

Anti-Obesity Effect of Proanthocyanidins from the Coat of Scarlet Runner Beans on High-Fat Diet-Fed Mice

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Abstract Proanthocyanidins are oligomeric or polymeric flavonoids found in several plants. They can be classified into two subgroups, namely A- and B-type proanthocyanidins. The coat of scarlet runner beans contains abundant proanthocyanidins (SRPAs) of the B-type configuration. To evaluate the efficacy of SRPAs as anti-obesity agents, we first examined their effect on lipase activity *in vitro* and found that they are potent inhibitors of this enzyme. Subsequently, we examined their effect on mice fed a high-fat diet. Male mice were assigned to the following seven-subject dietary groups: (1) high-fat diet; (2) high-fat diet supplemented with 0.5% SRPAs; (3) high-fat diet supplemented with 1.0% SRPAs, and; (4) standard chow for 15 weeks. SRPA supplementation decreased body weight gain; liver and kidney weight; perirenal, peritesticular, and periintestinal fat content; liver cholesterol level, and; serum neutral lipid and cholesterol levels. In contrast, the faecal lipid content and the liver anti-oxidative capacity increased. These findings suggest a potential use of SRPAs as a dietary supplement exerting anti-obesity effects through the inhibition of fat digestive enzymes.

Keywords: scarlet runner bean coat, anti-obesity, proanthocyanidin, lipase activity inhibition, fat digestion

Cite This Article: Zhaohong Ci, Chengyu Jiang, Shuo Feng, Shan Wu, Yang Cui, Yuki Sasaki, and Michiyuki Kojima, "Anti-Obesity Effect of Proanthocyanidins from the Coat of Scarlet Runner Beans on High-Fat Diet-Fed Mice." *Journal of Food and Nutrition Research*, vol. 6, no. 2 (2018): 103-109. doi: 10.12691/jfnr-6-2-6.

1. Introduction

An imbalance between energy intake and expenditure may result in the abnormal growth of adipose tissue, thereby leading to obesity [1]. Obesity is strongly associated with the metabolic syndrome, which is a risk factor for several lifestyle diseases, such as hyperlipidemia, hypertension, and diabetes [2]. As pancreatic lipase is a key enzyme for fat digestion, blocking fat decomposition and absorption by its inhibition is an effective approach for preventing obesity. Pancreatic lipase inhibitors from foods and other natural sources have been reported to suppress lipid absorption [3].

Proanthocyanidins belong to flavonoids and are oligomers or polymers of flavan-3-ols [4]. They can be classified into two subgroups, namely B-type proanthocyanidins, in which monomers are linked only with B-type bonds, and A-type proanthocyanidins, which have both A- and B-type bonds. The mean degree of polymerization (mDP) is a factor influencing the biological activities of proanthocyanidins. For example, polyphenolic compounds with higher mDP have been shown to possess greater antioxidant [5] and lipase inhibition activities [6]. Thiolytic is a method for

the structural characterization of proanthocyanidins, as it supposedly only breaks the single B-type bonds without affecting the doubly linked A-type bonds, resulting in the release of the terminal units and the formation of thioether derivatives from the extension units. For example, thiolytic in okra results in the release of underivatized epigallocatechin and epicatechin [7], whereas epigallocatechin and galocatechin are released as derivatives by the cleavage of B-type proanthocyanidins bonds of that takes place during thiolytic in pea and faba bean [8].

Scarlet runner bean (*Phaseolus coccineus* L.) is cultivated for its seeds (dried or fresh) but also as an ornamental plant [9]. The dry seeds of the plant are a valuable source of many nutrients, including proteins, starch, dietary fibers, and oligosaccharides [10], and are used in salads, soups, and amanatto in Japan. The seed coat of legume grains reportedly contains numerous types of phenolics, which have been suggested to play an important protective role against oxidative damage [11,12].

In the present study, we utilized thiolytic to structurally characterize the proanthocyanidins contained in the scarlet runner bean's coat (SRPAs). Moreover, we examined their ability to inhibit pancreatic lipase activity *in vitro* and their anti-obesity effect in high-fat diet-fed mice *in vivo*.

