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Antiobese Effects of Novel Saponins from Edible Seeds of Japanese Horse Chestnut (*Aesculus turbinata* BLUME) after Treatment with Wood Ashes

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Recently, we have identified novel saponins from edible seeds of Japanese horse chestnut (*Aesculus turbinata* BLUME) after processing the natural seeds with wood ashes to remove bitterness. We attempted to determine anti-obesity effects of those saponins from edible seeds as well as natural seeds. The purified individual components of saponins from natural and edible seeds inhibited pancreatic lipase in vitro. The potency was in the order of escins > desacylescins > deacetylescins. Escins Ib and IIb as well as deacetylescins Ib and IIb with the angeloyl moiety were more potent than the corresponding Ia and IIa series with the tigloyl moiety. Moreover, in vivo anti-obesity effects of the saponin fractions were monitored for 8 weeks in mice fed high-fat diets. Saponin fractions from both seeds significantly attenuated the elevation in body weight, the mass of peritoneal adipose tissues, and plasma triacylglycerol, which was accompanied by higher contents of undigested fats in feces without changes in food intake, indicating the effective inhibition of fat digestion in vivo. Taken together, saponin fractions including desacylescins and deacetylescins from edible seeds are potentially useful for the development of nutraceutical foods with anti-obesity effects and more attenuated bitter taste.

KEYWORDS: Saponin; escin; deacetylescin; desacylescin; Japanese horse chestnut; *Aesculus turbinata* BLUME; anti-obesity effect

INTRODUCTION

European horse chestnut seeds (*Aesculus hippocastanum* L.) have been shown to contain large amounts of mixed triterpenoidal saponins called escins (*1, 2*), which exhibit several biological activities such as inhibitory actions on the elevation in blood glucose (*1, 2*) and alcohol absorption (*1, 2*) as well as anti-inflammatory, anti-edematous, and capillaro-protective activities (*3–5*). However, Japanese horse chestnuts have been traditionally utilized as edible sources of food such as rice balls and rice cake in Japan. However, prior to utilization, the natural seeds are generally treated with wood ashes to remove harshness with the highly bitter taste due to the presence of escins. Recently, we found the chemical conversion of escins from natural seeds to the derivatives without acetyl or acyl moieties through alkaline hydrolysis during food processing with wood ashes (*6, 7*). Moreover, our laboratory has already provided the evidence that the resulting compounds, deacetylescins and desacylescins, have inhibitory effects on the elevation in blood glucose levels in mice in addition to much less bitter taste (*7, 8*).

Until now, we have established the methods for the fractionation, separation, and purification of each saponin component from natural or edible seeds (*6–8*). Moreover, we are now able to analyze all components of escins, deacetylescins, and desacylescins at the same time in a single run by reverse-phase high-performance liquid chromatography (HPLC). It is well known that obesity is one of the predominant risk factors for diseases such as diabetes, hypertension, and atherosclerosis. Since dietary lipids contain higher content of food energy, high-fat diets common in many Western countries lead to the development of obesity. Hence, much attention has been paid to the food factors that exhibit anti-obesity effects. In the present study, we focused on the nutraceutical activities of the novel saponins prepared from natural or edible seeds of Japanese horse chestnut with special reference to the anti-obesity effects of those saponins in mice fed a high-fat diet in vivo as well as the

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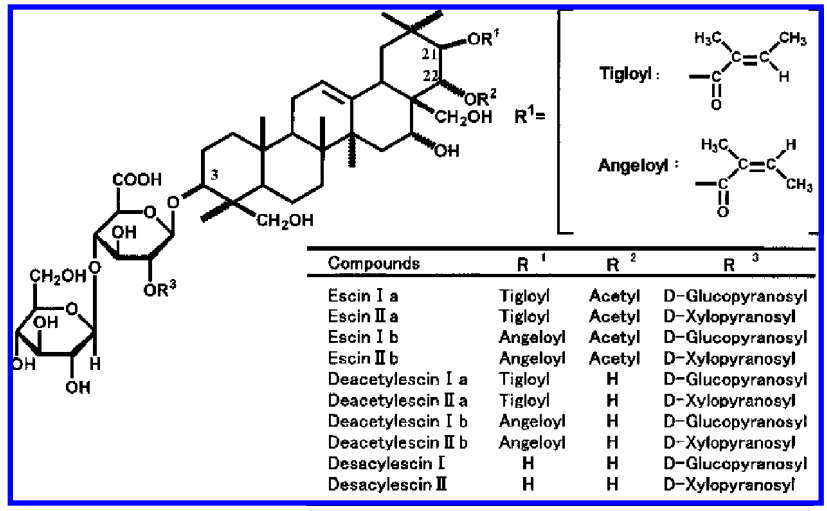

