

Effect of Water Extract of Germinated Brown Rice on Adiposity and Obesity Indices in Mice Fed a High Fat Diet

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Abstract

An extract obtained from germinated brown rice was evaluated for protective effects against the adverse effects of a chronic high fat diet. C57BL/6J mice were divided into three groups; normal diet + water group (ND), high fat diet + water group (HD), high fat diet + brown rice water extract group (HD + BE) and fed for 8 weeks (6 day/week). The ND group diet was 11 kcal fat %. The HD group and HD + BE group diet were 42 kcal fat %. Weight gains were not significantly different between groups. However, abdominal fat % was 1.6-fold higher in the HD group than HD + BE group. Feed consumption was significantly higher in the ND group than HD group. Serum triglyceride, total cholesterol and LDL-cholesterol increased with a high fat diet, but decreased by brown rice water extract administration to the high fat diet group. Serum HDL-cholesterol decreased by the high fat diet, but increased by the brown rice water extract administration. Also, serum HDL-cholesterol/total cholesterol ratio was 2.3-fold higher in the HD + BE than the HD. Liver triglyceride and total cholesterol were not significantly different among groups. These data suggest that brown rice water extract administration improves the serum lipid profiles of C57BL/6J mice.

Key words: high fat diet, brown rice water extract, lipid profiles

INTRODUCTION

Obesity has increased in recent years mainly due to the continuous increase in the intake of animal foods and decrease in activity levels (1). This will cause not only certain problems in appearance, but also with chronic diseases due to the fact that the accumulated fat in the body causes biochemical and physiological malfunctions (2). In addition, medical costs for the fat related diseases have increased from day to day, and the effort to decrease the percentage of body fat has become a worldwide interest. Developments of functional foods that emphasize improvements of body lipids are particularly important in the present time.

Brown rice has been added in various beverages and teas, and sold as a type of 'germinated brown rice'. It is known that the germinated brown rice presents an excellent assimilation, and increases useful elements when it is germinated, such as various vitamins, arabinoxylan, and γ -aminobutyric acid (GABA) (3). In addition, it is known that various components including phytic acid

and calcium can be separated due to the increase in phytases when plant's seeds are germinated, and then the absorptivity of the minerals into the body increased (4,5).

The presence of GABA in plants has been known for at least half a century (6). Although the role of GABA in plants is not yet clear, there is increasing interest in the utilization of GABA as a functional plant component. Several lines of evidence suggest that plant extracts containing high levels of GABA are effective for improving blood pressure regulation (7,8) and for recovery from alcohol-related symptoms (8-10). For example, the administration of the germinated brown rice water extract prevented ethanol-induced increases in liver triglyceride and total cholesterol concentrations (11). In addition, water extracts of germinated brown rice showed immune cell stimulating activities and apoptosis effects on some cancer cells (12,13). In this study, we evaluated protective effects of an extract from germinated brown rice against the adverse effects of chronic high fat diet.

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MATERIALS AND METHODS

Materials

DL-methionine, fiber, and choline bitartrate were purchased from Sigma Chemical Co. (St. Louis, USA). AIN-76 vitamin and mineral mixes were purchased from Harlan Teklard (Madison, USA), casein was from Cottee (Gordon, Australia), sucrose and corn oil were from Jeiljedang (Seoul, Korea) and corn starch was from Samyang genex (Seoul, Korea). Chitosan was supplied from El-Chitosan Korea Co., Ltd. in Jeonju, Korea. Brown rice (*Oryza sativa* L. cv. Dongjin) was obtained from a Jeonju market place, in Korea. All other reagents were purchased from commercial sources and were of the highest grade available.

Preparation of brown rice extracts

Brown rice was germinated by soaking it in 50 ppm chitosan in 5 mM glutamic acid solution at 25~26°C in the dark for 72 h as described (3,11). At 12 h intervals the germination solution was exchanged for freshly prepared solution. The germinated brown rice was air dried, frozen in liquid nitrogen, ground with a mortar and pestle, and then extracted by autoclaving in distilled water at 121°C for 30 min. The sample was centrifuged at 15,000×g at 4°C for 30 min and the supernatant was collected, passed through 0.45 µm filters and used as an extract.

