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Water Extract of *Houttuynia cordata* Thunb. Leaves Exerts Anti-Obesity Effects by Inhibiting Fatty Acid and Glycerol Absorption

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Summary *Houttuynia cordata* Thunb. is used in folk medicine for diuresis and detoxification. However, it has not yet been reported to have an anti-obesity effect. We found that the water extract of *H. cordata* leaves (WEH) inhibited the corn oil-induced increase in plasma triglyceride levels in mice. WEH also inhibited the oleic acid- and glycerol-induced increase in the levels of plasma nonesterified fatty acids and glycerol, respectively. Moreover, WEH had anti-obesity effects in mice with high-fat-diet-induced obesity. Therefore, WEH may be able to prevent or reduce obesity induced by a high-fat diet.

Key Words *Houttuynia cordata* Thunb., anti-obesity, fatty acid, glycerol, triglyceride

Obesity is an abnormal condition in which excessive triglycerides (TGs) accumulate in the adipose tissue. In Japan, the number of obese people older than 20 y (body mass index, >25) has been increasing yearly because of inadequate exercise, westernization of diet, and irregular eating habits. According to a national health and nourishment investigation conducted by the Ministry of Health, Labour and Welfare in 2007, about 1 of 4 Japanese adults is obese. Obesity is the most important risk factor for lifestyle-related diseases such as hypertension, type 2 diabetes and hyperlipemia (*1–4*). In particular, obesity causes an imbalance in the level of adipocytokines secreted by adipocytes, such as leptin, adiponectin, resistin, and plasminogen activator inhibitor-1, due to the excessive accumulation of visceral fat, and can cause metabolic syndrome (*5*, *6*). Furthermore, when symptoms worsen, metabolic syndrome can develop into atherosclerotic disease, which is associated with a very high mortality rate (*7*, *8*). Therefore, it is important to prevent or reduce obesity to achieve a better health-related quality of life.

Houttuynia cordata Thunb. (Saururaceae), which is called *dokudami* in Japanese, is widely distributed in eastern Asia, including China, Korea, and Japan, and is used in folk medicine for diuresis and detoxification. It contains many flavonoids (quercitrin, isoquercitrin, rutin, etc.), alkaloids (aristolactam B, norcepharadione B, splendidine, etc.), and volatile components of essential oils (methyl-*n*-nonyl ketone, lauraldehyde, β-myrcene, etc.) (*9–11*). Thus far, the extracts and components of *H. cordata* have been demonstrated to exhibit antioxidative, antiviral, antibacterial, antihypertensive and anti-inflammatory effects (*12–17*). However, these extracts have not yet been reported to have anti-obesity effects. Therefore, in this study we examined the anti-obesity action of *H. cordata*.

MATERIALS AND METHODS

Materials. The Free Glycerol Determination Kit and Lipase Kit S were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and DS Pharma Biomedical Co. Ltd. (Osaka, Japan), respectively. Porcine pancreatic lipase was obtained from ICN Biomedicals (Aurora, OH, USA). The mouse diet was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). The other materials were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of the water extract of H. cordata leaves (WEH). *H. cordata* leaves were collected from the premises of Tokyo University of Marine Science and Technology. The leaves were stored at -20°C until extraction. The water extract was obtained by boiling 4 kg of *H. cordata* leaves in 40 L of distilled water for 1 h. The extract was filtered and the filtrate was concentrated under reduced pressure and then lyophilized into a dry powder. The powder was stored at -20°C until use. The extract yield from the fresh leaves was about 5%.

