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Effect of a Proanthocyanidin-Rich Extract from Longan Flower on Markers of Metabolic Syndrome in Fructose-Fed Rats

HUI-YUN TSAI,[†] LIANG-YI WU,[‡] AND LUCY SUN HWANG*,[†]

Graduate Institute of Food Science and Technology, National Taiwan University, Number 1, Section 4, Roosevelt Road, Taipei 106, Taiwan, and Department of Bioscience Technology, Chung Yuan Christian University, 200, Chung Pei Road, Chung Li 320, Taiwan

Recent evidence strongly suggests that oxidative stress due to redox imbalance is highly associated with metabolic syndrome. The objective of this study was to evaluate the effect of the supplementation of longan flower water extract (LFWE), which showed powerful antioxidative activity in vitro, on markers of metabolic syndrome in a fructose-fed rat model. Male Sprague−Dawley rats were randomly divided into four groups: group C, fed with standard Purina chow; group F, fed with high-fructose diet (HF) alone; group L, fed with HF plus LFWE 125 mg/kg bw per day by gavage; and group H, fed HF plus LFWE 250 mg/kg bw per day by gavage. The dietary manipulation lasted for 14 weeks. Results of our study showed that rats fed with HF resulted in oxidative stress and affected the antioxidant status including plasma thiobarbituric acid and liver antioxidant enzyme activity. Treatment with LFWE significantly augmented the antioxidant system. HF was able to cause insulin resistance and elevation of the blood pressure. The supplementation of LFWE ameliorated insulin resistance by enhancing the expression of insulin signaling pathway related proteins, including insulin receptor substrate-1 and glucose transporter 4. LFWE supplementation was also found to decrease systolic blood pressure. These findings indicate that longan flower water extract may improve the symptoms of metabolic syndrome in fructose-fed rats.

KEYWORDS: Antioxidant; longan flowers; insulin resistance; blood pressure

INTRODUCTION

Metabolic syndrome is a cluster of disorders, including hyperglycemia, hyperinsulinemia, insulin resistance, hypertriglyceridemia, and hypertension. Insulin resistance is a prominent feature of metabolic syndrome and constitutes a major risk for type 2 diabetes and cardiovascular disease (*1*). Oxidative stress occurs when there is an imbalance between oxidant and antioxidant systems and accumulation and generation of reactive oxygen species (ROS) in the body. Abnormally high levels of ROS lead to damage to cellular membrane, tissues, and enzymes, an increase in lipid peroxidation, and development of insulin resistance and diabetes (*2*). Several epidemiological studies have shown that a negative correlation exists between the consumption of phenolic antioxidants and the risk of metabolic syndrome. In this study, we used a well-established rat model in which insulin resistance, hypertension, and dyslipidemia were induced by feeding animals with a high-fructose (HF) diet (*3*).

Plants contain many antioxidant compounds, such as tannins, flavonoids, and proanthocyanidins that have powerful antioxidant properties. Plant polyphenols are widely present in the diet and are thought to be important for human health. An increase in the consumption of antioxidants from these foods may reduce oxidative stress and chronic diseases (*4, 5*).

Longan (*Dimocarpus longan* Lour.) is the subtropical fruit of the Sapindaceae family, which is widely grown in China, Taiwan, and South East Asia. The fruit of longan is sweet and juicy and is consumed either fresh or as dried products. Longan fruits and seed extracts, containing high levels of gallic acid, corilagin, and ellagic acid, were found to have antioxidant activitiesy as effective as Japanese green tea extract (*6*). The flower has a fruity aroma that is used to prepare an infusion drink for refreshment. It is also used for the treatment of leucorrhea and kidney disorders in China (*7*). In our previous study, we have found that longan flower has a high content of polyphenolic compounds and possesses high antioxidant activity, which is attributed to (−)-epicatechin and proanthocyanidin A_2 (*8*). We have also reported that longan flower extract exhibited anti-inflammatory activity by suppressing the production of nitric oxide and prostaglandin E_2 in lipopolysaccharide-stimulated RAW 264.7 macrophages (*9*).

No scientific studies have reported the effect of longan flowers on insulin sensitivity. The present study was designed to evaluate the effects of the supplementation of the longan flower water

* To whom correspondence should be addressed. Tel: +886-2-33664114. Fax: +886-2-33664113. E-mail: lshwang@ntu.edu.tw.
[†] National Taiwan University.
[‡] Chung Yuan University.

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extract (LFWE) on insulin resistance, hypertension, oxidative stress, and insulin signaling pathway related proteins expression in a HF-fed rat model.

MATERIALS AND METHODS

Preparation of Longan Flower. Roasted male longan flowers were kindly provided by a longan farm (Tainan, Taiwan) in April 2006. Roasting was used to reduce moisture for storage and to enhance flavor. Dried longan flower was ground to a powder and passed through a 40 mesh sieve. The powder was soaked with a 50 v/w (mL/g) ratio of distilled water at 100 °C for 5 min, with occasional shaking to increase the extraction efficiency. The extract was then filtered through a #1 filter paper and freeze-dried to obtain lyophilized powder, LFWE. It was stored at −20 °C.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay. The DPPH assay was used to measure the free radical scavenging activity of LFWE. Briefly, the LFWE solutions in different final concentrations (0−30 μg/mL) were pipetted into a 96 well plate. Then, 250 μL of 100 μM DPPH methanol solution was added. The mixture was shaken and allowed to stand at room temperature for 90 min. Then, the absorbance was measured at 520 nm in a spectrophotometer (BIOTEK, Atlanta, GA). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. α-Tocopherol was used as the positive control.

