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Effects of *Momordica charantia* on insulin resistance and visceral obesity in mice on high-fat diet☆

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ABSTRACT

We examined the preventive effect of *Momordica charantia* L. fruit (bitter melon) on hyperglycemia and insulin resistance in C57BL/6J mice fed with a high-fat (HF) diet. Firstly, mice were divided randomly into two groups: the control group was fed low-fat (LF) diet, whereas the experimental group was fed with a 45% HF diet last for 12 weeks. After 8 week of induction, the HF group was subdivided into six groups and was given orally with or without *M. charantia* or rosiglitazone 4 weeks afterward. We demonstrated that bitter melon was effective in ameliorating the HF diet-induced hyperglycemia, hyperleptinemia, and decreased the levels of blood glycated hemoglobin (HbA1c) and free fatty acid (FFA) ($P < 0.01$, $P < 0.05$, $P < 0.05$, respectively), whereas increased the adipose PPARγ and liver PPARα mRNA levels. Additionally, bitter melon significantly decreased the weights of epididymal white adipose tissue and visceral fat, and decreased the adipose leptin and resistin mRNA levels. It is tempting to speculate that at least a portion of bitter melon effects is due to be through PPARγ-mediated pathways, resulting in lowering glucose levels and improving insulin resistance, and partly be through PPARα-mediated pathways to improve plasma lipid profiles. This is the first report demonstrating that bitter melon, is a food factor, but not a medicine, itself could influence dual PPARα/PPARγ expression and the mediated gene expression, is effective in ameliorating insulin resistance and visceral obesity.

1. Introduction

The incidence of diabetes has increased along with widespread lifestyle and dietary changes. Of particular importance may be proportion of fat in the diet. The chronic consumption of a high-fat (HF) diet is strongly associated with development of obesity [1] and can induce insulin resistance in human and animals [2–4]. It is clear that obesity constitutes a risk factor contributing to the development of Type 2 diabetes. Type 2 diabetes, which accounts for more than 90–95% of all diabetes, is characterized by two metabolic defects: including the majority of condition known as insulin resistance [5,6]. Both genetic and environmental factors play an important role in Type 2 diabetes. Obesity is associated with a decreased capacity of insulin to regulate glucose and lipid metabolism in the peripheral tissues. The increase in adipose tissue is accompanied by elevations of circulating free fatty acids (FFAs) levels [7]. However, studies in appropriate animal models will provide additional insights into the physiological effects of specific susceptibility genes [8]. The mouse model

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C57BL/6J mouse is reported to be susceptible to HF diet-induced obesity and Type 2 diabetes [9].

Recent advances have shown that adipose tissue not only stores excess energy in the form of fat but also secretes physiologically active substances called adipocytokines [10]. For instance, leptin is secreted from the enlarged adipose tissues in obesity [11]. The recently discovered resistin, however, highlights a direct mechanistic mediator from adipocytes to insulin resistance, thus linking obesity to diabetes [12]. Through these secreted products, adipocytes may affect the systemic metabolism, consequentially causing insulin resistance.

Thiazolidinediones (TZDs), as peroxisome proliferator-activated receptors (PPARs) ligands are heralded as a breakthrough in the pharmacological treatment of Type 2 diabetes. PPARγ represses the gene expression of leptin and resistin, all of which have been implicated in insulin resistance [13]. Collectively, activation of PPARγ mitigates insulin resistance. Fibrates are hypolipidemic drugs whose effects are mediated by the activation of PPARα. The administration of the drug also increases the fatty acid oxidation and the change may be involved in the reduction of fat deposits [14].

Recently, much attention has been focused on some food factors those may be beneficial for the prevention of body fat accumulation and possibly reduce the risk of diabetes. Although some drugs are used for the therapy of obese-related metabolic disease or possibly discussed as preventing body fat accumulation, there is little evidence that food factors themselves are beneficial for prevention of body weight gain and amelioration of insulin resistance.

Momordica charantia, also is referred to as bitter melon or bitter gourd, is a member of Cucurbitaceae family and has been used as a traditional anti-diabetic remedy for many years in countries such as China [15] and as a nourishing food, including nutrients: 94.3% water, 0.7% protein, 0.1% lipid, 3.1% carbohydrate, 1.2% cellulose, 0.5% ash, and a small quantity of 0.03% vitamin C [16]. The efficacy of bitter melon has also been reported in a number of animal studies, although many of them used insulin-dependent diabetes mellitus (IDDM) models [17–20]. Many components have been identified from bitter melon those possess hypoglycemic properties [21]. Bitter melon contains biologically active chemicals those include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins, and steroids [22,23]. The hypoglycemic chemicals of bitter melon are a mixture of steroidal saponins known as charantins and alkaloids [22]. A few isolated phytochemicals (charantins, a polypeptide-p, momordin Ic, oleanolic acid 3-*O*-monodesmoside, and oleanolic acid 3-*O*-glucuronide) of bitter melon have shown hypoglycemic activity [24–26]. Matsuda et al. reported that 2 important constituents including oleanolic acid 3-*O*-glucuronide and momordin Ic isolated from bitter melon exerted anti-hyperglycemic effect [26]. Recently, bitter melon is reported to suppress weight gain and have a potential for reduce adiposity [27]. And most of these studies were restricted to the normal animal model for a short period.

