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ARTICLE

Antiobesity and Hypolipidemic Effects of Polyphenol-Rich Longan (*Dimocarpus longans* Lour.) Flower Water Extract in Hypercaloric-Dietary Rats

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Plenty of polyphenols, i.e. phenolic acids and flavonoids, were found in longan flower water extract (LFWE) through spectrophotometric and HPLC analyses. Antiobesity and hypolipidemic effects of polyphenol-rich longan flower water extract (LFWE) were investigated in this study. Eight male rats per group were assigned randomly to one of the following dietary groups: (1) normal-caloric diet and pure water (NCD + NDW); (2) hypercaloric diet and pure water (HCD + NDW); (3) HCD and 1.25% (w/v) LFWE (HCD + 1.25% LFWE); (4) HCD and 2.5% (w/v) LFWE (HCD + 2.5% LFWE) for 9 weeks. Body weight, size of epididymal fat, serum triglyceride level and atherogenic index, and hepatic lipids were decreased ($p < 0.05$) in HCD rats by drinking 2.5% LFWE which may result from downregulated ($p < 0.05$) pancreatic lipase activity, and sterol regulatory element binding protein-1c (SREBP-1c) and fatty acid synthase (FAS) gene expressions, as well as upregulated ($p < 0.05$) LDL receptor (LDLR) and peroxisome proliferator-activated-receptor-alpha (PPAR-α) gene expressions, and also increased ($p < 0.05$) fecal triglyceride excretions. Therefore, polyphenol-rich LFWE indeed characterizes antiobesity and hypolipidemic effects *in vivo*.

KEYWORDS: Antiobesity; hypolipidemic effect; longan flower water extract; lipid homeostasis; pancreatic lipase activity; polyphenol

INTRODUCTION

Obesity has been regarded as a chronic disease by WHO and FDA since 1996. It is considered as one cause of heart disease, hypertension, diabetes, fatty liver, and is even related to certain cancers (*1*). Imbalanced fat or positive energy intake is one of the most important environmental factors resulting in obesity, and it could not only cause the accumulation of excessive body fat but also increase serum lipids. Although a plethora of research data related to molecular mechanisms regulating body weight is available to provide hope for antiobesity drugs, unsolvable side-effect problems remain (*2*, *3*). Currently, the market for antiobesity drugs contributes 2−6% of total health care costs in certain developed countries (*4*).

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Reduced cardiovascular and cerebrovascular diseases via high fruit and vegetable consumption have been reported, where polyphenols, i.e. flavonoids and phenolic acids, contribute partially (*5*). Natural polyphenols range from simple molecules (phenolic acids) to highly polymerized compounds (tannins). The health benefits of natural polyphenols from the plant kingdom have been proven in reducing body weight gain, lipid peroxidation, hypertension, hyperlipidemia, inflammation, DNA damage, and cancer (*6*−*10*). Yang et al. (*6*) investigated the effects of polyphenol-rich green, oolong, and black tea extracts on serum lipids of a hyperlipidemic animal model induced by a high-sucrose diet. All three kinds of tea extracts showed a hypolipidemic effect. In addition, only oolong and black tea extracts decreased weight gain in the 25-day experimental period.

Due to high phenolic compounds in roselle (*Hibiscus sabdariffa* L.) (*7*), Chemlali olive leaves (*10*), and litchi flowers (*Litchi chinensis* Sonn.) (*11*), they all exhibited cardiovascular protection. Moreover, polyphenols from plants have an affinity for proteins. Hot water extracts of various plant materials, such as leaves of *Acanthopanax sessiliflorus* and

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roots of *Salacia reticulate*, showed antiobesity effects via an inhibitory activity of pancreatic lipase, because of aggregation of lipase proteins (*12*, *13*). The longan (*Dimocarpus longans* Lour.) is an important economic fruit in Taiwan. Unpollinated longan flowers are generally regarded as disposable byproducts. Aqueous extracts of longan flowers were also found to possess a large number of phenolic compounds and flavonoids (*9*). People always steep tea or flowers in hot water as a normal drink and believe that those drinks benefit health. Moreover, commercial dried-longan-flower products have been also available in the market of Taiwan. Therefore, the antiobesity and hypolipidemic effects of longan flower water extract (LFWE) solution warrant further investigation.

Expressions of serum cholesterol clearance (i.e., LDL receptor), triglyceride biosynthesis (i.e., sterol regulatory element binding protein-1c, SREBP-1c; fatty acid synthase, FAS), and energy expenditure (i.e., peroxisome proliferator-activated receptor-alpha, PPAR-α; uncoupling protein-2, UCP2) regulate lipid homeostasis in serum and liver (*14*−*16*). Based on our literature search, the detailed mechanisms of LFWE solution on antiobesity and hypolipidemic effects remain nil. Therefore, the present study was designed to investigate the antiobesity and hypolipidemic effects of LFWE solution and elucidate its possible mechanisms via a rat model.

MATERIALS AND METHODS

Collection and Preparation of LFWE. Fresh longan (*Dimocarpus longans* Lour.) flowers were obtained from a local fruit farm (Taichung County, Taiwan). The flowers were dried in a hot-air dryer (Chi-Yeh Electric and Machinery Co., Taipei, Taiwan) at 40 °C for 16 h before water extraction. The 1.25 and 2.5% (w/v) LFWE solutions were obtained by steeping suitable quantities of dried longan flowers with boiled distilled water (100 °C) for 30 min, with a stir bar and laboratory stirrer/hot plate (model: PC420, Corning Inc.) for efficient extraction. The extract for one-week-consumption volume was then filtered through a No.1 filter paper and stored at −20 °C until feeding animals. The concentrations of LFWE solutions were chosen according to our preliminary study based on the rat's sense of taste.