Low-Density Lipoprotein (LDL) Oxidation Assay. Blood from a healthy adult volunteer was collected in a centrifuge tube and centrifuged (1800*g* for 15 min) to obtain serum. The LDL fraction (d = 1.019−1.063 g/mL) was isolated by density centrifugation in a ultracentrifuge using a 70 Ti rotor (Beckman, Palo Alto, CA), based on the method of Puhl et al. (*10*). The LDL layer was removed and dialyzed against 5 mM phosphate-buffered saline (PBS, pH 7.4). After dialysis, the cholesterol concentration in the LDL sample was determined using a cholesterol assay kit (E. Merck, Darmstadt, Germany). The LDL fraction was used immediately or purged with nitrogen and stored at 4 °C for less than 1 week. To evaluate the ability of LFWE to inhibit Cu^{2+}-induced LDL oxidation, conjugated dienes were monitored at 232 nm every 15 min. LDL was diluted with 5 mM PBS to a final concentration of 60 μg LDL fraction/mL and incubated at 37 °C in the presence of LFWE (1 μg/mL) and $CuSO_4$ (5 μM) for 16 h. The lag time was determined to be the intercept of the slopes for the lag and propagation phases and was compared with the control oxidized LDL. Trolox was used as the positive control.

Animal Experiment. Male Sprague−Dawley rats weighing 200−250 g were housed two to a cage in an air-conditioned room (22 ± 2 °C) on a 12 h light cycle (7:00 a.m. to 7:00 p.m.). These animals were maintained according to the guidelines established in Taiwan Government Guide for the Care and Use of Laboratory Animals. Rats were randomly divided into four groups (n = 8): group C, fed with standard Purina chow (#5001, Purina, St. Louis, MO; comprised of 23% protein, 56% carbohydrate, 4.5% fat, and 6% fiber); group F, fed with 60% HF diet (comprised of 21% protein, 60% fructose, 5% fat, and 8% fiber) alone; group L, fed with HF plus LFWE 125 mg (dissolved in 0.1 g LFWE/mL H_2O)/kg body weight (bw) per day by gavage (a low-dose group); and group H, fed HF plus LFWE 250 mg (dissolved in 0.2 g LFWE/mL H_2O)/kg bw per day by gavage (a high-dose group). LFWE was given at 6:00 p.m. everyday. Groups C and F were not given the sample (LFWE) but equal volumes of vehicle (H_2O) by gavage everyday for the same period. The dietary manipulation lasted for 14 weeks. Blood pressure was measured every week, and an oral glucose tolerance test (OGTT) was performed at 12 weeks of diet supplementation. At the end of the experiment, the rats were decapitated after overnight fasting, blood samples were collected in heparinized tubes, and the plasma was separated by centrifugation and stored at −20 °C until assayed for glucose, insulin, triglyceride, cholesterol, and thiobarbituric acid-reactive substances (TBARS). The liver was isolated and tested for glutathione reductase (GRd). The adipocytes were isolated from epididymal fat pads and tested for the expression of insulin signaling pathway related proteins, including insulin receptor substrate-1 (IRS-1), Akt, and glucose transporter 4 (GLUT 4).

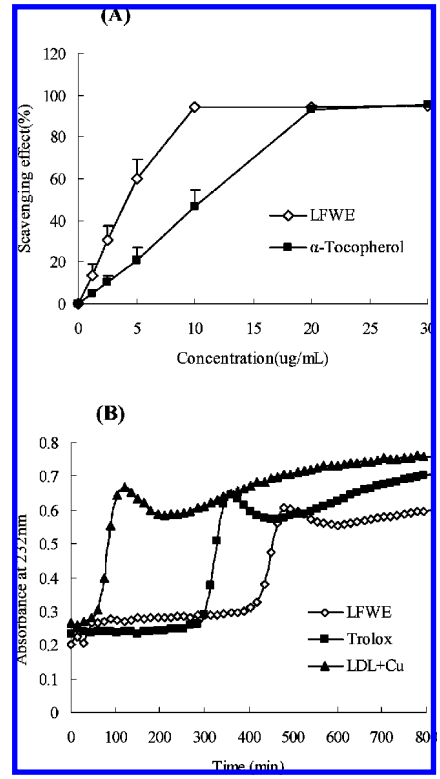

Figure 1. DPPH free radical scavenging effect of LFWE (**A**) and the inhibition of Cu^{2+}-induced oxidation of human LDL by LFWE (**B**).

Systolic Blood Pressure Measurement. Systolic blood pressure was measured by tail-cuff method using an automatic blood pressure monitoring system (BP-98A, Softron, Tokyo, Japan). The animals were kept in a warming element, which allowed the surrounding temperature to be increased to 37 °C, thus maintaining adequate circulation to allow reliable measurement of the systolic blood pressure. The average of five consecutive readings for accurate measurement was used for the blood pressure.