2. Materials and Methods

2.1. Materials

Scarlet runner beans were obtained from the Kawanishi Agricultural Cooperative Association (Obihiro, Japan). Diaion HP-20 columns and Sephadex LH-20 columns for chromatography were supplied by the Mitsubishi Chemical Corporation (Tokyo, Japan) and GE Healthcare Bio-Sciences AB (Uppsala, Sweden), respectively. All other reagents and chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless stated otherwise.

2.2. Extraction and Fractionation of Proanthocyanidins

Scarlet runner beans were immersed in distilled water and the coat was manually separated from the cotyledon, freeze-dried, grounded into powder, and extracted with 70% acetone. The mixture was filtered using Advantec No. 5 filters (Tokyo, Japan) and the filtrate was collected. The residues were subjected to another four rounds of 70% acetone extraction and filtration. Filtrates and soaking water were mixed, concentrated by rotary evaporation under vacuum, and purified by chromatography through Diaion HP-20 columns. The columns were washed with distilled water and then eluted with methanol. The SRPA-containing methanol fraction was concentrated by rotary evaporation under vacuum and used to supplement the mouse diet in the animal experiments. Moreover, a part of the concentrate was dissolved in ethanol and fractionated by Sephadex LH-20 column chromatography. The column was successively eluted with ethanol, methanol, and 60% acetone, to collect fractions I (fra.I), fraction II (fra.II), and fraction III (fra.III), respectively. The proanthocyanidin content of each fraction was determined by the HCl-butanol method [13], using cyanidin as the standard equivalent.

2.3. Thiolytic of SRPAs for RP-HPLC Analysis

Thiolytic was conducted according to a reported method with minor modification [14]. In a small glass vial, fra.II or fra.III (250 mg/mL) was mixed with HCl (0.1 % v/v in methanol) and 2-mercaptoethanol (5% v/v in methanol). The vial was sealed with an inert Teflon cap, heated at 40 °C for 60 min, and then stored at -20 °C until analysis. RP-HPLC (reversed-phase high-performance liquid chromatography) separation was conducted on C18 columns (250 mm × 4.6 mm) (Shimadzu Corporation, Tokyo, Japan), with SPD-10AD (Shimadzu Corporation, Tokyo, Japan) used for detection. Analysis was performed with (A) 0.1% (v/v) trifluoroacetic acid and (B) 0.1% (v/v) trifluoroacetic acid-acetonitrile. The elution protocol was as follows: 8% B (initial), 30% B for 30 min, 30% B for 50 min, and 8% B for 60 min at a flow rate of 1 mL/min. Detection was performed at 280 nm. The mDP of proanthocyanidins was calculated using the following formula:

$$mDP = 1 + \frac{\text{area of [catechin and epicatechin derivatives]}}{\text{area of [catechin and epicatechin]}} \quad (1)$$

2.4. Determination of Pancreatic Lipase Activity in Vitro

The assay for determining pancreatic lipase activity in vitro was carried out with a slightly modified version of the protocol described in Han et al. [15]. Briefly, 30 µL of 3 mg/mL pancreatic lipase and 0.45 mL of various concentrations of sample solution were pre-incubated for 10 min at 37 °C. After pre-incubation, a substrate solution containing glycerol triolein (80 mg), phosphatidylcholine (10 mg) and cholic acid (5 mg) in 9 mL of 0.1 M N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid buffer (pH 7.0) was added and incubated for 30 min at 37°C. Afterward, 2.5 mL of 1 M copper reagent and 5 mL chloroform were added to the reaction mixture, which was then centrifuged at 1,006 ×g for 10 min. The upper, aqueous phase was removed, whereas sodium diethyldithiocarbamate was added to the lower, free fatty acid-containing chloroform phase. The absorbance was measured at 440 nm and lipid concentrations were calculated using linoleic acid as a standard equivalent. Lipase inhibitory activity (%) was calculated from the following formula:

$$\text{Lipase inhib. activity(\%)} = \left(1 - \frac{FFA_{\text{sample}}}{FFA_{\text{control}}} \right) \times 100\% \quad (2)$$

where FFA_{sample} and FFA_{control} are the quantities of free lipids in the sample and the control, respectively.

