively against distilled water using a 100-Da molecular-weight-cutoff dialysis membrane. Small amounts of non-reacted Glc and impurities were removed following cation-exchange chromatography using a Dowex 50 cation-exchange resin. The structure of purified PGlc was determined by FT-IR and proton (^{1}H)/carbon (^{13}C) NMR spectroscopy [JNM-ECP-400 (400 MHz) spectrometer, JEOL, Japan]. PGlc was dissolved and then diluted with the medium. All other reagents were from Sigma Chemical (St. Louis, MO, USA) unless otherwise stated (Fig. 1).

PGlc: white solid; IR (KBr) ν_{max} 3412 (OH), 2864 (CH), 1578 (NH), 1309 (CN), 1200 (P=O), 1100~1010 (pyranose), 1050 (POC), 900 (PO); ^{1}H NMR (D_2O, 400 MHz) δ 5.33, 4.83 (1H, H-1$_\alpha$, H-1$_\beta$), 3.22, 3.0 (1H, H-2$_\alpha$, H-2$_\beta$), 4.0–3.47 (1H, H-3, 1H, H-4, 1H, H-5 and 2H, H_2-6), 4.7 (D_2O); ^{13}C NMR (D_2O, 400MHz) δ 89.40, 92.98 (C-1$_\alpha$, C-1$_\beta$), 54.4, 56.84 (C-2$_\alpha$, C-2$_\beta$), 69.40 (C-3), 75.22 (C-4), 75.22, 70.82 (C-5$_\alpha$, C-5$_\beta$), 63.90 (C-6).

2.2. Cell culture and adipocyte differentiation

Mouse 3T3-L1 preadipocytes were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 5% CO_2. One day after confluence (designated as "Day 0"), cell differentiation was induced with a mixture of methylisobutylxanthine (0.5 mM), dexamethasone (0.25 μM) and insulin (5 μg/ml) in DMEM containing 10% FBS. After 48 h (Day 2), the induction medium was removed and replaced by DMEM containing 10% FBS supplemented with insulin (5 μg/ml) alone. This medium was changed every 2 days. Samples were treated into culture medium of adipocytes on Day 0. After treatment with Glc and PGlc for 6 days, the adipose tissue was lysed for analysis. Cytotoxicity of PGlc was evaluated by MTT assay. Any significant toxic effect was not observed on the cells treated with PGlc up to a concentration of 200 μg/ml (data not shown). Therefore, the experiments were carried out up to a concentration of 200 μg/ml.

2.3. Measurement of TG content

Cellular TG contents were measured using a commercial TG assay kit (Triglyzyme-V, Eiken Chemical, Tokyo, Japan) according to the manufacturer's instructions. Cells were treated with PGlc with concentrations of 50, 100 and 200 μg/ml in 12-well plates during adipocyte differentiation for 6 days. The cells were washed twice with phosphate-buffered saline (PBS), scraped in 75 μl of homogenizing solution (154 mM KCl, 1 mM EDTA and 50 mM Tris, pH 7.4) and sonicated to homogenize the cell suspension. The residual cell lysate was centrifuged at 3000×*g* for 5 min at 4°C to remove fat layers. The supernatants were assayed for TG and protein contents. TG was normalized to protein concentration determined by the BSA as standard. Results were expressed as milligrams of TG per milligram of cellular protein.

