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Vitis thunbergii var. *taiwaniana* Extracts and Purified Compounds Ameliorate Obesity in High-Fat Diet-Induced Obese Mice

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ABSTRACT: The increasing prevalence of obesity continues to gain more attention worldwide. In this study, diet-induced obese mice were used to evaluate the antiobesity effects of extracts, fractions, and purified compounds from *Vitis thunbergii* var. *taiwaniana* (VTT). The C57BL/6J mice were fed a 5-week high-fat diet (HF) concurrently with ethanol extracts (Et-ext, 80 mg/kg) from roots (R), stems (S), and leaves (L) by oral gavage daily. Only R-Et-ext interventions showed significant weight reduction in mice compared with those in the HF group; however, mouse plasma contents of total cholesterols (TC), total triglycerides (TG) and low-density lipoproteins (LDL) of all three Et-ext intervened groups showed significant reductions compared with those in the HF group. Furthermore, intervention with the ethyl acetate-partitioned fraction (EA-fra, 60 mg/kg) from R-Et-ext but not the *n*-butanol-partitioned fraction or water fraction from R-Et-ext showed significant weight reduction in mice compared with those in the HF group. The same molecular weights of three resveratrol tetramers, (+)-hopeaphenol, (+)-vitisin A, and (−)-vitisin B, were isolated from the EA-fra of VTT-R. The (+)-vitisin A and fenofibrate (25 mg/kg) but not the (+)-hopeaphenol and (−)-vitisin B interventions showed significant weight reduction in mice compared with those in the HF group. The total feed intake among the HF groups with or without interventions showed no significant differences. The mouse plasma contents of TC, TG, LDL, free fatty acid, and plasma lipase activity of the three resveratrol tetramer-intervened groups showed reductions in the mice compared with those in the HF group. It was proposed that the lipase inhibitory activities of VTT extracts and purified resveratrol tetramers might contribute in part to the antiobesity effect, and these results suggested that VTT may be developed as functional food for achieving antiobesity objectives and requires further investigation.

KEYWORDS: *high-fat diet (HF), lipase, obesity, Vitis thunbergii var. taiwaniana (VTT), (+)-vitisin A*

INTRODUCTION

The increasing prevalence of obesity continues to gain more attention worldwide. It is estimated that more than one billion adults worldwide are overweight (body mass index (BMI) ≥ 25 kg/m^2) and at least 300 million of them are obese (BMI ≥ 30 kg/m^2). The WHO concluded that the main causes include sedentary lifestyles, high-fat and energy-dense diets, and increased urbanization.[1] Recent research showed that 86.3% of all American adults will become overweight or obese by the year 2030 [based on the 66.3% of obesity prevalence in a National Health and Nutrition Examination Study (NHANES) from 1999 to 2004], and 51.1% of them will be obese (based on 32.2% of obesity prevalence in NHANES from 1999 to 2004); in addition, the mean BMI will increase from 27.9 in the years 1999 to 2004 to 31.2 in the year 2030.[2] Obesity is a major contributing factor in over 0.3 million deaths per year in America and entails related economic costs of over $100 billion (US) per year.[3,4] It was estimated that the direct healthcare costs attributed to overweight and obesity (BMI ≥ 25 kg/m^2) will range from $861 to $957 billion US by 2030.[2] Obesity is based on excess fat deposited beneath the skin or surrounding the organs and may initiate inflammation and dyslipidemia, increase blood pressure, and decrease insulin sensitivity, accompanied by abnormal blood glucose, constituting a cluster of multiple metabolic risk criteria for cardiovascular diseases.[5,6] These metabolic risk criteria generally include (1) abdominal obesity (central obesity), (2) hypertriglyceridemia [fasting

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triglycerides (TGs) ≥ 150 mg/dL], (3) low serum high-density lipoprotein cholesterol (HDL ≤ 40 mg/dL), (4) elevated blood pressure (systolic ≥130 mmHg or diastolic ≥85 mmHg), and (5) high fasting blood glucose (≥110 mg/dL); among these, a diagnosis of three out of the five can be recognized as the metabolic syndrome.[6] Obesity associated with diabetes is considered not only to constitute a clinical problem, but also a public health issue in many countries; they are referred to as "the twin epidemics".[1] For obese individuals to prevent these metabolic risk factors leading to the onset of cardiovascular diseases and type 2 diabetes mellitus; weight loss from diet control and aerobic exercise are the first steps in lifestyle changes.[7] In fact, the commercial drugs for antiobesity treatments are very limited, such as orlistat approved in 1998[1,4] to inhibit pancreatic lipases and reduce fat absorption; however, the unpleasant side effects may involve oily spotting, fecal urgency, and abdominal pain.[1,8] Therefore, researchers seek extracts or compounds from natural resources with potential pancreatic lipase inhibitory activities accompanied by mild side-effects.[8] Fenofibrate synthesized in 1974 was proven to be a peroxisome proliferator-activated receptor α (PPARα) agonist, which regulated several gene expression-related lipid and lipoprotein metabolisms, and was later used for treating dyslipidemia,[9,10] hypertriglyceridemia, and obesity.[11−13]

A high-fat diet (HF) was reported to cause diet-induced obesity, dyslipidemia, insulin resistance, and high blood pressure in rodents.[14,15] Several studies focused on antiobesity activity from natural sources in HF-induced rodent models in two ways. In one, the sample was dosed concurrently with HF; such samples included pectin penta-oligosaccharides from hawthorn,[16] persimmon tannins,[17] *Akebia quinata* extracts,[18] and green tea extracts.[19] In the second, rodents were preinduced to obesity by HF for a time, and then the tested samples were dosed concurrently with HF; such samples included rutin and *o*-coumaric acid,[20] yam storage protein of dioscorin,[21] and fermented black soybean.[22] Hsu et al.[23] reported that the 70% ethanol extracts from the roots of *Vitis thunbergii* showed antiobesity effects in C57BL/6 mice at doses of 0.375% and 0.75% (W/W) in extract intervention concurrently with HF for 7 weeks; however, the group treated with 0.75% extracts showed lighter mice weights than those at the beginning, requiring further investigation. The small-leaf grape (the wild grape, *Vitis thunbergii* var. *taiwaniana*, VTT), a variety of *Vitis thunbergii*, has leaves and fruit smaller than those of the grape *Vitis vinifera*. The dried leaves of VTT are frequently used as tea substitutes in Taiwan, and the plant has been classified as an endemic herb by the Endemic Species Research Institute, Council of Agriculture, Taiwan, and also used in folk medicine for the treatment of hepatitis, jaundice, diarrhea, and arthritis.[24] The VTT ethanol extracts from tissue cultures[25] or stem[27] were reported to exhibit antihypertension activities using spontaneously hypertensive rats as models.