However, studies in appropriate animal models will provide additional insights into the physiological effects of specific susceptibility genes [8]. The mouse model C57BL/6J mouse is reported to be susceptible to HF diet-induced obesity and Type 2 diabetes [9]. Thus, the present study was designed to develop the disease animal model to examine the ameliorating effect of bitter melon on hyperglycemia and insulin resistance in C57BL/6J mice fed with a HF diet. As one of the possible mechanisms of bitter melon responsible for amelioration of insulin resistance, we also examined its effect on leptin level and the related genes expression.

2. Materials and methods

2.1. Preparation of extracts

M. charantia were purchased from the local market in August 2005 and were authenticated by the Graduate Institute of Chinese Pharmaceutical Sciences, China Medical University.

P fraction extracts: 100 kg of fresh fruit of bitter melon were grounded in an electric grinder, and were mixed with 2000 L water to decorate. After filtration, the aqueous extracts were concentrated to obtain water extract under reduced pressure at 40 °C. This was followed by water and *n*-butanol partition. The resulting *n*-butanol fraction was obtained for experiment. The extract yield was approximately 0.12%. It was stored at −20 °C until use for oral administration. The extracts was diluted and adjusted, then that was administrated orally to mice in a volume of 0.5 g, 1.0 g/kg body weight, respectively.

G fraction extracts: 100 kg of fresh fruit of bitter melon were grounded and were mixed with 50 L methanol to soak and extract in warning blender twice. The resulting methanol extract was followed by *n*-hexane partition, and the lower water layer obtained. The water layer was concentrated under reduced pressure at 40 °C and followed by ethyl acetate partition, and the water layer obtained. The water layer was followed by *n*-butanol partition, and the resulting *n*-butanol layer obtained for experiment. The extract yield was approximately 2.38%. It was stored at −20 °C until use for oral administration. The extracts was diluted and adjusted, then that was administrated orally to mice in a volume of 0.2 g, 1.0 g/kg body weight, respectively. Distilled water was administered in a similar volume to control mice.

2.2. Animals and diets

Male C57BL/6J mice, 5 week of age (National Laboratory Animal Breeding and Research Center, National Science Council, Taiwan, Republic of China) were used and maintained at 22 ± 3 °C under an automatic lighting schedule (08:00–20:00 h). After a 1-week acclimation period, mice were divided randomly into two groups. The control group (CON) was fed low-fat (LF) diet (Diet 12450B, Research Diets) whereas the experimental group was fed a 45% high-fat diet (Diet 12451, Research Diets) for 12 weeks. LF diet was composed of protein 20%, carbohydrate 70%, and fat 10%, whereas HF diet was composed of protein 20%, carbohydrate 35%, and fat 45% (of total energy, %kcal). After 8 week of induction, the experimental group was subdivided into six groups and was given orally with or without *M. charantia* or rosiglitazone 4 weeks afterward. During the last 4 weeks the control group (CON) and high-fat control (HF) mice were treated with vehicle only. The other groups were received bitter melon extract (including 0.5,

Table 1 – Composition of the high- and low-fat diets

Ingredient	LF	HF
Casein	800	800
L-Cystine	12	12
Corn starch	1260	291
Maltodextrin 10	140	400
Sucrose	1400	691
Cellulose, BW200	0	0
Soybean oil	225	225
Lard*	180	1598
Mineral Mix S10026	0	0
Dicalcium carbonate	0	0
Calcium carbonate	0	0
Potassium citrate, $1H_2O$	0	0
Vitamin Mix V10001	40	40
Choline bitartrate	0	0
FD&C Yellow Dye #5	0	
FD&C Red Dye #40		0
FD&C Blue Dye #1		
Total	4057	4057

Composition of the low-fat (LF) and high-fat (HF) diet is expressed as shown and as a percentage of total calories.
* There were no significant differences on body weight changes observed after bitter melon treatment as compared with the high-fat (HF) + vehicle (distilled water) group.

1.0 g/kg/day P extracts, or 0.2, 1.0 g/kg/day G extracts of bitter melon), or rosiglitazone 10 mg/kg, respectively. Body weight was measured weekly throughout the study. These dietary periods last for 12 weeks, and mice were maintained in accordance with the Animal Experiment Committee guidelines. The compositions of the experimental diets are shown in Table 1.

2.3. Collection of plasma, liver, and adipose tissue

After 12 week of treatment, mice were killed by decapitation and the blood was removed. The liver and white adipose tissues (WATs (including epididymal, mesenteric, and retroperitoneal WAT), and interscapular brown adipose tissue (BAT) were dissected according to the defined anatomical landmarks. The weights of tissues were measured. Visceral fat was defined as the sum of epididymal and retroperioneal WAT. They were then immediately frozen using liquid nitrogen and kept at −80 °C until use. The collected blood was kept at room temperature for 5 min for coagulation. Then, the plasma was obtained from the coagulated blood by centrifugation at 1600 × *g* for 15 min at 4 °C. The separation of the plasma was finished with 30 min. The plasma was immediately frozen at −80 °C until use.

2.4. Measurement of plasma lipid, glucose, insulin, and leptin levels

The plasma triglyceride (TG), total cholesterol (TC), and FFA concentrations were measured using commercial assay kits according to the manufacturer's directions (Triglycerides-*E* test, Cholesterol-*E* test, and FFA-C test, Wako Pure Chemical, Osaka, Japan). Plasma glucose level was measured by the glucose oxidase method (Model 1500; Sidekick Glucose Analyzer; YSI Incorporated, Yellow Springs, USA). Plasma insulin and leptin levels were measured by ELISA using a commercial assay kit according to manufacturer's directions (mouse insulin ELISA kit, Sibayagi, Gunma, Japan and mouse leptin ELISA kit, Morinaga, Yokohama, Japan). Percent HbA1c was measured with a Hemoglobin A1c kit (BioSystems S.A., Barcelona, Spain).

