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Research Paper

Platycodon grandiflorum modifies adipokines and the glucose uptake in high-fat diet in mice and L6 muscle cells

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Keywords
adipokines; glucose uptake; high-fat diet; L6 muscle cells; *Platycodon grandiflorum*

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Abstract

Objectives Obesity and diabetes have become the most common human health problems worldwide. Obesity's contribution to type 2 diabetes might be due to dysregulation of adipokines and glucose uptake.
Methods In this study, we performed in-vivo and in-vitro studies to evaluate the effects of *Platycodon grandiflorum* extract (PGE) on adipokines and glucose uptake. Before study, platycodin D concentrations were analysed by HPLC in PGE prepared in water, in 50% ethanol and in 80% ethanol, and we selected the 80% ethanol extract as the PGE for this study based on the HPLC results.
Key findings We found that inclusion of PGE in the high-fat diet (HFD) markedly attenuated food intake, body weight, epididymal fat weight, adipocyte size and blood glucose levels by the oral glucose tolerance test in mice, and maintained serum levels of adiponectin, resistin, leptin, fructosamine and triglycerides. Gene expression analysis revealed that PGE up-regulated adiponectin, and down-regulated TNF-α and leptin in fat tissue. In L6 muscle cells *in vitro*, PGE increased insulin-stimulated glucose uptake.
Conclusions We conclude that PGE may improve obesity in mice fed an HFD and glucose uptake in L6 muscle cells by modifying adipokines, and could offer clinical benefits as a supplement to treat obesity and diabetes.

Introduction

White adipose tissue (WAT) accumulation causes obesity, which is a major risk factor for type 2 diabetes and cardiovascular disease.[1] More than an energy storage depot, WHT plays a key role in the progression of insulin resistance through improper production of adipokines. The representative adipokines produced from adipose tissues include leptin, tumor necrosis factor (TNF)-α, resistin and adiponectin. The increased plasma leptin, TNF-α and resistin, and reduced plasma adiponectin are associated with insulin resistance.[2–4]

Studies on obesity in food science seek functional foods, food components and herbal extracts that can suppress appetite, retard body fat accumulation and promote weight loss.[5,6] Some plant-derived substances, such as *Zingiber officinale* rhizome extract, Shilianhua extract and American ginseng berry juice, may have beneficial effects in obesity.[7–9] Medicinal herb extracts are reported to lower blood glucose concentration and modify hyperlipidemia with less significant side effects than current drug therapies.[10,11]

Platycodon grandiflorum is a perennial plant of the Campanulaceae family and a source of triterpenoid saponins, carbohydrates and fibers.[12] As a herbal medicine, *P. grandiflorum* is shown to improve insulin resistance and lipid profile in rats with diet-induced obesity.[13,14] Platycodin D, which is a major component of *P. grandiflorum* and belongs to the oleanane type of triterpenoid saponin, possess anti-obesity and cholesterol-lowering properties.[13,15] However, there has been no study of the influence of *P. grandiflorum* on adipokines.

In the present study, we investigated for the first time the effects of *P. grandiflorum* extract (PGE) on potential mechanisms of PGE activities based on the expression of adipokines and glucose uptake in mice fed a high-fat diet (HFD) and in L6 muscle cells.

Materials and Methods

Preparation of the PGE

PGE, the oriental medicinal herb used in this experiment, is approved as an ingredient of food and has been used in oriental medicine to treat obesity in Korea. PGE was obtained from the Department of Pharmaceutical Preparation of Oriental Medicine, Oriental Medical Hospital, Kyung Hee University, Seoul, South Korea. The original source of the PGE was Kyung Hee Herb Pharm (WonJu, Korea). Drug quality was

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tested according to the standards of the Korea Food and Drug Administration and the standards of our hospital. A voucher specimen (code number HGGY/2009) was deposited in our department. Extractions were prepared from the dried PGE using three different solvents, which were water, 50% ethanol and 80% ethanol. In each case the dried PGE (1000 g) was added to 1500 ml solvent and boiled for 2 h at 100°C using a heating mantle. The sieve-filtered solution was then concentrated with a rotary evaporator (model NE-1, EYELA Co., Tokyo, Japan) and dried with a freeze dryer (model FD-1, EYELA Co., Tokyo, Japan). Each of these extracts was added to distilled water (1 g/10 ml) and boiled for 2 h at 95°C. The boiled solutions were centrifuged at 14 000 rpm for 20 min to obtain the supernatants.

