Original Research

Ethanol extract of Liuwei Dihuang reduces weight gain and visceral fat in obese-prone CD rats fed a high-fat diet

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Abstract

The current study investigated the effect and mechanisms of action of Liuwei Dihuang ethanol extract (LWDH-EE) on obesity and related metabolic phenotypes in male obese-prone CD rats. The rats were fed a high-fat diet and treated with 0 (obese control), 350 (EE350), or 700 (EE700) mg/kg/d of LWDH-EE in water once a day by gavage feeding for 10 weeks. The EE700 decreased body weight after 3 weeks of the treatment and the effect was maintained throughout the remaining study period. The EE700 also significantly reduced visceral fat and improved metabolic phenotypes by lowering the serum total cholesterol (T-C), non-high-density lipoprotein cholesterol, triacylglycerol, free fatty acids (FFA), and leptin levels. The EE350 reduced epididymal fat, serum T-C, and FFA but did not significantly affect other parameters. LWDH-EE dose-dependently increased fat and carbohydrate oxidations, energy expenditure, and the relative efficiency of fat oxidation for energy expenditure. EE350 and EE700 reduced food intake only in week 5 and did not affect the accumulative food intake in every week and the entire treatment period. Taken together, the results suggest that LWDH-EE is a potential therapeutic agent for the prevention of obesity possibly through a primary action of increasing energy metabolism and expenditure, along with a possible effect of decreasing energy intake.

Keywords: Liuwei Dihuang ethanol extract, weight gain, visceral fat, energy expenditure, blood lipids, hormones, obese rats

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Introduction

Obesity has doubled since 1960 and has now become a worldwide epidemic.[1] The prevalence of obesity has been increasing steadily among adults and also has been rapidly penetrating into the child and adolescent population since the past decade.[2] Obesity is associated with the development of numerous metabolic disorders, including primarily insulin resistance, diabetes, dyslipidemia, and hypertension.[3] These comorbidities contribute to the increased morbidity and mortality in obese people.[4] Although there is increased effort to address this alarming health threat, success has been marginal due to various challenges.[4] It is still demanding to develop new solutions for people with specific genetic characteristics, which determine the response to a given treatment, and different product preferences to prevent weight gain or induce weight loss. In this regard, herbal medicines have received an increasing attention in recent years.

A number of studies investigated the efficacy and mechanism of action of herbal medicines on body weight and body composition.[5,6] A recent review on the efficacy and safety of oriental herbal medicines that are composed of multiple herbs for treating human obesity has suggested that some oriental herbal medicines may be effective for the treatment of obesity when compared with conventional medicine or lifestyle control.[7] The interest in the traditional Chinese medicine is gaining rapidly not only in China and other Asian countries but also in the United States and Europe, especially used as an integrative medicine.[8] Liuwei Dihuang (LWDH), a classic Chinese herbal formula, has been used historically to treat a variety of ailments, especially the abnormality or insufficiency of liver and kidney functions. Recent studies have shown that LWDH is safe[9] and effective to protect against oxidative stress[10] and improve insulin resistance and diabetes mellitus.[11–13] In 2011, we demonstrated for the first time that oral administration of LWDH concentrated pills at a dose of 3500 mg/kg/d lowered body weight, reduced serum lipids, and improved insulin and leptin sensitivities in obese rats, without showing any adverse effects as assessed by liver enzyme tests.[14] LWDH formula consists of six herbs and the products in the market nowadays are manufactured in a form of the mixture of raw materials and crude extracts,