Table 1
Gene-specific primers used for RT-PCR and real-time RT-PCR

Gene	Direction	Sequence
PPARγ	Forward	5′-TTT TCA AGG GTG CCA GTT TC-3′
	Reverse	5′-AAT CCT TGG CCC TCT GAG AT-3′
SREBP1	Forward	5′-TGT TGG CAT CCT GCT ATC TG-3′
	Reverse	5′-AGG GAA AGC TTT GGG GTC TA-3′
C/EBPα	Forward	5′-TTA CAA CAG GCC AGG TTT CC-3′
	Reverse	5′-GGC TGG CGA CAT ACA GTA CA-3′
Pref-1	Forward	5′-CTA ACC CAT GCG AGA ACG AT-3′
	Reverse	5′-GCT TGC ACA GAC ACT CGA AG-3′
aP2	Forward	5′-TCA CCT GGA AGA CAG CTC CT-3′
	Reverse	5′-AAT CCC CAT TTA CGC TGA TG-3′
Leptin	Forward	5′-GGA TCA GGT TTT GTG GTG CT-3′
	Reverse	5′-TTG TGG CCC ATA AAG TCC TC-3′
LPL	Forward	5′-TCC AAG GAA GCC TTT GAG AA-3′
	Reverse	5′-CCA TCC TCA GTC CCA GAA AA-3′
FAS	Forward	5′-TTG CTG GCA CTA CAG AAT GC-3′
	Reverse	5′-AAC AGC CTC AGA GCG ACA AT-3′
HSL	Forward	5′-GAG GGA CAC ACA CACACC TG-3′
	Reverse	5′-CCC TTT CGC AGC AAC TTT AG-3′
Perilipin	Forward	5′-AAG GAT CCT GCA CCT CAC AC-3′
	Reverse	5′-CCT CTG CTG AAG GGT TAT CG-3′
TNFα	Forward	5′-AGG CCT TGT GTT GTG TTT CCA-3′
	Reverse	5′-TGG GGG ACA GCT TCC TTC TT-3′
β-Actin	Forward	5′-CCA CAG CTG AGA GGG AAA TC-3′
	Reverse	5′-AAG GAA GGC TGG AAA AGA GC-3′

2.4. Oil Red O staining

For Oil Red O staining [11], cells were washed gently with PBS twice, fixed with 3.7% fresh formaldehyde (Sigma) in PBS for 1 h at room temperature and stained with filtered Oil Red O solution (60% isopropanol and 40% water) for at least 1 h. After staining of lipid droplets with Red, the Oil Red O staining solution was removed and the plates were rinsed with water and dried. Images were collected on an Olympus microscope (Tokyo, Japan). Finally, the dye retained in the cells was eluted with isopropanol and quantified by measuring the optical absorbance at 500 nm.

2.5. RNA extraction and RT-PCR

Total cellular RNA was extracted from treated cells using Trizol reagent (Invitrogen, CA, USA) following the manufacturer's recommendations. Total RNA (2 μg) was converted to single-stranded cDNA using an RT system (Promega). The target cDNA was amplified using the sense and antisense primers shown in Table 1. The amplification cycles were 95°C for 45 s, 60°C for 1 min and 72°C for 45 s. After 30 cycles, the PCR products were separated by electrophoresis on 1.0% agarose gel for 30 min at 100 V. Gels were then stained with 1 mg/ml of ethidium bromide visualized by UV light using AlphaEase gel image analysis software (Alpha Innotech, CA, USA).

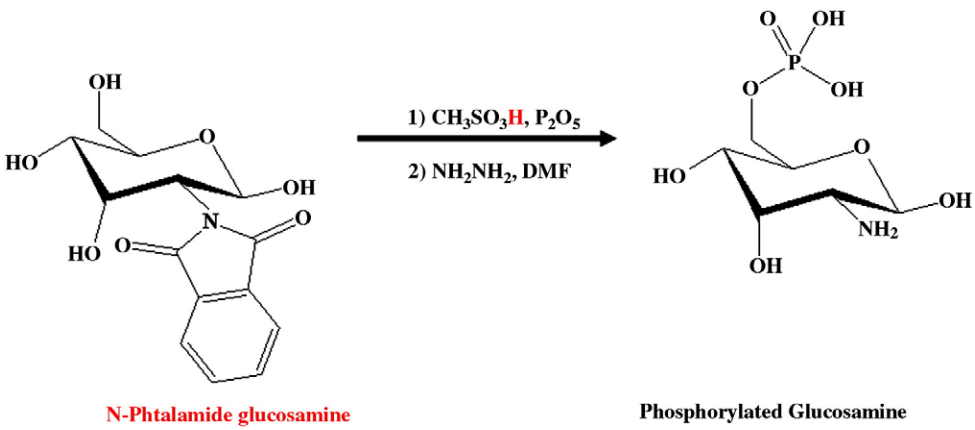

Fig. 1. Synthesis pathway of PGlc.