The oral administration of 95% ethanol extracts of VTT stems or VTT leaves (20 and 50 mg/kg) once a day concurrently with HF in preinduced obese Wistar rats showed no significant weight reductions compared with those with HF feeding only; however, the impaired glucose tolerance in obese rats was improved after VTT extract interventions.[26] Therefore, in this study, extracts, fractions, and purified compounds from VTT were dosed concurrently with HF. The C57BL/6J mice were fed with HF concurrently with ethanol extracts (Et-ext) from roots (R), stems (S), and leaves (L) by oral gavage daily to investigate the possibilities of antiobesity activity by VTT extracts. Furthermore, the Et-ext from VTT-R was fractionated into ethyl acetate-partitioned fraction (EA-fra), *n*-butanol-partitioned fraction (BuOH-fra), and water fraction (water-fra), and these were also used for the antiobesity experiments. Three resveratrol tetramers were purified from EA-fra of R-Et-ext and used for the antiobesity experiments, while fenofibrate was used for comparison. The plasma lipase activities of purified compound interventions *in vivo* were assayed to find the possible antiobesity mechanisms of VTT. It was proposed that lipase inhibitory activities might contribute in part to the antiobesity activities. These results suggested that VTT may be developed as a functional food for antiobesity, and this requires further investigation.

MATERIALS AND METHODS

Materials. The fenofibrate was purchased from Sigma Chemical Co. (St. Louis, MO). The HF diet for diet-induced obesity (in D12492, fat composition provided 60% of total 4057 kcal) was purchased from Research Diets, Inc. (NJ, USA). The standard mouse/rat chow was used as the normal diet (in 5P14 Diet, fat composition provide 12% of total calories, Prolab RMH2500) was purchased from PMI Nutrition International (MO, USA). The nonesterified fatty acids (NEFA) assay kit (FA115) was purchased from Randox Laboratories Ltd. (UK). The lipase activity assay kit (LIPASE-PS kit) was purchased from Trinity Biotech USA, NY (USA). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). For pure compound identification, optical rotation measurements were done with a JASCO DIP-180 digital spectropolarimeter; NMR spectra were analyzed in $CDCl_3$ at room temperature (rt) using a Varian Mercury plus 400 NMR spectrometer; electrospray ionization mass was determined on a VG platform electrospray mass spectrometer; thin-layer chromatography was performed on silica gel 60 F254 plates (Merck, Germany); silica gels (230−400 mesh ASTM, Merck) were used for column chromatography; the semipreparative HPLC was performed on a Hitachi L-7000 chromatograph with a Thermo Betasil C-18 column (5 μm, 250 mm × 10 mm).

Preparation of VTT Extracts and Fractions. The L, S, and R of VTT were collected in Taichung County, Taiwan, in July 2006; they were cultivated by Dr. Wen, Chi-Luan (Taiwan Seed Improvement and Propagation Station, Council of Agriculture, Taichung, Taiwan), and identified by Dr. Hsu, Tsai-Wen (Endemic Species Research Institute, Nantou, Taiwan). The voucher specimens were kept in the laboratory of Dr. Chi-I Chang, Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung, Taiwan, for reference. All the plant materials were oven-dried at 50 °C for 3 days and mechanically ground to a fine powder passing through a 10 mesh sieve. Each powder sample of L, S, and R (0.5, 0.5, and 3 kg, respectively) was extracted with ethanol (W/V = 1:5) at room temperature for 7 days, and the ethanol extract was filtered and evaporated under reduced pressure to get each Et-ext. For the fraction preparations, the powdered dried VTT roots (5.3 kg) underwent the above-mentioned method to get R-Et-ext (665.3 g), which was suspended in H_2O and then partitioned sequentially, using ethyl acetate (EA) and *n*-butanol (BuOH) as the solvents, respectively, to get EA-fra (507 g), BuOH-fra (20.7 g), and water-fra (97 g).

Compounds Isolated from R-Et-EA-Fra. The R-Et-EA-fra (285 g) was chromatographed on a silica gel column (150 cm × 12 cm), using solvent mixtures of *n*-hexane and EA with increasing polarity as eluents. Twenty-five fractions were collected. Stilbenoid compounds, rich in the *Vitis* genus plants, were considered as the active constituents. All 25 fractions were analyzed by HPLC method and monitored at 280 nm, the characteristic UV absorption wavelength of stilbenoids. Fraction 24 was found to have the highest absorption and was thus selected for further purification. Fraction 24 (65 g) was further chromatographed on a RP-18 column (5 cm × 60 cm) and eluted with water−methanol (1:1 to 0:1) to afford eight fractions (each 100 mL), fra 24A to fra 24H. The fra 24E was further purified by