Determination of Phytochemicals in LFWE. The amounts of total phenol contents, flavonoids, and condensed tannins were determined according to methods described by Liu et al. (*17*). Total phenol contents were determined by a method with Folin−Ciocalteu's phenol reagent using gallic acid as a standard and expressed as mg gallic acid equivalent (GAE)/100 mL extract. Total flavonoids were measured through a method with 10% $AlCl_3 \cdot H_2O$ solution using (+)-catechin as a standard and expressed as mg catechin equivalent (CE)/100 mL extract. Condensed tannins were expressed as mg CE/100 mL extract. Proanthocyanidin contents were read at 550 nm, on the basis of a colorimetric reaction with 10% $NH_4Fe(SO_4)_2$ after dissolution in hydrochloric acid (2 M) containing *n*-butanol (*18*). Total monomeric anthocyanins were determined using the differential pH method (ε = 26900) and expressed as mg cyanidin-3-glucoside equivalents (cy-3-gluE)/100 mL extract (*18*). Ascorbic acid was quantified according to the report of Klein and Perry (*19*). Composition and content of flavonoids and phenolic acids in the extracts were analyzed based on the previous method and procedures (*20*). The high performance liquid chromatography (HPLC) system is composed of a PrimeLine gradient model 500G HPLC pump system (Analytical Scientific Instruments, Inc., El Sobrante, CA) and an S-3210 photodiode-array detector (PDA) (Schambeck SFD GmbH, Bad Honnef, Germany). A Hypersil GOLD C_{18} column (250 × 4.6 mm, 5 μm; Thermo Fisher Scientific Inc.) and a gradient solvent system consisting of MeOH (solvent A) and dd H_2O with 9% glacial acetic acid (solvent B) (conditions: 5−17% A from 0 to 5 min and kept at 17% A from 5 to 25 min; 17−31% A from 25 to 40 min and kept at 31% A from 40 to 76 min; 31−40% A from 76 to 80 min and kept at 40% A from 80 to 120 min; flow rate = 0.8 mL/min) were used for separation of components, whose UV spectra were recorded from 220 to 450 nm. Phenolic acid compounds (gallic, gentisic, chlorogenic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic, sinapic, syringic, *p*-anisic and rosmarinic) and flavonoid standards (including catechin, epicatechin, rutin, naringin, myricetin, hesperidin, quercitrin, neohesperidin, eriodictyol, diosmin, morin, daidzein, quercetin, glycitein, narigenin, luteolin, genistein, hesperetin, kaempferol, apigenin and isorhamnetin) were purchased from Sigma Co. (St. Louis, MO). Those phenolic acid and flavonoid compounds were also run on the HPLC as standards to verify chemical compounds of LFWE.

Animal and Diets. The animal use and protocol was reviewed and approved by Chung Shan Medical University Animal Care Committee. Thirty-two male Sprague−Dawley (SD) rats of 9-week age were purchased from BioLACO Taiwan Co., Ltd., Taiwan. Each rat was housed in one cage in an animal room at 22 ± 2 °C with a 12/12 h light−dark cycle. Chow diets containing 48.7% (w/w) carbohydrate, 23.9% (w/w) protein, 5.0% (w/w) fat, 5.1% (w/w) fiber, and 7.0% ash (Laboratory Rodent Diet 5001, PMI Nutrition International/Purina Mills LLC.) and normal distilled water was provided for one week of acclimation. A chow diet was regarded as a normal-caloric diet (NCD) while a hypercaloric diet (HCD) was formulated as 48% (w/w) chow diets supplemented with 8% (w/w) corn oil (ICN Biomedicals, Inc., Irvine, CA) and 44% (w/w) condensed milk (Nestle Qingdao Ltd., China) (*21*). The protein, fat, and carbohydrate contents of the NCD and HCD were 23.9, 5.0, and 48.7%, as well as 14.9, 14.1, and 47.9%, accordingly. The NCD and HC contained 335 and 378 kcal/100 g diet, respectively. The normal distilled water and 1.25 and 2.5% (w/v) longan flower water extract (LFWE) were applied in the drinking solution in this study. Each group of 8 rats was randomly assigned to one of the following diet and drinking solution groups: (1) NCD and normal distilled water (NCD + NDW); (2) HCD and normal distilled water (HCD + NDW); (3) HCD and 1.25% (w/v) longan flower water extract (HCD + 1.25% LFWE); (4) HCD and 2.5% (w/v) longan flower water extract (HCD + 2.5% LFWE). All rats were fed the assigned diets and drinking solutions *ad libitum*. The experimental period lasted for 9 weeks. Body weight of rats was individually recorded every week. Weight increase (g) = initial body weight (g) − final body weight (g), and weight increase (%) = weight increase (g)/initial body weight (g) × 100%. Food and drinking water were changed daily. Food, energy, and water intakes were recorded for obtaining daily food (g), energy (kcal), and water intakes (mL) on a per rat daily basis.

Collection of Serum, Liver, Heart, Kidney, Epididymal and Perirenal Fats, and Feces. For ensuring the correction and stabilization of serum biochemical values not due to food digestion in rats, blood samples were collected via puncturing the retroorbital sinus with a capillary tube after all rats fasted overnight (approximately 14 h). At the end of the experiment (week 9), all feed was removed 14 h before killing. All rats were euthanized by CO_2. Liver, heart, kidney, and epididymal and perirenal adipose tissues from each rat were removed and weighed. Livers were stored at −80 °C for further analyses. Blood samples were also collected via decapitation. Serum was separated from blood samples by centrifugation 3000*g* for 10 min, and then stored at −80 °C. Feces were collected from each cage 48 h before the end of experiment and stored at −80 °C.