Compounds	R 1	R 2	R 3
Escin I a	Tigloyl	Acetyl	D-Glucopyranosyl
Escin II a	Tigloyl	Acetyl	D-Xylopyranosyl
Escin I b	Angeloyl	Acetyl	D-Glucopyranosyl
Escin II b	Angeloyl	Acetyl	D-Xylopyranosyl
Deacetylescin I a	Tigloyl	H	D-Glucopyranosyl
Deacetylescin II a	Tigloyl	H	D-Xylopyranosyl
Deacetylescin I b	Angeloyl	H	D-Glucopyranosyl
Deacetylescin II b	Angeloyl	H	D-Xylopyranosyl
Desacylescin I	H	H	D-Glucopyranosyl
Desacylescin II	H	H	D-Xylopyranosyl

Figure 1. Chemical structure of saponins isolated from natural and edible seeds of Japanese horse chestnut.

inhibitory activity of the purified components of saponins on pancreatic lipase in vitro.

MATERIALS AND METHODS

Materials. The seeds of the Japanese horse chestnut (*Aesculus turbinata* BLUME) were collected in forests of northern Hyogo Prefecture in Japan and identified as described before (*7*). Wood ashes were obtained from Makino Timber Industry (Okayama, Japan). β-Escin, triolein, Triglyceride E-Test Kit, Cholesterol E-test Kit, and Transaminase CII-Kit were supplied by Wako (Osaka, Japan). 4-Methylumbelliferyl oleate and porcine pancreatic lipase (type II) were purchased from Sigma (St. Louis, MO. USA). Diaion HP-20 and Chromatorex ODS 1024T for column chromatography were obtained from Nippon Rensui (Tokyo, Japan) and Fuji Silysia (Kasugai, Japan), respectively. Analytical and preparative columns of YMC-Pack ODS AM for reverse-phase HPLC are the products of YMC (Kyoto, Japan). Other chromatographic materials were obtained as described previously (*7*). Beef tallow, casein, cellulose, AIN-76 vitamin mixture, and AIN-76 mineral mixture were provided by Oriental Yeast (Tokyo, Japan). All other chemicals were of reagent grade and obtained from Wako or Nacalai Tesque (Kyoto, Japan).

Extraction, Fractionation, and Isolation of Saponins from Natural and Edible Seeds. Edible seeds of Japanese horse chestnut were prepared by treatment with wood ashes as described earlier (*7*). From natural and edible seeds of Japanese horse chestnut, escins and the derivatives were extracted with methanol and fractionated by absorption chromatography with Diaion HP-20 followed by reverse-phase column chromatography with Chromatorex ODS 1024T according to previous methods (*2, 7*). Starting separately with 2 kg of natural seeds and edible seeds, the recovery of the saponin fraction in natural seeds and edible seeds was calculated to be 3.0% and 0.78%, respectively, as dry weight. The saponin fraction either from natural or edible seeds was used for the anti-obesity tests of saponins in experimental mice fed high-fat diets as described below. The saponin from natural seeds comprised escins Ia, IIa, Ib, and IIb in a weight ratio of 1:0.79:0.93:0.43, respectively, as determined by reverse-phase HPLC as described below. However, the saponin fraction from edible seeds included deacetylescins Ia, IIa, Ib, and IIb, and desacylescins I and II in a weight ratio of 1:0.40:1:0.46:1.79:0.93, respectively.