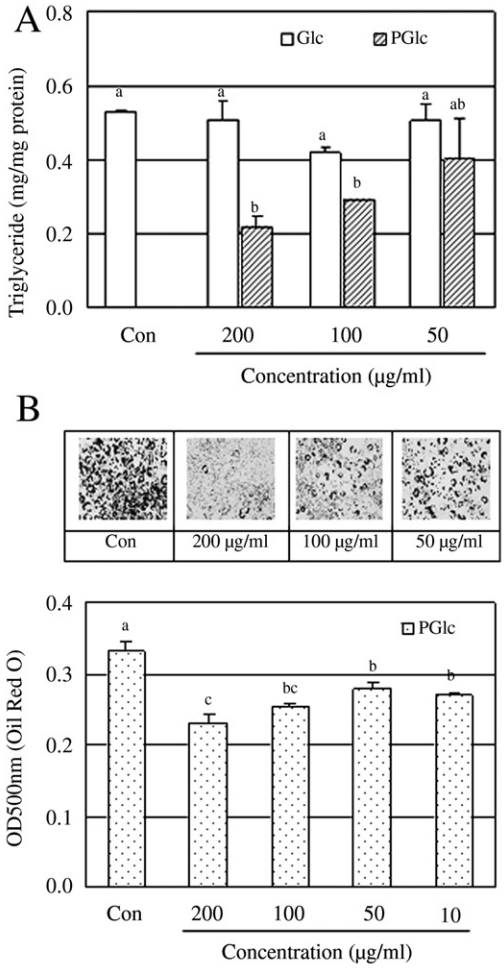

Fig. 2. Effects of Glc and PGlc on adipocyte differentiation in 3T3-L1 cells. Cells were cultured in the medium containing different concentrations from Day 0 to Day 6, and lipid accumulation was measured by (A) TG content and (B) Oil Red O staining. Means with the different letters (*a–c*) are significantly different ($P<.05$) by Duncan's multiple-range test. Con, untreated control adipocyte (0.5 mM methylisobutylxanthine, 0.25 μM dexamethasone and 5 μg/ml of insulin).

2.6. Real-time RT-PCR analysis

Total RNA was isolated from 3T3-L1 adipocytes using Trizol reagent (Invitrogen). For synthesis of first-strand cDNA, 1 μg of RNA was added to RNase-free water and oligo (dT), denaturated at 70°C for 5 min and cooled immediately. RNA was reverse transcribed in a master mix containing 1× RT buffer, 1 mM dNTP, 500 ng of oligo(dT), 140 U of MMLV reverse transcriptase and 40 U of RNase inhibitor at 42°C for 60 min and at 72°C for 5 min using an automatic Whatman thermocycler (Biometra, UK). One microliter of each RT reaction was amplified in a 25-μl PCR assay volume using a Master Mix kit containing HotStarTaq Plus DNA Polymerase, QuantiFast SYBR PCR buffer, dNTP mix, SYBR Green I dye and ROX dye (Quiagen, Germany). Quantitative SYBR Green real-time PCR was performed on Rotor gene 6000 (Corbett) using the following program: samples were incubated in an ExiCycler (Bioneer, Daejeon, Korea) for initial denaturation at 94°C for 10 min, followed by 40 PCR cycles. Each cycle proceeded at 94°C for 40 s, 59°C for 30 s and 72°C for 30 s. Relative quantification was calculated using the $2^{-(\Delta\Delta C_t)}$ method [12], where $\Delta\Delta C_t=(C_{t,target}-C_{t,actin})_{treated\ sample}-(C_{t,target}-C_{t,actin})_{control\ sample}$. To confirm amplification of specific transcripts, we produced melting curve profiles (cooling the sample to 40°C and heating it slowly to 95°C with continuous measurement of fluorescence) at the end of each PCR.

2.7. Western blot analysis

Western blotting was performed according to standard procedures. Briefly, cells were lysed in RIPA buffer containing 50 mM Tris–HCl, pH 8.0, 0.4% Nonidet P-40, 120 mM NaCl, 1.5 mM $MgCl_2$, 2 mM phenylmethylsulfonyl fluoride, 80 μg/ml of leupeptin, 3 mM NaF and 1 mM DTT at 4°C for 30 min. Cell lysates (50 μg) were separated by 12% SDS-PAGE, transferred onto a polyvinylidene fluoride membrane (Amersham Pharmacia Biotech, England, UK), blocked with 5% skim milk and hybridized with primary antibodies (diluted 1:1000). After incubation with horseradish peroxidase-conjugated secondary antibody at room temperature, immunoreactive proteins were detected using a chemiluminescent ECL assay kit (Amersham Pharmacia Biosciences, England, UK) according to the manufacturer's instructions. Western blot bands were visualized using an LAS-3000 Luminescent image analyzer (Fujifilm Life Science, Tokyo, Japan).