Inhibitory effects of pancreatic lipase. The inhibitory effects of lipase were measured using porcine pancreatic lipase and the Lipase Kit S. We pre-incubated 5 μL of pancreatic lipase (1 unit/mL), 2 μL of an esterase inhibitor (phenyl methyl sulfonyl fluoride), and 73 μL of a chromogenic reagent (5,5′-dithiobis-(2-nitrobenzoic acid)) in 96-well plates at 30°C for 5 min. We then added 10 μL of WEH solution (final concentration, 15.6–500 μg/mL) or distilled water as a control and 10 μL of substrate solution (dimercaprol tributyrate and sodium dodecyl sulfate) to each well, and incubated the mixtures at 30°C for 30 min ($n=3$). The reaction was stopped with 200 μL of stop reagent (sodium dodecyl sulfate), and the absorbance of the supernatant was

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measured at 405 nm. The substrate solution was added to a blank solution after addition of the stop reagent. The lipase activity was calculated as Lipase activity (%)=100×[(Absorbance of the test sample solution−Absorbance of the blank solution)/(Absorbance of the control solution−Absorbance of the blank solution)].

Animals. Male and female ddY mice (6 and 3 wk old, respectively) were obtained from Japan SLC, Inc. (Shizuoka, Japan). They were housed in cages (4 individuals/cage) under a 12 h/12 h light/dark cycle (lights from 8:00 to 20:00) in a room with controlled temperature and humidity (24±1°C and 50±2%, respectively). Animal studies were performed in accordance with Notification No. 88 of the Ministry of the Environment, Japan (2006) and the Guidelines for Animal Experimentation of Tokyo University of Marine Science and Technology, with the approval of the Animal Care and Use Committee of Tokyo University of Marine Science and Technology.

Loading test of TG and its metabolites. To evaluate whether WEH suppressed the absorption of TG and its metabolites, we administered TG, nonesterified fatty acid (NEFA), and glycerol to male ddY mice (6 wk old) that were fed a normal diet (ND) (MR stock; Nosan, Tokyo, Japan) for 1 wk and provided water ad libitum. The mice were fasted for 24 h and then divided into 2 or 4 groups (n=6, 8). Distilled water or WEH solution (20 mL/kg) was orally administered to each group before the oral administration of corn oil (8 mL/kg), oleic acid (8 mL/kg), or glycerol (0.6 mL/kg). The dosage of WEH ranged from 250 to 1,000 mg/kg. Blood was collected from the tail vein and centrifuged at 1,400×g at 4°C for 1 min to obtain plasma. The plasma TG, NEFA, and glycerol levels were measured using the Triglyceride E-test Wako Kit, NEFA C-test Wako Kit, and Free Glycerol Determination Kit, respectively, according to the protocols of the respective manufacturers. The area under the curve (AUC) was calculated using the time-course plot of the plasma TG level as an index of the total TGs absorbed.

Measurement of the amount of residual NEFA and glycerol in the small intestine. Emulsions were prepared by mixing 1 mL oleic acid, 5 mL distilled water, and 580 mg sodium cholate with or without 300 mg of WEH overnight at 37°C and 180 rpm. Male mice (6 wk old) were fed the ND for 1 wk and divided into 2 groups (n=3, 5) after being fasted for 24 h. After being anesthetized, their abdomens were opened and 1 mL of the emulsion or a 3% (v/v) glycerol solution was injected into the small intestine from the pylorus. Both sides of the small intestine, into which the emulsion or glycerol solution was injected, were surgically sutured. After 15 or 20 min, the sutured part was excised and the contents of the intestine were collected. The inside surface of the sutured part was washed with 5 mL saline, and the wash solution was mixed with the contents. We added 4 mL of hexane to the mixture to extract NEFA. This solution was vortexed and centrifuged at 3,000×g at 20°C for 20 min to collect the upper layer (hexane layer) containing oleic acid. This hexane extraction was repeated twice. The collected upper layer was dried using nitrogen gas. The mixture containing glycerol was dried using a rotary evaporator and a lyophilizer. Next, 1 mL of 2-propanol was added to the residue, and the resulting solution was used for the measurement of NEFA and glycerol levels.

