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Anti-hyperlipidemic activity of *Rhynchosia nulubilis* seeds pickled with brown rice vinegar in mice fed a high-fat diet

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Abstract

The abnormal content of blood lipids often results in metabolic diseases, such as hyperlipidemia and obesity. Many agents, including natural sources from traditional food, have been developed to regulate the blood lipid contents. In this study, we examined the anti-hyperlipidemic activity of *Rhynchosia nulubilis* seeds pickled with brown rice vinegar (RNSpBRV), a Korean traditional pickled soybean food. Since RNSpBRV is made of *R. nulubilis* seeds (RNS) soaked in brown rice vinegar (BRV), we compared the anti-adipogenic activity between RNS, BRV and solid fraction of RNSpBRV (SF-RNSpBRV), liquid fraction of RNSpBRV (LF-RNSpBRV). For this, the inhibitory effect of lipid accumulation in 3T3-L1 adipocyte was checked by adding methanol extracts of mixed RNS and BRV, LF-RNSpBRV, and SF-RNSpBRV. The addition of each methanol extract up to 1 mg/ml showed no cytotoxicity on 3T3-L1 adipocyte, and approximately 20% of the lipid droplet formation was suppressed with the methanol extract of BRL or SF-RNSpBRV. The highest suppression (42.1%) was achieved with LF-RNSpBRV. In addition, mice fed a high fat diet (HFD) supplemented with 5% RNSpBRV powder led to increased high density lipoprotein (HDL) cholesterol and lower blood glucose, triglyceride, and total cholesterol compared to mice fed with a HFD diet only. Interestingly, the size of the epididymis cells gradually decreased in HFD + 1% RNSpBRV- and HFD + 5% RNSpBRV-fed mice if compared those of HFD-fed mice. Taken together, these results provide evidence that RNSpBRV has a regulatory role in lipid metabolism that is related to hyperlipidemia.

Key Words: Hyperlipidemia, *Rhynchosia nulubilis* seeds, brown rice vinegar, high-fat diet, epididymis cell.

Introduction

Hyperlipidemia is a blood lipids disorder that involves abnormally elevated levels of any or all lipids in the blood. It is classified as primary hyperlipidemia, which is induced by genetic problems, and secondary hyperlipidemia, which is induced by other factors, such as disease, therapeutics, and abnormal diet [1,2]. An increase in blood lipids contents often results in abnormal blood clots, stroke, and myocardial infarction [3-7]. An excessive dietary intake is one of the key causes of hyperlipidemia and is closely related to obesity and diabetes [8-10].

Obesity patients showed elevated lipid concentrations in blood and enlarged fat masses [11,12]. Since white adipose tissue is the main energy reserve in the human body, the way of regulating the size and number of adipocytes has been considered as a means to overcome obesity [13]. Recent studies have shown that many natural agents induce anti-adipogenic activity. Green tea extracts, for example, have been reported as having anti-differentiation activity of preadipocytes and anti-obesity effects [14,15], and administration of perilla leaf extract reportedly ameliorates obesity and dyslipidemia induced by a high-fat diet [16]. Type I and II diabetes patients should be administrated insulin and therapeutics, respectively; however, repeated insulin treatments often induce hyperglycemia and anti-diabetes therapeutics have shown secondary effects [17,18]. For these reasons, there is an increased interest in developing alternative medicines, including natural herbs and traditional food extracts.

Rhynchosia nulubilis seed (RNS) is a kind of small black soybean and it is a species of legume native to East Asia. It has been used as an antioxidant in oriental medicine, and studies have documented its disease-ameliorating effects [19-21]. Black soybean has immune-modulating properties and antitumor effects [22], anthocyanins from black soybean seed coats have a protective effect on UVB-induced apoptotic cell death [23], and the polysaccharide of black soybean is reported to have myelopoiesis-promoting effects [24]. Daily intake of black soybean has also been reported as attenuating the risk of breast cancer [25]. In Korea, RNS has been widely used as a rice supplement and for making several combined foods. Among them, pickled RNS with brown rice vinegar (RNSpBRV) is a well-known RNS-based food that is considered to be a healthy food item because of its nutritional combination of RNS and BRV.

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Vinegar is used throughout the world as a food additive, preservative, and ingredient in salad dressing, and various types of vinegar can be made by using different crops. Vinegar has recently been targeted as a medical food because acetic acid, which is the main ingredient of vinegar, is reported to have anti-hyperlipidemic activity [26,27]. Since vinegar also has many other ingredients and RNSpBRV is composed of two main ingredients (RNS and BRV), it is possible that RNSpBRV has ameliorating anti-hyperlipidemic activity.

Although in Korea RNSpBRV is known to decrease blood lipids, there has been no published report on the anti-hyperlipidemic and anti-aidpogenic potential of RNSpBRV. Thus, the aims of this study were to investigate the anti-adipogenic activity of RNSpBRV *in vitro* and to evaluate the anti-hypherlipidemic effect of RNSpBRV on mice fed a high-fat diet.