OGTT. At 12 weeks, the rats were subjected to an OGTT. Briefly, after overnight fasting, a 0 min blood sample was taken by cutting the tail tip, then the glucose solution (1.5 g/kg) was immediately administered by gavage, and three more tail vein blood samples were taken at 30, 60, 90, and 120 min after glucose administration. The area under the curve (AUC) for glucose and insulin was calculated using the trapezoidal rule.

Biochemical Determinations. Blood samples were centrifuged at 2000*g* for 10 min at 4 °C. Then, the plasma was removed for the respective analytical determinations. The plasma glucose concentration was measured using a glucose analyzer (model 2300, Yellow Springs Instruments, OH). Plasma insulin levels were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Uppsala, Sweden). Plasma triglycerides and cholesterol were determined using commercial kits (E. Merck, Darmstadt, Germany) by enzymatic photocolorimetric methods. Lipid peroxidation products in plasma were determined by reaction with thiobarbituric acid as TBARS (*11*). TBARS were determined by the spectrophotometric method, using 1,1,3,3-tetramethoxypropane to establish the standard curve.

Preparation of Liver Homogenates. The liver samples were homogenized in potassium phosphate buffer (0.006 M KH_2PO_4, 0.004 M K_2HPO_4, and 1.15% KCl, pH 7.4) with a homogenizer to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 105000*g* for 60 min at 4 °C (Beckman, Palo Alto, CA). The supernatant obtained was used for biochemical analyses. The protein concentration was determined by a Bio-Rad protein assay kit using bovine serum albumin as the standard.

GRd Assay. Liver GRd was assayed by a reaction mixture containing 0.975 mL of 100 mM PBS, 1.1 mM $MgCl_2$, 5 mM oxidized GSSG, and 0.1 mM NADPH. Twenty-five microliters of liver homogenate was

added to trigger the NADPH conversion reaction. Changes in absorbance were monitored at 340 nm for 5 min at 25 °C. The specific enzyme activity of GR was expressed as nmol NADPH oxidized to $NADP^+$ per min per mg protein.

Adipocyte Tissue Processing. The epididymal fat pads were homogenized in ice-cold buffer containing 250 mM sucrose, 20 mM HEPES, 1 mM EDTA, and 1% protease inhibitor cocktail, pH 7.4. To remove the fat cake, the homogenate was centrifuged at 6000*g* for 10 min at 4 °C, and the supernatant was then aspirated and centrifuged at 250000*g* for 90 min at 4 °C. The pellet was resuspended in ice-cold buffer and stored at −80 °C until used in western immunoblotting.

Western Immunoblotting. The adipocyte tissue homogenates were subjected to electrophoresis on 6−12% sodium dodecyl sulfate (SDS) polyacrylamide gels and electrophoretically transferred to a PVDF membrane. The membranes were blocked for 1 h at room temperature with PBS buffer containing 5% skimmed milk and 0.5% Tween-20 (v/v) and incubated for 1 h at room temperature with the anti-IRS-1 (1:1500), anti-Akt (1:1000), anti-GLUT 4 (1:5000), and anti-β-actin (1:10000) antibodies, respectively, followed by incubation with HRP-conjugated secondary antibodies. The proteins were then developed using an enhanced chemiluminescence reagent kit (Perkin-Elmer, Waltham, MA) and exposed to X-ray film (Fujinomiya, Shizuoka, Japan). Quantification of the relative band intensity was performed by laser scanning densitometry.

Statistical Analysis. Data were presented as the means ± standard deviations (SDs). One-way analysis of variance followed by Duncan's multiple range test was used to analyze the effect of LFWE antioxidant activity in vitro. Student's *t* test was used to compare differences two groups in animal experiment. Differences between the two groups were considered to be statistically significant when the p value was <0.05.

RESULTS

Antioxidant Activity of LFWE in Vitro. Two antioxidant activity assays for the measurement of longan flowers water extract were conducted including DPPH radical scavenging and Cu^{2+}-induced human LDL oxidation. Longan flowers water extract demonstrated a concentration-dependent scavenging activity by quenching DPPH radicals and was compared with α-tocopherol as a positive control (**Figure 1A**). The IC_{50} values (defined as the concentration of test compound required to produce 50% inhibition) for DPPH scavenging by LFWE and α-tocopherol were 3.75 and 9.22 μg/mL, respectively. LFWE showed the greater DPPH radical scavenging activity than the positive control. The Cu^{2+}-induced human LDL oxidation method was used to evaluate the inhibitory effect of LFWE on LDL oxidation. Both LFWE and trolox prolonged the LDL oxidation lag phase as compared to LDL control (**Figure 1B**). The ΔT_{lag} values (defined as the difference in lag phase between the control and the sample) for LDL oxidation by LFWF and trolox were 352.1 and 224.4 min, respectively. LFWE showed the longest lag time, increased 57% than the positive control. On the basis of these results, it is evident that LFWE shows strong antioxidant activity in vitro.

Animal Characteristics. There were no differences in weight gain among different groups of rats throughout 14 weeks dietary manipulation, showing that the dietary supplementation of LFWE did not affect the growth (data not shown). The systolic blood pressure in the fructose group began to increase after 2 weeks (**Figure 2**), and significant differences were observed between the fructose and the control groups from week 2 to the end of the experiment, showing that HF supplementation resulted in elevated blood pressure. The administration of LFWE prevented the systolic blood pressure increment ($p < 0.05$). Each group compared to the fructose group had the following significant mean differences at the end of study: C, −20 mm Hg, L, −15 mm Hg; and H, −18 mm Hg.