Anti-obesity test. We formulated experimental diets based on the AIN-93M diet (Table 1) (*18*). To mimic a westernized diet rich in animal fat, we used beef tallow as the fat in the experimental high-fat diets (HFDs). WEH was administered to the mice as part of the HFD rather than mixed with the drinking water so that an anti-obesity effect could be more effectively demonstrated. Female mice (3 wk old) were fed a ND for 1 wk and then divided into 4 groups: ND, HFD, 1% WEH, and 2% WEH groups. Body weight and food intake were measured twice a week. After the mice were fed these diets for 11 wk, the mice were fasted overnight and

Table 1. Composition of mouse diets in the anti-obesity test.

Ingredient (g/kg)	Group			
	ND	HFD	1% WEH	2% WEH
β-Cornstarch	465.692	105.692	105.692	105.692
α-Cornstarch	155	155	155	155
Milk casein	140	140	140	140
Sucrose	100	100	100	100
Beef tallow	40	400	400	400
Cellulose	50	50	40	30
Mineral mix	35	35	35	35
Vitamin mix	10	10	10	10
L-Cystine	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5
tert-Butylhydroquinone	0.008	0.008	0.008	0.008
WEH	0	0	10	20
Calories (kcal/kg)	3,861	5,672	5,682	5,692

ND, nomal diet; HFD, high-fat diet; WEH, water extract of *Houttuynia cordata* Thunb. leaves.

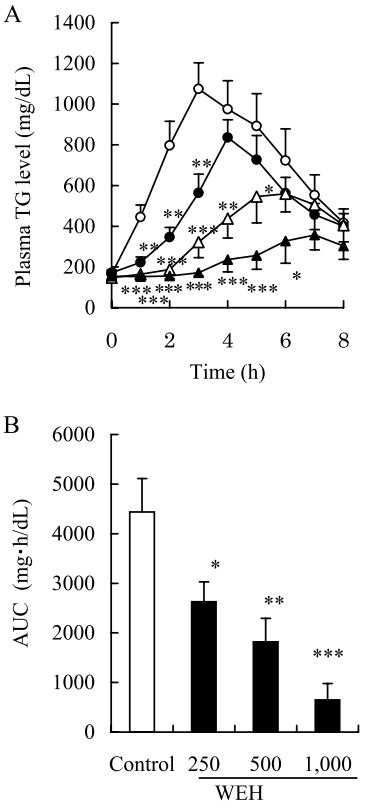

Fig. 1. Effect of water extract of *Houttuynia cordata* Thunb. leaves (WEH) on triglyceride (TG) absorption after oral administration of corn oil in mice. (A) Distilled water (20 mL/kg) and WEH (250, 500, and 1,000 mg/20 mL/kg) were administered to the mice in the control group (unfilled circles) and the WEH 250 mg/kg (filled circles), WEH 500 mg/kg (unfilled triangles), and WEH 1,000 mg/kg (filled triangles) groups, respectively, before the oral administration of corn oil (8 mL/kg). (B) The level of total absorbed TGs was evaluated using the area under the curve (AUC). Data (n=8) are presented as mean±SE. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.005$ vs. the control group.

blood was collected from the tail vein to measure blood parameters. The mice were killed by cervical dislocation, and the parauterine adipose tissue and liver were dissected and weighed. The TG and total cholesterol (TC) in the liver were extracted using the procedure described by Folch et al. (*19*), and their levels were measured with the Triglyceride E-test Wako Kit and Total Cholesterol E-Test Wako Kit.

Statistical analysis. Data were presented as mean± SE and analyzed by one-way ANOVA and the Dunnett test. Differences between groups were considered to be statistically significant at $p<0.05$.

RESULTS

Influence of WEH on fat absorption

The elevation of plasma TG levels was significantly lower in the WEH groups than in the control group 1–6 h after the administration of corn oil (Fig. 1A). The AUC for the WEH groups decreased in a dose-dependent manner (Fig. 1B). This shows that WEH had an inhibitory effect on fat absorption.