which explains the observed high dosage to decrease weigh gain.[14] Therefore, the effective or optimal dosage may be markedly reduced if the active fraction is identified. Moreover, the mechanism of action of LWDH on obesity is not well understood. In order to fully appreciate the potential weight-lowering benefits of LWDH, it is important to identify the active components/fractions of the concentrated pills and further elucidate the mechanism of action. Accordingly, we obtained water extract of the concentrated pills and investigated its antiobesity efficacy in obese rats.[15] The dose of 1200 mg/kg/d showed similar efficacy as the concentrated pills, whereas the dose of 600 mg/kg/d did not show any effects. Apparently, the effective dosage is still high and chemical analysis has revealed that this fraction contains a large amount of sugars but a small portion of five bioactive compounds.[16] Nevertheless, the ethanol extract contains one additional bioactive compound and much higher amounts of the five compounds identified in the water extract, and less amount of sugars.[16] Herein, we hypothesized that the fraction of ethanol extract (LWDH-EE) was the primary fraction of the LWDH concentrated pills that suppressed weight gain and fat deposit in obese rats.[14] We have determined the antiobesity potential of LWDH-EE on body weight gain and visceral fat deposition in obese-prone CD rats, which are developed from an outbred line of Crl:CD rats (Charles River stock of Sprague-Dawley) and develop obesity when fed a high-fat diet.[14] We have furthermore explored into the effects of LWDH-EE on energy intake, energy metabolism and expenditure, and obesity-related metabolic phenotypes, attempting to understand the mechanism of action of LWDH-EE on obesity and benefits on the management of obesity-related complications.

Materials and methods

Preparation of LWDH ethanol extract

LWDH is made from Radix Rehmanniae Preparata (prepared root of *Rehmannia glutinosa*, 160 g), Fructus Corni (FC, processed fruit of *Cornus officinalis*, 80 g), Cortex Moutan (root bark of *Paeonia suffruticosa*, 60 g), Rhizoma Dioscoreae (rhizome of *Dioscorea opposite*, 80 g), Poria (sclerotia of *Poria cocos*, 60 g), and Rhizoma Alismatis (rhizome of *Alisma plantago-aquatica,* 60 g).[17,18] The LWDH-EE was prepared as follows: the dry unpolished pills (6.9 kg) were milled and extracted twice with 95% EtOH with refluxing (30 min each, total EtOH 45 L). The solvent was removed under reduced pressure and dried at 70 °C to obtain LWDH-EE (1.12 kg; a yield of 16.2% from the concentrated pills). The preparation of the concentrated pills and following ethanol extract was conducted by Henan Wanxi Pharmaceutical Co. Ltd (Nanyang, Henan, P.R. China). The chemical analysis of LWDH-EE was carried out using nuclear magnetic resonance (NMR) and high-performance liquid chromatography-mass spectrometry diode array detector (HPLC-MS/DAD). The extraction and analytical methods, the chemical composition of the extract, chromatography of main peaks, and quantitative estimation of each bioactive compound were reported in 2012.[16]

Animal experiment

Male obese-prone CD rats, weighing 150–170 g, were obtained from Charles River Laboratories (Saint-constant, Québec, Canada) and housed individually in rat cages, with a 12-h light/dark cycle. The room lights were turned on at 6:00 am and off at 18:00 pm. After a week of acclimatization with free access to regular rodent chow and water, the rats (190–210 g) were randomly divided into three groups ($n = 12$/group). All rats were fed with the standard AIN-93 G diet modified to contain 2% cholesterol, 0.5% cholic acid, and 60% energy from fat (a mixture of lard and sunflower oil at 96:4, w/w). One group was used as the high-fat diet-induced obese control (high-fat control (HFC)) and the other two were treated with 350 mg/kg/d (EE350) and 700 mg/kg/d (EE700) of LWDH-EE, respectively. The obese resistant CD rats have been developed and well established by Charles River as the lean controls. They do not develop obesity when fed a high-fat diet as evidenced in our previous study compared with the obese-prone CD rats.[14] Therefore, we did not include the lean controls in the present study. LWDH-EE was dissolved in water and administered orally once a day by gavage between 10.00 am and 11.00 am for 10 weeks. The HFC group was gavaged with the vehicle. The dosage of LWDH-EE was determined based on the effective dose of the concentrated pills that lowered weight gain in obese-prone CD rats[14] and the yield of ethanol extract from the concentrated pills. Diet was prepared weekly and stored in a cold room. Rats were provided with fresh diet every day and food consumption was recorded accordingly. Body weight was measured every week. During the study, rats were monitored daily for any abnormal behaviors and activities that might be related to the treatments. After 10 weeks, rats were fasted overnight and anesthetized with inhalation of isoflurane (Pharmaceutical Partners of Canada Inc., Richmond Hill, ON). Blood samples were collected from the cardiac puncture into serum tubes. After centrifugation, serum was collected and stored at −80°C for later analysis. Mesenteric, epididymal, and perirenal fats were carefully dissected and weighed. The animal use and experimental protocols were approved by the Joint Animal Care and Research Ethics Committee of the University of Prince Edward Island and the National Research Council Canada – Aquatic and Crop Resource Development (Protocol number 09-028). The study was conducted following the guidelines of Canadian Council on Animal Care.

