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Water Extract of Liuwei Dihuang Reduces Weight Gain and Visceral Fat in Obese Rats

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Abstract: The present study was conducted to determine the effect and mechanism of action of Liuwei Dihuang (LWDH) on weight gain and visceral fat deposition in male obese-prone CD rats. The rats were divided into three groups and fed a high-fat diet (60 kcal% from fat). Two treatment groups received 600 (WE600) or 1200 (WE1200) mg/kg/d LWDH water extract dissolved in water by gavage feeding once a day for 10 weeks. The control rats were gavaged with the vehicle. Daily food intake and weekly body weight were recorded. Energy metabolism was measured using an indirect calorimeter during week 8 of the treatment. At the end of the study, rats were sacrificed. Immediately, visceral fat pads were dissected and weighed. Serum was collected for the measurement of blood lipids and hormones. It was found that WE1200 lowered body weight after 3 weeks of treatment and the effect was maintained throughout the remaining study period. WE1200 also lowered visceral fat mass, serum leptin, plasma free fatty acids and cholesterol, respectively. The energy expenditure was increased by WE1200 in both the light and dark periods. Oxygen consumption, carbon dioxide production and fat oxidation were increased in both light and dark periods, whereas carbohydrate oxidation increased only in the light period in the WE1200 group. Rats in the WE600 showed lower serum free fatty acids and leptin levels, while showing no effect on the other parameters compared to the control. These results demonstrated potential of using LWDH water extract to treat obesity and its related complications. The effect may be attributable to the increase of energy expenditure, decrease of food intake and improvement of leptin sensitivity.

Key words: Liuwei Dihuang, energy expenditure, food intake, leptin sensitivity, obesity, rat.

1. Introduction

Obesity has become an epidemic in the developed countries and also many developing countries. The rates of obesity and overweight are continuing to grow in adults, and unfortunately that the situation has been worsening by penetrating into the child and adolescent population since two decades ago. Obesity and overweight are associated with numerous metabolic complications, which include cardiovascular disease, hyperlipidemia, insulin and leptin resistance, type 2diabetes and hypertension [1]. Thus, obesity and overweight have emerged as a serious health threat, which increasingly impacts the quality of human life and imposes a heavy burden to the health care system.

There is a growing interest of natural health products in recent years to control body weight and improve disturbances clustered with the metabolic syndrome. In this regard, the Chinese traditional medicine has shed a light as a valuable resource in both information and starting materials [2]. Liuwei Dihuang (LWDH) is a classic Chinese herbal

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formulation that has been used for thousands of years to promote health or correct a cluster of symptoms including sweat, low fever, dizziness, tinnitus, emission, soreness in the lower back, without side effects being reported. LWDH is proven to nourish liver and kidney and restore or improve their functional insufficiencies, which are often associated with decreased metabolic rate and immune function [3, 4]. LWDH is one of prescriptions that have long been used for handling diabetic conditions in humans in China, Japan and other Asian countries [5]. Recent studies have generated scientific evidence demonstrating that LWDH can improve insulin resistance and diabetes in humans and animals [5, 6], and offers protection against early diabetic nephropathy in rats [7]. LWDH is prepared as a mixture of six herbs and there are several forms of LWDH being manufactured and marketed commercially in China. A most recent study showed for the first time that LWDH concentrated pills, when administered in water by gavage feeding, decreased weight gain, blood triacylglycerols (TAG) and improves insulin and leptin sensitivities in obese-prone CD rats [8]. As LWDH concentrated pills are a mixture of raw materials and crude water and ethanol extracts, bioactive components/fractions that are responsible for weight loss are not clear and moreover, the mechanisms of action have yet been elucidated.

It is well known that energy balance determines body weight [9]. Simply, a long term of higher energy intake over expenditure, or positive energy balance, results in the development of overweight and obesity [10]. Therefore, in the present study we obtained water extract (WE) of LWDH concentrated pills and investigated its effects on weight gain and visceral fat mass in obese rats. The objective of the current study was to determine whether the fraction of water extract is responsible for the observed weight-lowering effect of the LWDH concentrated pills. Moreover, we measured food intake, oxygen consumption, carbon dioxide production, fat and carbohydrate oxidations, and energy expenditure, as well as blood hormones related to appetite regulation and the control of caloric intake and energy metabolism, attempting to understand the underlying mechanisms.