2.8. Statistical analysis

Data were expressed as mean±S.E. values ($n=3$) and analyzed using the analysis of variance procedure of the Statistical Analysis System (SAS Institute, Cary, NC, USA). Significant differences between treatment means were determined using Duncan's multiple-range tests. Significance of differences was defined at the $P<.05$ level.

3. Results

3.1. Effect of PGlc on intracellular lipid accumulation in adipocytes

To explore the antiadipogenic potential of PGlc, we differentiated 3T3-L1 preadipocytes with PGlc for 6 days (from Day 0 to Day 6). Lipid accumulation as a major marker of adipogenesis was quantified by direct TG measurement and Oil Red O staining (Fig. 2). Treatment with PGlc reduced the TG content of differentiated adipocyte lysate in a dose-dependent manner ($P<.05$), but Glc did not. We also stained TGs of fully differentiated adipocytes with Oil Red O staining solution. The OD value of Oil Red O-eluted solution represents lipid droplet

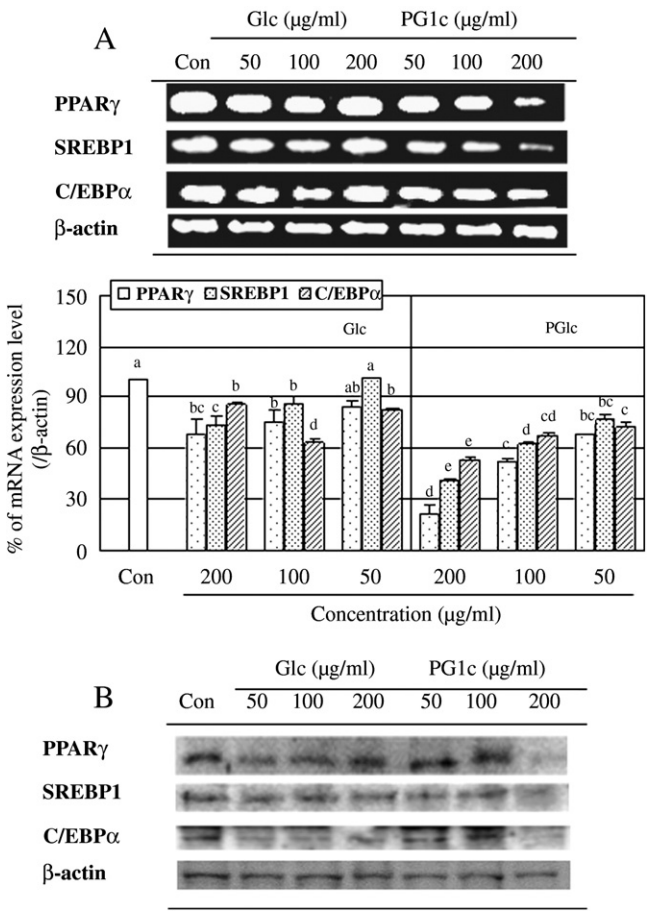

Fig. 3. Effects of Glc and PGlc on PPARγ, SREBP1 and C/EBPα mRNA (A) and protein (B) expressions in 3T3-L1 cells. Cells were cultured in the medium containing different concentrations of Glc and PGlc from Day 0 to Day 6. Means with the different letters (*a–e*) at each sample and mRNA are significantly different ($P<.05$) by Duncan's multiple-range test.

accumulation in the cytoplasm. PGlc inhibited lipid accumulation of treated cells in a dose-dependent manner ($P<.05$). The OD value of eluted dye decreased according to the increased concentrations during adipocyte differentiation.

3.2. Inhibition of adipogenesis

To determine whether PGlc affects the expression of transcriptional factors, we conducted RT-PCR and Western blotting analysis (Fig. 3). Treatment with PGlc reduced the size and intensity of the lytic zone on regulation of peroxisome proliferator-activated receptor-γ (PPARγ), differentiation-dependent factor 1/sterol regulatory element binding protein 1 (SREBP1) and CCAAT/enhancer binding protein-α (C/EBPα) mRNA, whereas down-regulation by Glc was not high compared with differentiated adipocytes. The inhibitory effects of PGlc exhibited a dose-dependent pattern. Treatment with PGlc also suppressed the protein expressions of PPARγ, SREBP1 and C/EBPα.