2.5. RNA extraction

Total RNA was extracted from the isolated of each mouse, using a Trizol Reagent kit (Molecular Research Center, Inc., Cincinnati, OH) according to the protocol provided by the manufacturer. The integrity of the extracted total RNA was examined by 2% agarose gel electrophoresis, and the RNA concentration was determined by the ultraviolet (UV) light absorbency at 260 and 280 nm (Spetrophotometer U-2800A, Hitachi). The quality of the RNA was confirmed by ethidium bromide staining of 18S and 28S ribosomal RNA after electrophoresis on 2% agarose gel containing 6% formaldehyde.

2.6. Relative quantitation of mRNA indicating gene expression

Total RNA (1 μg) was reverse transcribed to cDNA in a reaction mixture containing buffer, 2.5 mM dNTP (Gibco-BRL, Grand Island, NY), 1 mM of the oligo (dT) primer, 50 mM dithiothreitol, 40 U Rnase inhibitor (Gibco-BRL, Grand Island, NY), and 5 μL Moloney murine leukemia virus reverse transcriptase (Epicentre, USA) at 37 °C for 1 h, and then heated at 90 °C for 5 min to terminate the reaction. The polymerase chain reaction (PCR) was performed in a final 25 μL containing 1 U Blend Taq™-Plus (TOYOBO, Japan), 1 μL of the RT first-strand cDNA product, 10 μM of each forward (F) and reverse (R) primer, 75 mM Tris–HCl (pH 8.3) containing 1 mg/L Tween 20, 2.5 mM dNTP, and 2 mM $MgCl_2$. Preliminary experiments were carried out with various cycles to determine the nonsaturating conditions of the PCR amplification for all the genes studied. The primers are shown in Table 2.

The products were run on 2% agarose gels and stained with ethidium bromide. The relative density of the bands was evaluated using an AlphaDigiDoc 1201 software (Alpha Innotech, Co.). All the measured PCR products were normalized to the amount of cDNA of GAPDH in each sample.

2.7. Statistical analysis

The differences between groups were analyzed by comparison, using one-way analysis of variance (ANOVA), and in case of significance, a Dunnett test was also applied.

3. Result

3.1. Body weight, absolute adipose tissue weight, and body weight gain

All group mice started with similar mean body weights (20.3 ± 0.2 g). At week 8, the body weight of all the high-fat diet

Table 2 – Primers used in this study

Gene	Accession numbers	Forward primer and reverse primer	PCR product (bp)	Annealing temperature (°C)
PPARγ	EF062476.1	F: AGGCCGAGAAGGAGAAGCTGTTG R: TGGCCACCTCTTTGCTCTGCTC	275	55
Leptin	BC125245.1	F: AGTCTATCAACAGATCCTCACC R: CTCTGTGGAGTAGCCTGAAG	277	52
Resistin	NM022984.3	F: AGACTGCTGTGCCTTCTGGG R: CCCTCCTTTTCCTTTTCTTCCTTG	200	55
LPL	NM008509.1	F: GTACCTGAAGACTCGCTCTC R: AGGGTGAAGGGAATGTTCTC	156	55
PPARα	NM_011144	F: TATTCGGCTGAAGCTGGTGTAC R: GAAGAACTTCAACATGAACAA	270	55
GAPDH	XR003962.1	F: TGTGTCCGTCGTGGATCTGA R: CCTGCTTCACCACCTTCTTG	77	55

treated groups is significantly greater than the CON group. At week 12, the body weight in HF mice treated with vehicle is increased as compared with the CON group ($P < 0.001$), no statistical difference was found after treatment of bitter melon (Fig. 1). Body weight is determined by a balance between food intake and energy expenditure. We found that bitter melon had no suppressive effect on food intake in high-fat fed mice (Table 3). One of the explanations for the resistance to the gain of body weight may be malabsorption of food or increased total energy expenditure.

At week 12, all absolute adipose tissue (epididymal, mesenteric, retroperitoneal WAT, and visceral fat) weights, liver weights, interscapular BAT weights, and body weight gain were markedly greater in the HF group than in the CON group (epididymal WAT; 372.5%, mesenteric WAT; 127.7%, retroperitoneal WAT; 457.1%, and visceral fat; 392.3%, liver; 45.2%, interscapular BAT; 58.8%). Bitter melon and ROS treatment significantly suppressed the HF diet-induced increases in all of the absolute weight depots, liver weight, and interscapular BAT weights, except G1 treatment one explanation for this is the significantly increased PPARα and LPL mRNA level exerting the decreased circulating TG and resulting in visceral depots. All of bitter melon treatment significantly decreased the body weight gain (Table 4).