Materials and Methods

Chemicals

Dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich (cell culture grade, D2650, Yongin, Korea). HPLC grade Methanol (cat#:646377) and 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide (MTT) (cat#:M2003) were obtained from Sigma Aldrich (Yongin, Korea).

Preparation of RNS, BRV and RNSpBRV

Rhynchosia nulubilis seed and brown rice vinegar were purchased from a local Nonghyup market (Seoul, Korea). RNSpBRV was made by soaking RNS in BRV (1:2, w/v) at 4℃ for 1 month. For the 3T3-L1 cell differentiation inhibition assay, the liquid fraction of RNSpBRV and the solid fraction of RNSpBRV were separated, dried, powdered, and extracted with 90% methanol. Total RNSpBRV without separation was lyophilized and the resulting powder used for the animal study.

Cell culture

3T3-L1 adipocytes were obtained from the American Type Culture Collection and cultured in DMEM (BRL, Grand Island, NY) with 10% fetal bovine serum (BRL), 1% (v/v) 100 μM pyruvate, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. 3T3-L1 cells were differentiated according to Harmon *et al.* [28]. Briefly, confluent 3T3-L1 adipocytes were cultured for an additional 2 days, and differentiation was initiated by adding 0.5 mM 3-isobutyl-1-methylxanthine, 0.5 μM dexamethasone, and 10 μg/ml insulin to the culture medium. After 72 h in the culture, the medium was changed to DMEM with 10% FBS. The media was changed every 2 days until analysis on days 9-10. Each of the two methanol extracts, which were reconstituted in 1000 μg/ml stock solution in DMSO and filter sterilized, was added to the media after differentiation had been initiated. The final concentration of DMSO was adjusted to 0.1%. For the vehicle control, 0.1% DMSO was used instead of the test compound.

Measuring 3T3-L1 adipocyte differentiation

Following the 9-10 days of incubation with the extracts (described above), cells were washed with PBS, fixed in 4% paraformaldehyde for 20 min, and stained with 0.5% Oil red O solution (Sigma-Aldrich, Yongin, Korea) for 1 h at room temperature. After washing with water several times, isopropanol was added to resolve the Oil red O solution stained in lipid droplets. Absorbance was measured at 500 nm (Model 680 microplate reader, Bio-Rad, Hercules, CA).

Cytotoxicity assay

3T3-L1 adipocytes were seeded in a 96-well plate at a density of 1.5×10^5 cells/well. Twenty-four hours after plating, extracts were added to the cell culture medium at various concentrations to reach the final concentration from 10^2 to 10^7 ng/ml and left at 37℃ for 24 h. MTT solution (5 mg/ml) was then added, and the culture was incubated for 3 h, after which DMSO was added to dissolve the fromazan crystals. The absorbance at 540 nm was measured with a microplate reader (Model 680 microplate reader, Bio-Rad, Hercules, CA).

Animals

Two-week-old male ICR mice were purchased from Daehan Biolink (Eumseon, Korea). After 1 week of adaptation, mice were divided into three groups: high fat diet (HFD) (n = 5), HFD + RNSpBRV 1% (n = 5), and HFD + RNSpBRB 5% (n = 5). Mice in the HFD group were fed a high-fat diet *ad libitum* for 5 weeks. HFD + RNSpBRV 1%, and HFD + RNSpBRV 5% groups were fed a HFD supplemented with 1% and 5% RNSpBRV powder, respectively. The composition of each diet is shown in Table 1. Body weight and food intake were measured every 3 days and 1 day, respectively. Care and treatment of the animals conformed to the Sungkyunkwan University guidelines for the Ethical Treatment of Laboratory Animals (Sikgi 2012-02).

Animal treatment and plasma analysis

All mice were fasted for 16 h and then sacrificed under ether anesthesia. Tissue samples were dissected, rinsed with PBS, wiped with filter paper, and then weighed. Blood samples were collected from the abdominal aorta and centrifuged at 2500 g for 15 min at 4℃ to separate the serum. All samples were kept at -80℃ until analyzed. Blood glucose, total cholesterol, plasma triglyceride (TG), high-density lipoprotein (HDL) cholesterol, glutamic-oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT) concentrations were measured using a

Table 1. Ingredient composition of each diets (g/100g)

	HFD	HFD+RNSpBRV1%	HFD+RNSpBRV5%
Corn starch	16.8	16.8	16.8
Casein	20.0	20.0	20.0
Destrinized cornstarch	13.2	13.2	13.2
Sucrose	10.0	10.0	10.0
Mineral Mixture[a]	3.5	3.5	3.5
Vitamin mixture[a]	1.0	1.0	1.0
L-Cystine	0.3	0.3	0.3
Choline chloride	0.25	0.25	0.25
Tert-butylhydroquninone	0.0014	0.0014	0.0014
Cellulose	5.0	5.0	5.0
RNSpBRV	0.0	1.0	5.0
Lard	30.0	30.0	30.0

[a] AIN-9G mineral and AIN93G vitamin Mixture (Reeves, 1993)

commercial kit (Spotchem SP-4410, Arkray Inc, Kyoto, Japan). Low-density lipoprotein (LDL) cholesterol was manually calculated according to Friedewald WT *et al*. [29].