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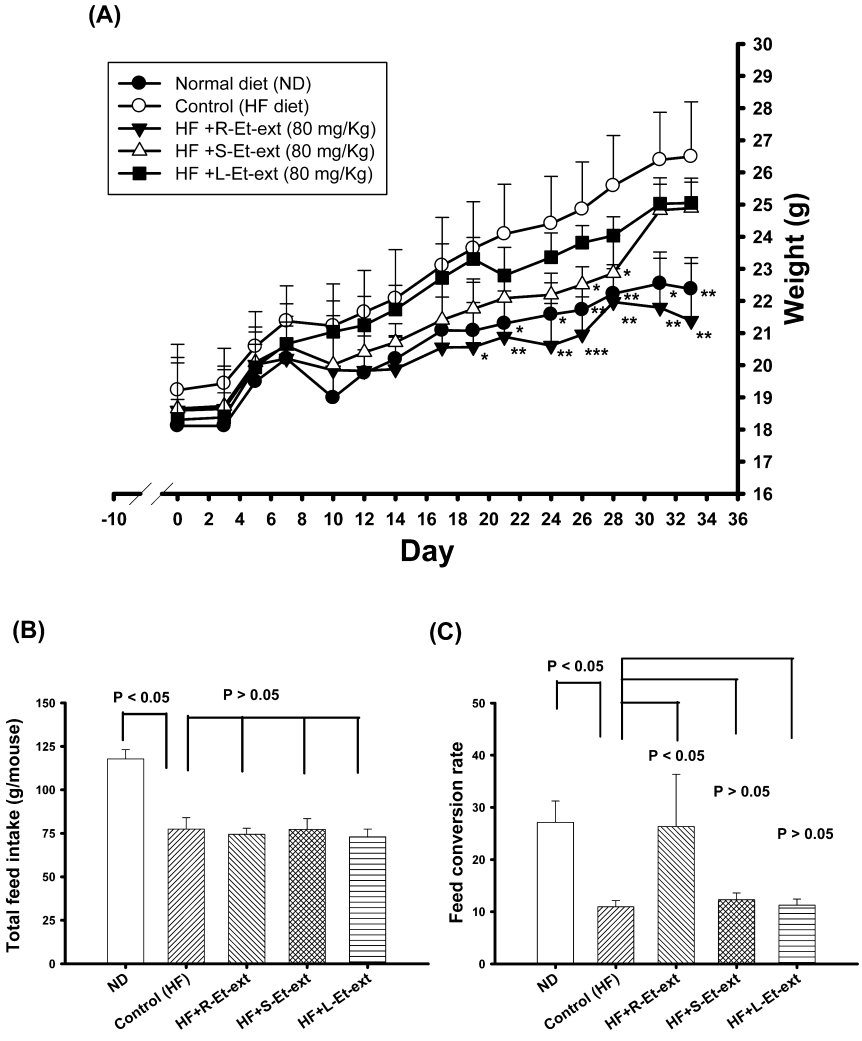

Figure 1. (A) Effects of ethanol extract (Et-ext) interventions from different parts of *Vitis thunbergii* var. *taiwaniana* (VTT) (VTT-R, roots; VTT-S, stems; VTT-L, leaves) on high-fat (HF) diet-induced obesity in C57BL/6 mice. The standard mouse/rat chow was used as the normal diet (ND) (Prolab RMH2500, 5P14 Diet). Each extract (80 mg/kg of body weight) was administered by oral gavage daily together with HF diets during the experiments. (B) Total feed intake at the end of experiment. (C) Feed conversion rate, [total feed intake (g)]/[mouse weight gain (g)], during experimental periods. The three-week-old C57BL/6J mice (N = 60) were acclimated for 1 week; the mice were randomly divided into five groups (N = 12 for each group) for 33 days. Values in extract-intervention animal experiments between treated group and the control group (HF only) or ND group and the control group (HF only) were analyzed using Student's t-test, and a difference compared with the control group (HF only) was considered statistically significant when $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

semipreparative HPLC using a Thermo Betasil C-18 column eluted with water−acetonitrile (9:1 to 1:1), 2 mL/min, to get (+)-hopeaphenol (39.1 mg, retention time, 43.7 min) and (+)-vitisin A (41.1 mg, retention time, 50.9 min), respectively. The fra 24F was further purified by semipreparative HPLC using a Thermo Betasil C-18 column eluted with water−acetonitrile (9:1 to 6:4), 2 mL/min, to get (−)-vitisin B (55.1 mg, retention time, 53.7 min). The three isolated purified compounds in the present research were the same as in a previous report, in which the compounds were isolated from VTT stems.[26] The above HPLC method was employed in the repeated injections for the collection of appropriate sample amount required for *in vivo* study.

Effects of VTT Extracts, Fractions, and Purified Compounds on HF-Induced Obesity in C57BL/6 Mice. Male C57BL/6J mice were purchased from the National Laboratory Animal Center (Taipei, Taiwan) and housed in wire-bottomed stainless steel cages in a temperature- and humidity-controlled room (at 22 °C) with a 12-h light/dark cycle; they had free access to feed and water. All animal experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee, Taipei Medical University (LAC-99-0136). In order to ascertain the effects of the VTT extracts on antiobesity, the three-week-old C57BL/6J mice (N = 60) were acclimated for 1 week; the mice were randomly divided into five groups (N = 12 for each group), including one normal diet (ND) group, one HF group as the control, and three VTT-Et-ext intervention groups (R-Et-ext, S-Et,-ext and L-Et-ext) for 33 days. In the Et-ext intervention groups, each extract (80 mg/kg) was administered by oral gavage daily, together with HF diets during the experiments. In order to determine the effects of VTT partitioned fractions from R-Et-ext on antiobesity, the three-week-old C57BL/6J mice (N = 50) were acclimated for 1 week; the mice were randomly divided into five groups (N = 10 for each group), including one ND group, one HF group as the control, and three fraction-intervention groups (EA-fra, BuOH-fra, and water-fra) for 36 days. In the fraction-intervention groups, EA-fra (60 mg/kg), BuOH-fra (5 mg/kg), and water-fra (15 mg/kg) were administered by oral gavage daily together with HF diets from the beginning to the end. To examine the effects of the purified compounds from R-Et-EA-fra on antiobesity, the three-week-old C57BL/6J mice (N = 45) were acclimated for 1 week; the mice were randomly divided into five groups (N = 9 for each group), including one HF group as the control and four intervention groups (fenofibrate, (+)-hopeaphenol, (+)-vitisin A, and (−)-vitisin B) for 38

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days. In the purified compound-intervention groups, each compound (25 mg/kg) was administered by oral gavage daily, together with HF diets during the experiments. The weights and feed intake of the mice were recorded during the experiments. The feed conversion was defined as a ratio of the amount of feed intake (g) divided by mouse weight gain (g) during the experimental periods. After being sacrificed, mouse organs (heart, liver and spleen) and adipose tissues (mesenteric fat and testicular fat) were collected and weighed for comparisons. The mouse blood was collected and assayed for TC, TG and LDL by the National Laboratory Animal Center (Nangang, Taipei). For plasma-free fatty acid content determination, the nonesterified fatty acids assay kit (FA115, Randox Laboratories Ltd., UK) was used and the absorbance at 570 nm was determined following the instructions by which the hydrogen peroxide was generated by the coupled enzymatic reactions of acyl-CoA synthetase and acyl-CoA oxidase. For determining the *in vivo* plasma lipase activity, the LIPASE-PS kit was used by following the instruction manual, which was mainly based on the assay of released glycerol. The released glycerol was further catalyzed by glycerol kinase and glycerol-3-phosphate oxidase to generate hydrogen peroxide, which was catalyzed by peroxidase in the presence of 4-aminoantipyrine. The colored product was assayed by using an ELISA reader (TECAN Sunrise microplate reader; Männedorf, Switzerland) with the increased absorbance at 550 nm.