Serum lipid analysis

Serum total cholesterol (T-C), high-density lipoprotein cholesterol (HDL-C,) and triacylglycerols (TAG) were analyzed using the enzymatic methods on a pointe-180 chemistry analyzer (Point Scientific Inc., Canton, MI), with 2.2%, 1.3%, and 6.5% intra-assay coefficients of variation, respectively. Non-HDL cholesterol (nonHDL-C) was calculated by subtracting HDL-C from T-C.[4] Serum free fatty acids (FFA) were measured using a commercial kit following the manufacturer's instructions (BioVision, Mountain View, CA), with 2% and 0.8% of intra- and inter-assay coefficients of variation, respectively.

Serum insulin, leptin, and adiponectin measurements

Insulin (Crystal Chem, Downer's Grove, IL), leptin, and adiponectin (Kamiya Biomedical, Seattle, WA) kits were used to measure the serum concentrations of these hormones, respectively, following the kit instructions. The concentration of each hormone was calculated in reference to the corresponding standard curve. The intra- and inter-assay coefficients of variation were 3.3% and 5.5% for insulin, 5.0% and 5.2% for leptin, and 9.3% and 10.5% for adiponectin, respectively.

Energy metabolism and expenditure

As all animals were fed the same diet and the groups had similar initial average body weights and standard deviations, an assumption was made that the different groups of rats had comparable baseline characteristics of energy metabolism and expenditure. Accordingly, the effect of LWDH-EE on energy metabolism and expenditure were measured following a steady treatment effect was reached. Briefly, in week 9 rats in each group were placed in cages equipped with a 20-channel open-circuit calorimetry system (Oxymax; Columbus Instruments International, Columbus, OH), with free access to the diet and water. Oxygen consumption (VO_2, mL/min/kg) and carbon dioxide production (VCO_2, mL/min/kg/) were measured every 45–60 s. Data were analyzed using the Clax software (Columbus Instruments International, Columbus, OH) after the deletion of values taken in the first 2 h. The final values represent a mean of multimeasurements for a period of 34 h. The following parameters were calculated and corrected for body weight as described elsewhere.[19]

$$\text{Respiratory exchange ratio (RER)} = VCO_2/VO_2$$

$$\text{Rate of fat oxidation (mg/min/kg)} = (1.689 * VO_2) - (1.689 * VCO_2)$$

$$\text{Rate of carbohydrate oxidation (mg/min/kg)} = (4.12 * VCO_2) - (2.91 * VO_2)$$

$$\text{Rate of energy expenditure (kJ/min/kg)} = (15.88 * VO_2) - (4.87 * VCO_2)$$

Statistical analysis

Data were analyzed with one-way analysis of variance (ANOVA) using the SAS statistical software version 9.2 (SAS Institute, NC). Two-way ANOVA was used to determine the difference of energy metabolism and expenditure between the light and dark periods. When a treatment effect was detected, differences among the group means were determined using the pair-wise comparisons of the least squares means test. Results are presented as means ± SEM, with the significance level being set at $P < 0.05$.

Results

Effect of LWDH-EE on body weight, visceral fat, and food intake in obese rats

Throughout the experimental period, there were no rats showing abnormal behaviors, activities, and illnesses. The oral administration of EE700 significantly decreased weight gain after 3 weeks of treatment and the effect was maintained throughout the remaining treatment period (Figure 1). At the end of the study, body weight was 9.5% lower ($P < 0.05$) in the EE700 group relative to the HFC group. The EE350 group tended to decrease body weight and had 7.2% lower ($P = 0.055$) final body weight compared with the HFC. As shown in Figure 2, EE700 decreased ($P < 0.05$) the percent weight (relative to body weight) of perirenal, epididymal, and mesenteric fats and the total fat of these three regions compared with the HFC group. The EE350 showed lower ($P < 0.05$) percent weight of epididymal fat but had no significant effect on percent weights of perirenal and mesenteric fats, and the percent weight of the total fat in these three regions.