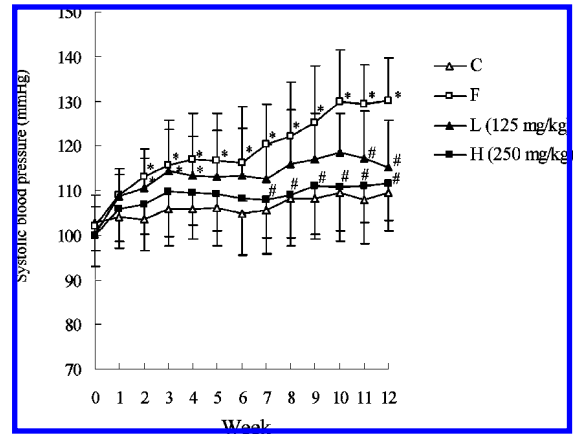

Figure 2. Systolic blood pressure changes in rats during 12 weeks of dietary manipulation. Rats of group C (△) were fed regular chow with H_2O; group F (□) was fed a HF diet with H_2O; group L (▲) was fed a HF diet with LFWE (125 mg/kg); and group H (■) was fed HF diet with LFWE (250 mg/kg). Values are shown as means ± SD ($n = 8$). $^*p < 0.05$ as compared with group C. $^\#p < 0.05$ as compared with group F.

OGTT. The response to an OGTT after 12 weeks of diet supplementation of LFWE is shown in **Figure 3A**. The plasma glucose levels were significantly elevated during OGTT in the F group as compared to the C group at 30 and 60 time points. In the H group, the plasma glucose levels were significantly decreased at 30 and 60 time points as compared to the fructose group and returned to normal levels. Plasma insulin concentrations were significantly higher at all time points throughout the OGTT in the F group as compared to the C group (**Figure 3B**). The plasma insulin concentrations in the H group were only higher at 30 and 60 time points as compared to the C group but returned to normal levels at 90 and 120 time points. The total AUCs for glucose and insulin are shown in **Figure 3C**,**D**. The plasma glucose and insulin AUCs were significantly increased in the F group as compared to the C group (glucose AUC: C, 311 ± 28 mg h/dL vs F, 336 ± 19 mg h/dL; insulin AUC: C, 1.99 ± 1.26 ng h/mL vs F, 5.03 ± 1.53 ng h/mL). After 12 weeks of administration of LFWE (250 mg/kg), the plasma glucose AUC (310 ± 19 mg h/dL) and insulin AUC (3.54 ± 0.89 ng h/mL) were significantly decreased as compared to the F group.

Biochemical Determinations. At sacrifice, there were no significant differences between the groups in bw, but epididymal fat pad weights and the relative epididymal fat pad weights were significantly higher in the F group. Epididymal fat pad weights and the relative epididymal fat pad weights were reduced after 14 weeks of treatment with LFWE (250 mg/kg) (**Table 1**). The concentrations of fasting plasma glucose, insulin, triglyceride, cholesterol, and TBARS were also analyzed (**Table 1**). Plasma glucose, insulin, triglyceride, and total cholesterol levels in the F group were significantly higher than in the C group. Plasma levels of glucose, insulin, and total cholesterol tend to be reduced in H group; no significant differences from either F or C groups were found.

Liver Oxidative Status. Figure 4 shows the GRd enzyme activity of the liver tissues. The GRd activity of the liver tissues was decreased significantly after 14 weeks of HF diet administration, relative to the control group. However, LFWE administration at the dose of 125 or 250 mg/kg exhibited a 1.22- and 1.34-fold increase of GRd activity, respectively, as compared to the fructose group. There were no differences in GPx activities among different groups after 14 weeks of dietary manipulation (data not shown).