2. Materials & Methods

2.1 Preparation and Chemical Profiling of LWDH Water Extract

LWDH concentrated pills are made from six herbs as reported previously [11]. For the preparation of LWDH water extract (WLDH-WE), the unpolished LWDH pills were milled and extracted twice with 95% ethanol, refluxing for 30 min each time with ethanol. The residue was then extracted twice with boiling water (30 min each time). The water was removed at reduced pressure and the extract was dried at 70°C to yield LWDH-WE. The preparation was conducted by the manufacturer of the concentrated pills (Henan Wanxi Pharmaceutical Ltd. Co., Nanyang, Henan, P.R. China). The chemical profiling of LWDH-WE was carried out at the National Research Council Canada (Charlottetown, PE, Canada) with NMR and HPLC-MS using the method reported elsewhere [11].

2.2 Animal Experiment

Thirty-six male obese-prone (OP) CD rats (Charles River Laboratories, Montréal, Québec, Canada), 150 to 170 g, were housed individually in cages in a temperature-controlled room with a 12-hour light: dark cycle. After one week of acclimation with free access to regular rodent chow and water, rats were weighed and randomly divided into 3 groups (n = 12/group). All rats were fed a casein-cornstarch-sucrose-based AIN-93G diet modified to contain 60 Kcal% from fat, 2% cholesterol and 0.5% cholic acid. The energy content is 5.45 kcal/g and fat was provided in a form of lard and sunflower oil mixture (96:4, w/w). LWDH-WE was dissolved in water and was administered orally once a day to two groups of rats at

doses of 600 (WE600) and 1200 (WE1200) mg/kg/d, respectively. The high-fat control group (HFC) was gavaged with the control vehicle. Weekly body weight and daily food intake were obtained throughout the study period. Rats were monitored for behaviours and activity. After 10 weeks of treatment, rats were fasted overnight and anesthetized with inhalation of isoflurane (Pharmaceutical Partners of Canada Inc., Richmond Hill, ON, Canada). Blood samples were collected from the cardiac puncture into serum tubes containing clot activator and plasma tubes containing 500 KIU aprotinin plus 1.25 mg EDTA-2Na/mL, respectively. After centrifugation, serum and plasma were collected, respectively and stored at -80°C for later analysis. For ghrelin assay, plasma was immediately treated with 1/10 volume of 1M HCl and stored at -80°C. Epididymal, perirenal and mesenteric 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 National Research Council Canada and the University of Prince Edward Island. The study was conducted in accordance with the guidelines of the Canadian Council on Animal Care.

2.3 Analysis of Plasma Lipids and Free Fatty Acids

Plasma total cholesterol (T-C), HDL cholesterol (HDL-C) and TAG were analyzed using the enzymatic methods on a pointe-180 chemistry analyzer (Point Scientific Inc., Canton, MI). Non-HDL cholesterol (nonHDL-C) was calculated by subtracting HDL cholesterol from the total cholesterol [12]. The levels of free fatty acids were measured in the serum *using* a free fatty acid quantification kit (BioVision, Mountain View, CA).

2.4 Serum Insulin, Leptin and Adiponectin Measurements

Serum insulin (Crystal Chem, Downer's Grove, IL), leptin and adiponectin (Kamiya Biomedical, Seattle, WA) levels were quantified following the manufacturers' instructions. Standards were run in parallel with the samples. The concentration of each hormone was calculated in reference to the corresponding standard curve and expressed as ng/ml.

2.5 Plasma Ghrelin, Peptide YY (PYY) and Neuropeptide Y (NPY)

Plasma levels of acylated ghrelin were quantified using a rat acylated ghrelin ELISA kit (Biovendor, Candler, NC). The antibody has 100% specificity for rat ghrelin and uses N-acetylcholinesterase (AchE)-Fab' conjugate to recognize only the acylated ghrelin. Plasma PYY levels were measured with an enzyme immunoassay kit (Yanaihara Institute, Shizuoka, Japan), and NPY levels measured with an ELISA kit (Millipore, Billerica, MA). The concentration was calculated in reference to the corresponding standard curve and presented as ng/ml.