2.5. Animals and Diets

Male ddy mice (Japan SLC, Inc., Shizuoka, Japan) were housed in plastic cages at 23 °C on a 12 h/12 h light/dark cycle, at a relative humidity of 60%. Mice had free access to standard chow and water for one week before the experiment. During the experiment, mice were fed a high-fat diet (Quick Fat; CLEA Japan, Inc., Tokyo, Japan) or standard chow (CE-2; CLEA Japan) to serve as controls. The high-fat diet had a total calorie content of 415.1 kcal/100 g and its composition (w/w %) was as follows: 24.0% crude protein, 14.6% crude fat, 2.7% crude fiber, 5.1% crude ash, 46.7% NFE (nitrogen-free extract), and 7.3% moisture [16]. Kishida et al. [17] used the same high-fat diet to induce obesity and type-2 diabetes in their study on the use of brown adipocytes to ameliorate insulin resistance. Standard chow had a total calorie content of 344.9 kcal/100 g and contained (w/w %) of 24.9% crude protein, 4.6% crude fat, 4.1% crude fiber, 6.6% crude ash, 51.0% NFE, and 8.9% moisture [17]. The study was approved by the regulatory authority of the National University Corporation Obihiro University of Agriculture and Veterinary Medicine, and it adhered to the standard principles described in the Guide for the Care and Use of Laboratory Animals.

2.6. Anti-obesity Evaluation of Proanthocyanidins in Mice Fed a High-Fat Diet

After adaptation, mice were divided into four groups, each with seven subjects: (1) high-fat diet group (HF); (2) high-fat diet with 0.5% SRPA (0.5% SRPAHF) group; (3)

high-fat diet with 1.0% SRPA (1.0% SRPAHF) group; (4) standard chow (SC) group. During the experiment, food and water were provided *ad libitum*. Food and water intake, body weight, and the weight of faeces were recorded for 15 weeks. Afterward, the mice were fasted, anesthetized with a Nembutal (pentobarbital) injection (0.75 $\mu\text{L/g}$ body weight), and dissected. Blood was collected from the heart and mixed quickly with EDTA-2Na (ethylenediaminetetraacetic acid disodium salt, 2-hydrate) as an anticoagulant. Liver and kidneys were weighted, frozen in liquid nitrogen, and stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Blood samples were centrifuged at $1000 \times g$ for 30 min to separate and the upper phase, i.e., the serum, was collected and stored at $-85\text{ }^{\circ}\text{C}$ until use for neutral lipid analysis and quantification of cholesterol (total, HDL, and LDL), alanine aminotransaminase (ALT), and aspartate transaminase (AST). The analysis of the serum samples was conducted by Obihiro clinical laboratory, Inc. (Obihiro, Japan).

Liver and faecal lipid concentrations were measured according to a previously reported method [18]. In brief, liver and faecal lipids were extracted by a chloroform/methanol solution (2:1, v/v). The extracts were dried under N_2 to constant weight and that weight value was recorded. Isopropanol was added, and liver and faecal cholesterol levels were determined by commercial kits (Wako Pure Chemical Industries, Ltd.).

The faecal bile acid content was measured as described previously [19]. Briefly, faeces were extracted by distilled water using sonication. The extract was centrifuged and the supernatants were collected. The bile acid content was determined from the supernatants using commercial kits (Wako Pure Chemical Industries, Ltd.).

Lipid peroxidation in liver was determined by measuring thiobarbituric acid-reactive substances (TBARS) with a slightly modified version of the protocol described in [19,20]. Briefly, 400 μL of 8.1% sodium dodecyl sulfate solution, 300 μL of 20% acetic acid (pH 3.5), and 500 μL of distilled water were successively added to 100 mg of liver. The mixture was homogenized using a Teflon homogenizer, and an equal volume of antioxidant solution (0.25 mg/mL copper sulfate) was added. After the mixture was incubated for 30 min at $37\text{ }^{\circ}\text{C}$, 950 μL was transferred to a capped test tube, to which 25 μL of 0.8% butylated hydroxytoluene acetic acid and 750 μL of 0.8% tertiary butyl alcohol were then added. The reaction mixture was placed for 1 h on ice, incubated at $100\text{ }^{\circ}\text{C}$ for 1 h, and then cooled in running water. Afterward, 0.5 mL of distilled water and 2.5 mL of n-butanol/pyridine (15:1) were added, mixed vigorously, and centrifuged at $1,500 \times g$ for 10 min to obtain the supernatant, which was measured at 532 nm. Lipid peroxidation was expressed as the amount of produced 1,1,3,3-tetraethoxypropane.