HPLC analysis for platycodin D concentration according to PGE extraction methods

The HPLC system used consisted of an Alliance 2690 Separation Module, a Waters 996 Photodiode Array Detector operated at 254 nm, and a Millenium[32] Chromatography Manager Version 3.2. Chromatographic separations were carried out on a Nucleosil C_{18} column (4.0 mm × 250 mm ID Waters Corporation, Milford, MA, USA) at ambient temperature. Platycodin D was purchased from Sigma Co. (St Louis, MO, USA). The methanol used was the product of J.T. Baker (Phillipsburg, NJ, USA). Acetic acid and n-hexane were of analytical grade (Yakuri Co., Osaka, Japan), and ultrapure water was used in this assay. The mobile phase consisted of 0.4% (v/v) acetic acid and 0.6% (v/v) methanol in water. The mobile phase was filtered through a 0.45 um membrane filter (Millipore, Bedford, MA, USA) and degassed before use. The flow rate was 1.0 ml/min. A 5-g portion of each PGE preparation (water, 50% ethanol and 80% ethanol extracts) was extracted twice by sonication in 30 ml of n-hexane and filtered. These residues were extracted again by sonication in 50 ml of methanol. The final residues were then filtered, concentrated with a rotary evaporator (EYELA Co., Tokyo) and adjusted to 20 ml with methanol. An aliquot (20 ul) of this sample solution was injected into the HPLC system.

The collection rates of the water, 50% ethanol and 80% ethanol extracts were 28.8%, 28.0% and 18.9%, respectively. The concentrations of platycodin D by HPLC analysis were 0.321 mg/dl in PGE extracted with water, 0.589 mg/dl in the 50% ethanol extraction and 1.060 mg/dl in the 80% ethanol extraction. Based on the HPLC results, we selected the 80% ethanol extract as the PGE for the in-vivo study.

Animals and diets

The animals used in this study were 2-month-old male ICR mice (Central Lab Animals, Inc., Korea) with an average body weight of 30 ± 5 g. The animal room was maintained at 22 ± 2°C and at ~40–70% relative humidity, with 12-h periods of light and dark. After 1 week of adaptation, the mice were fed an HFD (% kcal; carbohydrate : protein : fat = 20 : 20 : 60) for 6 weeks to induce obesity, except in the group fed a normal diet. The HFD-fed mice were randomly segregated into four groups: the HFD, HFD + PGE 1%, HFD + PGE 5% and HFD + acarbose groups. The diet for the PGE group contained 1% or 5% PGE and the diet for the acarbose group (positive control) contained 0.5% acarbose. A separate group was fed the standard diet.

Body weights were recorded once weekly and the total food consumption was recorded daily using an electronic scale (CAS 2.5D, Seoul, Korea). After 6 weeks the mice were killed and epididymal fat pad weights were recorded using an electronic scale. All experiments were carried out in accordance with guidelines from the Korean National Institute of Health Animal Facility. The Animal Care Committee at Kyung Hee University approved all protocols used in this study.

Blood collection and biochemical analysis

At the end of the experiment the food was removed and experiments were performed between 9 am and 12 pm. Blood was obtained by cardiac puncture under ether anesthesia and centrifuged immediately at 3000 × g for 15 min at 4°C to obtain plasma, which was stored at −70°C until analysis. Total protein, albumin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglyceride, fructosamine, adiponectin, leptin and resistin concentrations were analysed by Greenlab (Seoul, Korea).

Oral glucose tolerance test

To achieve a baseline blood glucose level, mice were fasted for 10 h in clean cages with free access to water from newly cleaned bottles. The fasting (baseline) blood glucose measurement was taken by applying a drop of tail blood to a One-Touch Ultra Glucometer (Lifescan Canada Ltd). The baseline blood glucose level was identical across all groups. A filter-sterilized solution of glucose (65 mg/ml) was then administered to each animal, in a bolus dose equivalent to 1.5 mg glucose/gram of body weight. Tail vein blood samples were withdrawn without anesthesia before (0 min) and at 30 and 60 min after the glucose administration. The oral glucose tolerance test (OGTT) was performed after 3 and 6 weeks of the HFD.

Histology and morphometric analysis of white adipose tissues

Epididymal adipose tissues were rapidly removed from each mouse. The tissues were immediately fixed in 10% neutral buffered formalin, embedded in paraffin and processed for

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histological analysis. All the tissues were sectioned (2 μm thick) and routinely stained using hematoxylin and eosin. A morphometric analysis was performed under an Olympus photomicroscope (Olympus BX-50, Olympus Optical, Tokyo, Japan). The adipocyte area was determined by capturing bright-field images and measuring a total of 200 cells per mouse.