Adipose tissue weight and adipocyte size

Epididymal white adipose tissue was weighed, fixed with 10% paraformaldehyde solution, and embedded in paraffin. Sections (4 μm thick) were cut and stained with hematoxylin and eosin. All images were taken with an optical microscope (Axioplan2 imaging; Zeiss, Jena, Germany), and the size of cells was calculated by using an image analyzer (Analysis 3.2; Softimaging System, Jena, Germany).

Statistical analyses

The results are presented as mean ± standard deviation, and the data were analyzed by one-way analysis of variation (ANOVA) using XLSTAT program (Addinsoft, NY, USA). It was followed by the Student-Newman-Keuls test. P values of 0.05 were considered significant.

Results

Cell viability and inhibition of lipid droplet formation on 3T3-L1 adipocytes

To check the inhibitory activity in adipocyte differentiation, methanol extracts of RNS, BRV, liquid fraction of RNSpBRV (LF-RNSpBRV), and solid fraction of RNSpBRV (SF-RNSpBRV) were used in the lipid droplet formation assay. An MTT assay was performed to determine the cytotoxicity of each extract. As shown in Fig. 1A, the addition of up to 1 mg/ml of each methanol extract showed almost no cytotoxicity on 3T3-L1 cell proliferation. Based on these data, 3T3-L1 adipocytes were exposed to each methanol extract (0.1 mg to 1 mg). Intracellular lipid droplets were stained with Oil Red O and quantitated to check

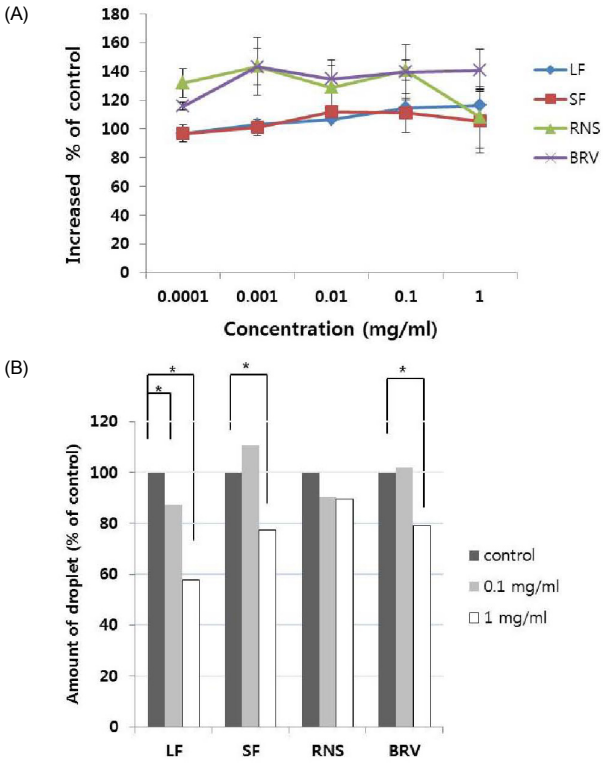

Fig. 1. The anti-adipogenic effect of methanol extract of RNS, BRV, SF-RNSpBRV, and LF-RNSpBRV. (A) 3T3 L1 adipocytes were treated with four types of methanol extracts (10^2 to 10^7 ng/ml) for 24 h. Cell viability was measured by an MTT assay. (B) Differentiated 3T3 L1 adipocytes were treated with four types of methanol extracts (0.1 mg/ml and 1 μg/ml) from the start of differentiation. Lipid accumulation rate was compared by calculating the Oil red O staining at OD = 500 nm. RNS and BRV mean *Rhynchosia nulubilis* seeds and brown rice vineagar respectively. LF and SF are the liquid fraction of RNSpBRV and the solid fraction of RNSpBRV, respectively. Each data value is expressed as the mean ± SE for at least three independent experiments. *P< 0.05 compared with the control.

the effect of each extracts on lipid accumulation. Reduced Oil red O staining occurred with treatment of BRV (20.7%, $P < 0.05$) as compared to the control cells. However, there was no significant suppression in cells treated with RNS. Interestingly, although methanol extraction of RNS seems to have little effect on lipid accumulation, methanol extracts of the solid fraction of RNS pickled in BRV (SF-RNSpBRV) suppressed lipid droplet formation (22.6%, $P < 0.05$). In addition, the highest suppressive effects (42.1%, $P < 0.05$) occurred in methanol extracts of LF-RNSpBRV-treated 3T3-L1 adipocytes (Fig. 1B). These results suggested that RNSpBRV have enhanced suppressive activity of lipid accumulation than RNS and BRV alone. Hence, we selected RNSpBRV for further animal studies.