2.6 Energy Metabolism and Expenditure

During week 8 of the treatment, rats in each group were placed in a 20-channel open-circuit calorimetry system (Oxymax; Columbus Instruments International, Columbus, OH) for 36 hr to measure energy metabolism. Rats in the calorimeter chambers had free access to food and water. A mass flow controller was used to measure gas concentration (mass) and flow rate in each chamber. The combination of zirconia oxide-based oxygen sensor and high speed beam non-dispersed infra-red carbon dioxide sensor provides the measurement of oxygen consumption (VO_2, ml/ kg/hr) and carbon dioxide production [VCO_2, (ml/kg/hr)] in every 45–60 sec. The relationship between oxygen consumption and carbon dioxide production is related to the energy content of foodstuff utilized. This calorific value (CV) is then applied to the volume of gases exchanged to compute heat production (Kcal/hr). Data were analyzed using the Clax software after discarding the values for the first 2 hr. The final values represent a mean of multi-measurements in a period of 34 hr. VO2 and

VCO2 values were normalized with respect to the animal body weight, corrected according to an effective mass value, and used to calculate the following parameters of energy metabolism as described previously [13].

Respiratory exchange ratio= VCO_2/VO_2

Rate of fat metabolism (mg⁄min⁄kg) = (1.689 * VO_2) – (1.689 *VCO_2)

Rate of carbohydrate metabolism (mg⁄min⁄kg) = (4.12*VCO2) – (2.91 *VO_2)

Rate of energy expenditure (kJ⁄min/kg) = (15.88*VO_2) – (4.87*vCO_2)

2.7 Statistical Analysis

Data were analyzed by one-way ANOVA using SAS 9.2 statistical software (SAS Institute, NC, USA). When a treatment effect was detected, differences among the group means were determined using the least squares means test, with significance level being set at $p < 0.05$. Results are presented as means ± SEM.

3. Results

3.1 Effect of LWDH Water Extract on Body Weight and Food Intake

All rats completed the study and none showed any abnormal behaviors and activities. After 3 weeks of treatment, body weight in the WE1200 group was 10% lower ($p < 0.05$) than the HFC group (Fig. 1). This effect maintained throughout the remaining treatment period. At the end of the 10-week treatment, the WE1200 group had 8.5% ($p < 0.05$) lower body weights than the control. The WE600 group tended to show consistently lower (approximately 3%) body weights than the control but did not reach the significant level. It is suggestive that LWDH-WE suppressed weight gain in obese rats in a dose-dependent manner. As shown in Table 1, rats in the WE1200 group had lower food intake at weeks 3 and 5, receptively, while having no effect in other weeks. The WE600 group did not affect food intake in any week throughout the treatment period. Accumulative food intakes showed a tendency of dose-dependent reduction over the 10-week treatment period after LWDH-WE supplementation although they did not differ among the three groups (supplemental Fig. 1).

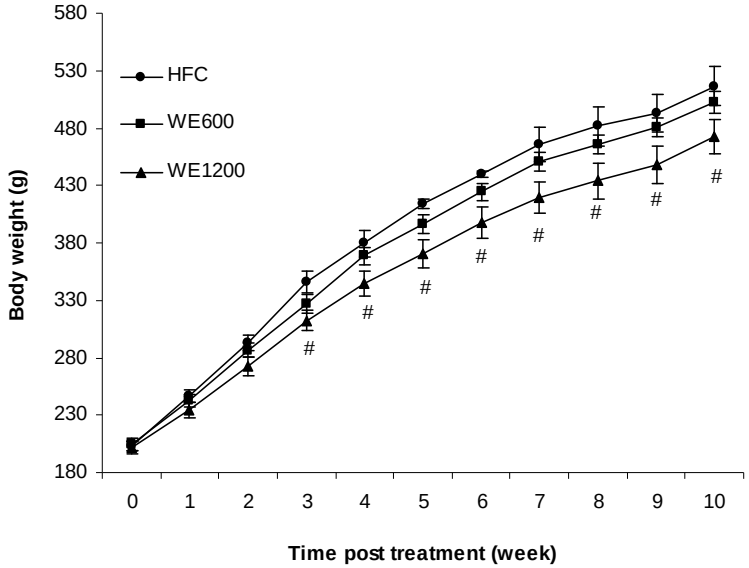
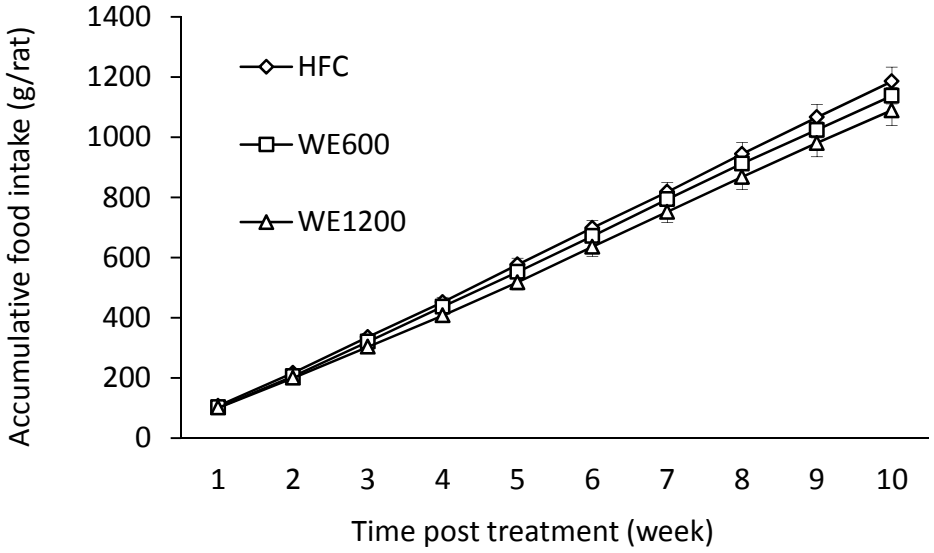