For the purification of each component of saponins and the related compounds from natural and edible seeds, HPLC analysis was conducted on a Shimadzu LC-2010A system equipped with a preparative HPLC column of YMC-Pack ODS AM (150 mm × 10 mm i.d.), which was eluted at a flow rate of 3 mL/min with a mobile phase of methanol/10 mM sodium phosphate buffer (pH 2.7) (62:38, v/v). The elution of saponins and the derivatives was detected by monitoring the absorbance at 230 nm. To confirm the purity of each component, an analytical column of YMC-Pack ODS AM (150 mm × 6 mm i.d.) was eluted at a flow rate of 0.8 mL/mL with the same mobile phase as that described above. These methods enabled us to isolate the purified components of escins Ia, IIa, Ib, and IIb from natural seeds and deacetylescins Ia, IIa, Ib, and IIb from edible seeds (**Figure 1**). To isolate the purified components of desacylescins I and II from edible seeds, the above preparative column of YMC-Pack ODS was eluted at a flow rate of 3 mL/min with a mobile phase of acetonitrile/10 mM sodium phosphate buffer (pH 2.7) (20:80, v/v). Moreover, for the determination of the composition of deacetylescins and desacylescins, these compounds were also separated on the preparative column using a gradient system of acetonitrile/10 mM sodium phosphate buffer (pH 2.7) from a volume ratio of 20:80 to 40:60 for 140 min.

Instrumental Analyses. The identification of the purified components of saponins was carried out by the nuclear magnetic resonance (NMR) analyses of ^{1}H NMR and ^{13}C NMR using the JNM-A400 FT-NMR (400 Hz) system (JEOL, Tokyo, Japan) as described before (*7*). The sample was dissolved in pyridine-d_5 containing 0.05% tetramethylsilane as an internal standard. Chemical structures of saponins and the related substances were also confirmed by electrospray ionization-mass spectrometry (ESI-MS) on a ThermoQuest LCQ Deca XP mass spectrometer at a positive ion mode for escins Ia, IIa, Ib, and IIb, and deacetylescins Ia, IIa, Ib, and IIb. Desacylescins I and II were analyzed in a negative mode of ESI-MS. The spectral data were comparable with the earlier studies (*2, 7*).

Assay of Pancreatic Lipase Activity. The inhibitory effect of escins and the derivatives on the enzyme activity of porcine pancreatic lipase was determined as described before (*7, 9*). Briefly, the enzyme (0.066 units with triacetin as a substrate according to the supplier) was incubated with 50 μM 4-methylumbelliferyl oleate as a substrate in the presence of increasing concentrations of the purified components of escins and the related compounds in a total volume of 200 μL by dissolving in 20 mM McIlvane buffer (pH 7.4) with 0.02% sodium deoxycholate. The sample to be tested was dissolved in 70% methanol and added to the reaction mixture to give a final methanol concentration of 3.5%. After the incubation at 37 °C for 20 min, the enzyme reaction was terminated by adding 1 mL of 0.1 M HCl and 2 mL of 0.1 M sodium citrate. The amount of 4-methylumbelliferone released by the lipase was determined fluorometrically at an excitation wavelength of 320 nm and an emission wavelength of 450 nm. In addition, lipase activity was also assayed by measuring the release of oleic acid from triolein as described earlier (*10*).

Anti-Obesity Tests of Saponin Fractions from Natural and Edible Seeds in Mice Fed High-Fat Diets. Female 3-week-old ICR mice were obtained from Shimizu Laboratory Supplies (Kyoto, Japan). The mice were housed at 23 °C on a 12 h/12 h light/dark cycle and had free access to standard MF chow (Shimizu, Kyoto, Japan) and water for 10 days for their adaptation. We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during this research. The experimental mice were divided into four groups after adaptation and fed the experimental

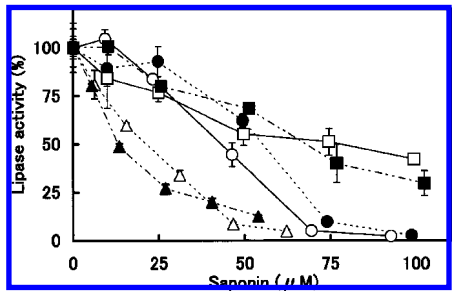

Figure 2. Effects of purified components of escins and desacylescins on pancreatic lipase activity. Symbols: ○, escin Ia; ●, escin IIa; △, escin Ib; ▲, escin IIb; □, desacylescin I; ■, desacylescin II. Data represent the mean ± SE ($n = 3$).