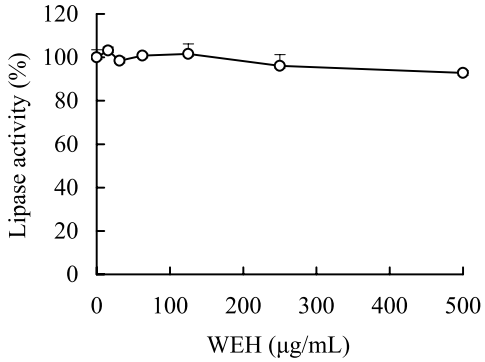

Fig. 2. Effect of WEH on pancreatic lipase activity in vitro. WEH: water extract of *Houttuynia cordata* Thunb. leaves. Data (n=3) are presented as the mean±SE.

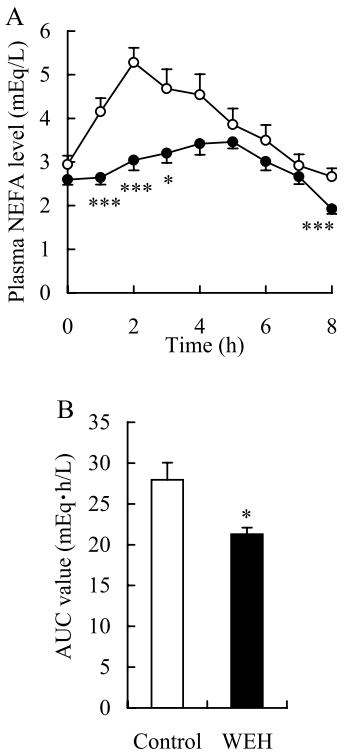

Fig. 3. Effect of WEH on the plasma NEFA level after the oral administration of oleic acid in mice. (A) Distilled water (20 mL/kg) and WEH (water extract of *Houttuynia cordata* Thunb. leaves, 1,000 mg/20 mL/kg) were administered to the mice in the control (unfilled circles) and WEH (filled circles) groups, respectively, before the oral administration of oleic acid (8 mL/kg). (B) The level of total absorbed nonesterified fatty acid (NEFA) was evaluated using the area under the curve (AUC). Data (n=8) are presented as the mean±SE. $^{*}p<0.05$, $^{***}p<0.005$ vs. the control group.

Influence of WEH on pancreatic lipase activity

The pancreatic lipase activity in the WEH groups was not significantly lower than that in the control group, which suggests that WEH had little influence on pancreatic lipase (Fig. 2).

Influence of WEH on the plasma NEFA and glycerol levels in the loading test in mice

The elevation of plasma NEFA levels in the WEH group was significantly lower than that in the control

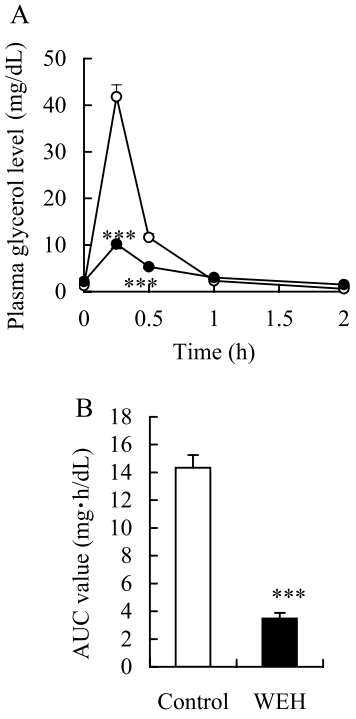

Fig. 4. Effect of WEH on the plasma glycerol level after the oral administration of glycerol in mice. (A) Distilled water (20 mL/kg) and WEH (water extract of *Houttuynia cordata* Thunb. leaves, 1,000 mg/20 mL/kg) were administered to the mice in the control group (unfilled circles) and WEH group (filled circles), respectively, before the oral administration of glycerol (0.6 mL/kg). (B) The level of total absorbed glycerol was evaluated using the area under the curve (AUC). Data (n=6) are presented as the mean±SE. ***p<0.005 vs. the control group.