Administration of RNSpBRV powder to mice fed a high-fat diet

Since RNSpBRV showed a suppressive role in lipid accumulation on 3T3-L1 adipocytes, RNSpBRV powder was prepared by lyophilizing total RNSpBRV without separating the liquid and solid fractions. As shown in Table 2, RNSpBRV supplementation did not affect body weight, food intake (Table 2), or selected

Table 2. Effect of RNSpBRV on food intake and body weight gain

	HFD	HFD+ RNSpBRV1%	HFD+ RNSpBRV5%
Initial body weight (g)	28.04 ± 1.82[a]	28.01 ± 1.41[a]	28.19 ± 1.53[a]
Final body weight (g)	41.59 ± 3.18[a]	41.26 ± 2.44[a]	41.23 ± 1.90[a]
Daily body weight gain (g)	0.39 ± 0.08[a]	0.38 ± 0.08[a]	0.35 ± 0.06[a]
Daily food intake (g)	4.00 ± 034[a]	4.10 ± 0.27[a]	3.88 ± 0.30[a]

ICR mice (n = 5 per group) were used for weight measurement for 5 weeks. Values represent the mean ± SD. The data were analyzed by ANOVA, and the different letters in same line indicate statistically significant difference (P< 0.05).

Table 3. Effect of RNSpBRV on Epididymis fat and each organ weights in HFD diet fed mice

	HFD	HFD+ RNSpBRV1%	HFD+ RNSpBRV5%
Epididymis fat (g)	1.68 ± 0.31[a]	1.70 ± 0.15[a]	1.47 ± 0.26[a]
Liver (g)	1.94 ± 0.18[a]	2.08 ± 0.23[a]	1.90 ± 0.26[a]
Kidney (g)	0.45 ± 0.04[a]	0.44 ± 0.05[a]	0.44 ± 0.03[a]
Spleen (g)	0.08 ± 0.01[a]	0.09 ± 0.01[a]	0.09 ± 0.01[a]
Heart (g)	0.16 ± 0.01[a]	0.16 ± 0.01[a]	0.17 ± 0.02[a]
Testis (g)	0.24 ± 0.05[a]	0.22 ± 0.02[a]	0.22 ± 0.03[a]

ICR mice (n = 5 per group) were used for weight measurement for 5 weeks. Values represent the mean ± SD. The data were analyzed by ANOVA, and the different letters in same line indicate statistically significant difference (P< 0.05).

organ weights (Table 3). However, administration of 5% RNSpBRV (160.3 ± 29.2 mg/dl) significantly reduced the level of blood glucose by 18.8% compared to the HFD group (197.0 ± 34.9 mg/dl). In addition, levels of total cholesterol (169.5 ± 30.8 mg/dl) and TG (67.8 ± 13.8 mg/dl) were significantly reduced

Table 4. RNSpBRV downregulated the blood glucose and improved lipid profile in HFD fed mice

	HFD	HFD+ RNSpBRV1%	HFD+ RNSpBRV5%
Glucose (mg/dl)	197.0 ± 34.9[a]	177.0 ± 14.9[a,b]	160.3 ± 29.2[b]
TG (mg/dl)	82.2 ± 12.5[a]	78.1 ± 15.6[a,b]	67.8 ± 13.8[b]
Total cholesterol (mg/dl)	206.7 ± 39.7[a]	203.1 ± 23.8[a,b]	169.5 ± 30.8[b]
HDL cholesterol (mg/dl)	116.0 ± 15.5[a]	146.2 ± 6.7[b]	137.4 ± 14.2[b]
LDL cholesterol (mg/dl)	74.6 ± 21.7[a]	41.3 ± 13.9[b]	18.5 ± 13.8[b]

ICR mice (n = 5 per group) were used for weight measurement for 5 weeks. Values represent the mean ± SD. The data were analyzed by ANOVA, and the different letters in same line indicate statistically significant difference (P< 0.05).

in mice fed RNSpBRV 5% by 18% and 17.6%, respectively, compared to the HFD groups (total cholesterol 206.7 ± 39.7 mg/dl; TG 82.2 ± 12.5 mg/dl). Interestingly, levels of HDL cholesterol were significantly increased in both 1% and 5% RNSpBRV-fed mice groups (146.2 ± 6.7 mg/dl, 137.4 ± 14.2 mg/dl, respectively) compared to HFD-fed mice (116.0 ± 15.5 mg/dl) (Table 4). In addition, administration of RNSpBRV showed the decreased LDL cholesterol levels in 1% and 5% RNSpBRV-fed mice groups (41.3 ± 13.9 mg/dl, 18.5 ± 13.8 mg/dl, respectively) compared to HFD-fed mice (74.6 ± 21.7 mg/dl).