diets for 8 weeks. The high-fat diet group was fed a diet containing 40% beef tallow as fat, 30% casein, 10% corn starch, 10% sucrose, 5% cellulose, 3.5% mineral mixture, 1% vitamin mixture, 0.3% methionine, and 0.2% choline. The groups of the high-fat diet supplemented with saponin fractions included 0.1% or 0.5% saponin fraction from natural seeds or 0.5% saponin fraction from edible seeds in place of casein. The control group was continuously given the standard laboratory chow, MF pellets, ad libitum. Body weight and the amount of food intake by the mice were measured every week. After 8 weeks of feeding the indicated diets, the mice were anesthetized with diethyl ether, the blood of the mice was withdrawn from the main vein with heparin as an anticoagulant, after which the mice were killed and dissected. Then, peritoneal adipose tissues and organs of liver and kidney were removed quickly and weighed. The plasma samples were analyzed for the levels of neutral fats (triacylglycerols), total cholesterol, glutamic oxaloacetic transaminase (GOT) activity, and glutamic pyruvic transaminase (GPT) activity. After 7 weeks of feeding the experimental diets, the total amounts of food intake and feces excreted by two mice in one cage were recorded during a period of 2 days. The recovered feces were used for the extraction of triacylglycerols by the method of Folch et al. (*11*), after which the triacylgycerols were determined by Triglyceride E-Test Kit (Wako).

Statistical Analysis. Data represent the mean ± standard error (S.E.) of 6 mice. Statistical significance was evaluated by one-way analysis of variation with Mini StatMate software (ATMS Co., Tokyo). Then, the differences between means were compared using Dunnett's post-test multiple comparisons. Differences were considered to be significant when $p < 0.05$.

RESULTS

Inhibitory Effects of Saponins from Natural and Edible Seeds of Japanese Horse Chestnut on Pancreatic Lipase Activity. Each component of saponins purified from natural and edible seeds was tested for its effect on pancreatic lipase activity using 4-methylumbelliferyl oleate as a substrate (**Figures 2** and **3**). All of the purified compounds exhibited an inhibitory effect on lipase activity. The similar results were obtained with triolein as a substrate of the digestive lipase (data not shown). When the potency of those compounds was compared on a molar basis, all of the escins derived from natural seeds clearly showed more inhibitory actions on the enzyme than deacetylescins and desacylescins from edible seeds. Among saponins from edible seeds, desacylescins I and II without both acyl moieties at C-21 and C-22 were more efficacious in inhibiting digestive lipase as compared with desacetylescins without an acetyl group at C-22. Therefore, the potency of saponins was in the order of escins > desacylescins > deacetylescins. Interestingly, both escins Ib and IIb, and deacetylescins Ib and IIb with an angeloyl moiety at C-21 exhibited more inhibitory effect than the corresponding escins Ia and IIa, and deacetylescins Ia and IIa with a tigloyl moiety at C-21, respectively.

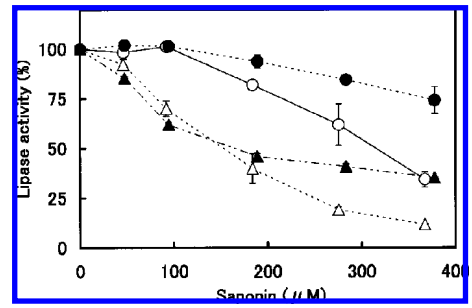

Figure 3. Effects of purified components of deacetylescins on pancreatic lipase activity. Symbols: ○, deacetylescin Ia; ●, deacetylescin IIa; △, deacetylescin Ib; ▲, deacetylescin IIb. Data represent the mean ± SE ($n = 3$).

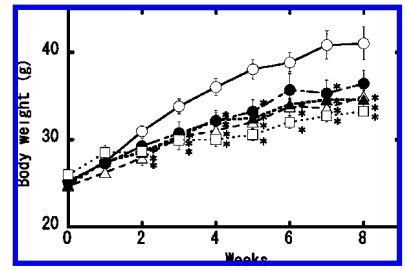

Figure 4. Suppression of body weight gain in mice fed a high-fat diet containing saponin fraction either from natural or edible seeds. For 8 weeks, mice were fed a high-fat diet supplemented with none (○), 0.1% saponin fraction from natural seeds (●), 0.5% saponin fraction from natural seeds (△), 0.5% saponin fraction from edible seeds (▲), or standard MF chow (□). Data represent the mean ± SE ($n = 6$). *$p < 0.05$, significantly different from mice fed a high-fat diet only (○).