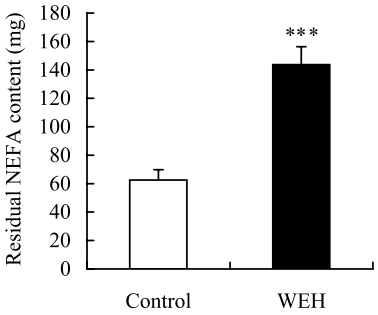

Fig. 5. Effect of WEH on the residual nonesterified fatty acid (NEFA) content in the lumen of the small intestine after injection of the emulsion in mice. One milliliter of the emulsion was injected into the mice in the control group without WEH (Water Extract of *Houttuynia cordata* Thunb. Leaves) (unfilled circles) and the WEH group with 50 mg of WEH (filled circles), respectively. Data (n=5) are presented as the mean±SE. ***p<0.005 vs. the control group.

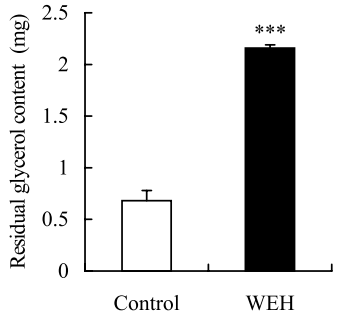

Fig. 6. Effect of WEH on the residual glycerol content in the lumen of the small intestine after the injection of glycerol in mice. One milliliter of 3% (v/v) glycerol was injected into the mice in the control group without WEH (Water Extract of *Houttuynia cordata* Thunb. Leaves) (unfilled circles) and the WEH group with 100 mg of WEH (filled circles), respectively. Data (n=3) are presented as the mean±SE. ***p<0.005 vs. the control group.

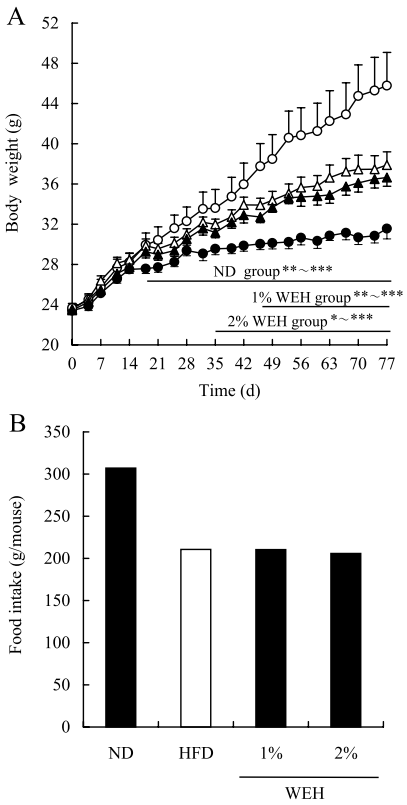

Fig. 7. Effect of WEH on the body weight (A) and food intake (B) in mice in which obesity was induced by the administration of HFD for 11 wk. Unfilled circles, high-fat diet (HFD) group; filled circles, normal-diet (ND) group; unfilled triangles, 1% WEH group; filled triangles, 2% WEH group. Data (n=8) are presented as the mean±SE. *p<0.05, **p<0.01, ***p<0.005 vs. the HFD group in Panel A. Data are presented as the total amount of food intake in each group in Panel B.

group 1–3 h after the administration of oleic acid (Fig. 3A). The AUC for the WEH group was significantly lower than that for the control group (Fig. 3B).

The elevation of plasma glycerol levels in the WEH group was significantly lower than that in the control group 15 and 30 min after glycerol administration (Fig. 4A). The AUC for the WEH group was significantly lower than that for the control group (Fig. 4B).

Influence of WEH on residual NEFA and glycerol contents in mouse small intestine

At 20 min after the injection of NEFA into the mouse intestine, the residual NEFA content in the small intes-

Table 2. Parameters associated with the lipid metabolism of mice.