Determination of Serum Lipid Parameters. Serum total cholesterol (TC), triglyceride (TG), and high-density-lipoprotein cholesterol (HDL-C) were measured by using commercial kits (Randox Laboratories Ltd., Antrim, U.K.). In the HDL-C analysis, low-density-lipoprotein (LDL), very-low-density-lipoprotein (VLDL), and chylomicrometer in serum were precipitated by an addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation (3000*g* for 10 min), the cholesterol concentration in the HDL fraction was determined by using the total cholesterol commercial kit (Randox Laboratories Ltd., Antrim, U.K.). Those methods are based on the detection of colored end-products at 500 nm. The atherogenic index (AI) was calculated by the formulation of (TC-HDL-C)/HDL-C (*22*).

Determination of Hepatic/Fecal Cholesterol and Triglyceride. Hepatic and fecal lipid extractions were measured according to the procedures of Tzang et al. (*23*). Briefly, hepatic and fecal lipids were extracted by a chloroform/methanol solution (2:1, v/v). The extract was dried under N_2 and resuspended in isopropanol via an ultrasonic cleaner (model: DC150H, Taiwan Delta New Instrument Co. Ltd., Taiwan) for efficient dissolution. Fecal cholesterol and triglyceride concentrations were measured using commercial kits (Randox Laboratories Ltd., Antrim, U.K.).

Measurement of Pancreatic Lipase Activity. A 0.3 g portion of pancreas was homogenized on ice in 0.6 mL of phosphate buffer saline

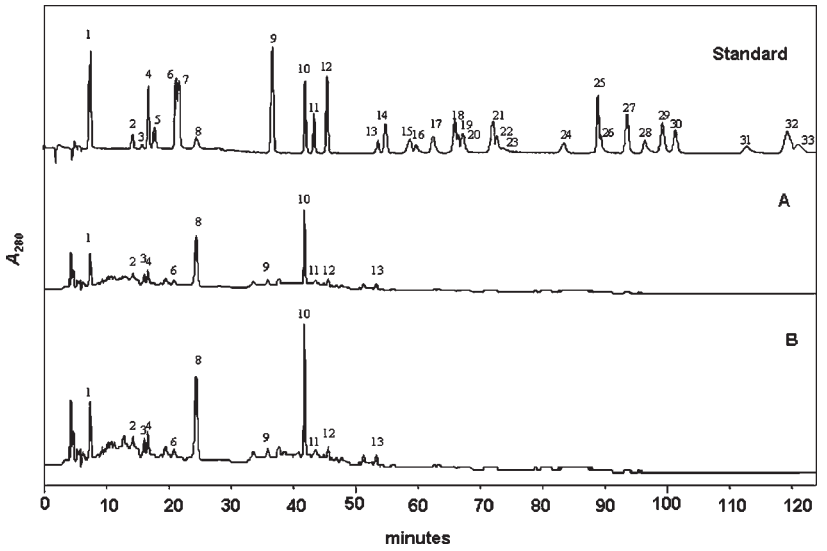

Figure 1. Chromatograms of flavonoids and phenolic acids in (**A**) 1.25% (w/v) and (**B**) 2.5% (w/v) longan flower water extract solution. 1 = gallic acid, 2 = catechin, 3 = gentisic acid, 4 = chlorogenic acid, 5 = *p*-hydroxybenzoic acid, 6 = vanillic acid, 7 = caffeic acid, 8 = epicatechin, 9 = *p*-coumaric acid, 10 = ferulic acid, 11 = sinapic acid, 12 = syringic acid, 13 = rutin, 14 = *p*-anisic acid, 15 = naringin, 16 = myricetin, 17 = hesperidin, 18 = rosmarinic acid, 19 = quercitrin, 20 = neohesperidin, 21 = eriodictyol, 22 = diosmin, 23 = morin, 24 = daidzein, 25 = quercetin, 26 = glycitein, 27 = narigenin, 28 = luteolin, 29 = genistein, 30 = hesperetin, 31 = kaempferol, 32 = apigenin, 33 = isorhamnetin.

(PBS, pH 7.0, containing 0.25 M sucrose) and centrifuged at 15000*g* at 4 °C for 15 min. The supernatant was collected for further analyses. The protein content in the supernatant was measured according to the procedures of a Bio-Rad protein assay kit (Cat. No.: 500-0006, Bio-Rad Laboratories, Inc., Hercules, CA) against bovine serine albumin standard. Pancreatic lipase activity was assayed by a commercial kit (Randox Laboratories Ltd., Antrim, U.K.). The values of pancreatic lipase activity for the rats in HCD + NDW, HCD + 1.25% LFWE, and HCD + 2.5% LFWE groups were expressed relatively to the average values for the rats in the NCD + NDW group, which was set to 100%.