Anti-Obesity Effects of Saponin Fractions from Natural and Edible Seeds in Mice Fed High-Fat Diets. The mice in the high-fat diet group significantly showed higher body weight than those in the control group fed standard laboratory chow (**Figure 4**). However, significant suppression of body weight gain was detectable in other groups of mice fed with high-fat diets containing saponin fractions from natural and edible seeds. The high-fat diet containing 0.5% saponin fraction from edible seeds was clearly effective in attenuating the gain of body weight. The attenuating effect of this diet group was almost similar to that found in the high-fat diet group with 0.5% saponin fraction from natural seeds. The high-fat diet with 0.1% saponin fraction from natural seeds also significantly reduced the gain of body weight with slightly less effect than the groups fed 0.5% saponin fraction from either seed. The resulting attenuating effect on body weight was not due to the reduced food intake because we did not recognize significant changes in food intake between the experimental groups after 8 weeks of feeding different diets.

To monitor the changes in whole body or organs in mice fed different diets during the feeding periods, we measured the weight of whole body, peritoneal adipose tissues, livers, and kidneys after 8 weeks (**Table 1**). The increased body weight in the high-fat diet group was appreciably higher than in the group of standard laboratory chow. In contrast, the diets supplemented with 0.5% saponin fractions from natural and edible seeds were significantly effective in attenuating the body weight and adipose mass. In sharp contrast, the weights of liver and kidney per 100 g of body weight did not differ significantly between the diet groups, suggesting no abnormal accumulation of fats in those organs.

Moreover, to determine whether different diets might affect the plasma parameters in mice, we also examined the changes

Table 1. Effects of Saponin Fractions from Natural and Edible Seeds on the Weight of Whole Body, Peritoneal Adipose Tissues, Liver, and Kidney in Mice Fed High-Fat Diets for 8 Weeks[a]

diet group	whole body (g)	peritoneal adipose tissues (g/100 g body weight)	liver (g/100 g body weight)	kidney (g/100 g body weight)
HF	41.03 ± 1.90	6.55 ± 0.87	3.52 ± 0.08	1.18 ± 0.02
HF + 0.1% SFNS	36.45 ± 1.51	4.02 ± 0.39*	3.36 ± 0.05	1.21 ± 0.02
HF + 0.5% SFNS	35.00 ± 1.56*	3.64 ± 0.46*	3.56 ± 0.04	1.11 ± 0.03
HF + 0.5% SFES	34.62 ± 1.00*	2.96 ± 0.78*	3.45 ± 0.40	1.16 ± 0.01
standard chow	33.24 ± 0.52*	2.12 ± 0.60*	3.42 ± 0.04	1.16 ± 0.01

[a] Abbreviations: HF, high-fat diet; HF + 0.1% SFNS, high-fat diet containing 0.1% saponin fraction from natural seeds; HF + 0.5% SFNS, high-fat diet containing 0.5% saponin fraction from natural seeds; HF + 0.5% SFES, high-fat diet containing 0.5% saponin fraction from edible seeds. Data represent the mean ± S. E. ($n = 6$). *$p < 0.05$, significantly different from mice fed a high-fat diet only.

Table 2. Effects of Saponin Fractions from Natural and Edible Seeds on the Plasma Parameters in Mice Fed High-Fat Diets for 8 Weeks[a]

diet group	triacylglycerols (mg/dL)	total cholesterols (mg/dL)	GOT (units/L)	GPT (units/L)
HF	126.7 ± 7.6	102.0 ± 12.2	119.6 ± 7.7	37.7 ± 13.2
HF + 0.1% SFNS	120.0 ± 17.3	130.7 ± 5.7	112.6 ± 11.7	53.0 ± 11.2
HF + 0.5% SFNS	95.4 ± 6.0*	145.3 ± 7.0*	69.0 ± 20.3*	25.2 ± 15.9
HF + 0.5% SFES	86.9 ± 9.3*	118.8 ± 9.2	70.2 ± 12.7*	20.5 ± 4.3
standard chow	84.0 ± 5.6*	113.9 ± 5.3	55.6 ± 5.7*	15.2 ± 1.4

[a] Data represent the mean ± S. E. ($n = 6$). *$p < 0.05$, significantly different from mice fed a high-fat diet only.