RNA extraction and real-time reverse transcriptase-polymerase chain reaction

Total RNA was isolated from the epididymal fat tissues of each mouse using Trizol (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. An aliquot of total RNA was reverse transcribed using MMuLV reverse transcriptase (Promega, Madison, WI, USA) and amplified using Taq DNA polymerase (Promega). Primers for the reverse transcription-polymerase chain reaction (RT-PCR) analysis were designed as follows: tumor necrosis factor (TNF)-α, sense 5′-TCTTCTCAAAATTCGAGTGACAAG-3′ and antisense 5′-GAGAACCTGGGAGTAGACAAGGTA-3′; adiponectin, sense 5′-ACCTACGACCAGTATCAGGAAAAG-3′ and antisense 5′-ACTAAGCTGAAAGTGTGTCGACTG-3′; leptin, sense 5′-AGTGGGAATGAGAAATCACTTAGC-3′ and antisense 5′-GTGTATTGCTTTCCATCAAGTGTC-3′; cyclophilin, sense 5′-TGGAGAGCACCAAGACAGACA-3′, and antisense 5′ GTCGACAATGATGACATCCTTCA-3′.

L6 cells culture

L6 muscle cells were cultured as monolayers in Dulbecco's modified Eagle's medium (DMEM) containing 1 mM pyruvate, 1 mM glutamine and 25 mM glucose. The medium was supplemented with 5% fetal bovine serum (FBS), 100 U/ml penicillin G, 100 μg/ml streptomycin and 25 μg/ml amphotericin B. Cells were maintained in an incubator with humidified air containing 5% CO_2 at 37°C.

The effects of PGE glucose uptake

Experiments were carried out in 24-well plates seeded at 5×10^5 cells/ml and maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. When cells reached confluence, the medium was changed to DMEM containing 0.5% FBS and 2.5 mM glucose for 24 h to induce differentiation and fusion of myoblasts into myotubes, and prevent further division. The myotubes were then incubated with test substances (PGE and/or insulin) for 24 h. Uptake of 2-deoxy-D-glucose (2DG) was then determined during a further 15-min incubation period. The monolayers were washed with glucose-free Krebs–Ringer bicarbonate (KRB) buffer at 22°C, then incubated with 1 ml of KRB containing 0.1 mM 2DG and 2-deoxy-D[^{3}H]glucose (0.2 uCi) for 15 min at 22°C. The buffer was removed and the cells were washed twice with ice-cold KRB buffer and lysed with 0.3 ml 1 M NaOH. Radioactivity in ^{3}H was determined in a liquid-scintillation counter (1450 MicroBeta TriLux, PerkinElmer).

Statistical analysis

All values were expressed as the mean ± SE The significance of differences between groups was determined using one-way analysis of variance (ANOVA), followed by a non-parametric post hoc test least significance difference. The area under the curve (AUC) of glucose in the OGTT was calculated from measurements taken before (0 min) and after (up to 60 min) glucose administration on the basis of the trapezoidal rule, which is a method used to approximate a definite integral by evaluating the integrand at two points. All P values were two-tailed and significance was set at $P < 0.05$. All calculations were performed using the SPSS for Windows, Version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Body weight and energy intake

The high-fat diet significantly increased the mouse body weights at 6 weeks compared to weights in the standard diet group (STD; 41.1 ± 0.98 g vs. 34.5 ± 0.54 g). The PGE at 1%, PGE at 5% and acarbose treatments significantly suppressed the body weight gains compared to those in the untreated HFD group (Table 1).

Food intakes by the HFD groups were lower than those in the STD group (3.03 ± 0.18 g vs. 4.61 ± 0.27 g), but the caloric intake of animals fed the HFD surpassed that of the STD group (70.40 ± 4.26 kJ vs. 45.92 ± 2.69 kJ). Administration of PGE and acarbose had no effect on food or caloric intake compared with the untreated HFD group (Table 1).

Fat mass, adipocyte size, lipid profiles and hepatic functions

Fat masses in the untreated HFD group were higher than those of the STD group, but PGE 5% and acarbose treatments significantly reduced fat accumulation in mice fed the HFD (Table 2).