Statistical Analyses. Data were expressed as mean ± SD. Multiple group comparisons in animal experiments under the same treatment time were performed using one-way analysis of variance (ANOVA) followed by the *post hoc* Tukey's test; the weight values and accumulated feed intakes in pure compound interventions that have not been indicated with the same letter were significantly different ($P < 0.05$). The different weight values in extracts- or fraction-intervention animal experiments or biochemical parameters between the treated group and the control group (HF only) and between the ND and the control group (HF only) were analyzed using Student's *t*-test; and any difference in comparison with the control group (HF only) was considered statistically significant when $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***). Statistical analysis was performed using the GraphPad Prism 5.0 software (San Diego, CA, USA).

RESULTS AND DISCUSSION

Antiobesity Activities of Ethanol Extracts in High-Fat Diet-Induced Obesity Mice Models. The Et-ext of VTT-R, VTT-S, and VTT-L at the same concentration of 80 mg/kg were dosed daily concurrently with HF to investigate the antiobesity activity compared with the HF only (the control). From the results of Figure 1A, mice fed with the standard chow (the normal diet) showed lighter average body weights and significant differences compared with those fed with HF from day 21 to day 33 (*$P < 0.05$; **$P < 0.01$). The R-Et-ext intervention at a dose of 80 mg/kg, but not S-Et-ext and L-Et-ext, showed reduced mice weight gains compared with those fed HF only (the control) from day 19 to day 33 and showed significant difference (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) during the feeding experiments. The total feed intake at the end of the animal experiments was checked, and results are shown in Figure 1B. The total feed intake in the ND group was higher and significantly different ($P < 0.05$) compared with the control; the intervention groups of R-Et-ext, S-Et-ext, and L-Et-ext showed no difference compared with the control ($P > 0.05$). Therefore, the reductions in mice weight gain with R-Et-ext interventions (Figure 1A) did not result from the reduction of feed intake. The feed conversion rate ([total feed intake]/[weight gain]) was calculated and is shown in Figure 1C. The feed conversion rate in the ND group and the R-Et-ext intervention group, but not the S-Et-ext and L-Et-ext groups, was significantly higher ($P < 0.05$) compared with the control. The data revealed that the R-Et-ext interventions might inhibit HF-induced obesity in mice more than S-Et-ext or L-Et-ext interventions under the feeding conditions.

The biochemical index changes in the mouse plasma after the Et-ext interventions are shown in Figure 2, including total cholesterols (TC), total triglycerides (TG), and low-density lipoproteins (LDL). From the results of Figure 2, it was clear that mice in the ND group and the intervention groups showed improved effects in regard to reduced total cholesterols (Figure 2A), total triglycerides (Figure 2B), and low-density lip-

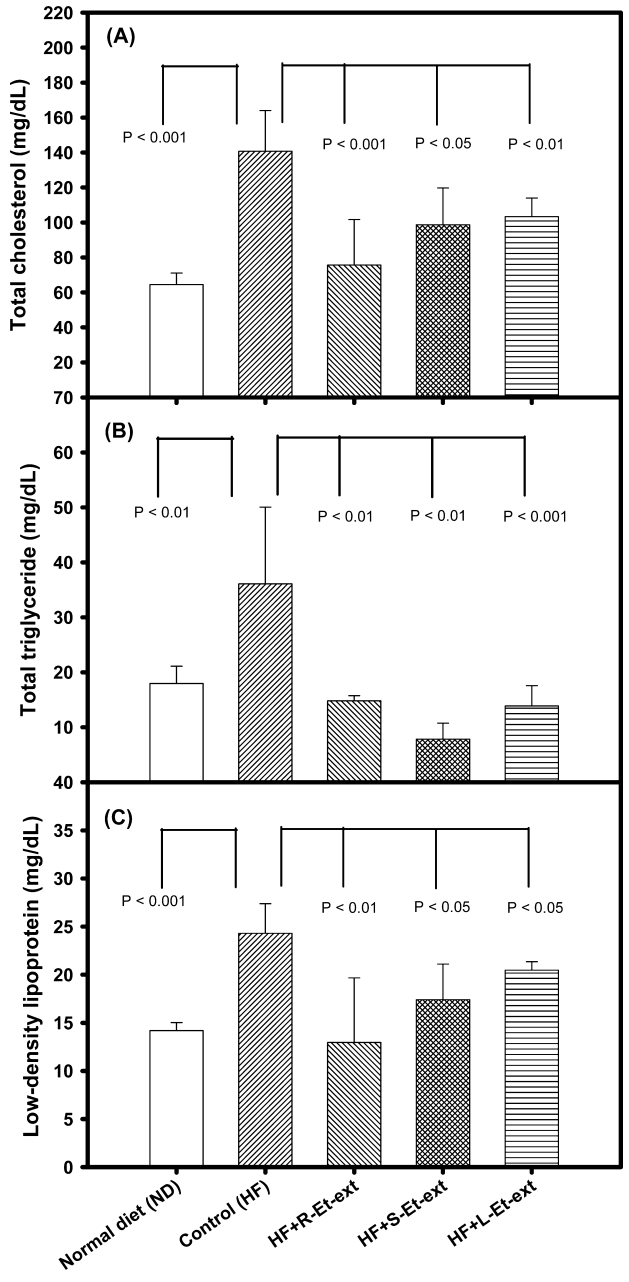

Figure 2. Effects of interventions with ethanol extract (Et-ext) from VTT-R, VTT-S, and VTT-L on plasma levels of obesity-related cardiovascular risk parameters, including (A) total cholesterol, (B) total triglyceride, and (C) low-density lipoprotein. Values in extract-intervention animal experiments were analyzed between treated group and the control group (HF only) or ND group and the control group (HF only) using Student's *t*-test, and a difference compared with the control group (HF only) was considered statistically significant when $P < 0.05$, $P < 0.01$, or $P < 0.001$.