Fig. 1 Effect of LWDH water extract on the body weights of obese-prone CD rats. HFC, rats fed the high-fat diet and gavaged with water; WE600, rats fed the high-fat diet and gavaged with 600 mg/kg/d of LWDH water extract dissolved in water; WE1200, rats fed the high-fat diet and gavaged with 1200 mg/kg/d of LWDH water extract dissolved in water. Data are means ± SEM (n = 12). # different from the HFC group ($p < 0.05$).



Supplemental Fig. 1 Accumulative food intake of rats treated with LWDH water extract. Accumulative food intake was calculated weekly and analyzed statistically using ANOVA with repeated measures. Data are means ±SEM (n = 12). There were no differences among the three groups ($p > 0.05$).

Table 1 Effect of LWDH water extract on food intake of obese-prone rats fed a high-fat diet.

Treatment	Time post treatment (week)									
	1	2	3	4	5	6	7	8	9	10
HFC#	15.3±0.5	15.6±0.4	17.0±0.8	16.6±0.8	17.8±0.7	17.3±0.7	17.1±1.0	17.9±1.2	17.3±1.0	17.0±0.8
WE 600	14.6±0.5	14.7±0.4	16.4±0.5	16.6±0.4	17.8±0.7	17.0±0.4	17.4±0.7	17.9±1.2	15.9±0.9	16.1±0.5
WE 1200	14.5±0.7	14.1±0.7	14.7±0.6*	14.8±0.9	15.7±0.8*	16.8±0.8	16.5±0.8	16.6±0.9	16.0±1.1	15.4±0.7

HFC, rats fed the high-fat diet and gavaged with water; WE600, rats fed the high-fat diet and gavaged with 600 mg/kg/d of LWDH water extract dissolved in water; WE1200, rats fed the high-fat diet and gavaged with 1200 mg/kg/d of LWDH water extract dissolved in water. Data are means ± SEM (g/d, n = 12). *Different from the HFC group ($p < 0.05$).

3.2 WE1200 Decreases Mesenteric, Perirenal and Epididymal Fat Depositions

The weight of visceral fat pads was calculated as a percentage of the corresponding body weight (Table 2). The data showed that WE1200 decreased ($p < 0.05$) relative weights of the perirenal, epididymal and mesenteric fats as well as the percentage of the total visceral fat mass (sum of these three regions) as the percentage of the corresponding body weight compared to the HFC group. WE600 did not significantly affect the relative weights of the perirenal, epididymal and mesenteric fats and the total visceral fat mass.