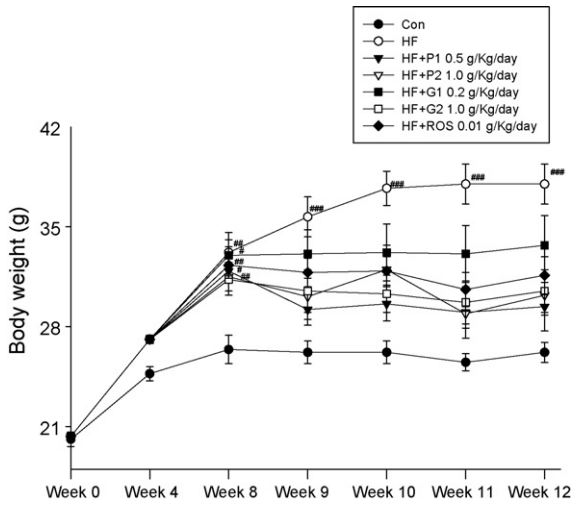

Fig. 1 – Body weight change. Mice were fed with 45% high-fat diet (HF) or low-fat diet (CON) for 12 weeks. At 8 weeks post-HF, the HF mice were treated with vehicle (water; p.o.), or extracts of *Momordica charantia*, or rosiglitazone (p.o.) accompanied with HF diet for 4 weeks. All values are means ± S.E. ($n = 9$). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with the control group at the same time by ANOVA coupled with Dunnett's test. There were no significant differences in body weight changes observed after bitter melon treatment.

3.2. *Plasma glucose, blood glycated hemoglobin (HbA1c), and insulin levels*

We evaluated the percent of hemoglobin nonenzymatically (percent HbA1c), as an integrated measure of long-term blood glucose regulation. At the beginning of the study, all of mice started with similar concentration (47.3 ± 1.4 mg/dL). At week 8, the glucose levels and percent HbA1c of the HF group were significantly greater than the CON group (110.2 ± 7.0 mg/dL vs. 56.8 ± 3.2 mg/dL, 53.6 ± 7.4% vs. 12.1 ± 2.0%, respectively) ($P < 0.001$, $P < 0.001$, respectively). At week 12, the levels of glucose, HbA1c and insulin were significantly greater in the HF group than in the CON group ($P < 0.001$, $P < 0.001$, $P < 0.001$, respectively). After treatment, all of bitter melon- and ROS-treated groups showed a significant reduction in plasma glucose and HbA1c as compared with the HF group. Insulin concentration caused a significant fall in P1 and ROS treated-groups, inversely (Table 5). We demonstrated that antihyperglycemic activity of bitter melon was comparable to ROS in high-fat fed mice.

3.3. *Plasma lipid*

As time past, the hypercholesterolemic phenomenon was deteriorated by HF diet. At week 8, the plasma TC and TG levels of HF group were significantly greater than the CON group (194.5 ± 1.8 mg/dL vs.146.1 ± 3.4 mg/dL; 219.6 ± 4.7 mg/dL vs. 123.0 ± 13.3 mg/dL) ($P < 0.001$, $P < 0.001$, respectively) (data not shown) which again demonstrated that the disease animal model was set-up, and afterwards we began to administrate of the experimental drug. At week 12, the TC, TG, FFA levels were 81.3%, 42%, and 95% greater in the HF group than in the CON

Table 3 – Food intake for 4 week in high-fat fed mice

Drug	Dose (g/kg/day)	Food intake (g/day/per mice)				
		2 week after HF or LF	1 week	2 week	3 week	4 week
CON		3.30 ± 0.02	3.21 ± 0.09	3.03 ± 0.25	3.11 ± 0.27	3.19 ± 0.12
FAT		$2.75 \pm 0.02^{\#\#\#}$	$2.48 \pm 0.06^{\#\#}$	$2.35 \pm 0.03^{\#}$	$2.30 \pm 0.17^{\#}$	$2.56 \pm 0.09^{\#\#\#}$
FAT + P1	0.5	$2.84 \pm 0.03^{\#\#\#}$	2.32 ± 0.17	2.31 ± 0.14	1.98 ± 0.07	2.44 ± 0.05
+P2	1.0	$2.79 \pm 0.06^{\#\#\#}$	2.23 ± 0.01	2.34 ± 0.03	2.13 ± 0.04	2.23 ± 0.02
FAT + G1	0.2	$2.41 \pm 0.02^{\#\#\#}$	2.29 ± 0.06	2.44 ± 0.13	2.11 ± 0.12	2.37 ± 0.01
+G2	1.0	$2.52 \pm 0.03^{\#\#\#}$	2.44 ± 0.03	2.52 ± 0.10	2.28 ± 0.12	2.51 ± 0.08
FAT + ROS		$2.81 \pm 0.01^{\#\#\#}$	2.36 ± 0.19	2.37 ± 0.20	2.16 ± 0.05	2.47 ± 0.04

We have investigated bitter melon once again on food intake for 4 week. Male C57BL/6J mice, 5 week of age were used and after a 1-week acclimation period, mice were divided randomly into two groups. The control group (CON) was fed low-fat (LF) diet whereas the experimental group was fed a 45% high-fat (HF) diet for 2 weeks, afterwards we began to administrate drug for 4 week accompanied with HF. All values are means ± S.E. ($n = 9$). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with the control group by ANOVA at the same time; bitter melon has no suppressive effect on food intake as compared with the high-fat (HF) + vehicle (distilled water) group. P1, P2, G1, G2, extracts of *Momordica charantia*.