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oproteins (Figure 2C), and significant differences ($P < 0.001$, $P < 0.01$, and $P < 0.05$) compared with the control. The present data showed that S-Et-ext, L-Et-ext, and R-Et-ext at 80 mg/kg could improve obesity-related cardiovascular risk parameters; R-Et-ext also showed reductions in the mouse weight gain induced by the HF. We previously reported that S-Et-ext, L-Et-ext, and R-Et-ext of VTT showed angiotensin converting enzyme inhibition, and the selected S-Et-ext showed antihypertensive activities against spontaneously hypertensive rats.[27] The oral administration of 95% Et-ext of VTT-S and VTT-L (20 and 50 mg/kg) daily concurrently with HF in preinduced obese Wistar rats was shown to improve the impaired glucose tolerance in obese rats.[26] It seems that the biological activities of VTT are dependent on the plant portions (roots, stems, or leaves) used. Therefore, the R-Et-ext was fractionated into EA-fra, BuOH-fra, and water-fra for the antiobesity experiments.

Antiobesity Activities of Partitioned Fractions from Root Ethanol Extracts in High-Fat Diet-Induced Obesity Mice Models. To evaluate the effects of partitioned fractions from R-Et-ext on antiobesity activities, the R-Et-ext (665 g) was suspended in H_2O and then sequentially partitioned to get EA-fra (507 g, 76%), BuOH-fra (20.7 g, 3%), and water-fra (97 g, 15%). Compared with the 80 mg/kg R-Et-ext used and based on the recoveries of each partitioned fraction, the doses used in the animal feeding experiments of EA-fra, BuOH-fra, and water-fra were 60 mg/kg, 5 mg/kg (2-fold recovered doses), and 15 mg/kg (1.25-fold recovered doses), respectively. The EA-fra, BuOH-fra, and water-fra were dosed daily concurrently with HF to investigate the antiobesity activity compared with the HF only (the control). From the results of Figure 3A, mice fed with the standard chow (the normal diet) showed lighter average body weights and significant differences compared with those fed with HF from day 26 to day 36 (*$P < 0.05$; **$P < 0.01$). The intervention with EA-fra, but not the BuOH-fra and water-fra, showed reduced mice weight gain compared with those fed HF only (the control) from day 26 to day 36 and showed significant difference (*$P < 0.05$; **$P < 0.01$) during the feeding experiments. From the results of Figures 1A and 3A, the EA-fra contained the main antiobesity activities of R-Et-ext. In analyzing the total feed intake (Figure 3B), it was found that the total feed intake in the ND group was higher and significantly different ($P < 0.01$) compared with the control; the intervention groups of EA-fra, BuOH-fra, and water-fra showed no difference compared with the control ($P > 0.05$), and the reductions in mice weight gain of EA-fra intervention (Figure 3A) did not result from lower feed intake. The calculated feed conversion rate ([total feed intake]/[weight gain]) is shown in Figure 3C. The feed conversion rate in the ND and EA-fra groups, but not BuOH-fra and water-fra groups, was significantly higher ($P < 0.01$) compared with the control, which meant that EA-fra might retard HF-induced obesity mice even with the same total feed intake among the intervention groups and the control. Reagan-Shaw et al.[28] suggested translating dose studies among animals to animals and animals to human trials based on body surface area normalization. From the results of Figures 1A and 3A, it was calculated that the human equivalent dose was about 6.5 mg/kg or 4.9 mg/kg of human body weight, respectively, from R-Et-ext (80 mg/kg of mouse) or R-Et-EA-fra (60 mg/kg of mouse) in the present experiment. An adult of 60 kg weight might have to consume about 400 mg of R-Et-ext or 300 mg of R-Et-EA-fra per day to achieve similar antiobesity benefits. This should be investigated further.

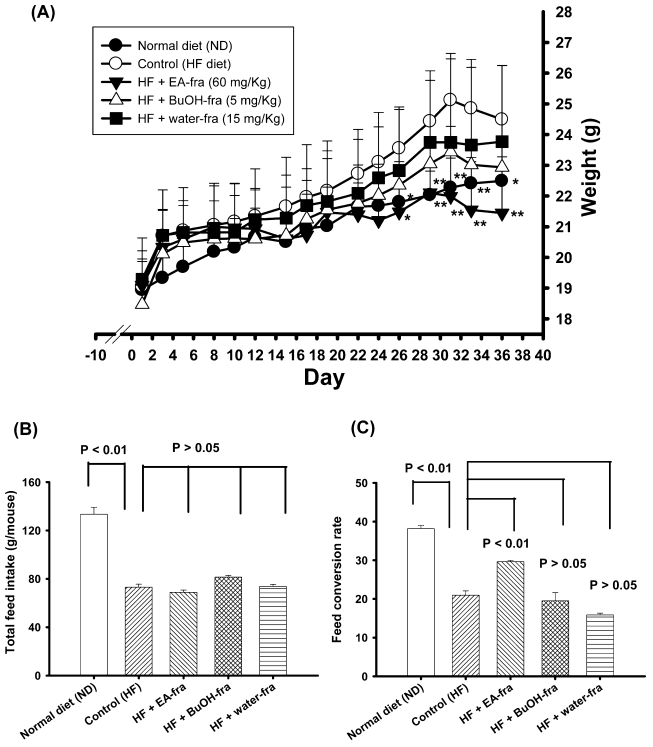

Figure 3. (A) Effects of interventions with different partitioned fractions (ethyl acetate-partitioned fraction, EA-fra; *n*-butanol-partitioned fraction, BuOH-fra; water fraction, water-fra) from R-Et-ext on high-fat (HF) diet-induced obesity in C57BL/6 mice. The EA-fra (60 mg/kg of body weight), BuOH-fra (5 mg/kg of body weight), and water-fra (15 mg/kg of body weight) were administered by oral gavage daily together with HF diets during the experiments. The standard mouse/rat chow was used as the normal diet (ND) (Prolab RMH2500, 5P14 Diet). (B) Total feed intake at the end of experiment. (C) Feed conversion rate, [total feed intake (g)]/[mouse weight gains (g)], during experimental periods. The three-week-old C57BL/6J mice ($N = 50$) were acclimated for 1 week; the mice were randomly divided into five groups ($N = 10$ for each group) for 36 days. Values in fraction-intervention animal experiments between treated group and the control group (HF only) or ND group and the control group (HF only) were analyzed using Student's *t*-test, and a difference compared with the control group (HF only) was considered statistically significant when $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