Both EE350 and EE700 reduced ($P < 0.05$) food intake in week 5 while showing no effect in other weeks (Table 1). The accumulative food intakes were not different ($P > 0.05$) in every week (Supplementary Figure 1). The total food intakes over the 10-week treatment period were 1089 ± 33, 1094 ± 30, and 1185 ± 47 g/rat for EE350, EE700, and HFC groups, respectively, and did not differ among the groups.

Effect of LWDH-EE on energy metabolism and expenditure in obese rats

All parameters used for energy metabolism and expenditure were corrected for the differences of body weight. Rats in the EE700 and EE350 groups had higher oxygen consumption ($P < 0.05$ for both groups) and carbon dioxide production ($P < 0.01$ and $P < 0.05$, respectively) as compared with control rats during the light period (Table 2).

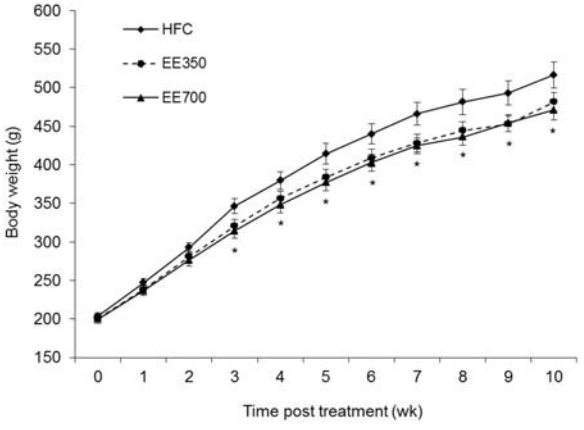

Figure 1 Effect of LWDH ethanol extract on body weight in obese-prone rats fed a high-fat diet. Obese rats were treated with gavage feeding of 350 or 700 mg/kg/d of LWDH ethanol extract in water for 10 weeks. Data are means ± SEM ($n = 12$ for each group).
HFC: high-fat control; EE350: 350 mg/kg/d of Liuwei Dihuang ethanol extract; EE700: 700 mg/kg/d of Liuwei Dihuang ethanol extract.
*Different from the HFC control ($P < 0.05$)

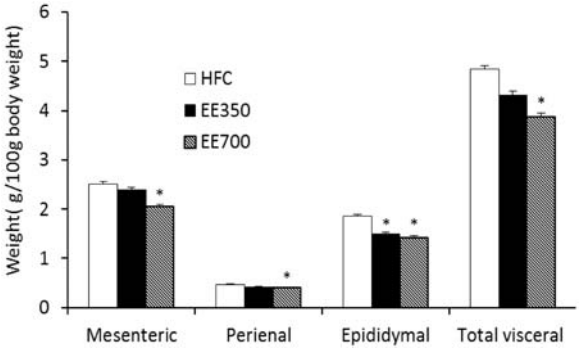

Figure 2 Effect of LWDH ethanol extract on the weight of visceral fats in obese-prone rats fed a high-fat diet. Rats were treated with gavage feeding of 350 or 700 mg/kg/d of LWDH ethanol extract for 10 weeks. Data are presented as means ± SEM ($n = 12$ for each group).
HFC: high-fat control; EE350: 350 mg/kg/d of Liuwei Dihuang ethanol extract; EE700: 700 mg/kg/d of Liuwei Dihuang ethanol extract.
*Different from the HFC control ($P < 0.05$)

The fat oxidation was increased ($P < 0.05$) in either EE700 or EE 350 group and carbohydrate oxidation increased ($P < 0.01$) in EE700 group only. Energy expenditure was increased by 10.7% ($P < 0.01$) in EE700 group and 6.5% ($P < 0.05$) in EE350 group. Similarly, during the dark period the EE700 and EE350 groups increased oxygen consumption and carbon dioxide production ($P < 0.05$) (Table 3). They increased carbohydrate oxidation ($P < 0.05$) while having no effect on fat oxidation. The EE700 and EE350 groups increased ($P < 0.05$) energy expenditure by 8.4% and 7.5%, respectively. The RER was unaffected in both the light and dark periods and the values were lower than 0.8. The oxygen consumption, carbon dioxide production, fat oxidation, carbohydrate oxidation, and energy expenditure were higher in the dark period than the light period (Supplementary Table 1). However, the degree of increase by LWDH-EE in all parameters was higher in the light period than the dark period (Tables 2 and 3).