Adipose tissue histopathology

To further investigate whether the administration of RNSpBRV is effective in adipose tissue formation *in vivo*, we measured the fat mass and adipocyte size in ICR mice. As showed in Table

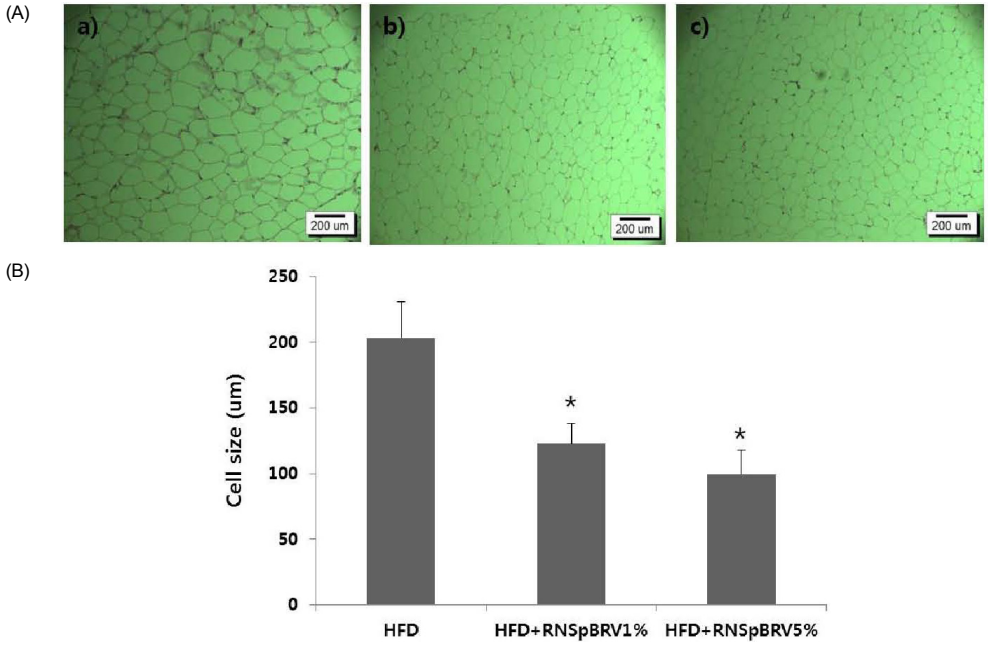

Fig. 2. Adipocyte size in ICR mice fed a high-fat diet alone or supplemented with RNSpBRV for 5 weeks. (A) Representative area of epididymal fat tissues of (a) HFD-, (b) HFD+1%RNSpBRV-, and (c) HFD+5%RNSpBRV-fed mice. Epididymal fat tissues were isolated, fixed, and embedded in paraffin. Epididymal fat adipocytes were visualized by hematoxylin and eosin staining. The scale bar represents 200 μm. (B) Epididymal fat adipocyte size was measured by using a microscope and quantified using an image analyzer. More than 100 cells in three representative areas were measured. Each data value is expressed as the mean ± SE. *P< 0.05 compared with the HFD fed mice group.

3, administration of RNSpBRV did not affect the total mass of epididymal fat tissue. However, the mean size of epididymal cells were significantly decreased in the 1% RNSpBRV-treated group (122.8 ± 14.8 μm) and was further reduced in the RNSpBRV5 group (99.6 ± 17.8 μm) compared to HFD-fed mice (203.2 ± 27.2 μm) (Fig. 2B). The total number of epididymal cell did not change (data not shown), but larger epididymal cells tended to decrease in size in RNSpBRV-administrated ICR mice (Fig. 2A).

Limitation of power calculation in vivo study

In our study, although administration of RNSpBRV (5% of ingredient) showed anti-hyperlipidemic activities such as improved lipid profile (Table 4) and size reduction of adipocyte in high fat diet fed mice (Fig. 2B), the amount of RNSpBRV (5% of ingredient) was not enough to reduce the body weight (Table 2) as well as fat weight compared with HFD fed mice (Table 3). These results may come from the limited sample size of RNSpBRV *in vivo* study.