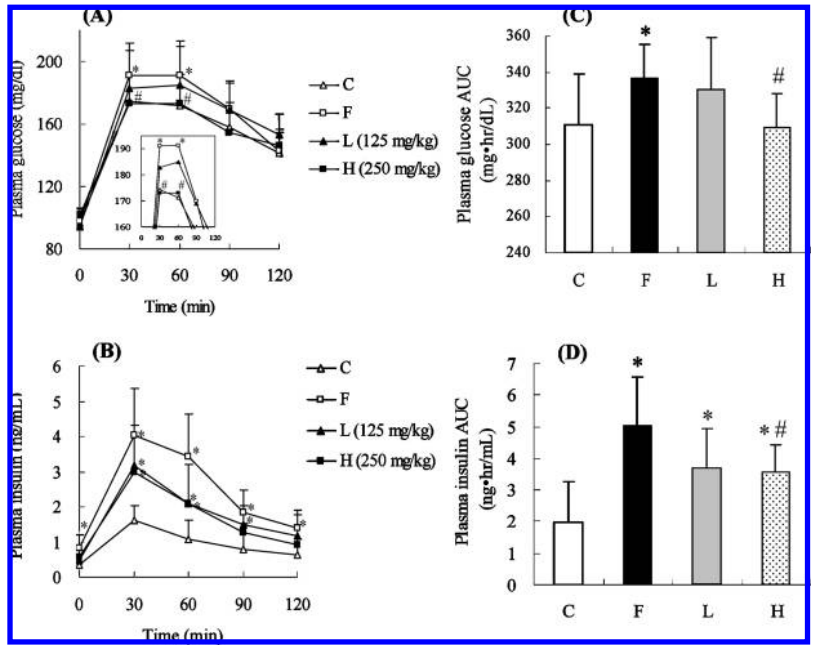

Figure 3. (**A**) Plasma glucose responses during an OGTT (1.5 g glucose/kg bw) performed after 12 weeks of dietary manipulation. (**B**) Plasma insulin responses during an OGTT. Values are shown as means ± SD ($n = 8$). (**C**) Area under the curve on plasma glucose in an oral glucose tolerance. (**D**) Area under the curve on plasma insulin in an oral glucose tolerance. Values are shown as means ± SD ($n = 8$). *$p < 0.05$ as compared with group C. #$p < 0.05$ as compared with group F.

Table 1. Epididymal Fat Pads Weight, Relative Epididymal Fat Pads Weight, and Metabolic Variables[a]

group	C	F	L	H
bw (g)	490.8 ± 70.6	492.2 ± 45	499.0 ± 42.1	484.5 ± 46.9
epididymal fat pads weight (g)	5.7 ± 1.9	8.1 ± 2.5*	8.5 ± 1.6*	7.2 ± 1.8
relative epididymal fat pads weight (g/100 g bw)	1.2 ± 0.3	1.7 ± 0.5*	1.7 ± 0.3*	1.5 ± 0.3
glucose (mg/dL)	114.4 ± 3.3	123.6 ± 7.4*	114.9 ± 13.1	114.3 ± 9.1
insulin (ng/mL)	0.26 ± 0.07	0.51 ± 0.17*	0.38 ± 0.09*	0.36 ± 0.20
triglyceride (mg/dL)	75.4 ± 17.7	141.5 ± 26.1*	142.4 ± 33.8*	120.3 ± 35.5*
cholesterol (mg/dL)	62.7 ± 10.4	82.8 ± 9.75*	85.0 ± 12.0*	79.7 ± 27.8
TBARS (nmol MDA/mg protein)	0.100 ± 0.013	0.120 ± 0.015*	0.115 ± 0.009*	0.096 ± 0.020#

[a] Values are shown as means ± SD ($n = 8$). *$p < 0.05$ as compared with group C. #$p < 0.05$ as compared with group F.

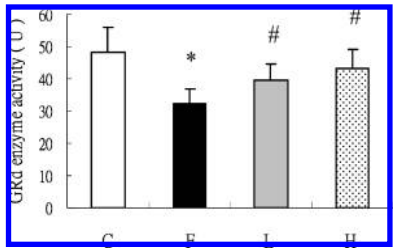

Figure 4. Liver GRd in rats after 14 weeks of dietary manipulation. Enzyme activity (U): The unit of GRd enzyme activity is nmol NADPH oxidized per min per mg protein. Values are shown as means ± SD ($n = 8$). *$p < 0.05$ as compared with group C. #$p < 0.05$ as compared with group F.

Insulin Signaling Pathway Related Proteins in Adipocytes. To evaluate the possible mechanism by which LFWE improved insulin resistance, we measured the insulin signaling pathway related proteins in adipocytes, including IR, IRS-1, Akt, and GLUT 4. Following 14 weeks of treatment, there were no differences in the expression of IR protein in adipocytes of fructose group and the control group (data not shown), and treatment with LFWE for 14 weeks did not modify these values (data not shown). The expression of IRS-1 protein in adipocytes of the fructose group was close to 38% of that in control group (**Figure 5A**). The 14 weeks of LFWE (125 mg/kg) treatment raised the IRS-1 protein expression in adipocytes to 2-fold of that in fructose group. Similarly, the IRS-1 protein expression in H group raised to 2.5-fold of that in fructose group, and the level was close to control group. The expression of Akt protein in adipocytes of the fructose group was close to 60% of that in control group (**Figure 5B**). After 14 weeks of LFWE treatments (125 or 250 mg/kg), the Akt protein expression did not increase, however. The expression of GLUT4 protein in adipocytes of the fructose group was close to 42% of that in control group (**Figure 5C**). After 14 weeks of LFWE treatment (125 mg/kg), the GLUT4 protein expression in adipocytes was raised to 1.3-fold of that in fructose group. The GLUT4 protein expression in H group was further increased to be significantly higher (1.5-fold) than the fructose group. However, the GLUT4 protein expression in both groups was still significantly lower than the control group.