Table 4 – Absolute tissue weight, body-weight gain over 4 week, and semiquantative RT-PCR analysis for LPL mRNA expression in adipose tissue

Parameter	CON	HF	HF + P1	HF + P2	HF + G1	HF + G2	HF + ROS
			0.5[a]	1.0[a]	0.2[a]	1.0[a]	0.01[a]
Absolute tissue weight (g)							
EWAT	0.512 ± 0.057	$2.418 \pm 0.112^{\#\#}$	$1.206 \pm 0.208^{***}$	$1.056 \pm 0.239^{***}$	$1.576 \pm 0.312^{*}$	$1.241 \pm 0.212^{***}$	$1.455 \pm 0.170^{**}$
MWAT	0.398 ± 0.040	$0.905 \pm 0.114^{\#\#\#}$	$0.490 \pm 0.108^{*}$	$0.436 \pm 0.070^{**}$	0.688 ± 0.150	$0.439 \pm 0.054^{***}$	$0.410 \pm 0.084^{**}$
RWAT	0.157 ± 0.023	$0.874 \pm 0.062^{\#\#\#}$	$0.473 \pm 0.100^{**}$	$0.433 \pm 0.064^{***}$	$0.601 \pm 0.118^{*}$	$0.443 \pm 0.082^{***}$	$0.458 \pm 0.061^{***}$
Visceral fat	0.669 ± 0.074	$3.292 \pm 0.169^{\#\#\#}$	$1.679 \pm 0.306^{***}$	$1.489 \pm 0.292^{***}$	$2.177 \pm 0.421^{*}$	$1.684 \pm 0.293^{***}$	$1.912 \pm 0.224^{***}$
Liver	1.017 ± 0.056	$1.477 \pm 0.136^{\#\#\#}$	$1.008 \pm 0.054^{***}$	$1.110 \pm 0.052^{**}$	$1.185 \pm 0.067^{*}$	$1.037 \pm 0.060^{***}$	$1.053 \pm 0.037^{***}$
BAT	0.144 ± 0.009	$0.229 \pm 0.035^{\#\#}$	$0.141 \pm 0.0139^{*}$	$0.144 \pm 0.015^{*}$	0.183 ± 0.024	$0.147 \pm 0.018^{*}$	$0.153 \pm 0.012^{*}$
Weight gain (g)	-0.21 ± 0.12	$4.81 \pm 0.54^{\#\#\#}$	$-2.67 \pm 0.82^{***}$	$-2.84 \pm 0.52^{***}$	$0.04 \pm 0.41^{***}$	$-2.60 \pm 0.43^{***}$	–
LPL mRNA	1.802 ± 0.110	1.988 ± 0.280	1.363 ± 0.338	1.363 ± 0.338	$4.510 \pm 0.581^{***}$	$3.401 \pm 0.230^{*}$	2.154 ± 0.334

All values are means ± S.E. ($n = 9$). $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with the control group by ANOVA; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the high-fat (HF) + vehicle (distilled water) group. Total RNA (1 μg) isolated from tissue was reverse transcripted by MMLV-RT, 10 μL of RT products were used as templates for PCR. Signals were quantitated by image analysis; each value was normalized by GAPDH. P1, P2, G1, G2, extracts of *Momordica charantia*. EWAT: epididymal white adipose tissue; RWAT: retroperioneal white adipose tissue; MWAT: mesenteric white adipose tissue; visceral fat: EWAT + RWAT.

[a] Dose (g/kg/day).

group (but there were no statistical significance in TG levels). Bitter melon has no dose-independent effect in TC, TG and FFA, one explanation is due to the complex integredients. Nevertheless, bitter melon and ROS suppressed the HF diet-induced increases in the TC, TG, and FFA concentrations (Table 5).

3.4. *Leptin concentration and epididymal WAT leptin and resistin mRNA levels*

At week 12, leptin concentrations, the epididymal WAT leptin, and resistin mRNA level was greater in the HF group than in the CON group. Bitter melon- and ROS-treated groups showed a significant decrease in blood leptin concentration and WAT leptin and resistin mRNA expression as compared with the HF group (except P1 has no statistical difference in the leptin mRNA level) (Table 5; Figs. 2 and 3). Through these secreted products, bitter melon may affect the systemic metabolism, consequentially improving insulin resistance.

3.5. *Epididymal WAT PPARγ and lipoprotein lipase (LPL) and hepatic PPARα mRNA levels*

The epididymal WAT PPARγ and liver PPARα mRNA level was lower in the HF group than in the CON group. After treatment, only at high dose of bitter melon the PPARγ mRNA level was greater than the HF group, and the PPARα mRNA level was greater in all of the bitter melon treated-groups than in the HF group (Figs. 4 and 5). It has been suggested that LPL was a key enzyme in the metabolism of triglyceride-rich lipoprotein. At the end of studies, the results of measured LPL mRNA showed the G fractional extracts significantly increased LPL mRNA expression in adipose tissue (Table 5). Recently, the metabolic regulator fibroblast growth factor 21(FGF21) has antidiabetic properties in animal models of diabetes and obesity, and PPARα is a key regulator of hepatic FGF21 [28]. Further studies on the regulation of FGF21 by bitter melon will also be investigated.

Table 5 – Blood glucose levels, blood glycated hemoglobin (HbA1c), plasm free fatty acid (FFA), total cholesterol (TC), triglyceride (TG), leptin, and insulin levels at week 12