3.3. Effect of PGlc on preadipocyte factor 1, adipocyte fatty acid binding protein, fatty acid synthase, lipoprotein lipase and leptin mRNA expression

We further studied whether PGlc regulates the expressions of the adipogenic target genes adipocyte fatty acid binding protein (aP2), fatty acid synthase (FAS), lipoprotein lipase (LPL) and leptin, as well as the preadipocyte factor 1 (Pref-1) gene characteristic of an undifferentiated state. Treatment with PGlc inhibited the expression levels of FAS, LPL and leptin mRNA in a dose-dependent manner (Fig. 4). Moreover, treatment with PGlc during adipocyte differentiation induced significant up-regulation of Pref-1 mRNA and down-regulation of the expression level of aP2 mRNA (Fig. 5).

3.4. Effect of PGlc on lipolysis

As the lipolytic response during differentiation of adipocytes, the gene expression levels of perilipin, hormone-sensitive lipase (HSL) and tumor necrosis factor-α (TNFα) were determined using RT-PCR (Fig. 6). Treatment with PGlc up-regulated HSL mRNA expression, but not perilipin mRNA expression. Also, treatment with PGlc induced down-regulation of TNFα mRNA expression compared with fully differentiated adipose tissue.

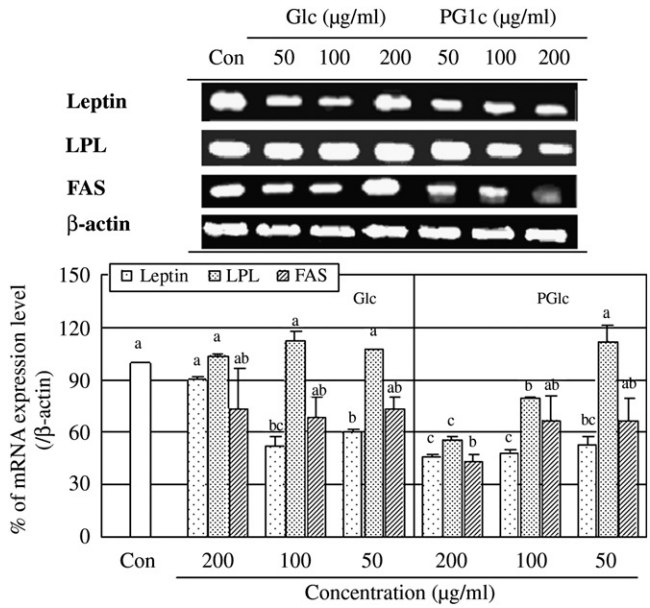

Fig. 4. Effects of Glc and PGlc on leptin, LPL and FAS mRNA expressions in 3T3-L1 cells. Cells were cultured in the medium containing different concentrations of Glc and PGlc from Day 0 to Day 6. Means with the different letters (*a–c*) at each sample and mRNA are significantly different ($P<.05$) by Duncan's multiple-range test.

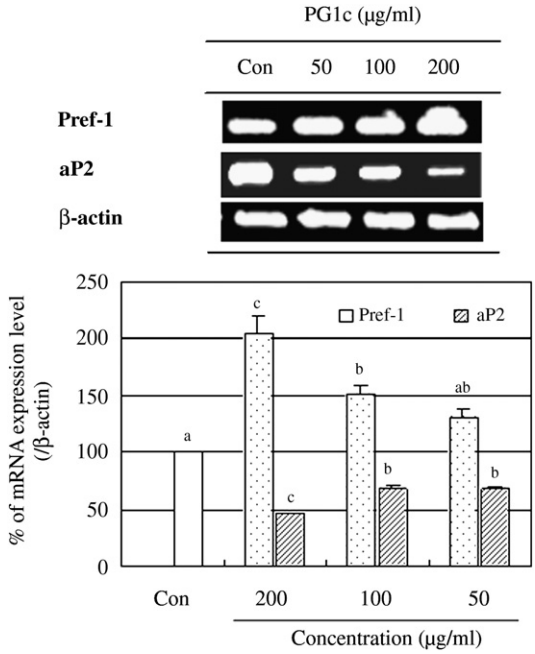

Fig. 5. Effect of PGlc on Pref-1 and aP2 mRNA expressions in 3T3-L1 cells. Cells were cultured in the medium containing different concentrations of PGlc from Day 0 to Day 6. Means with the different letters (*a–c*) at each mRNA are significantly different ($P<.05$) by Duncan's multiple-range test.