Hepatic mRNA Expressions of LDL Receptor (LDLR), Sterol Regulatory Element Binding Protein-1c (SREBP-1c), Fatty Acid Synthase (FAS), Peroxisome Proliferator-Activated Receptor-Alpha (PPAR-α), Uncoupling Protein-2 (UCP2), and Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH). Total RNA was isolated from the stored frozen liver tissues by using the protocol described by RNeasy Mini Kits (Qiagen, Valencia, CA). Reverse transcription was carried out with 2 μg total RNA, 8 μL of reaction buffer, 2 μL of dNTPs, 4.8 μL of $MgCl_2$, 4 μL of Oligo-dT (10 pmol/L) and 200 U of RTase (Promega, Madison, WI) with diethyl pyrocarbonate (DEPC) H_2O in a final volume of 40 μL at 42 °C for 1 h. After a heat inactivation, 1 μL of cDNA product was used for a PCR amplification. The appropriate primers of target genes were designed for rat's LDL receptor, SREBP-1c, FAS, PPAR-α, UCP2, and GAPDH as follows: LDLR sense 5′-GGAGGACTCTGTTCCGAGGAA-3′, antisense 5′-GAGCTAGCTGCTTCTCATCCTC-3′; SREBP-1c sense 5′-GAGCTAGCTGCTTCTCATCCTC-3′, antisense 5′-CGCACACAGGGCTAGGCGGG-3′; FAS sense 5′-TTGCCCGAGTCAGAGAAC-3′, antisense 5′-CGTCCACAATAGCTTCATAGC-3′; PPAR-α sense 5′-GGACAAGGCCTCAGGGTACC-3′, antisense 5′-CCACCATCTTGGCCACAAGC-3′; UCP2 sense 5′-TCAGCCTCGATGTTCCCAGC-3′, antisense 5′-AGCCAGGGTCTAGGGGAAGA-3′; GAPDH sense 5′-GACCCCTTCATTGACCTCAAC-3′, antisense 5′-GGAGATGATGACCCTTTTGGC-3′. The size of reaction products is as follows: for LDLR, 301 bp; SREBP-1c, 412 bp; FAS, 779 bp; PPAR-α, 461 bp; UCP2, 403 bp; GAPDH, 264 bp. GAPDH was used as an internal control in all reactions. The PCR amplification was performed using a DNA thermal cycler (ASTEC PC-818, ASTEC Co., Ltd., Fukuka, Japan) under the following conditions. LDLR: 30 cycles at 94 °C for 1 min, 57 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C. SREBP-1c, FAS, and UCP2: 35 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C. PPAR-α: 33 cycles at 94 °C for 1 min, 57 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C. GAPDH: 25 cycles at 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C. The final products were subjected to electrophoresis on a 2% agarose gel and detected by ethidium bromide staining via a UV light. The relative expression levels of the mRNAs of the target genes were normalized using the GAPDH internal standard.

Statistical Analysis. The sample size for the experiment was determined by power analysis arbitrarily set between 80 and 90% in order to detect an effect at the 5% significant level (*24*). The experiment was conducted using a completely random design (CRD). Data were analyzed using analysis of variance (ANOVA). A significant difference was used at the 0.05 probability level, and differences between treatments were tested using the least significant difference (LSD) test. All statistical analyses of data were performed using SAS (SAS Institute, Inc., 2002).

RESULTS

Phytochemical Contents in LFWE. Contents of total phenols, total flavonoids, condensed tannins, proanthocyanidins, and ascorbic acid in the 2.5% (w/v) LFWE were 242.32 mg GAE/100 mL, 68.32 mg CE/100 mL, 47.24 mg CE/100 mL, 52.45 mg/100 mL, and 3.04 mg/100 mL, respectively. Meanwhile, contents of total phenols, total flavonoids, condensed tannins, proanthocyanidins, and ascorbic acid in 1.25% (w/v) LFWE were 125.47 mg GAE/100 mL, 35.20 mg CE/100 mL, 24.21 mg CE/100 mL, 27.24 mg/100 mL, and 1.60 mg/100 mL, respectively. No monomeric anthocyanins were detected in the extracts. Flavonoids, such as (+)-catechin, epicatechin, and rutin, and phenolic acids including gallic acid, gentisic acid, chlorogenic acid, vanillic acid, *p*-coumaric acid, ferulic acid, sinapic acid and syringic acid were found in the LFWE (**Figure 1**). Epicatechin (18.72 and 36.93 mg/100 mL for the 1.25 and 2.5% (w/v) LFWE, respectively) and gentisic acid (20.07 and 39.34 mg/100 mL for the 1.25 and 2.5% (w/v) LFWE, respectively) were the highest levels of flavonoid and phenolic acid in LFWEs, respectively (**Table 1**). Then, amounts of flavonoids and phenolic acids in the 1.25% (w/v) LFWE were 51.28 and 29.88 mg/100 mL, respectively, as well as 97.05 and 58.89 mg/100 mL in 2.5% (w/v) LFWE, respectively (**Table 1**).

Effects of Rat Performance and Sizes of Liver, Heart, Kidney, and Epididymal and Perirenal Fat by Drinking LFWE Solutions. Regardless of treatment types of drinking solutions, a normal-caloric diet (NCD) resulted in lower ($p < 0.05$) body weight of

rat in the final experiment than a hypercaloric diet (HCD) (**Table 2**). The weight increase in g and % of HCD + 2.5% LFWE rats were lowered 26.4 g and 5.9% ($p < 0.05$), respectively, when compared to HCD rats. However, no ($p > 0.05$) differences on the weight increases (g and %) were observed between HCD + 1.25% LFWE and NCD + NDW groups. Although higher ($p < 0.05$) food and water intakes of rats during the experimental period were observed in the NCD + NDW group, calculated energy intakes were no different ($p > 0.05$) among groups. After sacrificing rats at the end of the experiment, liver, heart, kidney, epididymal and perirenal fat of rats were collected, and their sizes relative to body weight were also calculated (**Table 2**). No differences ($p > 0.05$) on the relative sizes of liver, heart, and kidney were observed. Generally, HCD increased ($p < 0.05$) the relative sizes of epididymal and perirenal fat, but drinking 2.5% LFWE solution in HCD rats showed a smaller (p 0.05) size of epididymal fat when compared to those only drinking NDW.

Table 1. Contents of Flavonoids and Phenolic Acids in 1.25 and 2.5% (w/v) LFWE Determined by HPLC[a]

		contents (mg/100 mL)	
compound	retention time (min)	1.25% (w/v) LFWE	2.5% (w/v) LFWE
gallic acid	7.59	8.92 ± 0.07	15.97 ± 0.18
(+)-catechin	14.35	8.54 ± 0.18	16.73 ± 0.45
gentisic acid	15.65	20.07 ± 1.11	39.34 ± 1.22
chlorogenic acid	16.81	1.94 ± 0.05	3.85 ± 0.09
vanillic acid	21.25	1.87 ± 0.04	3.68 ± 0.12
epicatechin	24.54	18.72 ± 0.73	36.93 ± 1.29
p-coumaric acid	36.63	0.67 ± 0.02	1.24 ± 0.42
ferulic acid	41.64	14.53 ± 0.48	26.49 ± 0.88
sinapic acid	43.04	1.93 ± 0.06	3.78 ± 0.14
syringic acid	45.19	1.35 ± 0.05	2.70 ± 0.09
rutin	53.28	2.62 ± 0.07	5.23 ± 0.21
phenolic acid amount		51.28	97.05
flavonoid amount		29.88	58.89
total amount		81.16	155.94

[a] The data are given as mean ± SEM ($n = 3$).