Discussion

Hyperlipidemia can be managed by diet, exercise, anti-hyperlipidemic therapeutics, and insulin treatment. Because insulin therapy and anti-hyperlipidemic agent treatment have side effects, efforts have focused on finding safer and more effective natural anti-hyperlipidemic sources [30,31]. In this study, we investigated the anti-hyperlipidemic effect and anti-adipogenic activity of RNSpBVP. Since RNSpBRV is made by soaking RNS in BRV, we first compared the inhibitory effects in lipid accumulation of 3T3-L1 adipocytes by adding methanol extracts of RNS, BRV, SF-RNSpBRV, and LF-RNSpBRV. Interestingly, although methanol extracts of BRV showed significant anti-adipogenic activity, administration of LF-RNSpBRV showed increased anti-lipid accumulation activity in 3T3-L1 adipocyte. In addition, methanol extracts of SF-RNSpBRV also showed anti-adipogenic activity despite no significant change in cells treated with RNS (Fig. 1B). These results suggest that RNSpBRV has an anti-lipid accumulating effect in differentiated 3T3-L1 adipocytes and RNSpBRV has more anti-adipogenic potential than RNS and BRV. Because lipogenesis is an important process in lipid homeostasis [32,33], investigation of anti-adipogenic activity is a valuable method for determining anti-hyperlipidemic potential. Until now, many natural sources which contain anti-adipogenic activity have been screened by phytochemical studies. For examples, methanol extract of *idesia polycarpa* showed inhibitory effect in adipocyte differentiation [34]. Various Korean cereals such as *setaria italic beauvois* and *panicum miliaceum* L. were revealed to have anti-adipogenic activities [35] and phenolic compounds with anti-adipogenic activity were isolated from the aerial parts of *pulsatilla koreana* [36]. However, to our knowledge, our study is the first investigation of anti-adipogenic activity of RNSpBRV, a Korean RNS-based food. Because anti-adipogenic activity occurred with both SF-RNSpBRV and LF-RNSpBRV, we investigated regulating the effects of blood lipids levels *in vivo* using RNSpBRV powder. Daily supplement of 1% and 5% RNSpBRV powder for 5 weeks did not decrease body weight or organ weight (including the liver) in HFD-fed mice (Table 3). In addition, there were no significant differences in GOT and GPT between HFD-fed and HFD + RNpBRP 5%-fed mice (data not shown). These data suggest that, although not sufficient for decreasing body weight of HFD-fed mice, administration of up to 5% RNSpBRV powder does not induce liver toxicity.

It has been reported that increasing TG and decreasing HDL cholesterol levels are highly associated with obesity [37] and high level of TG was reported to be a risk factor in cardiovascular disease [38]. High levels of cholesterol and LDL cholesterol increase the risk of coronary heart disease [39,40]. However, high HDL cholesterol has a role in transporting excess cholesterol to the liver [39]. Because RNSpBRV showed anti-adipogenic activity *in vitro*, we next investigated the effect of RNSpBRV in the control of blood glucose, TG, total cholesterol, and HDL cholesterol *in vivo*. As indicated in Table 4, RNSpBRV showed a regulatory role for blood glucose and lipids content. Although a 1% RNSpBRV supplement did not appear to be sufficient for downregulating blood glucose, TG, and total cholesterol levels of HFD-fed mice, when the quantity of RNSpBRV was increased to 5%, levels of blood glucose, TG, and total cholesterol were decreased compared to the HFD-fed mice. Moreover, HDL cholesterol content significantly increased even in the HFD + 1% RNSpBRV-fed groups. Interestingly, LDL cholesterol was significantly decreased in RNSpBRV fed groups. These results demonstrate that RNSpBRV has a strong anti-hyperlipidemic potential.

It is well known that hyperlipidemia is closely linked to obesity. Since adipose tissue, which is an active endocrine and paracrine organ, is where fat accumulation occurs, measuring the cell number and size of adipocytes in adipose tissue has been used to determine the degree of obesity. Actually, administration of germinated brown rice extract to obesity mice showed anti-obesity effect with decreased size of adipose tissue [41]. Rapha diet which containing silkworm pupa peptide, *Garcinia cambogia*, white bean extract, mango extract, raspberry extract, cocoa extract and green tea extract also showed the anti-obesity effect with reduced fat weight and decreased size of epididymal, perirenal and mesenteric adipocyte [42]. In this study, although the total weight of epididymis fat did not differ among groups (Table 3), we found that administration of RNSpBRV reduced the size of epididymis fat cells when compared to those of HFD-fed mice (Fig. 2). This result strongly supports the fact that administration of RNSpBRV could regulate the hyperlipidemia related adipocyte cell metabolism *in vivo*.

Metabolic disease, such as hyperlipidemia and obesity, is gradually increasing, and numerous pharmaceutics for “curing” these diseases have been developed. Natural compounds are an attractive source for treating metabolic disease because of their

lower potential to cause side effects, such as headache and heart attack. For these reasons, recent studies have focused on investigating the anti-hyperlipidemic and anti-obesity properties from natural herbs. Traditional foods are good sources for screening the unknown effects of agents controlling disease. In addition, it has great advantage in adapting to human. In this report, we examined the anti-adipogenic effects of RNSpBRV, a Korean traditional food, using 3T3-L1 adipocytes. We investigated the anti-hyperlipidemic effects of RNSpBRV in mice fed a HFD. Although the amount of RNSpBRV that we used in these experiments (1-5% of ingredients) seemed to be insufficient for showing its full effects in reducing body and epididymis fat weight, it clearly showed an effect on lowering blood glucose, TG, and total cholesterol levels of HFD-fed mice and reduced the size of epididymis fat cells. This is a limitation of our study that insufficient amounts of RNSpBRV were used *in vivo* study resulted in reduced power of RNSpBRV. Therefore, further study is needed to increase the sample size of RNSpBRV *in vivo* experiments and to determine the chemical identity of RNSpBRV and the molecular mechanism that regulates adipocyte differentiation and lipid metabolism.