The average adipocyte size was larger in the HFD group than that in the STD group, and PGE 1%, PGE 5% and acarbose significantly decreased the size of adipocytes (Figure 1). Total cholesterol and HDL-cholesterol were higher in the HFD group than in the STD group (Table 2). PGE 5% significantly decreased plasma triglyceride. Total cholesterol, HDL-cholesterol and LDL-cholesterol concentrations were unchanged by all treatments.

The liver enzymes GOT and GPT were measured in plasma as indicators of liver damage. GOT activity was significantly lower in the PGE 1%, PGE 5% and acarbose groups than in

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Table 1 Effects of PGE on body weight, energy intake and carbohydrate metabolism in a mouse model of diet-induced obesity

	STD	HFD	HFD+PGE1 %	HFD+PGE 5%	HFD+acarbose
Body weight (g)					
Initial weight	31.5 ± 0.31	32 ± 0.39	31.2 ± 0.92	30.7 ± 0.45	31.5 ± 0.45
Final weight	34.5 ± 0.54	41.1 ± 0.98###	38.5 ± 0.72*	33.8 ± 0.89***	33.7 ± 0.87***
Weight gain	3 ± 0.37	9.1 ± 0.81###	7.3 ± 0.72	3.1 ± 0.84***	2.2 ± 0.84***
Intake (day/mouse)					
Food intake (g)	4.61 ± 0.27	3.03 ± 0.18###	3.07 ± 0.17	3.5 ± 0.32	2.66 ± 0.17
Energy intake (kJ)	45.92 ± 2.69	70.40 ± 4.26###	71.29 ± 4.04	81.37 ± 7.41	61.90 ± 3.92
Carbohydrate metabolism					
Fructosamine (μmol/L)	213.3 ± 7.27	245 ± 8.42##	209.67 ± 8.79**	211.8 ± 5.67**	214.25 ± 7.05*
OGTT (AUC)					
3 weeks	12107.25 ± 441.7	14057.25 ± 760.2#	12741.75 ± 654.6	10844.25 ± 533.7**	9366.75 ± 403.3***
6 weeks	11937.75 ± 516.1	15143.25 ± 1072.0#	12602.25 ± 789.3	11474.25 ± 721.6*	11072.25 ± 522.7**

Data are presented as the mean ± SE ($n = 10$). STD, standard diet group; HFD, high-fat diet group; HFD + PGE1%, high-fat diet supplemented with *Platycodon grandiflorum* extract (PGE) 1%; HFD + PGE5%, high-fat diet supplemented with PGE 5%; HFD + acarbose, high-fat diet supplemented with acarbose drug; OGTT, oral glucose tolerance test; AUC., area under the curve. Plasma glucose AUC values were calculated at 0–60 min after oral glucose administration (1.5 mg glucose/gram body weight). #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ compared with the STD group; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the HFD group.

Table 2 Effects of PGE on adipokines, fat mass, lipid profiles and hepatic function in diet-induced obesity

	STD	HFD	HFD+PGE1%	HFD+PGE5%	HFD+acarbose
Adipokine (ng/mL)					
Adiponectin	226.71 ± 56.9	74.7 ± 13.76##	111.71 ± 28.74	222 ± 37.97**	235.27 ± 63.06*
Resistin	0.27 ± 0.02	0.42 ± 0.05#	0.28 ± 0.02*	0.22 ± 0.02***	0.26 ± 0.01**
Leptin	0.47 ± 0.08	0.62 ± 0.07	0.58 ± 0.07	0.37 ± 0.09*	0.62 ± 0.07
Adipose tissue					
Epididymal fat mass (g)	0.57 ± 0.05	1.19 ± 0.14##	1.2 ± 0.11	0.78 ± 0.05*	0.44 ± 0.06***
Adipocyte size (μm^2)	4382 ± 1359	10198 ± 2536	7469 ± 3696.	6228 ± 2821	5465 ± 2068
Lipid (mg/dL)					
Total cholesterol	140.78 ± 6.76	210.38 ± 14.54##	206 ± 14.22	211.6 ± 7.98	186.41 ± 6.13
HDL cholesterol	92.84 ± 2.8	131.34 ± 7.83##	124.53 ± 7.09	125.4 ± 3.66	126.09 ± 3.13
LDL cholesterol	10.11 ± 1.21	11 ± 1.05	9.33 ± 1.29	12.4 ± 1.63	10 ± 1.13
Triglyceride	103 ± 13.44	95.75 ± 21.49	84.67 ± 15.48	52.3 ± 6.03*	71.29 ± 6.9
Liver function					
Liver mass (g)	1.45 ± 0.05	1.57 ± 0.05	1.59 ± 0.05	1.35 ± 0.05*	1.11 ± 0.05***
Total protein (mg/dL)	5.69 ± 0.18	5.45 ± 0.13	5.2 ± 0.15	5.32 ± 0.06	5.59 ± 0.05
Albumin (mg/dL)	1.89 ± 0.06	1.79 ± 0.06	1.68 ± 0.05	1.8 ± 0.02	1.87 ± 0.02
GOT (IU/L)	111.33 ± 12.23	128.38 ± 18.14	88.56 ± 5.34*	68.7 ± 5.44**	83.88 ± 9.35*
GPT (IU/L)	36.33 ± 4.07	43.75 ± 11.13	29.11 ± 3.2	27.5 ± 2.55	29.59 ± 9.08