Table 1 Effect of LWDH ethanol extract on food intake (g) of obese-prone rats fed a high-fat diet

	Time post treatment (week)									
	1	2	3	4	5	6	7	8	9	10
HFC[a]	15.4 ± 0.6	15.6 ± 0.5	17.0 ± 0.8	16.6 ± 0.8	17.9 ± 0.7	17.4 ± 0.7	17.1 ± 1.0	18.0 ± 1.2	17.4 ± 1.0	17.0 ± 0.9
EE350	15.1 ± 0.7	14.6 ± 0.7	16.4 ± 0.8	15.7 ± 0.7	15.2 ± 0.6*	15.8 ± 0.7	15.1 ± 0.7	16.3 ± 0.6	16.0 ± 1.0	15.5 ± 0.5
EE700	15.0 ± 0.7	15.1 ± 0.7	15.4 ± 0.7	15.9 ± 0.8	15.6 ± 0.6*	15.9 ± 0.5	15.7 ± 0.6	16.1 ± 0.4	15.4 ± 0.4	15.5 ± 0.6

LWDH: Liuwei Dihuang; HFC: high-fat control; EE350: 350 mg/kg/d of Liuwei Dihuang ethanol extract; EE700: 700 mg/kg/d of Liuwei Dihuang ethanol extract.
[a]HFC, rats fed the high-fat diet and gavaged with water; EE350, rats fed the high-fat diet and gavaged with 350 mg/kg/d of LWDH ethanol extract in water; EE700, rats fed the high-fat diet and gavaged with 700 mg/kg/d of LWDH ethanol extract in water. Data are presented as means ± SEM ($n = 12$).
*Different from the HFC group, $P < 0.05$.

Table 2 Effect of LWDH ethanol extract on energy metabolism during the light period in obese-prone rats fed a high-fat diet

	VO_2 (mL/min/kg)	VCO_2 (mL/min/kg)	RER	RFO (mg/min/kg)	RCO (mg/min/kg)	REE (kJ/min/kg)	RFO/RsEE	RCO/REE
HFC[a]	19.25 ± 0.54	15.00 ± 0.41	0.7799 ± 0.0022	7.16 ± 0.23	5.82 ± 0.19	232.59 ± 6.61	0.0308 ± 0.0009	0.0251 ± 0.0026
EE350	20.57 ± 0.41*	16.00 ± 0.33*	0.7782 ± 0.0020	7.72 ± 0.17*	6.06 ± 0.25	248.76 ± 17.48*	0.0310 ± 0.0011	0.0244 ± 0.0030
EE700	21.64 ± 0.39*	17.03 ± 0.31**	0.7869 ± 0.0022	7.79 ± 0.17*	7.18 ± 0.76**	260.68 ± 16.68**	0.0299 ± 0.0010*	0.0276 ± 0.0028*

LWDH: Liuwei Dihuang; HFC: high-fat control; EE350: 350 mg/kg/d of Liuwei Dihuang ethanol extract; EE700: 700 mg/kg/d of Liuwei Dihuang ethanol extract; VO_2: oxygen consumption; VCO_2: carbon dioxide production; RER: respiratory exchange ratio; RCO: rate of carbohydrate oxidation; RFO: rate of fat oxidation; REE: rate of energy expenditure.
[a]HFC, rats fed the high-fat diet and gavaged with water; EE350, rats fed the high-fat diet and gavaged with 350 mg/kg/d of LWDH ethanol extract in water; EE700, rats fed the high-fat diet and gavaged with 700 mg/kg/d of LWDH ethanol extract in water. Data are presented as means ± SEM ($n = 12$).
*$P < 0.05$, **$P < 0.01$ compared with the HFC group.