DISCUSSION

In our previous report, it was shown that LFWE contained high levels of polyphenols (total polyphenol, 548.2 ± 12.7 mg/g; total flavonoids, 139.3 ± 0.2 mg/g; and proanthocyanidins, 112.5 ± 5.2 mg/g) (*9*). Polyphenols are common constituents of foods and the major antioxidants of our diet. Several studies demonstrated that treatment with antioxidants like α-lipoic acid,

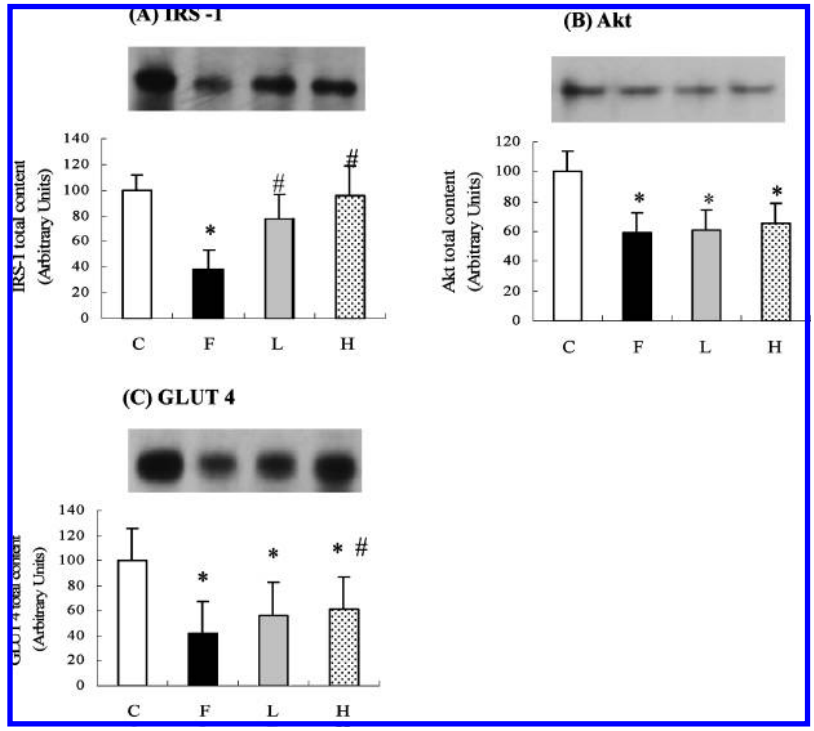

Figure 5. Quantitation of insulin signal transduction pathway related protein expression (**A**) IRS-1, (**B**) Akt, and (**C**) GLUT 4 of adipocytes isolated from rats after 14 weeks of dietary manipulation. Values are shown as means ± SD ($n = 4$). $^{*}p < 0.05$ as compared with group C. $^{\#}p < 0.05$ as compared with group F.

vitamin E, vitamin C, or glutathione improves insulin sensitivity in insulin-resistant individuals and patients with type 2 diabetes (*12*−*14*).

We selected the DPPH assay and the inhibition of human LDL oxidation assay to assess the antioxidant activity of LFWE. Both assays showed that LFWE had powerful antioxidative capacity. Longan seed extract was found to be as effective as Japanese green tea extract (*6*). However, our current study indicated that DPPH scavenging activity of LFWE ($IC_{50} = 3.75$ μg/mL) is fairly higher than that of seed extract ($IC_{50} = 11.6$ μg/mL) and Japanese green tea extract ($IC_{50} = 10.2$ μg/mL). It indicated that the flowering part of the longan plant had the strongest antioxidant activity. LDL oxidation is considered to be a key mechanism in atherosclerosis (*15*). We observed that LFWE effectively increased lag time for LDL oxidation. The flower rich in polyphenols such as *Hibiscus sabdariffa* L. and *Punica granatum* resulted in reduced susceptibility of LDL to oxidation. The extracts of *Hibiscus* flowers preventing LDL oxidation may due to its anthocyanin components (*16*). Pomegranate polyphenols were shown in humans and atherosclerotic apolipoprotein E-deficient mice to reduce the capacity of macrophages to oxidatively modify LDL and significantly decreased the atherosclerotic lesions (*17*).

In animal studies, chronic fructose feeding can induce tissue oxidative stress and provides a model of metabolic disorders including hyperglycemia, hyperinsulinemia, hypertension, and hypertriglyceridemia. It has been recommended that elevation of blood pressure in fructose-fed rats is sequential to the development of insulin resistance and hyperinsulinemia (*18, 19*). LFWE supplementation decreases the fructose-induced blood pressure elevations and ameliorates insulin resistance. It has been reported that corilagen in the longan seed could lower the blood pressure of spontaneously hypertensive rats (SHR) through blocking noradrenaline release and (or) by direct vasorelaxation (*20*). Geraniin has also been shown to reverse the orthostatic hypotension in SHRs that were stimulated through an effect on the noradrenergic nerve and subsequent release of noradrenaline (*21*). Corilagen and geraniin have also been isolated from longan flower (*22*). We suggest that these two hydrolyzable tannins may play a role in decreasing blood pressure in our study.

Evidence indicates that dietary fructose has destructive effects by causing both depletion of antioxidant defenses and elevation of free radical production (*23*). Faure et al. observed an impairment of the antioxidant defense systems in rats fed a HF diet, and supplementation with the antioxidant, vitamin E, could improve the oxidative defense system and have a beneficial effect on insulin sensitivity (*24*). Our study clearly showed that a HF diet lead to an oxidative stress, as indicated by the higher levels of plasma TBARS and decreased liver GRd enzyme activity in HF group. However, supplementation of LFWE significantly ameliorated these phenomena. These results suggest that LFWE had strong in vivo antioxidant properties, and the protective effect against oxidative stress may contribute to the increased insulin sensitivity in the fructose-fed rats.