Data are presented as the mean ± SE ($n = 10$). STD, standard diet group; HFD, high-fat diet-fed group; HFD+PGE1%, high-fat diet supplemented with *Platycodon grandiflorum* extract (PGE) 1%; HFD+PGE5%, high-fat diet supplemented with PGE 5%; HFD+acarbose, high-fat diet supplemented with acarbose drug; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ compared with the STD group; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the HFD group.

the untreated HFD group (Table 2). GPT activity did not differ significantly among the groups. The average liver weight in the HFD group was increased compared to that in the STD group, and PGE 5% and acarbose significantly decreased the weight of liver (Table 2).

Carbohydrate metabolism

Mice on the HFD exhibited significantly higher plasma fructosamine than those in the STD group (245.00 ± 8.42 μmol/l vs. 213.30 ± 7.27 μmol/l). At week 6, HFD-fed mice treated with PGE 1%, PGE 5% and acarbose showed significantly lower fructosamine concentrations.

The blood glucose patterns of mice on the STD, HFD, HFD+PGE 1%, HFD+PGE 5% and HFD+acarbose diets following oral glucose administration at 3 and 6 weeks are presented in Figure 1. HFD-fed mice showed impaired glucose tolerance (Figure 2). The blood glucose levels increased gradually and reached a plateau at 30 min in all groups.

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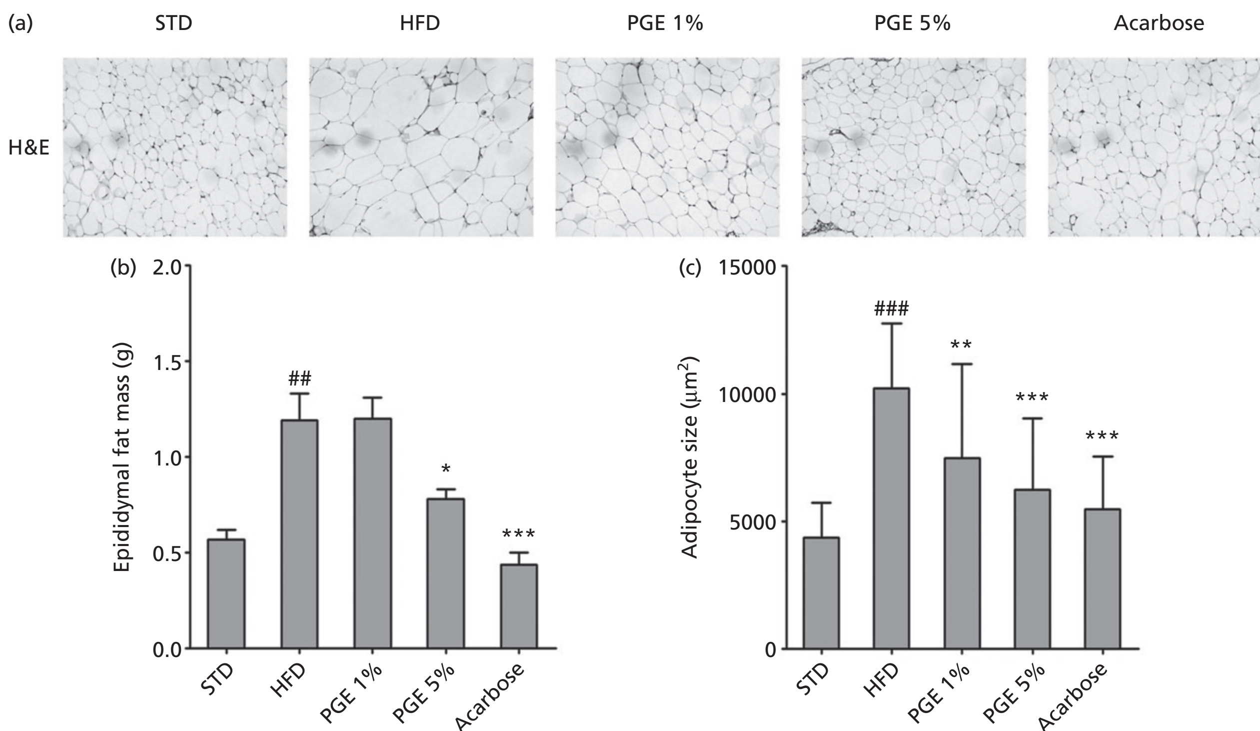