Changes of Serum Lipids and Cholesterol Profile, as Well as Liver and Fecal Cholesterol and Triglyceride Levels, by Drinking LFWE Solutions. Serum triglyceride (TG) and total cholesterol (TC) levels of rats after 9 weeks of feeding are shown in **Table 3**. Serum TG and TC were increased ($p < 0.05$) in HCD groups when compared to those of the NCD group, while serum HDL-C was decreased in HCD groups. Drinking 2.5% LFWE normalized ($p < 0.05$) serum TG and HDL-C in HCD rats similar to those of NCD rats. Due to serum HDL-C normalized by drinking 2.5% LFWE solution in HCD groups, a higher ($p < 0.05$) atherogenic index [(TC-HDL-C)/HDL-C, AI] of HCD groups, except HCD + 2.5% LFWE, was calculated. Higher ($p < 0.05$) liver cholesterol level was measured only in the HCD + NDW groups among all groups while liver triglyceride levels were apparently increased ($p < 0.05$) by HCD (**Table 3**). Our results indicated that both 1.25 and 2.5% LFWE lowered ($p < 0.05$) liver cholesterol in the HCD groups, but the lowering ($p < 0.05$) effect of liver triglyceride levels was observed only in the 2.5% LFWE group. Fecal triglyceride and cholesterol outputs were also measured in the present study (**Table 3**). Drinking LFWE in HCD rats resulted in the increased ($p < 0.05$) fecal triglyceride levels and daily output when compared to those only drinking NDW. Although no difference ($p > 0.05$) on fecal cholesterol levels among all groups was analyzed, higher ($p < 0.05$) daily fecal cholesterol outputs were calculated in HCD groups drinking LFWE solution.

Effect of Lipid Homeostasis by Dinking LFWE Solutions. Higher ($p < 0.05$) pancreatic lipase activities were observed in HCD rats (HCD + NDW, HCD + 1.25% LFWE, and HCD + 2.5% LFWE) compared to NCD rats (**Figure 2**). However, drinking 1.25 and 2.5% LFWE solutions apparently inhibited ($p < 0.05$) 37.8 and 41.5% pancreatic lipase activities, respectively, in HCD rats. Regulations of gene expressions about serum and liver lipids of rats were shown in **Figure 3**. The LDLR mRNA expressions were upregulated ($p < 0.05$) 1.47 and 1.76 times of HCD rats drinking 1.25 and 2.5% LFWE solutions compared to those only drinking NDW, respectively. HCD rats also showed higher ($p < 0.05$) SREBP-1c and FAS gene expressions than NCD rats (NCD + NDW); however, 2.5% LFWE

Table 2. Growth Performance, and Relative Sizes of Live, Heart, Kidney, Epididymal Fat, and Perirenal Fat of the Experimental SD Rats[a]

group	NCD + NDW	HCD + NDW	HCD + 1.25% LFWE	HCD + 2.5% LFWE
		Growth Performance		
initial body wt (g)	393.21 ± 4.76 a	395.48 ± 7.45 a	397.18 ± 4.32 a	393.33 ± 4.37 a
final body wt (g)	504.81 ± 11.72 c	539.93 ± 9.17 ab	545.59 ± 12.29 a	511.49 ± 3.31 bc
wt increase (g)	111.62 ± 7.39 b	144.45 ± 6.29 a	148.40 ± 12.29 a	118.15 ± 3.91 b
wt increase (%)	27.99 ± 1.64 b	36.65 ± 1.76 a	37.44 ± 3.22 a	30.71 ± 1.74 b
food intake (g/rat/day)	28.46 ± 0.51 a	24.96 ± 0.68 b	25.73 ± 0.67 b	24.72 ± 0.57 b
energy intake (kcal/rat/day)	95.34 ± 1.72 a	94.36 ± 2.57 a	97.29 ± 2.53 a	93.46 ± 2.17 a
water intake (ml/rat/day)	45.36 ± 1.75 a	39.57 ± 3.01 b	37.97 ± 0.75 b	36.74 ± 0.63 b
polyphenol intake (mg/rat/day)	0	0	30.82	57.29
polyphenol intake (mg/kg BW/day)	0	0	56.48	112.01
		Relative size (g/100 g BW)		
liver	2.40 ± 0.03 a	2.36 ± 0.05 a	2.38 ± 0.04 a	2.39 ± 0.07 a
heart	0.34 ± 0.02 a	0.34 ± 0.01 a	0.31 ± 0.01 a	0.32 ± 0.00 a
kidney	0.64 ± 0.01 a	0.62 ± 0.02 a	0.64 ± 0.03 a	0.62 ± 0.01 a
epididymal fat	1.83 ± 0.09 c	2.44 ± 0.18 a	2.22 ± 0.07 ab	2.09 ± 0.09 bc
perirenal fat	2.70 ± 0.11 b	3.91 ± 0.37 a	3.62 ± 0.28 a	3.63 ± 0.27 a