Parameter	CON	HF	HF + P1 0.5	HF + P2 1.0	HF + G1 0.2	HF + G2 1.0	HF + ROS 0.01
			0.5[a]	1.0[a]	0.2[a]	1.0[a]	0.01[a]
Glucose level (mg/dL)	66.6 ± 3.9	126.0 ± 12.3$^{\#\#\#}$	87.5 ± 3.5**	72.7 ± 2.3***	89.3 ± 6.7**	87.2 ± 4.5**	91.5 ± 7.9*
HbA1c (%)	8.85 ± 0.57	13.96 ± 0.45$^{\#\#\#}$	9.24 ± 0.25**	9.64 ± 0.99*	9.53 ± 0.29*	10.02 ± 0.64*	10.16 ± 0.14*
FFA (meq/L)	0.42 ± 0.03	0.82 ± 0.06$^{\#\#\#}$	0.56 ± 0.08*	0.63 ± 0.06	0.60 ± 0.04*	0.85 ± 0.07	0.52 ± 0.09*
TC (mg/dL)	147.4 ± 8.2	267.3 ± 17.5$^{\#\#\#}$	183.0 ± 25.8*	219.2 ± 11.1	176.0 ± 15.1*	185.6 ± 23.3*	211.3 ± 21.8
TG (mg/dL)	127.3 ± 10.4	181.3 ± 12.1	154.0 ± 9.8	136.7 ± 6.7*	144.3 ± 4.5*	140.3 ± 14.7	137.2 ± 6.3*
Leptin (μg/mL)	1.21 ± 0.32	10.74 ± 0.17$^{\#\#\#}$	4.16 ± 1.81**	3.26 ± 1.04***	5.49 ± 1.21*	4.01 ± 0.84***	2.92 ± 0.51***
Insulin (μg/L)	89.2 ± 9.2	261.3 ± 47.8$^{\#\#\#}$	84.4 ± 34.1*	181.5 ± 27.7	245.8 ± 26.0	188.2 ± 34.1	136.1 ± 18.2*

All values are means ± S.E. (n = 9). $^{\#\#\#}P < 0.001$ compared with the control group by ANOVA; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the high-fat (HF) + vehicle (distilled water) group. P1, P2, G1, G2, extracts of *Momordica charantia*.
[a] Dose (g/kg/day).

4. Discussion

Our study results demonstrated that bitter melon extract effectively controlled hyperglycemia and hyperinsulinemia by significantly reducing blood glucose and insulin levels in C57BL/6J mice on a HF diet. Moreover, bitter melon exerted its effects similar to conventional insulin sensitizers, which act as a PPARα and PPARγ agonist. Also, the level of blood glycosylate HbA1C, which is a maker of long-term control of blood glucose, is significantly decreased.

Although we have mentioned P and G fraction extraction manner in materials and method, we have reminded that P extract ranged widely, even though G fraction was included. G extract ranged narrowly and was composed of containing aglycone compounds, such as goyaglycoside, momordicoside, and goyasaponin [29]. More polar ingredients were extracted in P extraction manner including triterpene such as oleanolic acid and its glycosides (oleanolic acid 3-*O*-glucuronide and momordin Ic) and conjugated linoleic acid, phytol, and charantins. Oleanolic acid 3-*O*-glucuronide and momordicoside is belonged to pentacyclic triterpene and have shown hypoglycemic activity [26]. Thus, P extract is more effective than G extract in causing a significant fall in plasma glucose levels and both of P and G extract exerting anti-diabetic effect.

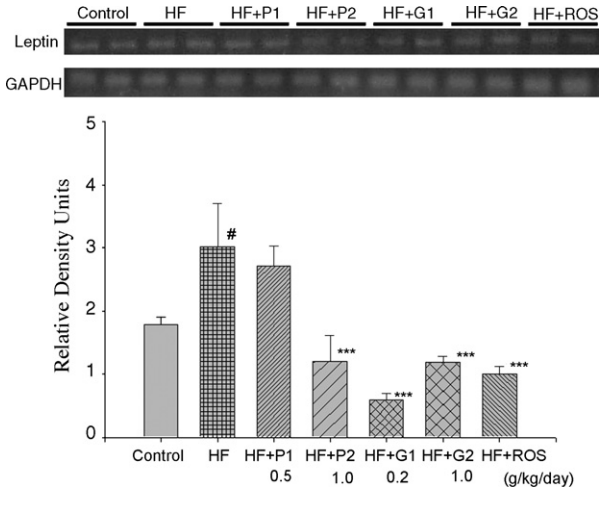

Fig. 2 – Semiquantative RT-PCR analysis for leptin mRNA expression in adipose tissue. Total RNA (1 μg) isolated from tissue was reverse transcripted by MMLV-RT, 10 μL of RT products were used as templates for PCR. Signals were quantitated by image analysis; each value was normalized by GAPDH. All values are means ± S.E. (n = 9). $^{\#}P < 0.05$ compared with the control group; $^{***}P < 0.001$ compared with the high-HF (HF) + vehicle(distilled water) group. P1, P2, G1, G2, extracts of *Momordica charantia*.

C57BL/6J is a commonly used laboratory strain, and is susceptible to HF diet-induced Type 2 diabetes. Previous others results have shown that hyperglycemia develops within 1 month of introduction of a HF diet in C57BL/6J mouse, and diabetes/obesity syndrome worsens with time and with increasing obesity, moreover, C57BL/6J mice fed a HF diet at 16 weeks had developed to the increased abdominal fat mass [11,30], and our study result at week 12 was in accordance with these. In addition, it has been proposed that WAT is associated with energy storage [31], whereas BAT is correlated with energy expenditure [32]. Both of WAT and BAT mass was significantly decreased by bitter melon treatment demonstrating that bitter melon exerts the relationship with energy homeostasis.

The present study proved that bitter melon, traditionally used as an anti-diabetic herb, is effective to improve insulin resistance in a mouse model of Type 2 diabetes possibly by decreasing blood glucose and improving lipid metabolism through the regulation of PPARs-mediated pathway. PPARs are ligand-activated transcription factors belonging to the nuclear receptor superfamily, and PPAR ligands include fatty acids and eicosanoids [33].