Figure 1 Light microscopic appearance of hematoxylin and eosin stained adipocytes (A), epididymal fat (B) mass and adipocyte size (C) in white fat tissue of mice fed the STD, HFD, HFD+PGE1%, HFD+PGE5% and HFD+acarbose diets. Data are presented as the mean ± SE (n = 10). ##$P < 0.01$, ###$P < 0.001$ compared with the STD group; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the HFD group.

Glucose levels of the HFD group were significantly higher than those of STD group. The treatments with PGE 5% and acarbose resulted in significantly lower plasma glucose concentrations at 30 and 60 min (Figure 1), and lower $AUC_{0–60}$ values than in the untreated HFD group (Table 1).

Gene expression and plasma levels of adipokines in white adipose tissue

The plasma levels of adiponectin in the untreated HFD group were lower than those in the STD group and were normalized by the PGE 5% and acarbose treatments (Table 2). The plasma levels of resistin and leptin in the untreated HFD group were higher than those in the STD group and all treatments normalized resistin levels. The PGE 5% treatment significantly decreased circulating plasma leptin (Table 2), whereas other treatments did not significantly affect it.

The expression of TNF-α, adiponectin and leptin mRNAs was measured by RT-PCR. As shown in Figure 3, the TNF-α and leptin mRNAs were highly expressed in the HFD group at 6 weeks, while the adiponectin showed lower expression than in the STD group. In the PGE 5% and acarbose groups at 6 weeks, the TNF-α and leptin mRNAs were significantly lower and the adiponectin mRNA was higher than in the untreated HFD group.

Glucose uptake

L6 myotubes incubated for 24 h with PGE (20 and 30 μg/ml) showed an increase in 2DG uptake of 1.2 fold (Figure 4). This did not differ significantly from 2DG uptake induced by 10^{-6}M insulin. At high concentrations, however, PGE (40 and 50 μg/ml) increased 2DG uptake by 1.7 fold, which did differ significantly from the uptake induced by 10^{-6}M insulin (Figure 4).

Discussion

Obesity is a serious and rapidly growing public health problem in most countries around the world.[16] Obesity, the accumulation of excessive body fat, can lead to various metabolic disorders, including fatty liver, hyperlipidemia, hypertension, arteriosclerosis and impaired glucose tolerance.[17–21] The accumulation and expansion of white adipose tissue causes obesity and induces infiltration by macrophages, which increase levels of pro-inflammatory cytokines. PGE is reported to improve insulin resistance and the serum lipid profile in rats with diet-induced obesity.[13,14,22] Zhao *et al*. reported that platycodin-enriched diets can lower circulating cholesterol and whole-body cholesterol content, and reduce cardiovascular disease risk by mechanisms that do not involve cholesterol absorption or synthesis.[13] Kim *et al*. showed that