2.7. Statistical Analysis

Values are expressed as the mean \pm standard error (S.E.M). Differences were evaluated by one-way ANOVA, followed by Tukey's studentized range (Honestly Significant Difference) test, using SAS v9.3 (SAS Institute Inc., Cary, NC, USA). Differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Proanthocyanidin Content per SRPA Fraction

The 70% acetone extract of the scarlet runner beans' coat was rich in proanthocyanidins (180 mg/g seed coat), much higher than the reported content of pea, lentil, and faba bean (3.36, 3.29, and 6.54 mg/g seed, respectively) [8]. We performed Sephadex LH-20 column chromatography to obtain three fractions: Fra.I, Fra.II, and Fra.III. No proanthocyanidins were detected in Fra.I, whereas the proanthocyanidin content in Fra.II and Fra.III amounted to 47% and 53% of total weight, respectively (Figure 1). According to Saito et al. [21], Fra.I contains monomeric phenolic compounds; Fra.II, polyphenols with a low degree of polymerization; and Fra.III, polyphenols with a high degree of polymerization.

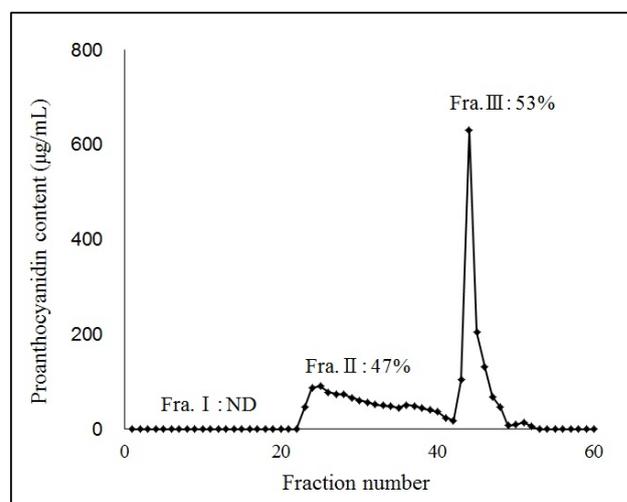


Figure 1. SRPA fraction was applied to an LH-20 column for the successive elution of Fra.I (ethanol fraction, 1–20); Fra.II (methanol fraction, 21–40), and; Fra.III (60% Acetone fraction, 41–60). ND, not detected

3.2. Thiolytic Analysis of Fra.II and Fra.III

To investigate the composition and calculate the mDP values for Fra.II and Fra.III, thiolytic cleavage was carried out and the reaction products were analyzed by RP-HPLC (Figure 2). During the thiolytic cleavage of proanthocyanidins, terminal units are released as free flavan-3-ols, whereas all extension units are attacked by the nucleophilic agent (2-mercaptoethanol was used in this study) to generate the respective derivatives. We detected (+)-catechin and (–)-epicatechin monomers in Fra.II and Fra.III, indicating that these constituted the terminal units of the corresponding proanthocyanidins. Moreover, the (–)-epicatechin derivative was the compound with the largest peak area, suggesting that (–)-epicatechin was the main constituent compound for proanthocyanidins in both fractions. Regarding mDP, Fra.III had a higher value (9.6) than Fra.II (3.8). In both fractions, proanthocyanidins were of the B-type configuration. B-type proanthocyanidins were previously found in pea, lentil, and faba bean, and their mDPs were determined at 5–8 by thiolytic cleavage [8].