	Group			
	ND	HFD	1% WEH	2% WEH
Adipose tissue (mg/g of BW)	28.0±4.5***	98.0±14.3	61.9±3.5**	50.0±4.6***
Liver (mg/g of BW)	36.4±1.8	31.8±1.3	34.0±1.0	34.4±0.5
Hepatic TG (mg/g of liver)	95±5***	160±6	136±5*	101±7***
Hepatic TC (mg/g of liver)	7.6±0.2	9.0±0.8	6.7±0.6**	6.4±0.4**
Plasma TG (mg/dL)	98±7***	131±5	107±5***	106±3***
Plasma TC (mg/dL)	130±5***	197±17	133±10**	141±6**
Plasma NEFA (mEq/L)	1.58±0.06***	1.87±0.04	1.50±0.05***	1.47±0.03***
Plasma glycerol (mg/dL)	4.2±0.2***	5.5±0.2	4.4±0.1***	4.0±0.2***
Plasma glucose (mg/dL)	78.1±3.5**	93.6±4.6	73.3±2.8**	75.4±1.1**

ND, normal diet; HFD, high-fat diet; WEH, water extract of *Houttuynia cordata* Thunb. leaves; TG, triglyceride; TC, total cholesterol; NEFA, nonesterified fatty acid.

Data are presented as the mean±SE (n=8). *p<0.05, **p<0.01, ***p<0.005 vs. the HFD group.

tine in the WEH group was significantly higher than that in the control group (Fig. 5).

At 15 min after the injection of glycerol into the mouse intestine, the residual glycerol content in the small intestine in the WEH group was significantly higher than that in the control group (Fig. 6).

Influence of WEH on body-weight gain and food intake in mice fed the HFD for 11 wk

The body weight in the HFD group was significantly higher than that in the ND group on the 18th day of feeding. This increase in body weight was suppressed to a significantly greater extent in the 1% and 2% WEH groups than in the HFD group after days 46 and 35, respectively (Fig. 7A). There was very little difference in food intake between the HFD and 1% and 2% WEH groups (Fig. 7B).

Effects of WEH on parauterine adipose tissue, liver weight, hepatic lipid content, and plasma parameters in mice fed the HFD

The weight of parauterine adipose tissue in both the 1% and 2% WEH groups was significantly lower than that in the HFD group (Table 2). In contrast, there was no difference in liver weight among the 4 groups. The hepatic TG and TC levels in the 1% and 2% WEH groups were significantly lower than those in the HFD group. The levels of plasma TG, TC, NEFA, glycerol, and glucose in both the 1% and 2% WEH groups were also significantly lower than those in the HFD group.

DISCUSSION

Several studies are currently being conducted to identify food materials that can help prevent and reduce obesity. In this study, we found that WEH inhibited the elevation of plasma TG levels in mice that were administered oil (Fig. 1). We also studied the mechanism that underlies this anti-obesity effect of WEH.

WEH inhibited fat absorption in a dose-dependent manner. Interestingly, the time of the peak plasma TG level was delayed as the dosage of WEH increased. This is probably because the active ingredients of WEH are absorbed from the small intestine faster than TG. The absorbability of TG increases with a decrease in the amount of active ingredients in the intestinal lumen. If we consider that an effective dose remains in the intestinal lumen longer as the dosage increases and that elevation of the plasma TG level is gradually hindered, the time of the peak is thought to be delayed in proportion to the dosage of WEH. The elevation of the plasma TG level was also not suppressed by treatment with WEH at 125 mg/kg (data not shown). Therefore, the minimal effective concentration was 250 mg/kg.