[a] The data are given as mean ± SEM ($n = 8$). Mean values with different letters were significantly different ($p < 0.05$). NCD + NDW: normal-caloric diet + normal distilled water. HCD + NDW: hypercaloric diet + normal distilled water. HCD + 1.25% LFWE: hypercaloric diet + 1.25% (w/v) longan flower water extract. HCD + 2.5% LFWE: hypercaloric diet + 2.5% (w/v) longan flower water extract. Polyphenol intake (mg/rat/day) was calculated via a formulation of total polyphenols in NDW (0 mg/100 mL), 1.25, or 2.5% LFWEs × the average water intake in NDW, 1.25 and 2.5% LFWE solution, respectively. Polyphenol intake (mg/kg BW/day) was calculated via a formulation of polyphenol intake in each group/average final body weight in each group, respectively.

downregulated ($p < 0.05$) SREBP-1c and FAS gene expressions in HCD rats. Besides, LFWE also normalized ($p < 0.05$) PPAR-α mRNA expressions in HCD rats, while there was a tendency toward higher UCP2 gene expressions in HED rats drinking LFWE solutions.

DISCUSSION

Obesity is regarded as an emergent disease in developed countries. Increasing consumption of higher energy-dense, i.e. high fat or carbohydrate foods, is considered as a major reason to induce obesity. The obesity induced by high-fat/carbohydrate diets was summarized with the following reasons: (1) the high palatability; (2) the increased insulin secretion and lower energy cost for fat deposition; (3) the concomitant apparent lack of stimulation of fat oxidation by fat intake (*25*). Therefore, fat content is one of the main factors influencing the energy density of diets, and an increase in energy density was shown to result in passive overconsumption in humans and rats, which in turn promotes the development of obesity (*26*−*29*). A high-protein diet is prone to reduce food intake but increase protein rather than lipid deposition in animals, thus reducing energy intake and body weight (*30*). Furthermore, Levin and Dunn-Meynell (*21*) indicated that rats fed with an obesity-inducing diet (8% corn oil, 44% sweetened condensed milk, and 48% rat chow) which is the same as our formulation of HCD have increased arcuate nucleus neuropeptide Y (NPY) expression but reduced central leptin sensitivity. In our study, no ($p > 0.05$) differences in food and energy intakes were calculated (**Table 2**). Hence, it is speculated that the obesity of rats induced by feeding HCD in the present study should be due to its high-fat contents and prone to lipid rather than protein deposition.

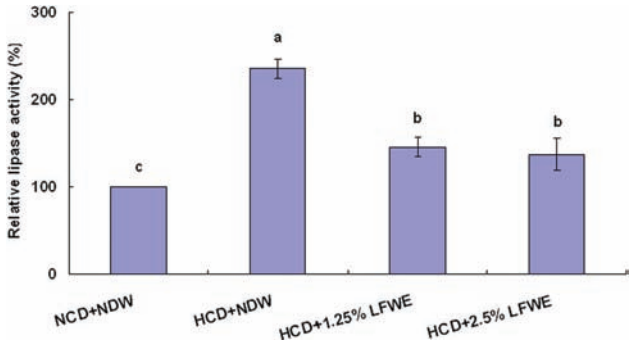

Figure 2. Relative pancreatic lipase activity of the experimental SD rats. The data are expressed as mean ± SEM ($n = 8$); a−c, columns with unlike letters differ significantly ($p < 0.05$). NCD + NDW: normal-caloric diet + normal distilled water. HCD + NDW: hypercaloric diet + normal distilled water. HCD + 1.25% LFWE: hypercaloric diet + 1.25% (w/v) longan flower water extract. HCD + 2.5% LFWE: hypercaloric diet + 2.5% (w/v) longan flower water extract. The values of pancreatic lipase activity for the rats in HCD + NDW, HCD + 1.25% LFWE, and HCD + 2.5% LFWE groups were expressed relatively to the average values for rats in the NCD + NDW group, which was set to 100%.

Longan flowers are rich in polyphenols, i.e. phenolic acids, flavonoids, condensed tannins, and proanthocyanindins, but no anthocyanins and ascorbic acid (*9*). Our results also correspond to theirs; however, a very low amount of ascorbic acid could be detected in our LFWE. Our data showed that epicatechin, genistisic acid and ferulic acid are the major components in our LFWE. Hsieh and colleagues (*31*) demonstrated that epicatechin is the major antioxidant compound in longan flowers. Besides, Tsai et al. (*32*) reported that longan flower water extract can augment antioxidant system and improve the symptoms of metabolic syndrome in high-fructose-dietary rats. Moreover, antiobesity and hypolipidemic effects of polyphenols were documented in previous studies (*6*, *12*, *13*). Hence, the antiobesity and hypolipidemic effects of LFWE were investigated in this study.

Generally, high-fat diets increase body weight, and sizes of organ or adipose tissues, which lead to some chronic diseases, i.e. hypertension, hyperlipidemia, diabetes, and fatty liver. It was evidenced that naturally occurring polyphenols own an inhibition of lipase activity (*33*, *34*). A suppression of pancreatic lipase and an enhancement of lipolysis were regarded as an efficient way to control body weight (*12*). In addition, reductions of the hepatic triglyceride and visceral fat deposition in rats fed tea catechins or hot-treated tea catechins were attributed to decreasing activities of hepatic fatty acid synthase and malic enzyme (*35*). Hence, due to the plenty of phytochemical contents in the LFWE, it could be speculated that the decreased ($p < 0.05$) body weight increase and size of epididymal fat of HCD rats drinking 2.5% LFWE solution (**Tables 2** and **3**) were partially due to the suppression of pancreatic enzymes or hepatic enzymes related to fatty acid biosynthesis.