Table 3. Effects of Saponin Fractions from Natural and Edible Seeds on Food Intake and Excretion of Triacylglycerols in Feces during 2 Days after Mice Were Fed High-Fat Diets for 7 Weeks[a]

diet group	food intake (g/2 days)	excreted feces (2 g/2 mice/2 days)	triacylglycerols in feces (g/2 mice/2 days)	composition of triacylglycerols in feces (%)
HF	12.44 ± 1.24	2.60 ± 0.56	0.90 ± 0.15	34.6
HF + 0.1% SFNS	10.79 ± 0.29	2.07 ± 0.32	0.92 ± 0.12	44.4
HF + 0.5% SFNS	9.46 ± 0.82	2.35 ± 0.12	1.69 ± 0.29*	71.9
HF + 0.5% SFES	12.01 ± 0.19	3.22 ± 0.24	1.64 ± 0.06*	50.9

[a] Data represent the mean ± S. E. ($n = 6$). *$p < 0.05$, significantly different from mice fed a high-fat diet only.

in the levels of triacylglycerols, total cholesterols, and GOT and GPT activities (**Table 2**). The marked increase in the plasma level of triacylglycerols was observed in the high-fat diet group. The elevated levels were significantly reversed in the high-fat diet groups with 0.5% saponin fractions from natural and edible seeds to the lower levels almost equivalent to the control diet. Feeding the high-fat diet without saponins caused a greater increase in GOT activity than standard chow. However, the supplementation of 0.5% saponin fractions from natural and edible seeds significantly abolished increased enzyme activity in the high-fat diet only. The similar effects on GPT activity appeared to be observed with the saponins, but that was not significantly different between the diet groups. High-fat diet or the saponin fraction from edible seeds had no appreciable effect on the plasma levels of total cholesterol, although the slight increase was recognized in the high-fat diet group with 0.5% saponin fraction from natural seeds.

Effects of Saponin Fractions on Food Intake and Digestion of Triacylglycerols. To investigate the influence of saponin fractions on food intake and digestion of dietary fats by mice, we analyzed for the amounts of food intake, stool excretion, and triacylglycerols in feces (**Table 3**). There were almost no significant differences among the test groups with respect to their food intake. Some decrease in food intake appeared to be detectable in the diets with 0.1% or 0.5% saponin fraction from natural seeds at the level of no statistical significance. In addition, the amounts of stool excretion were not significantly different between the test groups. In sharp contrast, mice fed high-fat diets with 0.5% saponin from natural seeds and edible seeds showed appreciably higher levels of triacylglycerols in feces than the control mice fed high-fat diet only, supporting the inhibitory action of those saponins on the digestion and absorption of neutral fats in vivo.

DISCUSSION

As a target of anti-obesity action of saponins from natural or edible seeds, the effect of those compounds on pancreatic lipase should be evaluated in vitro. Until now, orlistat, a pancreatic lipase inhibitor, has been shown to be efficacious in inhibiting obesity and related symptoms by stimulating fat excretion in feces through the inhibition of digestive lipase in the small intestine (*12*). Here, we provided the evidence for the inhibitory action of saponins from natural and edible seeds on the activity of pancreactic lipase. The potency was found to be in the order of escins > desacylescins > deacetyl escins, suggesting the importance of acyl moieties at C-21 and an acetyl moiety at C-22 for more inhibitory activity. It should be noted that both escins Ib and IIb, and deacetylescins Ib and IIb with angeloyl moiety at C-21 exerted more potent inhibition on pancreatic lipase than escins Ia and IIa, and deacetylescins Ia and IIa with tigloyl moiety at C-21. Considering chemical structures of geometric isomers at C-21, the tigloyl moiety has a double bond with cis configuration in terms of methyl groups. In contrast, the angeloyl group has a double bond with trans configuration. These facts led us to assume that the b-type saponins with

angeloyl moiety might enter the active center of pancreatic lipase due to the trans configuration, resulting in a more inhibitory effect. Interestingly, desacylescins without both acyl and acetyl moieties more potently inhibited pancreatic lipase activity than deacetylescins without acetyl group only. This result is apparently different from our related study on the effect of escins and their derivatives on the elevation in blood glucose levels since desacylescins were less potent in suppressing glucose absorption than corresponding deacetylescins at the same dose (*7*). The varied efficacy would reflect the difference in the sensitivity of digestive enzymes to each component of saponins. At present, the mechanism for the specificity of those saponins on the digestive lipase remains unclear. Further studies have yet to be done at the molecular level.