Compounds Isolated from R-Et-EA-fra and Used for Antiobesity Activities in High-Fat Diet-Induced Obesity Mice Models. The EA partitioned fractions from R-Et-ext were selected to isolate compounds for further animal studies. Stilbenoid compounds, rich in the *Vitis* genus plants, are considered to be their active constituents. Three resveratrol tetramers with the same molecular weight (Figure 4A), (+)-hopeaphenol, (+)-vitisin A, and (−)-vitisin B, were isolated and identified by comparing their physical and spectral data (specific rotation, MS, and NMR), which were the same as a previous report from VTT-S-Et-ext.[26] (+)-Hopeaphenol, (+)-vitisin A, and (−)-vitisin B at the same concentration of 25 mg/kg were dosed daily concurrently with HF to investigate the antiobesity activity compared with the HF only (the control). The (+)-vitisin A was previously reported to have antihypertensive activities (10 mg/kg) against spontaneously hypertensive rats.[27] Therefore, it was calculated to use a similar

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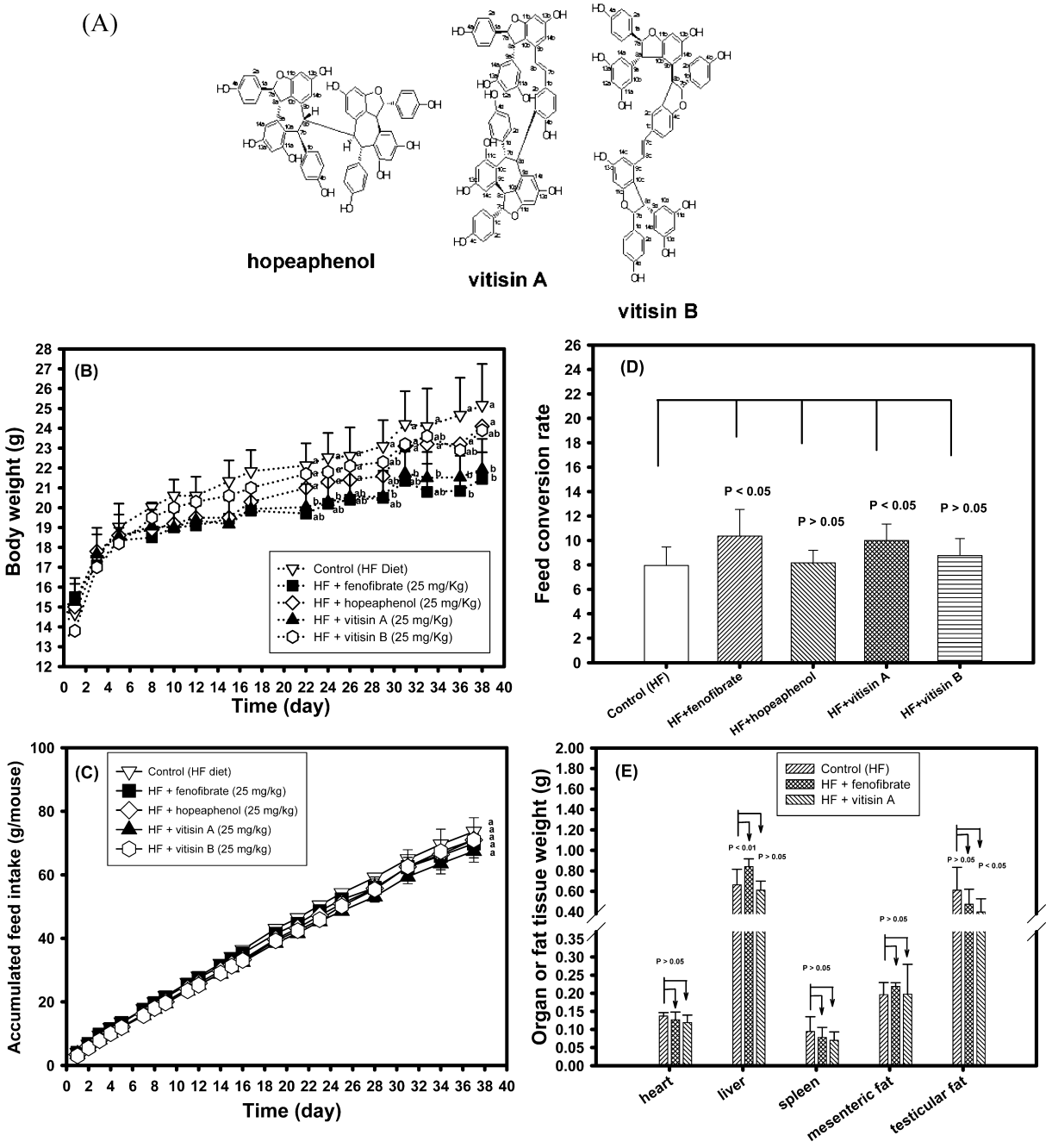

Figure 4. (A) Chemical structures of three resveratrol tetramers with the same molecular weight, (+)-hopeaphenol, (+)-vitisin A, and (−)-vitisin B, isolated from the EA-fra of VTT-R-Et-ext. (B) Effects of (+)-hopeaphenol, (+)-vitisin A, and (−)-vitisin B on high-fat (HF) diet-induced obesity in C57BL/6 mice; fenofibrate was used for comparisons. Each compound (25 mg/kg of body weight) was administered by oral gavage daily together with HF diets during the experiments. (C) Accumulated feed intake during animal experiments. (D) Feed conversion rate [total feed intake (g)]/[mouse weight gains (g)], during experimental periods. (E) After mice were sacrificed, mouse organs (heart, liver, and spleen) and adipose tissues (mesenteric fat and testicular fat) were collected and weighed for comparisons. Multiple group comparisons in animal experiments under the same treated times were performed using one-way analysis of variance (ANOVA) followed by the *post hoc* Tukey's test, and weight values and accumulated feed intake in pure compound interventions that have not been indicated with the same letter were significantly different ($P < 0.05$). Other values between treated group and the control group (HF only) were analyzed using Student's *t*-test, and a difference compared with the control group (HF only) was considered statistically significant when $P < 0.05$, $P < 0.01$, or $P < 0.001$.