ROS have been reported to interfere with insulin signaling cascade leading to impaired glucose utilization (*25*). Therefore, we also determined the effect of LFWE on insulin signaling cascade related protein levels in adipocytes. It is confirmative that IRS-1 tyrosine phosphorylation in response to insulin stimulation generally increases the association of IRS-1 with the phosphatidylinositol 3-kinase, resulting in increased phosphatidylinositol 3-kinase activity, which in turn leads to activation of protein kinase B or Akt, ultimately, stimulating the GLUT4 translocation, to enhance the insulin-stimulated glucose disposal (*26, 27*). In our study, it seems that excess fructose may interfere with insulin action by altering the quantity of insulin signals. Therefore, we suggested that any observed increases in IRS-1 related signals in adipocyte of fructose-fed rats after LFWE treatment would provide strong evidence for the beneficial effects on impaired insulin action. Considering that the regulation of glucose

uptake in adipocyte via GLUT4 is a fundamental action of insulin and this process is impaired in type 2 diabetes (*2*), our data showed that the GLUT 4 protein expression was significantly increased in fructose-fed rats by LFWE treatment.

Similar phenomena have been reported for other antioxidant materials. The cinnamon extract containing polyphenols with doubly linked procyanidin type A polymers has been reported to increase insulin sensitivity (*28*) and affect the IR and IRS-1 insulin signaling proteins expression in animal model (*29*). The gallic acid present in pomegranate flower has been known to be responsible for the activation of PPAR-γ and GLUT 4, a potential mechanism for the antidiabetic action (*30*). Both of these compounds also exist in longan flowers (*9*). Conceivably, these phenolic compounds found in longan flower may function as antioxidants and have beneficial effects on insulin action in our experimental model.

In conclusion, our results suggest that LFWE shows powerful antioxidant activity and may improve the oxidative stress, hypertension, and symptoms of metabolic syndrome. The beneficial effects of LFWE are associated with amelioration of defective insulin action on specific postreceptor insulin signaling related to IRS-1 and GLUT 4 proteins expression. From this viewpoint, LFWE could become a natural dietary substance for the treatment of insulin resistance and metabolic syndrome.

ABBREVIATIONS USED

LFWE, longan flower water extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl; LDL, low-density lipoprotein; HF, high fructose; bw, body weight; GRd, glutathione reductase; TBARS, thiobarbituric acid-reactive substances; IRS-1, insulin receptor substrate-1; GLUT 4, glucose transporter 4; OGTT, oral glucose tolerance test; AUC, area under curve.

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LITERATURE CITED

(1) Meerarani, P.; Badimon, J. J.; Zias, E.; Fuster, V.; Moreno, P. R. Metabolic syndrome and diabetic atherothrombosis: Implications in vascular complications. *Curr. Mol. Med.* **2006**, *6*, 501–514.

(2) Evans, J. L.; Goldfine, I. D.; Maddux, B. A.; Grodsky, G. M. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction. *Diabetes* **2003**, *52*, 1–8.

(3) Lê, K. A.; Tappy, L. Metabolic effects of fructose. *Curr. Opin. Clin. Nutr. Metab. Care* **2006**, *9*, 469–475.

(4) Jin, L.; Xue, H. Y.; Jin, L. J.; Li, S. Y.; Xu, Y. P. Antioxidant and pancreas-protective effect of aucubin on rats with streptozotocin-induced diabetes. *Eur. J. Pharmacol.* **2008**, *582*, 162–167.

(5) Sicard, P.; Delemasure, S.; Korandji, C.; Grand, A. S.; Lauzier, B.; Guilland, J. C.; Duvillard, L.; Zeller, M.; Cottin, Y.; Vergely, C.; Rochette, L. Anti-hypertensive effects of Rosuvastatin are associated with decreased inflammation and oxidative stress markers in hypertensive rats. *Free Radical Res.* **2008**, *42*, 226–36.

(6) Rangkadilok, N.; Sitthimonchai, S.; Worasuttayangkurn, L.; Mahidol, C.; Ruchirawat, M.; Satayavivad, J. Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. *Food Chem. Toxicol.* **2007**, *45*, 328–336.

(7) Hsu, H. Y. *Illustrations of Chinese Herb Medicine of Taiwan*; Chinese Herb Medicine Committee National Health Administration: Taipei, Taiwan, R.O.C., 1972; p 168.

(8) Hsieh, M. C.; Shen, Y. J.; Kuo, Y. H.; Hwang, L. S. Antioxidative activity and active components of longan (*Dimocarpus longan* Lour.) flower extracts. *J. Agric. Food Chem.* **2008**, *56*, 7010–7016.

(9) Ho, S. C.; Hwang, L. S.; Shen, Y. J.; Lin, C. C. Suppressive effect of a proanthocyanidin-rich extract from longan (*Dimocarpus longan* Lour.) flowers on nitric oxide production in LPS-stimulated macrophage cells. *J. Agric. Food Chem.* **2007**, *55*, 10664–10670.

(10) Puhl, H.; Waeg, G.; Esterbauer, H. Methods to determine oxidation of low-density lipoproteins. *Methods Enzymol.* **1994**, *233*, 425–441.