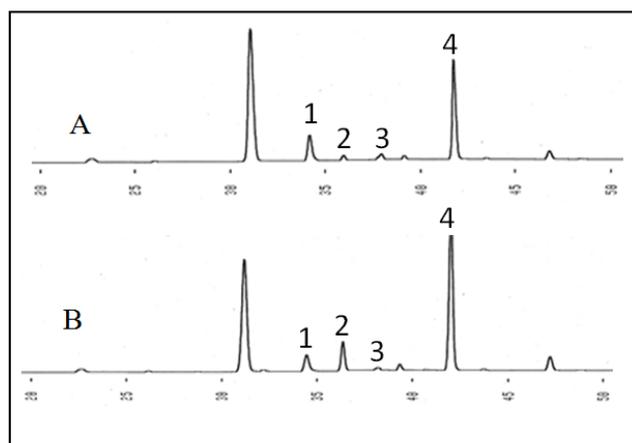


Figure 2. RP-HPLC chromatogram (280 nm) of the products of Fra.II (A) and Fra.III (B) thiolysis. Peaks: 1, (+)-catechin; 2, (+)-catechin derivative; 3, (-)-epicatechin; 4, (-)-epicatechin derivative

3.3. Inhibitory Effects of SRPAs on Lipase Activity in Vitro

Polyphenols from litchi flowers have been previously demonstrated to exert an inhibitory effect on porcine pancreatic lipase [22]. Generally, the suppressive effect of polyphenols on lipase activity is attributed to their affinity for proteins, which leads to the aggregation of the enzyme [23]. The effects of SRPAs (total, Fra.II, and Fra.III) on pancreatic lipase activity were investigated. All three demonstrated a significant ($p < 0.05$) dose-dependent suppressive effect, with IC_{50} values of 4.12 ± 0.22 , 3.88 ± 0.35 , and 1.84 ± 0.46 $\mu\text{g/mL}$, respectively (Table 1). Since Fra.III contains proanthocyanidins with a higher degree of polymerization compared to those of Fra.II, the fact that the former had a stronger lipase inhibitory activity compared to the latter indicates that more highly polymerized proanthocyanidins have more potent inhibitory activities. As a means of comparison, we also analyzed the lipase inhibitory activity of epigallocatechin gallate, which has been reported to be a stronger lipase inhibitor than catechin, gallic acid, and epicatechin. [24]. We calculated its IC_{50} value at 18.01 ± 1.39 $\mu\text{g/mL}$, thus it is a weaker lipase inhibitor than SRPAs.

Table 1. SRPA IC_{50} Values

	IC_{50} ($\mu\text{g/mL}$)
SRPAs (total)	4.12 ± 0.22^b
Fra.II	3.88 ± 0.35^b
Fra.III	1.84 ± 0.46^c
Epigallocatechin Gallate	18.01 ± 1.39^a

Values represent mean \pm S.E.M. Differences between values superscripted with different letters were significant ($p < 0.05$).

3.4. Effects of SRPAs on Body Weight Gain, Organs, and Adipose Tissue in Mice Fed a High-Fat Diet

To assess the anti-obesity effects of SRPAs, mice were fed with different diets (see Subsection 2.5, “Animals and Diets”) for 15 weeks and various parameters were measured at the end of that period. Results are shown in Table 2.

The mice of the HF group displayed the highest gain of body weight, whereas, as expected, SC mice exhibited the lowest gain of body weight. As made obvious by the results of the 1.0% SRPAHF, supplementation with SRPAs was clearly effective in attenuating the gain of body weight ($p < 0.05$ vs. the HF group), lowering it to a level close to the one seen in the SC group. A similar suppression of body weight gain was reported in studies investigating the effects of the consumption of polyphenols from green tea [25] and seaberry leaf [26]. In contrast, no significant difference was observed in our study among the different diet groups with respect to food consumption, water intake, and faecal weight.

We also investigated whether supplementation with SRPAs for 15 weeks affects the weight of organs and adipose tissue. Both 0.5% and 1.0% SRPAHF had lower liver and kidney mass, as well as less perirenal fat content, compared to the HF group. At 1.0% supplementation, SRPAs also suppressed the increasing effect of high-fat diet on peritesticular and periintestinal fat content, compared to the HF group. Similar decreases in adipose tissue weight have been reported for seaberry leaf polyphenols by Nishi et al. [26]. The decrease in body weight gain by SRPAs might be responsible for the normalization of these organs and tissues [3].