dose based on body surface area normalization[28] in antiobesity experiments of HF-induced mice models. It was calculated that the mouse equivalent dose was about 20 mg/kg of body weight, and 25 mg/kg was used in the present intervention experiments. Fenofibrate at the same concentration was used for comparisons. It was also reported that fibrates are considered more effective than statins in lowering triglyceride and raising HDL levels, due to their agonist effect on PPARα, and have a primary indication in hypertriglyceridemia in humans.[7] Mancini et al.[11] reported that fenofibrate (320 mg/kg body weight) treatments for the second month concurrently with HF for two months or fenofibrate (320 mg/kg body weight) treatments concurrently with HF for two months in Wistar rats could have a final body weight and white adipose tissue mass similar to

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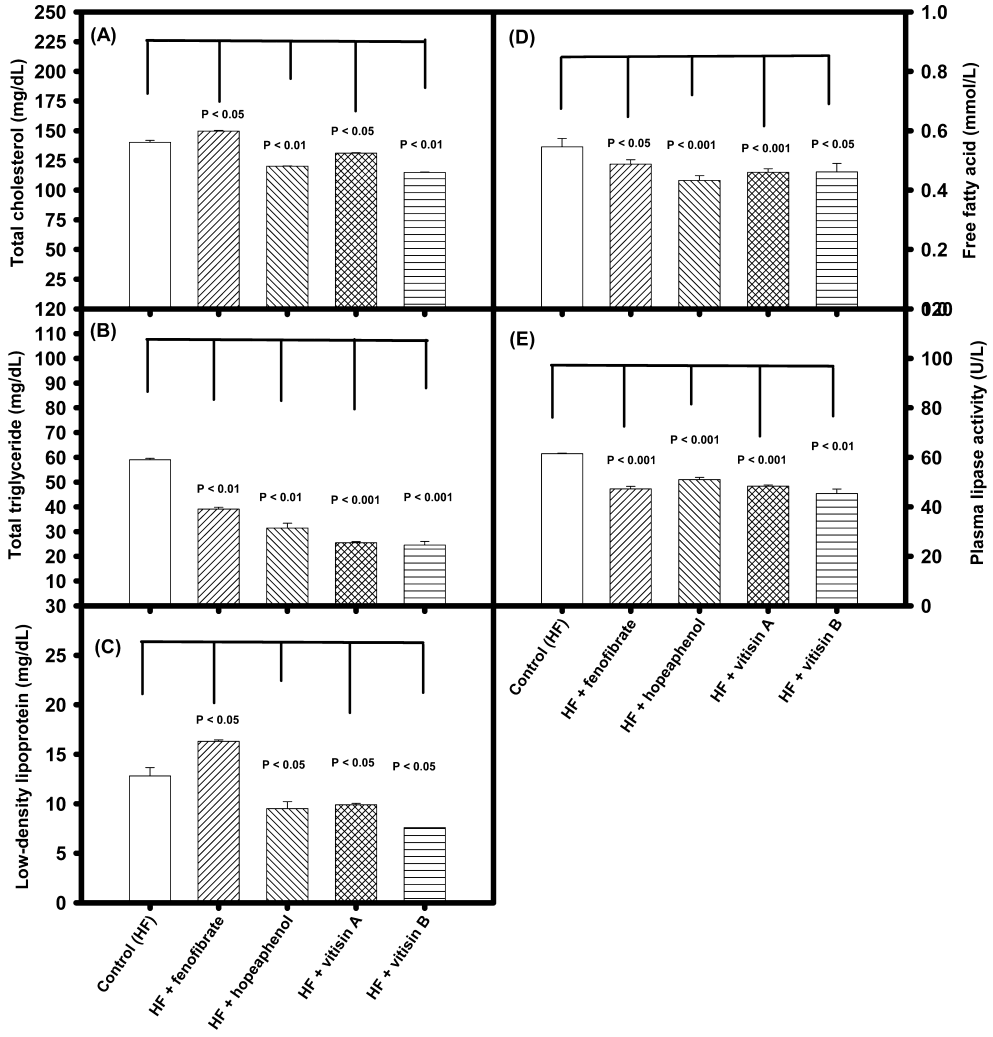

Figure 5. Effects of (+)-hopeaphenol, (+)-vitisin A, (−)-vitisin B, and fenofibrate interventions on plasma levels of obesity-related cardiovascular risk parameters, including (A) total cholesterol, (B) total triglyceride, (C) low-density lipoprotein, (D) free fatty acid, and (E) plasma lipase activity (U/L). Values in extract-intervention animal experiments were analyzed between treated group and the control group (HF only) using Student's *t*-test, and a difference compared with the control group (HF only) was considered statistically significant when $P < 0.05$, $P < 0.01$, or $P < 0.001$.