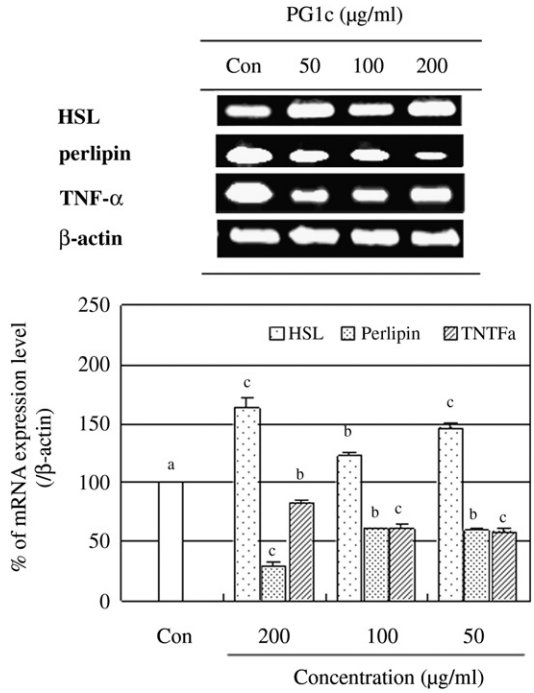

Fig. 6. Effect of PGlc on HSL, perilipin and TNFα mRNA expressions in 3T3-L1 cells. Cells were cultured in the medium containing different concentrations of PGlc from Day 0 to Day 6. Means with the different letters (*a–c*) at each mRNA are significantly different ($P<.05$) by Duncan's multiple-range test.

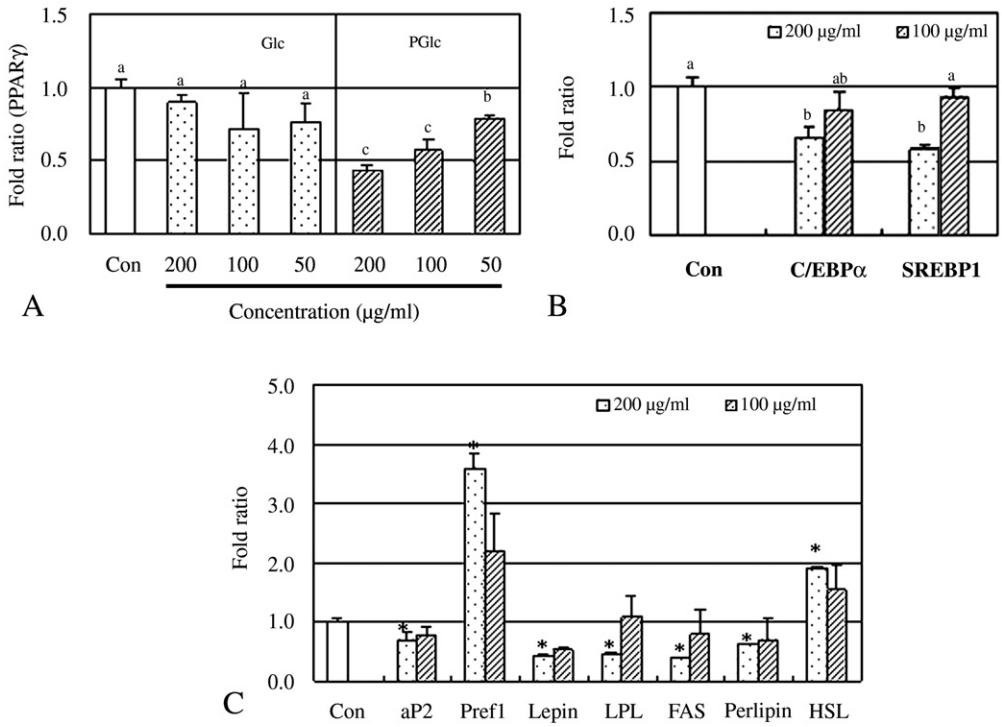

Fig. 7. Effect of PGlc on real-time PCR analysis in 3T3-L1 cells. Cells were cultured in the medium containing different concentrations of PGlc from Day 0 to Day 6. Means with the different letters (*a*–*c*) at each mRNA are significantly different ($P<.05$) by Duncan's multiple-range test. *$P<.05$.