Supplementation with 1.0% SRPAs significantly decreased liver lipid and cholesterol content. These results are in agreement with those of Ali et al. [27] who reported that rats fed a high-fat diet supplemented with cocoa polyphenols displayed decreased liver weight, as well as lower liver lipid and cholesterol content, compared with mice fed a high-fat diet only. We also observed that supplementation with 0.5 or 1.0% SRPAs significantly increased the lipid and total cholesterol content of faeces. Our results are similar to those of Uchiyama et al. [3], who reported that supplementation of high-fat diet with 5% black tea polyphenols increased faecal triglyceride content. We conclude that the inhibition of lipase activity by SRPAs reduced fat decomposition and, by extension, the absorption of free fatty acids. Thus, more fat was excreted, whereas less fat was accumulated.

3.5. Effects of SRPAs on Serum and Liver Lipid Peroxides in Mice Fed a High-Fat Diet

We also examined how the different diets affected various serum parameters associated with obesity including the levels of neutral lipids, cholesterol (total, HDL, and LDL), ALT, and AST. There were no significant changes in ALT and AST levels among the different diet groups.

In contrast, as seen in Table 3, both the 0.5% SRPAHF and the 1.0% SRPAHF group displayed significantly lower levels of neutral lipids and LDL cholesterol compared with the HF group. Moreover, supplementation with 1.0% SRPAs suppressed the increasing effect of high-fat diet on HDL cholesterol. Total cholesterol levels were significantly lower and the HDL:LDL ratio was significantly higher in the 1.0% SRPAHF group compared to the HF group. Similar decreases in total plasma cholesterol have been reported for proanthocyanidins isolated from the seed shells of Japanese horse chestnut [24] and black tea [3]

and may be attributed to a decrease in the micellar solubility of cholesterol, as has been previously shown in a study using Reishi extracts [28]. In turn, the decrease in micellar solubility may result from specific interactions between proanthocyanidins and phosphatidylcholine, as was reported for epigallocatechin gallate [29].

To investigate possible changes in the liver antioxidant capacity, we analyzed liver lipid peroxide content by measuring the abundance of TBARS in the different diet groups. Both the 0.5% SRPAHF and the 1.0% SRPAHF group had significantly lower liver lipid peroxide levels compared with the HF group (Table 3), indicating that SRPAs increase the antioxidant capacity of the liver. This result is in agreement with that of a previous study in which supplementation of a high-fat diet with buckwheat miso decreased lipid peroxide levels compared with high-fat diet alone [19].

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Table 2. Total Body, Organ, and faecal weight Measurements for the Four Dietary Groups

	HF	0.5% SRPAHF	1.0% SRPAHF	SC
Food consumption (g/day)	5.88 ± 0.42	5.67 ± 0.41	5.78 ± 0.58	6.03 ± 0.25
Water intake (mL/day)	6.45 ± 0.40	6.39 ± 0.47	6.40 ± 0.51	6.61 ± 0.35
Initial body weight (g)	42.97 ± 1.86	41.88 ± 2.97	42.33 ± 2.26	42.29 ± 3.28
Final body weight (g)	55.00 ± 2.88 ^a	51.94 ± 5.32 ^{a,b}	49.34 ± 5.51 ^{a,b}	47.43 ± 2.51 ^b
Body weight gain (g/15 weeks)	15.56 ± 7.35 ^a	11.66 ± 3.27 ^{a,b}	9.52 ± 2.50 ^b	8.36 ± 1.64 ^b
Liver weight (g)	2.23 ± 0.20 ^a	1.83 ± 0.08 ^b	1.79 ± 0.12 ^b	1.75 ± 0.12 ^b
Liver lipid content (mg/g)	111.13 ± 7.25 ^a	111.52 ± 8.16 ^a	95.68 ± 7.11 ^b	80.55 ± 9.86 ^c
Liver cholesterol (mg/g)	7.86 ± 1.73 ^a	4.81 ± 0.92 ^b	5.15 ± 1.28 ^b	3.59 ± 0.59 ^b
Kidney weight (g)	0.85 ± 0.174 ^a	0.67 ± 0.05 ^b	0.67 ± 0.01 ^b	0.71 ± 0.03 ^b
Perirenal fat (g)	0.77 ± 0.16 ^a	0.49 ± 0.15 ^b	0.41 ± 0.16 ^b	0.45 ± 0.06 ^b
Periintestinal fat (g)	1.79 ± 0.47 ^a	1.34 ± 0.36 ^{a,b}	1.06 ± 0.33 ^{b,c}	0.71 ± 0.16 ^c
Peritesticular fat (g)	2.71 ± 0.37 ^a	2.29 ± 0.84 ^{a,b}	1.75 ± 0.79 ^b	1.57 ± 0.36 ^b
Faecal weight (g/day)	1.06 ± 0.08	1.05 ± 0.07	1.16 ± 0.02	1.13 ± 0.01
Faecal lipid content (mg/day)	83.99 ± 19.35 ^b	78.84 ± 9.12 ^b	103.46 ± 11.35 ^a	102.88 ± 11.68 ^a
Total faecal cholesterol content (mg/day)	3.43 ± 0.57 ^c	4.22 ± 0.44 ^c	5.37 ± 1.13 ^b	7.94 ± 0.97 ^a
Faecal bile acid (μmol/day)	0.28 ± 0.05	0.31 ± 0.08	0.30 ± 0.05	0.32 ± 0.12