3.3 WE1200 Alters Fat and Carbohydrate Metabolisms and Energy Expenditure

It should be noted that all parameters measured for energy metabolism were normalized to the effective body mass. In both the light and dark periods, rats in the WE1200 group had higher ($p < 0.05$) oxygen consumption and carbon dioxide production compared to controls (Tables 3 and 4). During the light period, WE1200 increased ($p < 0.05$) fat and carbohydrate oxidations and energy expenditure. In the dark period, WE1200 enhanced ($p < 0.05$) fat oxidation and energy expenditure, whereas did not significantly affect carbohydrate oxidation. The respiratory exchange ratio (RER) was unaffected in both the light and dark

periods, and the values were lower than 0.8, indicating that fat oxidation was predominant, in line with the nature of the high-fat diet. It was interestingly noted that energy metabolism and expenditure were higher in the dark period than the light period. Nevertheless, the magnitude of the increase upon LWDH-WE supplementation was larger in the light period than in the dark period. For example, WE1200 increased fat oxidation, carbohydrate oxidation, and energy expenditure by 7%, 15%, and 9% in the light period while by 7%, 3% and 6% in the dark period, respectively. As LWHD-WE promoted the oxidation of both fat and carbohydrate, the ratio of energy expenditure to fat oxidation rate or carbohydrate oxidation rate was calculated. It was shown that LWDH-WE did not alter the efficiency of fat or carbohydrate oxidation towards energy expenditure, meaning that the increased energy expenditure by LWDH-WE was due to the increases in both fat and carbohydrate oxidations.

3.4 LWDH Water Extract Reduces Serum Cholesterol and Free Fatty Acids

As illustrated in Fig. 2, WE1200 decreased ($p < 0.05$) serum T-C and nonHDL-C levels as compared with the HFC group, whereas WE600 had no effect. Either dose of LWDH-WE had no effect on serum HDL-C levels and TAG levels. The levels of serum free fatty acids were decreased ($p < 0.05$) in both WE1200 and WE600 groups relative to the HFC group (Table 5).

Table 2 LWDH water extract reduces the relative weight of visceral fat mass in obese-prone rats fed a high-fat diet.

	HFC[#]	WE600	WE1200
Mesenteric[&]	2.50±0.18	2.35±0.16	2.01±0.15[*]
Perirenal	0.47±0.01	0.39±0.02	0.39±0.03[*]
Epididymal	1.86±0.08	1.72±0.13	1.44±0.13[*]
Total fat	4.83±0.25	4.56±0.28	3.85±0.26[*]

[&]Each value is the percentage of the body weight. [#] HFC, rats fed the high-fat diet and gavaged with water; WE600, rats fed the high-fat diet and gavaged with 600 mg/kg/d of LWDH water extract dissolved in water; WE1200, rats fed the high-fat diet and gavaged with 1200 mg/kg/d of LWDH water extract dissolved in water. Data are percentages of the body weights and presented as means ± SEM (g, n = 12). [*]Different from the HFC group ($p < 0.05$).

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

	VO2 (ml/min/kg)	VCO2 (ml/min/kg)	RER	RFO (g/min/kg)	RCO (g/min/kg)	EE (kJ/min/kg)	EE/RFO	EE/RCO
HFC[#]	19.25±0.54	15.08±0.411	0.7799±0.0022	7.16±0.23	5.82±0.19	232.58±6.61	32.54±0.29	40.19±1.18
WE 600	19.62±0.58	15.40±0.46	0.7870±0.0032	7.06±0.22	6.52±0.306	236.41±7.04	33.56±0.43	36.80±1.59
WE 1200	21.04±0.40[*]	16.49±0.35[*]	0.7833±0.0032	7.69±0.13[*]	6.70±0.35[*]	253.86±4.71[*]	33.05±0.47	38.78±1.66

Table 4 Effect of LWDH water extract on energy metabolism during the dark period in obese-prone rats fed a high-fat diet.

	VO2 (ml/min/kg)	VCO2 (ml/min/kg)	RER	RFO (g/min/kg)	RCO (g/min/kg)	REE (kJ/min/kg)	EE/RFO	EE/RCO
HFC[#]	21.35±0.82	16.59±0.59	0.7787±0.0036	8.02±0.40	6.26±0.18	258.15±10.24	32.45±0.54	41.56±1.97
WE 600	21.37±0.30	16.75±0.23	0.7839±0.0040	7.80±0.20	6.82±0.35	257.86±3.82	33.17±0.57	39.24±2.87
WE1200	22.68±0.46[*]	17.59±1.21[*]	0.7859±0.0073	8.59±0.27*	6.45±0.43	274.55±5.69[*]	32.12±0.63	44.41±3.17

[#] HFC, rats fed the high-fat diet and gavaged with water; WE600, rats fed the high-fat diet and gavaged with 600 mg/kg/d of LWDH water extract dissolved in water; WE1200, rats fed the high-fat diet and gavaged with 1200 mg/kg/d of LWDH water extract dissolved in water. VO2, volume of oxygen; VCO2, volume of carbon dioxide; RER, respiratory exchange ratio; RCO, rate of carbohydrate oxidation; RFO, rate of fat oxidation; REE, Rate of energy expenditure. Data are presented as means ±SEM (n = 12). [*]Different from the HFC group ($p < 0.05$).