References

1. Boullart AC, de Graaf J, Stalenhoef AF. Serum triglycerides and risk of cardiovascular disease. Biochim Biophys Acta 2012;1821: 867-75.
2. Chait A, Brunzell JD. Acquired hyperlipidemia (secondary dyslipoproteinemias). Endocrinol Metab Clin North Am 1990;19:259-78.
3. Diaz JA, Ballard-Lipka NE, Farris DM, Hawley AE, Wrobleski SK, Myers DD, Henke PK, Lawrence DA, Wakefield TW. Impaired fibrinolytic system in ApoE gene-deleted mice with hyperlipidemia augments deep vein thrombosis. J Vasc Surg 2012;55:815-22.
4. Gong WH, Zheng WX, Wang J, Chen SH, Pang B, Hu XM, Cao XL. Coexistence of hyperlipidemia and acute cerebral ischemia/reperfusion induces severe liver damage in a rat model. World J Gastroenterol 2012;18:4934-43.
5. Oravec S, Krivosikova Z, Krivosik M, Gruber K, Gruber M, Dukát A, Gavorník P. Lipoprotein profile in patients who survive a stroke. Neuro Endocrinol Lett 2011;32:496-501.
6. Huang J, Qian HY, Li ZZ, Zhang JM. Comparison of clinical features and outcomes of patients with acute myocardial infarction younger than 35 years with those older than 65 years. Am J Med Sci 2013;346:52-5.
7. Chen K, Li W, Major J, Rahaman SO, Febbraio M, Silverstein RL. Vav guanine nucleotide exchange factors link hyperlipidemia and a prothrombotic state. Blood 2011;117:5744-50.
8. Maraki MI, Aggelopoulou N, Christodoulou N, Anastasiou CA, Toutouza M, Panagiotakos DB, Kavouras SA, Magkos F, Sidossis LS. Lifestyle intervention leading to moderate weight loss normalizes postprandial triacylglycerolemia despite persisting obesity. Obesity (Silver Spring) 2011;19:968-76.
9. Gupta N, Shah P, Goel K, Misra A, Rastogi K, Vikram NK, Kumari V, Pandey RM, Kondal D, Wasir JS, Bhardwaj S, Gulati S. Imbalanced dietary profile, anthropometry, and lipids in urban Asian Indian adolescents and young adults. J Am Coll Nutr 2010; 29:81-91.
10. Singh RB, Rastogi SS, Rao PV, Das S, Madhu SV, Das AK, Sahay BK, Fuse SM, Beegom R, Sainani GS, Shah NA. Diet and lifestyle guidelines and desirable levels of risk factors for the prevention of diabetes and its vascular complications in Indians: a scientific statement of the International College of Nutrition. Indian Consensus Group for the Prevention of Diabetes. J Cardiovasc Risk 1997;4:201-8.
11. Fujioka K. Management of obesity as a chronic disease: nonpharmacologic, pharmacologic, and surgical options. Obes Res 2002; 10 Suppl 2:116S-123S.
12. Devlin MJ, Yanovski SZ, Wilson GT. Obesity: what mental health professionals need to know. Am J Psychiatry 2000;157:854-66.
13. Rayalam S, Della-Fera MA, Baile CA. Phytochemicals and regulation of the adipocyte life cycle. J Nutr Biochem 2008;19:717-26.
14. Cunha CA, Lira FS, Rosa Neto JC, Pimentel GD, Souza GI, da Silva CM, de Souza CT, Ribeiro EB, Sawaya AC, Oller do Nascimento CM, Rodrigues B, de Oliveira Carvalho P, Oyama LM. Green tea extract supplementation induces the lipolytic pathway, attenuates obesity, and reduces low-grade inflammation in mice fed a high-fat diet. Mediators Inflamm 2013;2013:635470.
15. Hasumura T, Shimada Y, Kuroyanagi J, Nishimura Y, Meguro S, Takema Y, Tanaka T. Green tea extract suppresses adiposity and affects the expression of lipid metabolism genes in diet-induced obese zebrafish. Nutr Metab (Lond) 2012;9:73.
16. Kim MJ, Kim HK. Perilla leaf extract ameliorates obesity and dyslipidemia induced by high-fat diet. Phytother Res 2009;23: 1685-90.
17. McCrimmon RJ, Frier BM. Hypoglycaemia, the most feared complication of insulin therapy. Diabete Metab 1994;20:503-12.
18. Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. Diabetes Care 2005; 28:1083-91.
19. Yim JH, Lee OH, Choi UK, Kim YC. Antinociceptive and anti-inflammatory effects of ethanolic extracts of Glycine max (L.) Merr and Rhynchosia nulubilis seeds. Int J Mol Sci 2009; 10:4742-53.
20. Lim SY. Comparison of effect of various types of soybeans on mutagenicity and growth of human cancer cell lines. J Life Sci 2010;20:1532-7.
21. Jin CY, Han MH, Park C, Hwang HJ, Choi EA, Choi YH. Sarijang enhances maturation of murine bone marrow-derived dendritic cells. J Life Sci 2011;21:1789-94.
22. Liao HF, Chou CJ, Wu SH, Khoo KH, Chen CF, Wang SY. Isolation and characterization of an active compound from black soybean [Glycine max (L.) Merr.] and its effect on proliferation and differentiation of human leukemic U937 cells. Anticancer Drugs 2001;12:841-6.
23. Tsoyi K, Park HB, Kim YM, Chung JI, Shin SC, Shim HJ, Lee WS, Seo HG, Lee JH, Chang KC, Kim HJ. Protective effect of anthocyanins from black soybean seed coats on UVB-induced apoptotic cell death in vitro and in vivo. J Agric Food Chem 2008;56:10600-5.
24. Liao HF, Chen YJ, Yang YC. A novel polysaccharide of black soybean promotes myelopoiesis and reconstitutes bone marrow after 5-flurouracil- and irradiation-induced myelosuppression. Life Sci 2005;77:400-13.
25. Do MH, Lee SS, Jung PJ, Lee MH. Intake of fruits, vegetables,