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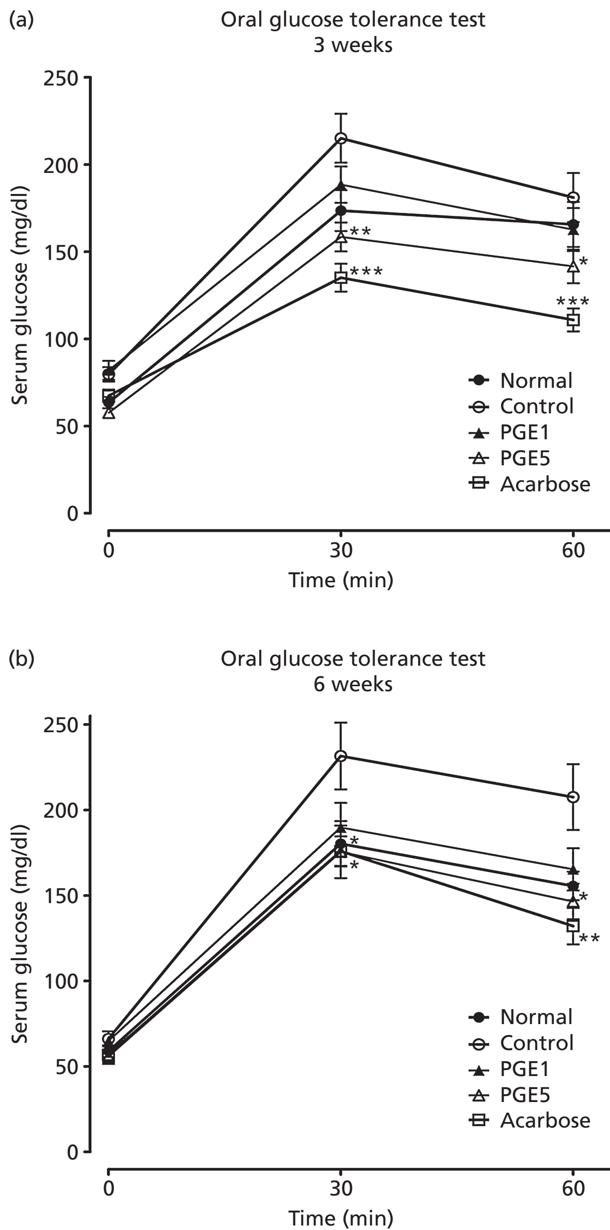

Figure 2 Oral glucose tolerance at 3 (A) and 6 (B) weeks in mice fed the STD, HFD, HFD+PGE1%, HFD+PGE5% and HFD+acarbose diets. Glucose (1.5 mg/g body weight) was administered orally at the time indicated by the arrow, and blood glucose levels were determined at 0, 30 and 60 min thereafter. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with the HFD group.

PGE may be useful in preventing and treating metabolic disorders characterized by hyperinsulinemia, such as non-insulin-dependent diabetes mellitus, syndrome X and coronary artery disease.[14] In this study we investigated the effects of PGE on obesity, glucose tolerance and adipokines such as adiponectin, TNF-α, resistin and leptin expression in a mouse model of obesity induced by an HFD. In this model,

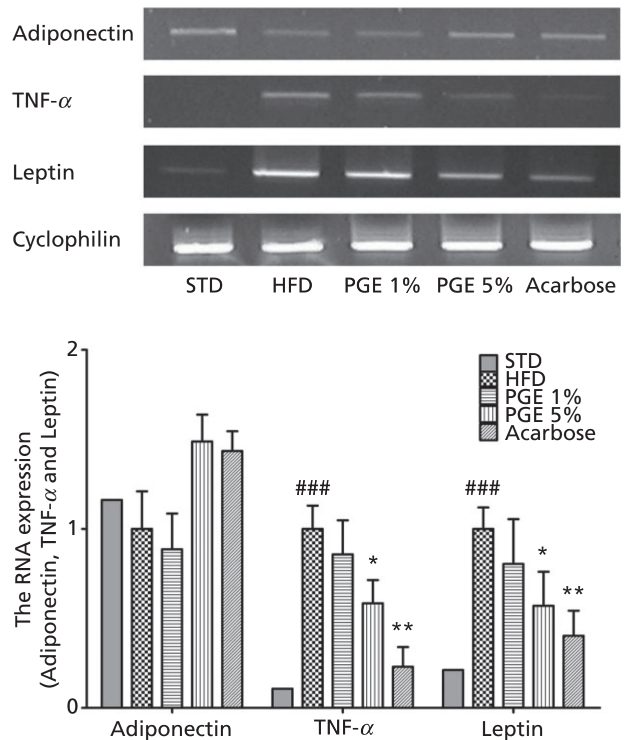

Figure 3 Adiponectin, TNF- α and leptin mRNA expression in fat tissue of mice fed the STD, HFD, HFD+PGE1% and HFD+PGE5% diets. $^{###}P < 0.001$ compared with the Normal group, $*P < 0.05$, $**P < 0.01$ compared with the HFD group.