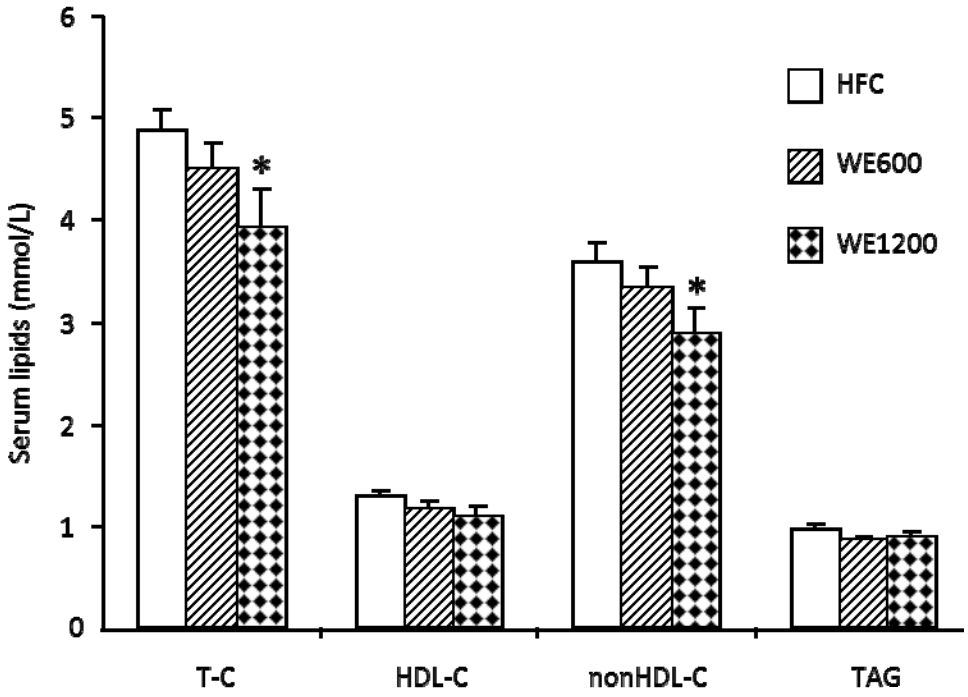


Fig. 2 Effect of LWDH water extract on serum total cholesterol (T-C), HDL cholesterol (HDL-C), nonHDL cholesterol (nonHDL-C) and triacylglycerols (TAG) in obese-prone rats fed a high-fat diet. Data are means ±SEM (n = 12). [*]Different from the HFC group ($p < 0.05$).

Table 5 Serum concentration of free fatty acids, leptin, insulin, ghrelin, neuropeptide Y (NPY), and peptide YY (PYY) in obese rats treated for 10 weeks with LWDH water extract.

	FFA[#] (mmol/ml)	Leptin (ng/ml)	NPY (ng/ml)	PYY (ng/ml)	Ghrelin (ng/ml)	Insulin (ng/ml)	Adiponectin (μg/ml)
HFC[&]	0.67±0.02	7.35±0.12	0.32±0.01	3.12±0.06	1.69±0.22	4.04±0.49	15.3±1.1
WE600	0.62±0.01*	6.89±0.20*	0.30±0.01	2.96±0.08	2.14±0.19	3.73±0.35	12.6±1.7
WE1200	0.60±0.01*	6.85±0.21*	0.31±0.03	2.91±0.10	2.25±0.19	3.33±0.44	12.1±1.0

[#]FFA, free fatty acids, NPY, neuropeptide Y; PYY, peptide YY. [&]HFC, rats fed the high-fat diet and gavaged with water; WE600, rats fed the high-fat diet and gavaged with 600 mg/kg/d of LWDH water extract dissolved in water; WE1200, rats fed the high-fat diet and gavaged with 1200 mg/kg/d of LWDH water extract dissolved in water. Data are presented as means ± SEM (n = 12). [*]Different from the HFC group ($p < 0.05$).

3.5 LWDH Water Extract Reverses Leptin Resistance

Serum leptin concentration was decreased ($p < 0.05$) in rats supplemented with either dose of LWDH-WE (Table 5). Serum insulin and adiponectin levels were not affected by either dose of LWDH-WE (Table 5), with large variations being observed.