and soy foods in relation to breast cancer risk in Korean women: a case-control study. Nutr Cancer 2007;57:20-7.
26. Yamashita H, Fujisawa K, Ito E, Idei S, Kawaguchi N, Kimoto M, Hiemori M, Tsuji H. Improvement of obesity and glucose tolerance by acetate in Type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Biosci Biotechnol Biochem 2007;71: 1236-43.
27. Fushimi T, Suruga K, Oshima Y, Fukiharu M, Tsukamoto Y, Goda T. Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. Br J Nutr 2006;95: 916-24.
28. Harmon AW, Harp JB. Differential effects of flavonoids on 3T3-L1 adipogenesis and lipolysis. Am J Physiol Cell Physiol 2001;280:C807-13.
29. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18:499-502.
30. Seo EY, Ha AW, Kim WK. alpha-Lipoic acid reduced weight gain and improved the lipid profile in rats fed with high fat diet. Nutr Res Pract 2012;6:195-200.
31. Sung JH, Chon JW, Lee MA, Park JK, Woo JT, Park YK. The anti-obesity effect of Lethariella cladonioides in 3T3-L1 cells and obese mice. Nutr Res Pract 2011;5:503-10.
32. Choi WH, Gwon SY, Ahn J, Jung CH, Ha TY. Cooked rice prevents hyperlipidemia in hamsters fed a high-fat/cholesterol diet by the regulation of the expression of hepatic genes involved in lipid metabolism. Nutr Res 2013;33:572-9.
33. Wang H, Chen G, Ren D, Yang ST. Hypolipidemic activity of okra is mediated through inhibition of lipogenesis and upregulation of cholesterol degradation. Phytother Res. Forthcoming 2013.
34. Lee M, Lee HH, Lee JK, Ye SK, Kim SH, Sung SH. Anti-adipogenic activity of compounds isolated from Idesia polycarpa on 3T3-L1 cells. Bioorg Med Chem Lett 2013;23:3170-4.
35. Park MY, Seo DW, Lee JY, Sung MK, Lee YM, Jang HH, Choi HY, Kim JH, Park DS. Effects of Panicum miliaceum L. extract on adipogenic transcription factors and fatty acid accumulation in 3T3-L1 adipocytes. Nutr Res Pract 2011;5:192-7.
36. Liu Q, Ahn JH, Kim SB, Hwang BY, Lee MK. New phenolic compounds with anti-adipogenic activity from the aerial parts of Pulsatilla koreana. Planta Med 2012;78:1783-6.
37. Paccaud F, Schlüter-Fasmeyer V, Wietlisbach V, Bovet P. Dyslipidemia and abdominal obesity: an assessment in three general populations. J Clin Epidemiol 2000;53:393-400.
38. Malloy MJ, Kane JP. A risk factor for atherosclerosis: triglyceride-rich lipoproteins. Adv Intern Med 2001;47:111-36.
39. Ansell BJ, Watson KE, Fogelman AM, Navab M, Fonarow GC. High-density lipoprotein function recent advances. J Am Coll Cardiol 2005;46:1792-8.
40. Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. Circulation 2001;104:365-72.
41. Ho JN, Son ME, Lim WC, Lim ST, Cho HY. Anti-obesity effects of germinated brown rice extract through down-regulation of lipogenic genes in high fat diet-induced obese mice. Biosci Biotechnol Biochem 2012;76:1068-74.
42. Kim J, Kyung J, Kim D, Choi EK, Bang P, Park D, Kim YB. Anti-obesity effects of Rapha diet(R) preparation in mice fed a high-fat diet. Lab Anim Res 2012;28:265-71.