PPARγ is mainly present in adipose tissue, and plays a key role in glucose homeostasis and differentiation of fat cells [34,35]. PPARγ ligand activates PPARγ. TZD, a synthetic PPARγ ligand, significantly increased insulin sensitivity via PPARγ, actually display improved insulin sensitivity on a HF diet [36]. In NIDDM patients, TZD, a synthetic PPARγ ligand, significantly increased insulin sensitivity [37]. Lee et al. [38] have suggested that PPARγ ligand up-regulated the expression of genes involved in glucose uptake of adipocytes and lipid storage of adipocytes. We observed that bitter melon increased adipose tissue PPARγ mRNA expressions. Therefore, we assumed that bitter melon used in our study behaved

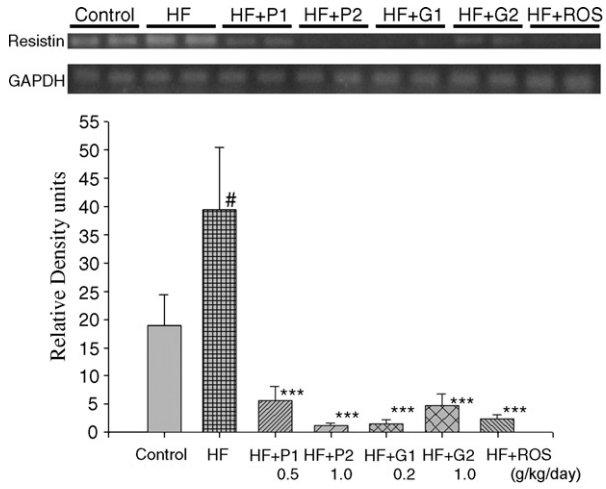

Fig. 3 – Semiquantative RT-PCR analysis for resistin mRNA expression in adipose tissue. Total RNA (1 μg) isolated from tissue was reverse transcripted by MMLV-RT, 10 μL of RT products were used as templates for PCR. Signals were quantitated by image analysis; each value was normalized by GAPDH. All values are means ± S.E. (n = 9). $^{\#}P < 0.05$ compared with the control group; $^{***}P < 0.001$ compared with the high-HF (HF) + vehicle (distilled water) group. P1, P2, G1, G2, extracts of *Momordica charantia*.

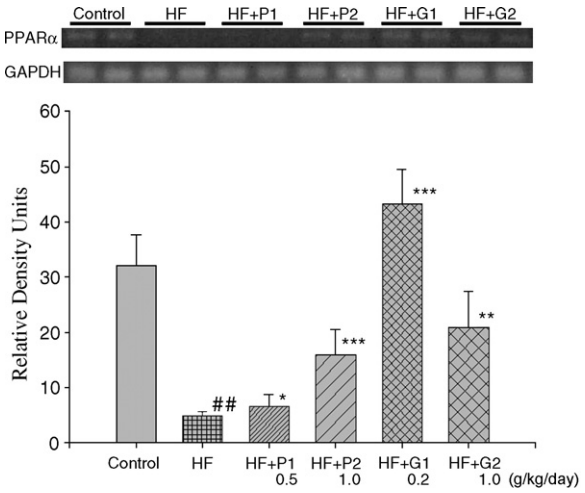

Fig. 5 – Semiquantative RT-PCR analysis for PPARα mRNA expression in liver tissue. Total RNA (1 μg) isolated from tissue was reverse transcripted by MMLV-RT, 10 μL of RT products were used as templates for PCR. Signals were quantitated by image analysis; each value was normalized by GAPDH. All values are means ± S.E. (n = 9). $^{\#\#}P < 0.01$ compared with the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the high-HF (HF) + vehicle (distilled water) group. P1, P2, G1, G2, extracts of *Momordica charantia*.

similar to several PPARs ligands. Also, the increased expression of LPL and a significant decrease in blood TG and FFA by bitter melon extract is possibly mediated by increased expression of PPARs.

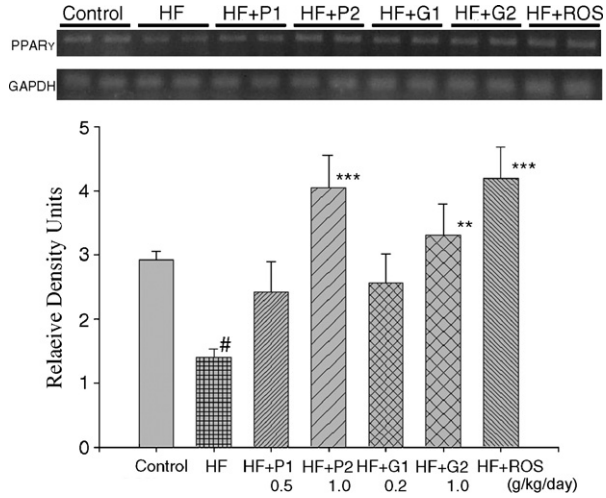

Fig. 4 – Semiquantative RT-PCR analysis for PPARγ mRNA expression in adipose tissue. Total RNA (1 μg) isolated from tissue was reverse transcripted by MMLV-RT, 10 μL of RT products were used as templates for PCR. Signals were quantitated by image analysis; each value was normalized by GAPDH. All values are means ± S.E. (n = 9). $^{\#}P < 0.05$ compared with the control group; $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the high-HF (HF) + vehicle (distilled water) group. P1, P2, G1, G2, extracts of *Momordica charantia*.