Table 3 Effect of LWDH ethanol extract on energy metabolism during the dark period in obese-prone rats fed a high-fat diet

	VO_2 (mL/min/kg)	VCO_2 (mL/min/kg)	RER	RFO (mg/min/kg)	RCO (mg/min/kg)	REE (kJ/min/kg)	RFO/REE	RCO/REE
HFC[a]	21.35 ± 0.82	16.59 ± 0.59	0.7787 ± 0.0036	8.02 ± 0.40	6.26 ± 0.18	258.15 ± 10.24	0.0309 ± 0.0016	0.0247 ± 0.0045
EE350	23.01 ± 0.39*	18.02 ± 0.30*	0.7840 ± 0.0031	8.44 ± 0.20	7.27 ± 0.31*	277.79 ± 4.85*	0.0304 ± 0.0014	0.0262 ± 0.0038
EE700	23.34 ± 0.36*	18.34 ± 0.26*	0.7892 ± 0.0022	8.28 ± 0.19	7.92 ± 0.17*	279.82 ± 4.54*	0.0296 ± 0.0010*	0.0284 ± 0.0028*

HFC: high-fat control; EE350: 350 mg/kg/d of Liuwei Dihuang ethanol extract; EE700: 700 mg/kg/d of Liuwei Dihuang ethanol extract; VO_2: oxygen consumption; VCO_2: carbon dioxide production; RER: respiratory exchange ratio; RCO: rate of carbohydrate oxidation; RFO: rate of fat oxidation; REE: rate of energy expenditure.
[a]HFC, rats fed the high-fat diet and gavaged with water; EE350, rats fed the high-fat diet and gavaged with 350 mg/kg/d of LWDH ethanol extract in water; EE700, rats fed the high-fat diet and gavaged with 700 mg/kg/d of LWDH ethanol extract in water. Data are presented as means ± SEM ($n = 12$).
*$P < 0.05$ compared with the HFC group.

Table 4 Effect of LWDH ethanol extract on serum lipids and obesity-related hormones in obese-prone rats

	T-C (mmol/L)	HDL-C (mmol/L)	nonHDL-C (mmol/L)	TAG (mmol/L)	FFA (mmol/mL)	Glucose (mmol/L)	Leptin (ng/mL)	Insulin (ng/mL)	Adiponectin (μg/mL)
HFC[a]	4.87 ± 0.21	1.29 ± 0.04	3.15 ± 0.16	0.97 ± 0.05	0.67 ± 0.02	7.19 ± 0.39	7.35 ± 0.12	4.04 ± 0.49	15.31 ± 1.13
EE350	4.19 ± 0.31*	1.15 ± 0.07	2.90 ± 0.34*	0.85 ± 0.06	0.54 ± 0.06*	7.43 ± 0.27	7.21 ± 0.19	3.90 ± 0.57	15.39 ± 1.35
EE700	3.89 ± 0.19**	1.20 ± 0.09	2.38 ± 0.18*	0.82 ± 0.04*	0.46 ± 0.03**	6.61 ± 0.42	6.75 ± 0.12*	3.48 ± 0.61	14.08 ± 1.17

HFC: high-fat control; EE350: 350 mg/kg/d of Liuwei Dihuang ethanol extract; EE700: 700 mg/kg/d of Liuwei Dihuang ethanol extract; T-C: total cholesterol; HDL: high-density lipoprotein; HDL-C: HDL cholesterol; nonHDL-C: non-HDL cholesterol; TAG: triacylglycerols; FFA: free fatty acids.
[a]HFC, rats fed the high-fat diet and gavaged with water; EE350, rats fed the high-fat diet and gavaged with 350 mg/kg/d of LWDH ethanol extract in water; EE700, rats fed the high-fat diet and gavaged with 700 mg/kg/d of LWDH ethanol extract in water. Values are means ± SEM ($n = 12$).
*$P < 0.05$, **$p < 0.01$ compared with the HFC group.

For example, EE700 increased fat oxidation, carbohydrate oxidation, and energy expenditure by 9%, 23%, and 12% in the light period while by 3%, 13% and 8% in the dark period, respectively. As LWDH ethanol extract (LWDH-EE) promoted the oxidations of both fat and carbohydrate, we calculated the relative efficiency of fat oxidation or carbohydrate oxidation for energy supply. The results revealed that the high dose of LWDH-EE significantly increased the relative efficiency of fat oxidation while decreasing the relative efficiency of carbohydrate oxidation toward energy expenditure. Because larger increases in carbohydrate oxidation than fat oxidation occurred following the LWDE-EE supplementation, the absolute contributions of both oxidations to energy expenditure were actually increased at similar magnitudes, which were 11% and 12% in the light period and 10% and 8% in the dark period, respectively. However, fat oxidation supplied approximately 75% of the total energy, indicating that increased fat oxidation must have played a much greater role than carbohydrate oxidation in lowering weight gain and visceral fat mass.