those fed with the standard diets, and these two fenofibrate treatments could significantly increase liver weights compared with those of HF-treated and standard diet-treated rats. From the results of Figure 4B, the same molecular weight of these three resveratrol tetramers showed different antiobesity activities. It is clear that the intervention with (+)-vitisin A at a dose of 25 mg/kg, but not (+)-hopeaphenol and (−)-vitisin B, showed reduced mice weight gain compared with those fed HF only (the control) from day 22 to day 38 and showed significant difference ($P < 0.05$) during the feeding experiments. The fenofibrate showed generally reduced mice weight gain compared with those fed the HF only (the control) from day 22 to day 38 but only showed significant difference ($P < 0.05$) at days 36 and 38, which might be due to the variabilities of the experiment animals. At days 36 and 38, (+)-vitisin A and fenofibrate showed similar antiobesity activities, and both compound interventions showed significant difference compared with the HF only ($P < 0.05$). This is the first report that the (+)-vitisin A exhibited antiobesity activity *in vivo*. (+)-Vitisin A was also reported to exhibit yeast α-glucosidase inhibitory (IC_{50}, 1.22 μM) and anti-dipeptidyl peptidase-IV (IC_{50}, 90.75 μM) activities, which were associated with improvements in impaired glucose tolerance.[26] The R-Et-ext in the present research and S-Et-ext[26] were reported to isolate (+)-vitisin A. From quantification results of (+)-vitisin A through HPLC-UV method, the content of (+)-vitisin A in R-Et-ext and S-Et-ext was 42.6 and 0.19 mg/g, respectively. The content of (+)-vitisin A in R-Et-ex is about 224 times higher than that in S-Et-ext; therefore, it might be one major reason for R-Et-ext, but not S-et-ext, exhibiting antiobesity activity (Figure 1A).

The peptides of Arg-Phe and Ile-His-Arg-Phe derived from the rice glutelin protein showed antiobesity activities due to anorexigenic effects in lowering food intake in rodent models.[29,30] The accumulated feed intake among the intervened groups was checked during animal experiments (Figure 4C). It was found that the trends of accumulated feed intake among groups were very similar and showed no significant difference among groups ($P > 0.05$) at the end of the experiments. Therefore, the reductions in mice weight gain by (+)-vitisin A and fenofibrate interventions (Figure 4B) did not result from the reduction of feed intake. The feed conversion rate ([total feed intake]/[weight gain]) was calculated and is shown in Figure 4D. The same as Figures 1C and 3C, the feed

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conversion rate of (+)-vitisin A and fenofibrate interventions was significantly higher ($P < 0.05$) compared with that of the control. Figure 4E shows the weights of organ and fat tissues in the HF groups and fenofibrate- and (+)-vitisin A-intervention groups. The weights of heart, spleen, and mesenteric fat tissues in fenofibrate- and (+)-vitisin A-intervention groups showed no significant difference ($P > 0.05$) compared with those of the control; the weights of testicular fat tissues in fenofibrate- and (+)-vitisin A-intervention groups were somewhat lower, but only the latter showed significant difference ($P < 0.05$) compared with those of the control. The weights of livers in the fenofibrate-intervened groups were higher and significantly different ($P < 0.01$) compared with those in the control, which were similar to reports of Mancini et al. using fenofibrate-intervention in male Wistar rats concurrently with HF.[11]

The biochemical index changes in mice plasma after resveratrol tetramer or fenofibrate interventions are shown in the Figure 5, including TC, TG, LDL, free fatty acid, and plasma lipase activities. From the results of Figure 5, it is clear that the resveratrol tetramer-intervention mice showed improved effects, that is reduced total cholesterols (Figure 5A), total triglycerides (Figure 5B), low-density lipoproteins (Figure 5C), free fatty acid (Figure 5D), and plasma lipase activities (Figure 5E), and also showed significant differences ($P < 0.001$, $P < 0.01$, and $P < 0.05$) compared with the control. The fenofibrate interventions were shown to significantly reduce total triglycerides ($P < 0.01$, Figure 5B), free fatty acid ($P < 0.05$, Figure 5D), and plasma lipase activities ($P < 0.001$, Figure 5E) in mice plasma; however, total cholesterols ($P < 0.05$, Figure 5A) and low-density lipoproteins ($P < 0.05$, Figure 5C) increased compared with those in the control. The present data show that the same molecular weight of resveratrol tetramers, (+)-hopeaphenol, (+)-vitisin A, and (−)-vitisin B, and fenofibrate at 25 mg/kg could improve obesity-related cardiovascular risk parameters in plasma; (+)-vitisin A and fenofibrate also reduced the mouse weight gain induced by HF diets. The real mechanisms of VTT or (+)-vitisin A for antiobesity are not clear at present. Pan et al.[31] reported that 50% VTT-Et-ext interventions exhibited LDL and TC lowering activities in plasma and lipid lowering activities in livers of hypercholesterolemic rabbits, possibly through the regulation of AMPK-ACC pathway. Kim et al.[32] reported that (+)-vitisin A (IC_{50}, 5.0 μM) could inhibit 3T3-L1 preadipocyte differentiations by Oil Red O stains *in vitro*, and (+)-vitisin A could block the cell cycles of the 3T3-L1 preadipocyte by arresting G1 phases. It will be possible to use microarray chips to analyze the upregulation and downregulation of genes associated with lipid syntheses and lipid metabolisms with or without VTT or (+)-vitisin A interventions. It was proposed that the plasma lipase inhibitory activities of (+)-hopeaphenol, (+)-vitisin A, (−)-vitisin B, and fenofibrate might contribute in part to their antiobesity activities, and the *in vitro* lipase inhibitory activities and related kinetic analyses will be investigated in the future. The fenofibrate was proven to act as a PPARα agonist to regulate gene expression associated with lipid and lipoprotein metabolism. Possibly (+)-hopeaphenol, (+)-vitisin A, and (−)-vitisin B also acted as PPARα activators; this will need further investigation by surface plasmon resonance to demonstrate that these compounds interact directly with the PPARα ligand binding domain. It will be possible to use microarray chips to analyze the upregulation and down-regulation of genes associated with lipid syntheses and lipid metabolism with or without VTT or (+)-vitisin A interventions.

In conclusion, this is the first report of animal experiments showing that VTT-R-Et-ext, EA-fra of R-Et-ext, and vitisin A isolated from EA-fra exhibited antiobesity activities by reducing mice weight gain induced by HF. Therefore, (+)-vitisin A might have biological activities against metabolic syndrome disorders, including antiobesity, antihypertension,[27] and improvements in impaired glucose tolerance.[26] In addition, Et-ext from VTT-R, VTT-S, and VTT-L and hopeaphenol, vitisin A, and vitisin B interventions lowered obesity-related cardiovascular risk parameters in plasma. The interventions with (+)-hopeaphenol, (+)-vitisin A, and (−)-vitisin B also reduced the plasma lipase activities. The current results suggest that VTT may be developed as a functional food for antiobesity activity, and this requires further investigation.

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Notes

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