Most studies suggest that leptin enhances insulin sensitivity, there are several reports describing that leptin reduces insulin singaling in adipose tissue [39,40]. Surprisingly, TZDs reduce leptin mRNA levels in adipocytes. As leptin increases insulin sensitivity, the down-regulation of leptin expression by TZDs is probably due to other factors than its effect on insulin signaling [41,42]. The leptin gene is regulated by C/EBPα and it is found that PPARγ activation by TZDs antagonizes the C/EBPα-mediated transactivation of leptin promotor [43]. Thus, we speculated that bitter melon might have the same action mode like TZDs on the improvement of insulin sensitivity.

There are several reports suggesting that either obesity or a HF diet can result in the development of leptin resistance in rodents [44–46]. In this study, blood leptin levels were elevated by a HF diet and were positively correlated with the increase of visceral fat weight and body weight. The reduction in blood leptin concentrations along with significant reductions in epididymal and retroperitoneal adipose depot mass and visceral fat weight is in agreement with previous reports of the relationship with adipocyte production and secretion of leptin is positively corrected to adipose tissue mass [41,47]. It was also observed in our studies that bitter melon extract significantly reduced leptin concentrations. Since visceral obesity is thought to play a major role in the pathogenesis of metabolic syndrome [48], bitter melon is likely to be useful in the treatment of metabolic syndrome associated with visceral adiposity, such as hyperlipidemia, insulin resistance, and Type 2 diabetes.

It is believed that TZDs influence the secretion of an adipocyte factor that influences glucose uptake and/or

metabolism. Candidate factors include FFA and the recently discovered hormone resistin. Resistin was isolated as a negative target of PPARγ agonist and was proposed to serve as a possible mediator between obesity and Type 2 diabetes [14]. Resistin was first found to impair insulin action and to induce insulin resistance [14]. Our results are in line with Steppan et al. showing that a HF diet and obesity increased resistin expression, and the production of resistin is decreased by TZDs treatment [14]. According to the present results, we concluded that at least a portion of bitter melon effects is due to be through PPARγ-mediated pathways, including resistin and leptin, resulting in lowering glucose levels and improving insulin resistance. Moreover, bitter melon functions as a TZD and plays an important role in the efficacy of antidiabetes.

TZD-activated PPARγ shifts glucose and FFA into adipocyte, thus decreases glucose and FFA levels, an effect corroborated by the observation that PPARγ agonists induce body weight gain [42] and both adipocyte hypertrophy and hyperplasia [49], these happen only in subcutaneous fat, as opposed to visceral fat [50] in rodents. Additionally, visceral obesity has a strong association with insulin resistance [51]. TZD-activated PPARγ initiates visceral adipocytes apoptosis resulting in the circulating FFA is trapped subcutaneously and insulin resistance is mitigated [52]. Bitter melon suppressed visceral fat accumulation and decreased FFA levels and amelioratated insulin resistance in mice model with NIDDM on HF diets. Body weight gain was not found in our rosiglitazone treatment possibly by the reason of different dose or short duration of medication. It is well established that FFA are abnormally elevated in conditions such as Type 2 diabetes and obesity, and FFA represents a link between increased body fat and insulin resistance [53]. Finally, our results suggest that treatment of bitter melon in HF-fed mice effectively improves glucose homeostasis and exerts potent insulin sensitizing effect.

PPARα agonists are known to stimulate mitochondrial oxidation and cellular uptake of FFA by modifying the expression of genes such as acyl-CoA synthetase gene and fatty acid transport protein gene [54,55]. Ersten et al. [56] reported that, in a fasting state, cellular uptake and oxidation of fatty acids liberated from fat tissues occurs with increased liver PPARα expression. Pharmacological stimulation with synthetic PPARα ligands such as fibrates also up-regulates genes involved in fatty acid oxidation and cellular uptake of FFA [57–59]. PPARα ligands also increase the expression of the LPL gene [60], resulting in hypotriglyceridemic effect. Our results suggest that bitter melon improves plasma lipid profiles by stimulating fatty acid oxidation through PPARα-mediated pathways.

Our results demonstrate that bitter melon substantially reduces adipose tissue mass in mice fed with a HF diet. PPARα has been proposed to play a central role in a pathway that, under conditions of excess dietary energy, serves to minimize fat storage in the central organs at the expense of white adipose tissue [61]. Due to the involvement of PPARα in energy homeostasis, we speculate that bitter melon may activate PPARα in liver, leading to reductions in adipose mass, body weight gain, and hyperlipidemia in C57BL/6J mice fed with a HF diet. Since lipids that accumulate in adipose tissue are largely derived from circulating TG [62] and liver is a major target tissue for lipid and lipoprotein metabolism, bitter melon may be able to mobilize fat from adipose tissue by increasing fat catabolism in the liver. According to our results, the increased fatty acid oxidation and possibly decreased TG synthesis in liver effectively decreased adipose tissue mass, resulting in the regulation of visceral obesity.

5. Conclusion

Bitter melon extract significantly increased insulin sensitivity and improved hyperglycemia. We demonstrated that bitter melon could influence both PPARα/PPARγ and mediated gene expression, thus leads to suppress body weight gain and reduce visceral fat accumulation, and decrease the levels of TG and FFA. We speculated that bitter melon could not only regulate PPARα-mediated pathway, which induce liver fatty acid oxidation, thereby lower blood lipid effect, but also influence PPARγ-mediated pathway, which regulate adipocytokine gene expression, resulting in improving insulin resistance and effectively controlling hyperglycemia. Since we used a HF-induced obesity and NIDDM model, we speculated that bitter melon might be another choice in the protection against visceral obesity and Type 2 diabetes.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflict of interest.

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