3.6 Effect of LWDH Water Extract on Serum Ghrelin, PYY and NPY Levels

Fasting plasma PYY (3–36) levels tended to decrease ($p = 0.07$) in the WE1200group compared to the HFC group. Fasting serum acylated ghrelin and NPY levels were not different among the groups (Table 5).

3.7 Chemical Composition of LWDH Water Extract

As reported recently [11], the NMR data indicate that mono- or oligosaccahrides are the main components of LWDH-WE, along with low contents of phenolics, iridoid glycosides, and other compounds.

Analysis with C-18 column on HPLC revealed five specific compounds, which are gallic acid, 5-hydroxymethyl furfural, sweroside, loganin and paeoniflorin. Further analysis with HILIC column on the HPLC system, along with ELSD and MS information revealed that glycosides of 5-hyrdoxymethyl furfural and gallic acid, saccharide monomers, dimmers, and trimers were the major components.

4. Discussion

Development of obesity is a result of extended energy surplus, which is attributed to either increased energy intake or decreased energy expenditure, or the combination of both factors [14, 15]. Accordingly, treatment of obesity can be achieved through managing either of these two factors, and a better treatment effect would be expected if both the intake and expenditure of energy had been modulated favourably. In the present study, all rats were provided with the same diet, thus the decreases of food intake by WE1200 in weeks 3 and 5, together with the tendency of dose-dependent reduction over the treatment period, suggest that LWDH-WE might have affected appetite and satiation and consequently energy consumption, resulting in the reductions of body weight and fat deposition. This finding is in line with our previous study showing that LWDH concentrated pills reduces food intake and body weight in the same obese rat model[8]. Similar findings have been reported on several other herbs or herbal formulae. For example, animal studies in obese mice showed that the oral administration of parasitic loranthus ethanol extract and galega herb reduced food intake and body weight [16, 17]. Human clinical trials demonstrated that a South American herbal preparation containing YerbeMaté, Guarana and Damiana induced weight loss in obese subjects by reducing hunger feeling and meal size via delaying gastric emptying [18, 19].

Control of caloric intake involves gut hormones such as ghrelin, PYY and neurotransmitter NPY. Ghrelin is a stomach-derived peptide. It is acylated with a medium-chain fatty acid and displays a broad range of activity, from the central control of food intake to peripheral functions such as gastric emptying and insulin secretion [20–23]. PYY, a peptide produced by L cells of the small intestine and rectum. It is secreted after meal ingestion and released into the blood stream to inhibit gut motility and is proposed to stimulate a powerful central satiety response [24, 25]. NPY is a neuron transmitter, which is secreted by hypothalamus and functions to stimulate eating and fat storage [26]. Its expression in the brain is regulated by energetic status and ghrelin and PYY signals. LWDH-WE did not show any significant effect on fasting blood levels of ghrelin, PYY and NPY, in agreement, to certain extent, with previous studies in obese Zucker rats and diet-induced obese mice that fasting blood levels of these hormones were not altered after weight loss [27, 28]. Nevertheless, it is reported that the postprandial blood levels of these gut hormones are significantly different between obese and lean rats or mice, and are regulated by the intake of macronutrients [27, 28]. Evidence suggests that postprandial rather than fasting blood levels of ghrelin, PYY and NPY play an important role in regulating food intake and energy metabolism in rodents [29]. A prospective study on appetite-regulating hormones under fasting and postprandial conditions would help to elucidate whether LWDH has a regulatory effect on appetite and satiation.

In addition to energy intake, energy metabolism and expenditure are crucial in energy balance and weight control. Oxygen is an essential molecule in substrate oxidation, which converts the nutrients into carbon dioxide and water, when completely oxidized, and produce energy for various biological functions, body temperature maintenance and external activities. Therefore, oxygen consumption and carbon dioxide production are commonly used to estimate metabolic