On the basis of the above observations on the inhibition of the pancreatic lipase in vitro, it was conceivable that saponins from natural and edible seeds would have anti-obesity effects on mice in vivo fed high-fat diets supplemented with either saponin fraction. In this study, we found the significant suppression of body weight gain when mice were fed for 8 weeks with high-fat diets containing 0.1% and 0.5% saponin fraction from natural seeds as well as the high-fat diet with 0.5% saponin fraction from edible seeds. Furthermore, the anti-obesity effect of the saponins can be supported by the suppression of increases in the weight of peritoneal adipose tissues and the plasma levels of triacylglycerols in obese mice fed a high-fat diet. However, we did not recognize any unusual pathological symptoms in mice fed with high-fats with saponins from natural or edible seeds. For example, the decreased weight of peritoneal adipose tissues was not accompanied by the accumulation of neutral fats in the liver and kidney. A marked increase in plasma GOT activity was evident in obese mice fed a high-fat diet, indicating the damage of hepatic tissues. In sharp contrast, feeding saponins from natural and edible seeds in the high-diet fats reversed GOT activity to the level of the control group fed the standard chow. Taken together, saponins from both natural and edible sources would be useful as anti-obesity factors without exerting harmful effects in animal experiments.

Previously, we reported that the saponin fraction from natural seeds was 10-fold bitterer than that from edible seeds to human taste (*8*). Hence, it might be possible to consider that the effect was caused by a reduction in the food intake of mice fed saponins, especially from natural sources. However, in this study, this possibility can be clearly excluded because there were no significant differences between the groups of mice in terms of the amounts of food intake. Mice could be insensitive to the bitter taste of saponins. As predicted, we observed a more appreciably increased excretion of triacylglycerols in the feces of mice fed high-fat diets with saponins from natural and edible seeds than obese mice fed the high-fat diet only. This is reflected by the reduction in fat digestion and the increase in the contents of neutral fats in feces in the groups fed with saponins. These findings are supportive of the effective inhibition of pancreatic lipase by saponins in the digestive tracts in mice in vivo. Our in vitro studies showed that the inhibitory action of escins from natural seeds was more potent than that of desacylescins and deacetylescins from edible seeds. However, both saponin fractions from natural and edible seeds exhibited significant antiobese effects at similar levels. This might be accounted for by the efficacious doses of both saponins in animal experiments in vivo. Alternatively, effects other than the inhibitory action of saponin on pancreatic lipase might be involved in vivo.

Recent studies have also described anti-obesity effects of certain saponins and related compounds. These include the action of teasaponins to prevent the obesity induced in mice fed high-fat diets as well as the inhibitory action on pancreatic lipase activity in vitro (*10*). Moreover, diosicin, a saponin family, isolated from the root of *Dioscorea nipponica*, Makino, a perennial herb, has been shown to reduce fat absorption through the inhibition of pancreatic lipase (*9*). Apart from the anti-obesity action through the inhibition of lipase activity, some ginsenosides, saponins isolated from the roots of ginseng, have been reported to enhance adipose differentiation in cultured systems (*13*), suggesting the specific action of aglycone groups in some saponins with unique chemical structures after circulation in the body. More investigations needs to be done to unravel the structural basis of the specificity of saponins and related derivatives.

In summary, saponins from natural and edible seeds of Japanese horse chestnut are effective not only in inhibiting pancreatic lipase in vitro but also in exerting anti-obesity effects in vivo through the suppression of digestion and absorption of fats. These results imply the potential usefulness of desacylescins and deacetylescins, which are obtained during food processing of the seeds, in the utilization as nutraceutical anti-obesity factors with attenuated bitter taste.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; ESI-MS, electrospray ionization-mass spectrometry; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; S.E., standard error; HF, high-fat diet; HF + 0.1% SFNS, high-fat diet containing 0.1% saponin fraction from natural seeds; HF + 0.5% SFNS, high-fat diet containing 0.5% saponin fraction from natural seeds; HF + 0.5% SFES, high-fat diet containing 0.5% saponin fraction from edible seeds.

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