We believe that WEH suppressed fat absorption in the small intestine through 2 mechanisms: (1) inhibition of the activity of digestive lipases, which hydrolyze TG to NEFA and monoglycerides or glycerol, and (2) decrease in the uptake of these hydrolysates by intestinal epithelial cells. Fat absorption has often been reported to be suppressed by lipase inhibition (*20*–*23*). Therefore, we investigated whether WEH affected pancreatic lipase activity in vitro. However, the reaction rate of lipase did not change after WEH treatment (Fig. 2). Next, we examined whether WEH affected the blood level of NEFA and glycerol after they were administered to mice. The elevation in plasma NEFA and glycerol levels was markedly suppressed by WEH treatment (Figs. 3 and 4). These results confirmed that WEH did not inhibit the hydrolysis of TG by lipase and suppressed the absorption of hydrolysates of TG, such as NEFA and glycerol.

In addition, we found that the residual NEFA and glycerol levels in the small intestine increased after WEH injection (Figs. 5 and 6). Therefore, WEH inhibited the uptake of NEFA and glycerol from intestinal epithelial cells. NEFA is incorporated into intestinal epithelial cells through fatty acid translocase (FAT)/CD36 and plasma membrane fatty acid-binding protein (FABPpm) (*24*, *25*). Glycerol is transported from the brush border membrane of intestinal epithelial cells through channels composed of a protein called aquaporin-7 (AQP7) (*26*, *27*). WEH was thought to suppress the uptake of NEFA and glycerol by blocking FAT/CD36 and/or FABPpm, and also by suppressing AQP7. Copper has been shown to inhibit glycerol transport through AQPs (*26*,

28, *29*), and tetraethylammonium, an amine, has been shown to block AQP1 (*30*). *H. cordata* contains copper and many types of amines (*10*, *31*, *32*). In fact, copper and amines were detected in WEH by flame and the Dragendorff reaction (*33*, *34*), respectively (data not shown). Therefore, these water-soluble and small-molecular-weight components are thought to suppress glycerol absorption via AQP7. Some previous studies have shown that NEFA uptake is inhibited by sulfo-*N*-succinimidyl esters, fatty alcohol, and other NEFA derivatives (*35*, *36*). There have been no reports on the inhibition of NEFA uptake by natural water-soluble compounds. Since the pathways for NEFA and glycerol uptake are different, the components in WEH that inhibit NEFA uptake should be different from those that inhibit glycerol uptake. In addition, WEHs obtained after extraction times of 30 min, 1 h, and 2 h showed almost the same inhibitory effect on TG absorption (data not shown). This result suggested that the active components were not easily resolved by heat. While many lipase inhibitors are commercially available for preventing obesity, there are no compounds that suppress hydrolysate absorption. Therefore, WEH is a novel functional food for preventing and reducing obesity.

The anti-obesity effect of WEH was evaluated using obese mice that were fed a HFD with or without WEH for 11 wk. Female mice (3 wk old), but not male mice (6 wk old), were used in the experiment because it was easier to induce obesity in female mice by the HFD than in male mice (data not shown), and female mice (3–4 wk old) have been used in anti-obesity tests in previous studies (*23*, *37*, *38*). While there was hardly any difference in food intake between the HFD and WEH groups, the body-weight gain in the WEH groups was significantly lower than that in the HFD group (Fig. 7). This anti-obesity effect was confirmed after the long-term intake of WEH, which had an inhibitory effect on NEFA and glycerol absorption. We found that the weight of adipose tissue, the levels of hepatic and plasma lipids, and the level of glucose, all of which increased after HFD intake, decreased with WEH treatment (Table 2). It is well known that obesity causes hyperlipidemia and hyperglycemia (*1*, *4*). We believe that WEH can relieve these pathologic symptoms. Interestingly, the plasma glycerol level increased after HFD intake, and this increase was suppressed by WEH treatment, probably due to the inhibition of glycerol absorption by WEH.

In conclusion, WEH exerted anti-obesity effects by inhibiting NEFA and glycerol absorption from the small intestine in mice. Currently, there are no functional foods or supplements that inhibit the absorption of TG hydrolysates. WEH might be one such food that prevents and reduces obesity.

Acknowledgments

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