Effect of LWDH-EE on serum lipids and hormones of obese rats

The serum T-C was significantly lower in the EE350 ($P < 0.05$) and EE700 ($P < 0.01$) than the HFC group (Table 4). EE700 also reduced ($P < 0.05$) nonHDL-C and TAG, while showing no effect on HDL cholesterol concentrations. EE350 reduced ($P < 0.05$) nonHDL-C levels, without significant effects on HDL cholesterol and TAG concentrations. Moreover, LWDH-EE showed a dose-dependent effect to lower the concentration of serum FFA.

Serum leptin concentrations were decreased following LWDH-EE supplementation in a dose-dependent pattern and significantly lower ($P < 0.05$) in rats of the EE700 group compared with the control (Table 4). Serum insulin or adiponectin levels were not significantly altered by either dose of LWDH-EE, which might be due to large variations observed within the groups.

Discussion

Obesity develops as a result of the extended period of energy surplus, which is ascribed to increased energy intake and/or decreased expenditure.[20,21] LWDH-EE supplementation reduced food intake only in week 5 and did not affect the accumulative food intake over the entire treatment period. In comparison with the LWDH concentrated pills,[14] LWDH-EE showed a much weaker effect on food/energy intake as evidenced by the finding that LWDH concentrated pills reduced food intake after 2 weeks and the effect remained throughout the remaining treatment period. It is suggestive that food or energy intake played a minimal role in lowering weight gain of rats treated with LWDH-EE.

It is well known that energy expenditure is critical in the development of obesity. Obese-prone CD rats develop obesity as a result of reduction in energy expenditure, relevant to the etiology of human obesity.[22,23] It is interesting to see that dietary supplementation of LWDH-EE reversed this alteration by increasing energy metabolism and expenditure. Because LWDH-EE enhanced both fat and carbohydrate oxidations, it is not surprising to note that RER was unaltered, in agreement with a previous study.[24] LWDH-EE differently modulated the relative efficiency of fat and carbohydrate oxidations for energy supply. It increased fat oxidation but decreased carbohydrate oxidation efficiencies for energy expenditure. However, as a larger increase occurred in carbohydrate oxidation compared with fat oxidation following LWDE-EE supplementation, the absolute contributions of fat and carbohydrate oxidations to energy expenditure were increased at similar magnitudes. Because fat oxidation supplied approximately 75% of the total energy within the body due to the nature of the high-fat diet provided, the increase in fat oxidation must have played a greater role than the increase in carbohydrate oxidation in lowering body weight gain and visceral fat deposition following the LWDH-EE treatment. This finding strongly supports the emerging evidence that decreased fat oxidation is the leading cause of obesity and therefore, increasing fat oxidation is the primary approach to preventing or treating obesity.[25] In accordance with the eating behaviors and activity patterns of rats,[26] fat and carbohydrate oxidations and energy expenditure were higher in the dark period than in the light period. However, a larger magnitude of enhancement in energy metabolism and expenditure by LWDH-EE in the light period than the dark period implies that LWDH-EE exerted a stronger effect on the basal metabolic rate. These results demonstrated that LWDH-EE reduced weight gain and visceral fat mass in obese rats primarily through promoting fat and carbohydrate oxidations, basal metabolic rate, and energy expenditure. Additional studies may be needed to determine whether

LWDH-EE affects the digestion and absorption of nutrients, especially fat and carbohydrates.