3.5. Effect of PGlc on real-time PCR analysis of mRNA expression

During differentiation of adipocytes, the effect of PGlc on gene expression was determined using real-time PCR (Fig. 7). Treatment with PGlc induced dose-dependent down-regulation of PPARγ compared with differentiated adipocytes without sample treatment, while treatment with Glc did not exhibit any effect on regulation of PPARγ expressions. Therefore, real-time PCR analysis was carried out for PGlc, because there was no significant difference in PPARγ expressions between different concentrations of Glc. The expression levels of SREBP1, C/EBPα, aP2, FAS, LPL and leptin mRNA were down-regulated by treatment with PGlc. The expression levels of Pref-1 and HSL mRNA were up-regulated by treatment with PGlc. These results are in line with the results of PCR analysis.

4. Discussion

Obesity has been recognized worldwide as one of the most serious socioeconomic health problems. Obesity is the heavy accumulation of fat in the body's fat cells to such a serious degree that greatly increases the risk for obesity-associated diseases, such as type 2 diabetes, hypertension, cancer, osteoarthritis and heart disease, among others [2]. It is known that adipocyte differentiation and the amount of fat accumulation are associated with the occurrence and development of obesity [4]. Adipocytes play a vital role in lipid homeostasis and energy balance by relating to TG storage and free fatty acid release. For these reasons, many studies have been conducted to search for new health-beneficial materials for obesity or weight control. Evans et al. [13] suggested the following as possible mechanisms in reference to antiobesity actions: reducing incorporation of glucose and free fatty acids into TG; increasing oxidation of glucose and/or fatty acids; and increasing lipolysis. In this study, we synthesized PGlc as a sample and investigated its effects on lipid accumulation in cultured 3T3-L1 adipocytes by measuring TG contents and Oil Red O staining as indicators of lipid accumulation. We also examined the mRNA expression of several genes associated with adipogenesis and lipolysis during differentiation. Reduction of TG content during differentiation of adipocytes was more effective when treated with PGlc, compared with Glc. Treatment with PGlc reduced the OD value of Oil Red O-eluted solution in the cytoplasm of treated cells in a concentration-dependent manner, which means that PGlc inhibits adipogenesis during adipocyte differentiation. There is no report on how PGlc works on adipogenesis or lipolysis in 3T3-L1 adipocytes. However, our study resulted in a decrease in lipid formation. Therefore, it would be interesting to study the action mechanism of PGlc in 3T3-L1 adipocytes.

Adipocyte differentiation includes a series of programmed changes in specific gene expressions. Adipogenesis can be induced through the action of several enzymes, such as FAS, ACC, acyl-CoA synthetase and glycerol-3-phosphate acyltransferase. The expressions of these genes are regulated by transcription factors, such as PPARγ, C/EBPα and SREBP1, which are known to be critical activators for adipogenesis and showed early changes in gene expression during adipocyte differentiation [14–16]. PPARγ and C/EBPα, two central transcriptional regulators, are induced prior to the transcriptional activation of most adipocyte-specific genes. They play vital roles in adipocyte differentiation and coordinate expression of genes involved in creating or maintaining the phenotype of adipocytes [17]. Overexpression of these transcription factors can accelerate adipocyte differentiation. SREBP1 is known to critically cross-activate a ligand binding domain of PPARγ and promote the production of an endogenous PPARγ ligand [18]. SREBP1 also regulates the expression of the enzymes involved in lipogenesis and fatty acid desaturation [19]. The phosphorylation effect of Glc on the expression of these transcriptional factors was determined. Although Glc did not have any effect on down-regulation of PPARγ, SREBP1 and C/EBPα expressions, PGlc induced a significant down-regulation in a dose-dependent manner compared with differentiated adipocytes without sample treatment. Treatment with PGlc also induced down-regulated protein expressions of PPARγ, SREBP1 and C/EBPα in a dose-dependent manner. The down-

regulation of SREBP1 and C/EBPα by treatment with PGlc might reduce fatty acid synthesis, as well as the synthesis and activity of PPARγ, which may result in inhibition of lipid accumulation by blocking adipogenesis.