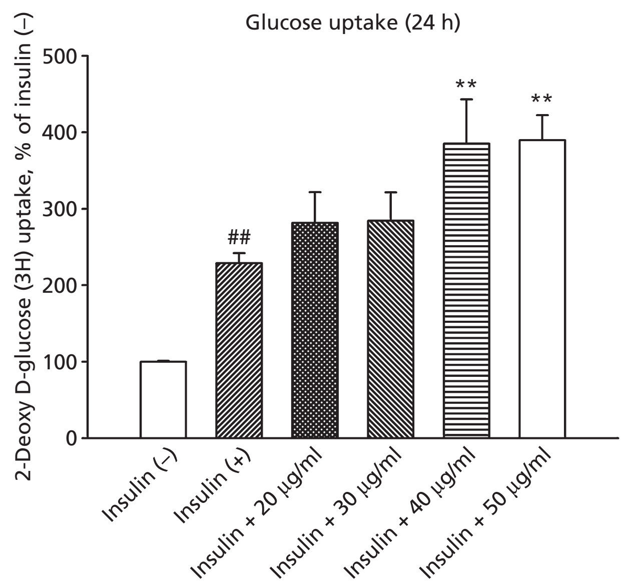

Figure 4 Uptake of 2DG by L6 myotubes after incubation for 24 h with 10^{-6}M insulin or with PGE at 20, 30, 40 and 50 μg/ml. $^{##}P < 0.01$ compared with the Normal group, $**P < 0.01$ compared with the HFD group.

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PGE treatment significantly suppressed obesity development, with corresponding effects on body weight, fat accumulation and related metabolic parameters.

Adipose tissue produces and secretes adipokines or adipocytokines, including adiponectin, leptin and resistin.[23] Adiponectin and leptin, derived from fat depots, are involved in energy homeostasis.[24] Adiponectin is present in plasma at a very high concentration compared to other adipocytokines. Its production falls, however, as visceral fat accumulates, i.e. plasma levels are inversely correlated with visceral adiposity.[25] Low adiponectin is closely associated with type 2 diabetes, lipid disorders, hypertension and certain inflammatory diseases.[25] Leptin levels may indicate nutritional status, and leptin activities may explain how fat mass influences food intake and energy expenditure through appetite and metabolic activity.[26] Circulating levels of leptin and adiponectin track the degree of obesity. In humans, leptin levels increase and adiponectin levels decrease in obesity.[27] Resistin is mainly concentrated in and secreted from fat tissue. Resistin is also associated with hypothalamic and peripheral control of lipid metabolism and in the regulation of food intake.[28] In this study, PGE treatment significantly decreased resistin and leptin, and increased adiponectin in plasma as compared to levels in animals fed the HFD without PGE. The adiponectin mRNA expression was also significantly higher, while TNF-α and leptin mRNAs were lower in HFD-fed mice given the PGE supplement. Adiponectin modulates a number of metabolic processes involving glucose regulation and fatty acid catabolism.[29] TNF-α, expressed in and secreted by adipose tissue, varies with the degree of adiposity and the associated insulin resistance.[30] TNF-α reduces insulin sensitivity by impairing insulin signal transduction.[31] At the hypothalamic level, TNF-α promotes a catabolic condition and inhibits food intake.[32] TNF-α can also regulate leptin mRNA and protein expression.[33] In the present experimental system, PGE significantly suppressed expression of TNF-α and leptin mRNAs, and increased adiponectin mRNA expression in fat tissue, changes which correlate with loss of body weight. These findings suggest that the oral administration of PGE may not only suppress body fat accumulation but may also promote degradation of accumulated fat.

To increase cellular glucose uptake is a major target in managing diabetes and resolving insulin resistance. In the L6 muscle cells, PGE increased glucose uptake in response to insulin, which may be related to the regulation of adipokines by PGE. The PGE feeding also increased oral glucose tolerance. This suggested that PGE may improve insulin resistance, lower plasma insulin concentration and normalize glucose tolerance by increasing glucose uptake and adiponectin expression, and decreasing resistin, TNF-α and leptin.

Conclusion

In conclusion, PGE supplementation significantly improved weight gain, fat accumulation and impairment of glucose tolerance as measured by OGTT, maintained serum levels of fructosamine and triglycerides, markedly attenuated serum levels of adiponectin, resistin, leptin and up-regulated adiponectin mRNA, and down-regulated TNF-α and leptin mRNA in WAT. PGE also increased insulin-stimulated glucose uptake in L6 muscle cells. These findings suggested that PGE may improve obesity in mice fed an HFD and glucose uptake in L6 muscle cells by modifying adipokines, and could offer clinical benefits as a supplement or component of a drug used to treat obesity and diabetes.

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