The reduction in blood FFA by LWDH-EE is in line with the increased fat oxidation, decreased visceral fat mass, and blood TAG. FFA cause lipotoxicity and its levels in the blood are closely associated with the circulating lipids and many diseases clustered in the metabolic syndrome. Therefore, normalizing blood levels of FFA has long been of interest in managing metabolic disorders, especially in obesity, diabetes, and insulin resistance.[27] In obese humans and rodents, blood leptin concentrations are elevated, resulting in leptin resistance and reduced capability to regulate properly appetite/satiation and energy metabolism.[28] The observed dose-dependent reduction in serum leptin levels by LWDH-EE could be a direct effect of the extract on leptin sensitivity. However, a secondary consequence of weight reduction or confounded effect of both mechanisms cannot be ruled out as a decrease in body weight generally improves leptin sensitivity. LWDH concentrated pills improved insulin sensitivity in obese-prone CD rats.[14] This effect was however not seen in the LWDH-EE fraction, which might be attributed to large variations observed within groups.

LWDH-EE dose-dependently lowered the plasma levels of T-C, nonHDL-C, and TAG levels and thus would benefit the cardiovascular system by reducing risk for developing atherosclerosis,[29] in addition to the benefits that are associated directly with lower body weight. As dyslipidemia is associated with excess body fat[30] and a weight reduction improves dyslipidemia,[29] it is not possible to elucidate under the present experimental conditions whether LWDH-EE possesses a direct effect of lowering blood cholesterol and TAG levels.

We have conducted chemical analysis of LWDH-EE using NMR and HPLC-MS/DAD and identified six main bioactive compounds, gallic acid, 5-hydroxymethyl furfural, sweroside, loganin, paeoniflorin, and paeonol, in agreement with other reports.[18,31] The chromatography and quantity of each compound are reported elsewhere in 2012.[16] Although not well understood, gallic acid and paeoniflorin are reported to have antiobesity activities[32–35] and lower blood lipids.[32,34] Paeonol has antioxidant and anti-inflammatory properties and regulates lipid metabolism.[36] Loganin is protective against hyperglycemia-induced inflammation and oxidative stress and glucolipotoxicity in liver and kidney.[37,38] Sweroside possesses anti-inflammatory activities.[39] It is not established whether inflammation and oxidative stress are among the causes of obesity, the inhibition of chronic inflammation and improvement of antioxidant capacity are demonstrated to be beneficial to obese subjects by reducing obesity-related complications.[40] In comparison with the water extract, ethanol extract contains 4–9-folds of gallic acid, 5-hydroxymethyl furfural, sweroside, loganin, and paeoniflorin. In addition, the ethanol extract contains paeonol that is not detectable in the water extract.[16] The differences in the chemical composition and the content of bioactive compounds between water and ethanol extracts of LWDH concentrated pills may explain the observed differences of antiobesity efficacy in obese rats.

Accumulating evidence suggests that a modest weight reduction, such as 5–10%, is sufficient to improve or even abolish many of the known obesity-related complications.[41] In the present study, EE350 and EE700 reduced body weight gain in obese rats by 7% and 10%, respectively, indicating that LWDH-EE is potentially a promising therapeutic agent on obesity and overweight. After dose translation from rats to humans,[42] the daily dosage is 3.9 and 7.9 g, respectively, for a 70 kg person. Moreover, as LWDH-EE contains considerable amounts of sugar monomers, dimers, and trimmers,[16] the dosage could be further decreased by removing saccharides, which are considered irrelevant to the weight-lowering effect of LWDH-EE.

Data presented in this study demonstrate that ethanol extract is the main active antiobesity fraction of LWDH concentrated pills. It suppressed weight gain and visceral fat accumulation in obese rats primarily through increasing fat and carbohydrate oxidations, energy metabolism, and expenditure, although a reduction in food intake might be involved. Additional benefits of LWDH-EE are attributed to the improvement of metabolic phenotypes, including lowering blood cholesterol, TAG, FFA, and leptin levels. Preventive and therapeutic potentials of LWDH-EE on obesity are rewarding. More studies are warranted to test on a more pure product and to optimize the composition of the six bioactive compounds.

Author contributions: All authors participated in the design, interpretation of the studies, and review of the manuscript. SN conducted the experiments, data acquisition, analysis and interpretation, and drafted the manuscript. JZ obtained the test material from Wanxi Pharmaceutical Co. Ltd, Nanyang, Henan, P.R. China, interpreted the data of chemical profile of the test material, and revised manuscript. YW designed the experiment, participated in the experiments, data acquisition, analysis and interpretation, and improvement of the manuscript.

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