PGlc inhibited adipogenesis in 3T3-L1 cells through down-regulating PPARγ, SREBP1 and C/EBPα expressions. PPARγ and C/EBPα synergistically activate the downstream adipocyte-specific gene promoters, such as aP2, FAS, LPL and leptin [20]. Thereby, we investigated the effect of PGlc on regulation of the adipogenic target genes aP2, FAS, LPL and leptin, as well as Pref-1. Pref-1 is known as an adipogenesis inhibitor and serves as a marker for preadipocytes. Pref-1 is characteristic of an undifferentiated state and is down-regulated during adipocyte differentiation [21]. A remarkable down-regulation of Pref-1 expression induces an increase in the expressions of C/EBPα and PPARγ [22]. The aP2 gene is the terminal differentiation marker of adipocytes and plays a central role in the pathway that links obesity to insulin resistance and fatty acid metabolism. Treatment with PGlc during adipocyte differentiation induced up-regulation of Pref-1 mRNA and down-regulation of aP2 mRNA. Moreover, the expression levels of FAS, LPL and leptin mRNA were also dose dependently suppressed by treatment with PGlc. Traditionally, FAS has been seen as a terminal marker of adipocyte differentiation. Activated PPARγ and SREBP1 are involved in a metabolic cascade, leading to expression of FAS and clearly able to cross-activate the FAS promoter [23]. LPL catalyzes the hydrolysis reactions of TG in which plasma TG is metabolized to free fatty acids for TG synthesis with adipose cells [24]. Adipose tissue LPL involves the extent of the TG depot in fat cells, and the high regulation of LPL activity in adipocyte is closely linked with obesity per se [25]. Leptin is exclusively secreted in adipose tissue in proportion to its TG stores and cell size in adipose tissue and may be important in the development and extent of obesity [25]. Leptin concentration in the serum is positively associated with adipose tissue mass [26]. Therefore, leptin is used as an indicative marker of obesity. These results suggest that phosphorylation of Glc might suppress differentiation of adipocytes and adipogenesis through C/EBPα-, SREBP1- and PPARγ-mediated adipogenesis mechanisms related to the downstream adipocyte-specific gene promoters, including aP2, FAS, LPL and leptin.

Since PGlc inhibited lipid formation during adipocyte differentiation, we also explored whether the reduction effect of PGlc on lipid accumulation is associated with lipolysis. Lipolysis includes some critical processes, such as phosphorylation of perilipin and HSL translocation into lipid droplets [27]. HSL mediates the hydrolysis of TG into fatty acids and glycerol. Moreover, the cytokine TNFα has been known to accumulate adipose tissue mass and is related to inducing lipolysis and apoptosis of adipocytes [28]. Overexpression of TNFα in adipose tissue is proportional to the extent of the fat depot. The expression levels of perilipin, HSL and TNFα during differentiation of adipocytes were determined as the lipolytic response. Treatment with PGlc up-regulated HSL mRNA expression and suppressed the expression levels of TNFα mRNA compared with fully differentiated adipose tissue. We can assume that the inhibition of PGlc on lipid accumulation is not mediated by perilipin, but by HSL and TNFα.

In conclusion, our results reveal that PGlc inhibits adipocyte differentiation and adipogenesis in 3T3-L1 cells. Moreover, at the molecular level, PGlc enhances the inhibited regulation of PPARγ, SREBP1 and C/EBPα and adipogenic-specific promoter genes. Therefore, our study suggests that PGlc may also be involved in direct binding to PPARγ to increase its activity, in addition to promoting PPARγ, SREBP1 and C/EBPα expressions through adipogenesis mechanisms related to the downstream adipocyte-specific gene promoters, including aP2, FAS, LPL and leptin. However, further molecular mechanism of PGlc on the transcription factor in adipogenesis or apoptosis of adipocytes through signaling pathways remains to be elucidated.

Acknowledgments

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