Polyphenol-rich green tea extracts have a greater hypolipidemic effect than oolong and black tea extracts, where it could be due to the lowest fat absorption in rats drinking green tea extracts (*6*). A promotion of cholesterol catabolism and inhibition of intestinal absorption of cholesterol were associated with hypolipidemic and antiatherogenic effects of apple phenols (*8*);

Table 3. Serum Triglyceride, Cholesterol, HDL-C and Atherogenic Index (AI), and Liver and Fecal Cholesterol and Triglyceride of the Experimental SD Rats[a]

group	NCD + NDW	HCD + NDW	HCD + 1.25% LFWE	HCD + 2.5% LFWE
serum				
triglyceride (mg/dL)	143.14 ± 4.66 b	167.43 ± 4.88 a	168.69 ± 6.98 a	146.06 ± 3.37 b
total cholesterol (mg/dL)	66.01 ± 4.31 a	67.14 ± 6.17 a	63.82 ± 2.60 a	68.62 ± 4.54 a
HDL-C (mg/dL)	50.36 ± 2.64 ab	40.66 ± 4.02 c	43.16 ± 3.52 bc	52.84 ± 2.54 a
AI	0.31 ± 0.05 b	0.66 ± 0.06 a	0.48 ± 0.07 a	0.30 ± 0.04 b
liver				
triglyceride (mg/g tissue)	7.47 ± 0.24 c	21.94 ± 0.53 a	20.82 ± 0.44 a	16.08 ± 0.71 b
cholesterol (mg/g tissue)	1.70 ± 0.11 b	2.12 ± 0.10 a	1.72 ± 0.04 b	1.71 ± 0.11 b
feces				
triglyceride (mg/g dry feces)	7.21 ± 0.08 ab	6.68 ± 0.01 b	7.31 ± 0.16 a	7.34 ± 0.24 a
triglyceride (mg/rat/day)	12.75 ± 0.88 ab	11.72 ± 0.43 b	14.29 ± 0.62 a	14.17 ± 0.19 a
cholesterol (mg/g dry feces)	6.52 ± 0.60 a	6.73 ± 0.56 a	6.71 ± 0.43 a	6.74 ± 0.31 a
cholesterol (mg/rat/day)	11.31 ± 0.37 b	11.71 ± 0.60 b	13.02 ± 0.13 a	13.37 ± 0.45 a

[a] AI: atherogenic index, (TC-HDL-C)/HDL-C. The data are given as mean ± SEM ($n = 8$). Mean values with different letters were significantly different ($p < 0.05$). NCD + NDW: normal-caloric diet + normal distilled water. HCD + NDW: hypercaloric diet + normal distilled water. HCD + 1.25% LFWE: hypercaloric diet + 1.25% (w/v) longan flower water extract. HCD + 2.5% LFWE: hypercaloric diet + 2.5% (w/v) longan flower water extract.

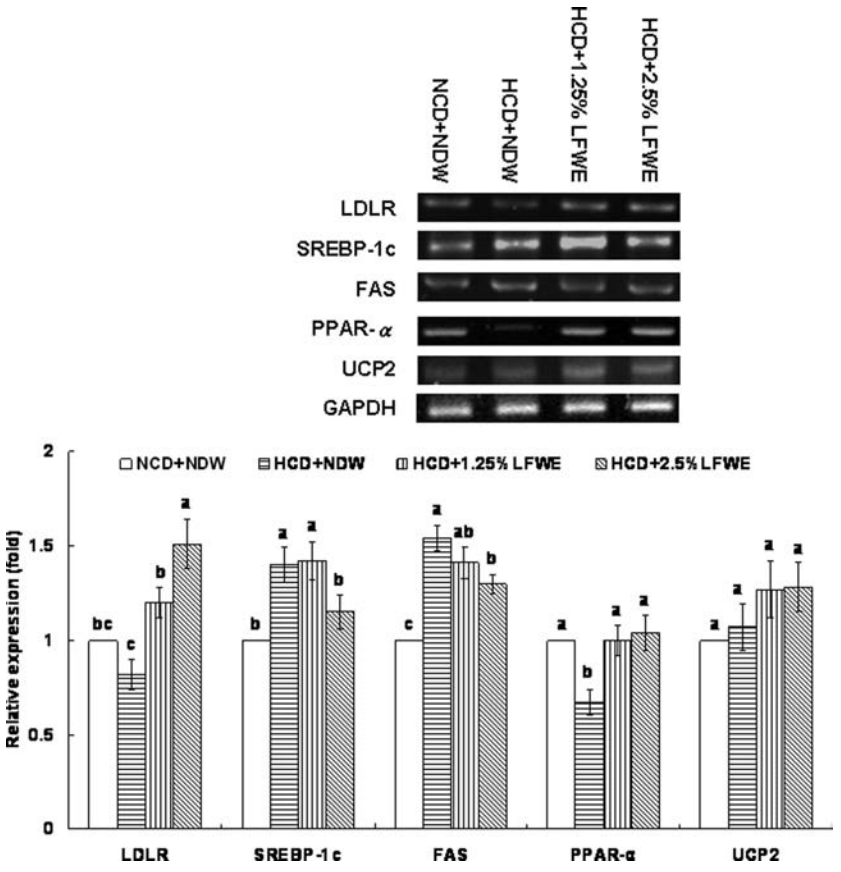

Figure 3. LDL receptor (LDLR), sterol regulatory element binding protein-1c (SREBP-1c), fatty acid synthase (FAS), peroxisome proliferator-activated receptor-alpha (PPAR-α), and uncoupling protein-2 (UCP2) mRNA expressions of the experimental SD rats. The data are expressed as mean ± SEM ($n = 8$); a−c, columns with unlike letters differ significantly in each gene ($p < 0.05$). NCD + NDW: normal-caloric diet + normal distilled water. HCD + NDW: hypercaloric diet + normal distilled water. HCD + 1.25% LFWE: hypercaloric diet + 1.25% (w/v) longan flower water extract. HCD + 2.5% LFWE: hypercaloric diet + 2.5% (w/v) longan flower water extract. The values of LDLR, SREBP-1c, FAS, PPAR-α, and UCP2 mRNA were normalized to the value of GAPDH, and values for the rats in HCD + NDW, HCD + 1.25% LFWE, and HCD + 2.5% LFWE groups were expressed relatively to the average values for rats in the NCD + NDW group, which was set to 1.