rate and energy expenditure. Fat and carbohydrate are the primary nutrients for energy supply and their oxidations are critical in energy homeostasis, fat storage, body composition and body weight [30]. The relationship between carbon dioxide production and oxygen consumption is also an indicator of which fuel (lipids or carbohydrates) that is predominantly used for energy within the body [15]. WE1200 increased oxygen consumption, carbon dioxide production, and energy expenditure in obese rats in both light and dark periods, providing strong evidence that LWDH-WE reduced weight gain in obese rats by up regulating energy metabolic rate and expenditure. LWDH-WE increased fat oxidation in both the light and dark periods and carbohydrate oxidation only in the light period, indicating that fat oxidation might have played a greater role than carbohydrate oxidation in decreasing weight gain and visceral fat mass by LWDH-WE. There is emerging evidence demonstrating that reduced fat oxidation is the leading cause to the development of obesity [31]. The observed higher metabolic rate and energy expenditure are in accordance with the eating and activity patterns of rats [32]. Furthermore, the larger magnitude of increases in the metabolic rate and energy expenditure during the light period than the dark period suggests that LWDH-WE might have decreased body weight and fat deposition primarily through boosting basic metabolic rate.

It is well-known that leptin plays an important role in energy metabolism and body weight control by regulating food intake and energy expenditure [33, 34]. In obese humans and rodents, the circulating leptin levels are elevated, resulting in leptin resistance and reduced capability to regulate properly appetite/satiation and energy metabolism [33]. The improvement of leptin resistance is beneficial to weight loss and in return, a weight reduction improves leptin sensitivity and thus lowers blood leptin levels. Therefore, the significant reductions of serum leptin upon LWDH-WE supplementation could be attributable to the direct role of LWDH-WE on leptin sensitivity. However, the data of the present study cannot exclude the possibilities that decreased serum leptin levels were a result of the secondary effect of weight reduction following LWDH-WE treatment or a result of the confounded effects of LWDH-EE on both leptin sensitivity and body weight.

It is well established that many metabolic abnormalities associated with obesity are caused by excessive free fatty acids released from adipose tissue into the blood stream [35]. Free fatty acids cause lipotoxicity and their circulating levels are associated with blood lipids and many diseases. Accordingly, normalizing or decreasing blood free fatty acids has been of great interest in managing metabolic disorders, especially diabetes and insulin resistance [36]. Thus, the decreased serum free fatty acids by the high-dose of LWDH-WE were considered beneficial to the management of metabolic disorders and complications associated with obesity. The reduction of serum free fatty acids might be a result of increased fat oxidation and thus fat mass. Similar effects of LWDH-WE were reported in diabetic rats [37]. The decrease in fat deposition by LWDH-WE might have also been related to the increased carbohydrate oxidation and energy expenditure, which reduced the amount of substrate for fatty acid and triacylglycerol syntheses. Amongst the complications of obesity, dyslipidemia has long been accepted as a primary risk factor in developing atherosclerosis and cardiovascular diseases [38]. WE1200 significantly lowered serum total and non HDL cholesterol levels in accordance with previous reports [39, 40]. In high-fat diet-induced hyperlipidemic male SD rats, the oral administration of LWDH-WE significantly lowered serum total cholesterol, LDL cholesterol and TAG levels while raising HDL cholesterol levels [39]. Further, in a clinical study LWDH pills lowered blood cholesterol and TAG levels in patients with nephrotic syndrome [40].

The chemical profiling of LWDH water extract is in agreement with the published results, except for saccharides that were not reported previously [41–43]. It is not surprising to see this discrepancy as previous studies analyzed methanol extracts while the current study was focused on water extract. Several studies have demonstrated that gallic acid [44, 45] and paeoniflorin [46] prevent weight gain, inhibit adipocyte proliferation, induce adipolysis, and lower blood lipids. To our knowledge, saccharides do not have weight- and lipid-lowering properties. It is not known about the functions of other bioactive compounds contained in LWDH-WE, including 5-hydroxymethyl furfural, sweroside, and loganin, on energy intake and metabolism. Thus, more studies are warranted to determine the effect and mechanism of action of each bioactive compound in LWDH-WE in modulating food intake and energy metabolism and importantly to optimize the combination of these individual bioactive compounds to produce maximal benefits of decreasing body weight and improving body composition and metabolic complications.

5. Conclusion

LWDH-WE suppressed weight gain and visceral fat deposition, improved leptin sensitivity, and lowered blood cholesterol and non-esterified fatty acids, which are the collective characteristics of the metabolic syndrome. Increases in oxygen consumption, carbon dioxide production, fat and carbohydrate oxidations and energy expenditure, along with decrease in energy intake, explain the reductions of body weight and visceral fat mass by LWDH-WE. The results have demonstrated the potential of LWDH-WE for the management of body weight, body composition and metabolic syndrome.

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