(11) Fraga, C. G.; Leibovitz, B. E.; Tappel, A. L. Lipid peroxidation measured as thiobarbituric acid-reactive substance in tissue slices: characterization and comparison between homogenates and microsomes. *Free Radical Biol. Med.* **1988**, *4*, 144–161.

(12) Paolisso, G.; Gambardella, A.; Tagliamonte, M. R.; Saccomanno, F.; Salvatore, T.; Gualdiero, P.; D'Onofrio, M. V.; Howard, B. V. Does free fatty acid infusion impair insulin action also through an increase in oxidative stress. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 4244–4248.

(13) Hennig, B.; Meerarani, P.; Ramadass, P.; Watkins, B. A.; Toborek, M. Fatty acid-mediated activation of vascular endothelial cells. *Metabolism* **2000**, *49*, 1006–1013.

(14) Jacob, S.; Lehmann, R.; Rett, K.; Häring, H. U. Oxidative stress and insulin action: A role for antioxidants. *Antioxid. Diabetes Manage.* **2000**, 319–338.

(15) Diaz, M. N.; Frei, B.; Vita, J. A.; Keaney, J. F. Antioxidants and atherosclerotic heart disease. *N. Engl. J. Med.* **1997**, *337*, 408–416.

(16) Chang, Y. C.; Huang, K. X.; Huang, A. C.; Ho, Y. C.; Wang, C. J. Hibiscus anthocyanins-rich extract inhibited LDL oxidation and oxLDL-mediated macrophages apoptosis. *Food Chem. Toxicol.* **2006**, *44*, 1015–1023.

(17) Aviram, M.; Dornfeld, L.; Kaplan, M.; Coleman, R.; Gaitini, D.; Nitecki, S.; Hofman, A.; Rosenblat, M.; Volkova, N.; Presser, D.; Attias, J.; Hayek, T.; Fuhrman, B. Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. *Drugs Exp. Clin. Res.* **2002**, *28*, 49–62.

(18) Hwang, I.; Ho, H.; Hoffman, B.; Reaven, G. M. Fructose induced insulin resistance and hypertension in rats. *Hypertension* **1987**, *10*, 512–516.

(19) Reaven, G. M.; Lithell, H.; Landsberg, L. Hypertension and associated metabolic abnormalities: The role of insulin resistance and the sympathoadrenal system. *N. Engl. J. Med.* **1996**, *334*, 374–381.

(20) Lin, T. C.; Hsu, F. L.; Cheng, J. T. Antihypertensive activity of corilagin and chebulinic acid, tannins from Lumnitzera racemosa. *J. Nat. Prod.* **1993**, *56*, 629–632.

(21) Hsu, F. L.; Lu, F. H.; Cheng, J. T. Influence of acetonylgeraniin, a hydrolyzable tannin from Euphoria longana, on orthostatic hypotension in a rat model. *Planta Med.* **1994**, *60*, 297–300.

(22) Lin, J. H.; Tsai, C. C. Phenolic constituents from the flowers of *Euphoria longana* Lam.Chin. *Pharm. J.* **1995**, *47*, 113–121.

(23) Delbosc, S.; Paizanis, E.; Magous, R.; Araiz, C.; Dimo, T.; Cristol, J. P.; Cros, G.; Azay, J. Involvement of oxidative stress and NADPH oxidase activation in the development of cardiovascular complications in a model of insulin resistance, the fructose-fed rat. *Atherosclerosis* **2005**, *179*, 43–49.

(24) Faure, P.; Rossini, E.; Lafond, J. L.; Richard, M. J.; Favier, A.; Halimi, S. Vitamin E improves the free radical defense system potential and insulin sensitivity of rats fed high fructose diets. *J. Nutr.* **1997**, *127*, 103–107.

(25) Rudich, A.; Tirosh, A.; Potashnik, R.; Hemi, R.; Kanety, H.; Bashan, N. Prolonged oxidative stress impairs insulin-induced glut4 translocation in 3t3-l1 adipocytes. *Diabetes* **1998**, *47*, 1562–1569.

(26) Maddux, B. A.; See, W., Jr.; Goldfine, A. L.; Goldfine, I. D.; Evans, J. L. Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by mircomolar concentrations of alpha-lipoic acid. *Diabetes* **2001**, *50*, 404–410.

(27) Birnbaum, M. J. Turning down insulin signaling. *J. Clin. Invest.* **2001**, *108*, 655–659.

(28) Anderson, R. A.; Broadhurst, C. L.; Polansky, M. M.; Schmidt, W. F.; Khan, A.; Flanagan, V. P.; Schoene, N. W.; Graves, D. J. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. *J. Agric. Food Chem.* **2004**, *52*, 65–70.

(29) Qin, B.; Nagasaki, M.; Ren, M.; Bajotto, G.; Oshida, Y.; Sato, Y. Cinnamon extract (traditional herb) potentiates in vivo insulin-regulated glucose utilization via enhancing insulin signaling in rats. *Diabetes Res. Clin. Pract.* **2003**, *62*, 139–148.

(30) Huang, T. H.; Peng, G.; Kota, B. P.; Li, G. Q.; Yamahara, J.; Roufogalis, B. D.; Li, Y. Anti-diabetic action of Punica granatum flower extract: Activation of PPAR-gamma and identification of an active component. *Toxicol. Appl. Pharmacol.* **2005**, *207*, 160–169.

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