Values represent mean ± S.E.M. In each column, differences between values superscripted with different letters were significant ($p < 0.05$).

Table 3. Serum and Liver Lipid Peroxide Measurements for the Four Dietary Groups

	HF	0.5% SRPAHF	1.0% SRPAHF	SC
Netural lipids (mg/dL)	111.00 ± 30.76 ^a	63.60 ± 30.62 ^b	47.40 ± 15.18 ^b	41.80 ± 12.11 ^b
Total cholesterol (mg/dL)	194.20 ± 27.43 ^a	152.40 ± 38.28 ^{a,b}	139.20 ± 20.90 ^b	137.40 ± 11.22 ^b
HDL cholesterol (mg/dL)	141.60 ± 26.60 ^a	118.60 ± 25.69 ^{a,b}	110.00 ± 17.58 ^b	109.20 ± 4.66 ^b
LDL cholesterol (mg/dL)	31.60 ± 5.90 ^a	22.00 ± 7.31 ^b	17.00 ± 4.64 ^{b,c}	12.00 ± 2.92 ^c
HDL:LDL	4.47 ± 0.55 ^c	5.71 ± 0.82 ^{b,c}	6.73 ± 1.48 ^b	8.76 ± 1.38 ^a
AST (U/L)	187.2 ± 43.24	143.40 ± 52.63	141.00 ± 47.37	138.40 ± 18.12
ALT (U/L)	25.60 ± 3.85	23.60 ± 6.11	23.60 ± 5.90	31.00 ± 8.89
Liver lipid peroxides (nmol/g)	265.34 ± 14.90 ^a	220.37 ± 39.20 ^b	203.86 ± 22.70 ^b	196.77 ± 17.05 ^b

Values represent mean ± S.E.M. In each column, differences between values superscripted with different letters were significant ($p < 0.05$).

4. Conclusions

The coat of scarlet runner beans contains abundant proanthocyanidins with single B-type bonds, which inhibit pancreatic lipase activity in vitro and are effective anti-obesity agents in vivo, as shown by experiments where mice were fed a high-fat diet supplemented with SRPAs. These observations indicate that this part of the seed may serve as a source for the development of nutraceuticals with anti-obesity activity.

Acknowledgements

We acknowledge the financial support given by the Obihiro University of Agriculture and Veterinary Medicine, as well as Iwate University, for the completion of the study.

Statement of Competing Interests

We declare that we do not have any conflict of interest.

List of Abbreviations

ALT, alanine aminotransaminase; AST, aspartate transaminase; EDTA 2Na, ethylenediaminetetraacetic acid disodium salt, 2-hydrate; Fra.I, fractions I; Fra.II, fraction II; Fra.III, fraction III; HDL, high-density lipoprotein; HF, high-fat diet; LDL, low-density lipoprotein; mDP, mean degree of polymerization; NFE, nitrogen-free extract; RP-HPLC, reversed-phase high-performance liquid chromatography; SC, standard chow; SRPAs, scarlet runner beans' coat proanthocyanidins; TBARS, thiobarbituric acid-reactive substances; 0.5% SRPAHF, high-fat diet supplemented with 0.5% SRPAs; 1.0% SRPAHF, high-fat diet supplemented with 1.0% SRPAs

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