meanwhile, an improvement of lipid profiles (HDL-C and AI) by supplementing apple phenols was reported (*36*). Our data demonstrated that LFWE not only reduced serum TG but also improved the serum cholesterol profile (AI) despite no difference in serum TC (**Table 3**). An HCD always couples with a higher lipid accumulation in the liver. Yang, Wang, and Chen (*6*) observed that increased liver triglyceride contents by a high-sucrose diet can be decreased by high polyphenol-rich green, oolong, and black tea extracts; hence, they assumed that this decrease may be related to the lesser fat absorption. Similarly, a higher fecal lipid excretion is highly associated with lower serum lipid levels, thus alleviating the hepatic lipid accumulation in hamsters (*23*). Therefore, it is speculated that the triglyceride-lowering effect of LFWE solutions on serum and liver lipids was partially associated with higher fecal triglyceride excretions (**Table 3**).

Pancreatic lipase can digest triglyceride in consumed food into monoglyceride and fatty acid for absorption (*4*). Polyphenols from plants have an affinity for proteins. A previous study indicated that apple polyphenol can block the triglyceride absorption via inhibiting pancreatic lipase activity *in vitro* (*34*). Hot water extracts of various plant materials, such as leaves of *Acanthopanax sessiliflorus* and roots of *Salacia reticulate*, also show antiobesity via an inhibitory activity of pancreatic lipase, because of aggregation of lipase proteins (*12*, *13*). **Table 1** shows that our LFWE also contains a plethora of polyphenols. Hence, we believe that the suppression of pancreatic lipase activities in HCD rats by drinking LFWE may be due to phenolic acids and flavonoids in LFWE solutions. Besides, more fecal triglyceride outputs also could be explained by the lower pancreatic lipase activity in HCD rats drinking LFWE solutions (**Table 3** and **Figure 2**).

LDLR plays an important role in clearance of cholesterol levels from blood, and its gene expression is downregulated by high-fat/cholesterol diets (*23*). Based on our data, increased LDLR gene expression in the HCD rats drinking LFWE was associated with the improved AI of HCD rats (**Figure 2** and **Table 3**). SREBP-1c and FAS are in charge of triglyceride biosynthesis. Generally, a body lipid accumulation might be from exogenous (i.e., diets) and endogenous (i.e., triglyceride biosynthesis) sources. The downregulation of SREBP-1c and FAS expressions can decrease deposition of visceral fat, and serum and liver triglyceride levels (*35*, *37*). Moreover, the pancreatic lipase activity of HCD rats was decreased by drinking LFWE solutions, which might result in a lesser absorption of exogenous lipids and increased the fecal lipid outputs in NCD groups, thereby lowering liver and serum triglyceride levels (**Figure 2** and **Table 3**). Interestingly, 2.5% LFWE solution also showed downregulation of SREBP-1c and FAS in HCD rats, which means the rate of lipid biosynthesis was lowered. Increased PPAR-α and UCP2 expressions results in higher β-oxidation in livers, thus increasing energy expenditure and decreasing the lipid level in the serum and liver (*38*, *39*). Therefore, it is supposed that lower size of epididymal fat, serum and liver lipids result from a combined effect of normalization of hepatic PPAR-α gene expression (**Figure 2**), suppression of

pancreatic activity (**Figure 1**) and SREBP-1c and FAS gene expressions (**Figure 2**), and higher fecal triglyceride output (**Table 3**).

It has been suggested that the total polyphenol intake per day needs to reach 1 g (*40*, *41*). Scalbert and Williamson (*41*) indicated that fruit and beverage (i.e., fruit juice, tea, coffee, and wine) are the main polyphenol dietary sources, but vegetables, dry legumes, and cereals only contribute a lesser extent. Our data indicated that 2.5% LFWE has the best characteristics on antiobesity and hypolipidemic effects in HCD rats. Via the total amount of polyphenols (155.94 mg/dL, phenolic acid + flavonoids) in 2.5% LFWE solution (**Table 1**), as well as water intake (36.74 mL/rat/day) and average final body weight (511.49 g) of HCD + 2.5% LFWE rats (**Table 2**), the dose of rats is calculated as 112.01 mg/kg body weight. Based on the formulation of dose translation from animal to human [HED (mg/kg body weight) = animal dose (mg/kg) × animal K_m/human K_m (rat K_m, 6; human K_m, 37)] (*42*), an adult human equivalent dose (body weight: 60 kg) is 18.16 mg/kg body weight, which is equal to approximately 1.09 g/day. Therefore, LFWE could be a good daily polyphenol intake substance for human. Furthermore, although glucuronization, sulfation, and methylation, the major metabolites for polyphenols, have been identified via phase II enzyme pathway, the bioefficacy of polyphenol phase II metabolites is different between human and rats (*43*). Hence, the bioavailability of LFWE polyphenols warrants further investigation.

Overall, the present study demonstrated that LFWE showed antiobesity and hypolipidemic effects via a combined effect of decreased exogenous lipid absorption, normalization of hepatic PPAR-α gene expression, suppression of pancreatic activity and SREBP-1c and FAS gene expression, and higher fecal triglyceride output. After a calculation from HCD rats drinking 2.5% LFWE solution, an adult human equivalent dose (60 kg) is 18.16 mg/kg body weight, which is equal to approximately 1.09 g/day. Therefore, LFWE solutions could be a good source of daily polyphenol intake; meanwhile, also shown were characteristics of antiobesity and hypolipidemic effects.

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