Analysis of GABA and free amino acids

GABA and free amino acids were fractionated from the water extracts using modified procedures of Baum et al. (14) and analyzed by an amino acid analysis system (Waters, USA) after 6-aminoquioly-N-hydroxysuccinimidyl carbonate (AQC) derivatization as described (15).

Animal and diets

Male mice that were in the C57BL6/J class and of average weights of 25~27 g were purchased from Jackson Laboratories (Bar Harbor, USA). The test groups consisted of three different diet groups, such as normal diet + water group (ND), high fat diet + water group (HD), and high fat diet + brown rice water extract group (HD + BE), and each group was bred for 8 weeks. All mice were individually housed in stainless steel cages with a randomized complete block design in an environmentally controlled facility: temperature (23 ± 1°C), humidity (53 ± 2%) and a 12-h light-dark cycle. Each test group was given access to water and feed without any regulations during the breeding period.

The composition of the test diet and application of the extracted solution are presented in Table 1. Purified raw materials based on the standard for rodents, such as the specification of AIN-76 white mice, were used

Table 1. Composition of experimental diets in each group and subgroup

Ingredient	Normal diet	High fat diet	
	DW ¹⁾	DW	BE ²⁾
Casein	20.0	20.0	20.0
Sucrose	50.0	35.0	35.0
Starch	15.0	-	-
Corn oil	5.0	-	-
Beef tallow	-	35	35
Cellulose	5.0	5.0	5.0
Mineral mixture	3.5	3.5	3.5
Vitamin mixture	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2
DL-methionine	0.3	0.3	0.3
Total	100.0	100.0	100.0
Brown rice water extract	-	-	10 µL/g BW ³⁾

¹⁾DW: Distilled water.

²⁾BE: Brown rice water extract.

³⁾BW: Body weight.

as a test diet. The average diet group was supplied with 11 kcal fat %, the high fat diet group was supplied with 42 kcal fat %, and the brown rice diet group was supplied with 10 µL of water extract per each mouse g weight. The diet for each test group was supplied as a manner of oral medication in the morning for 8 weeks, and non-application groups were supplied with the same amount of distilled water (Table 1). The amount of intake was measured only once every two days at a fixed time for the residual feed during the test period, and the remaining feed was used to calculate the amount of daily intake. In addition, the weights were measured once every week.

Prior to sacrificing the mice, food was withheld for 12 h. Each mouse was anesthetized with ether. Blood samples were collected by orbital venipuncture and stored in ice water for 1 h. Serum was prepared by centrifugation at 1,100×g for 15 min at 4°C, and stored at -80°C until analyses. Liver was removed and rinsed with phosphate buffered saline solution, wiped with a paper towel and stored at -80°C.

Analysis of lipids

The total cholesterol in the serum was measured by applying an enzyme method using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea), and a HDL-cholesterol fraction was prepared by the dextran sulfate-Mg⁺⁺ method (16). The LDL-cholesterol level was calculated by the Friedwald method (17). The triglyceride in the blood and liver tissue was enzymatically measured with a commercial kit (Asan Pharmaceutical Co., Seoul, Korea), and the total amount of lipids in the blood and liver tissue was measured by the sulfo-phospho-vanillin meth-

od (18) using a commercial kit (Kokusai Pharmaceutical Co., Kobe, Japan). Liver lipids were extracted from liver tissues according to the method of Folch et al. (19).

Statistical analysis

Data from individual experiments were expressed as the mean \pm standard deviation. SAS version 8 (SAS Institute, Cary, USA) was used for all statistical analyses. Significances of differences among the three groups were determined using Duncan's multiple range test and the accepted level of significance was $p < 0.05$ (20).

RESULTS AND DISCUSSION

Weight gain, feed consumption and feed efficiency ratio

There is a report that overweight people generally consume more caloric energy than that of average people (21). Other studies indicate that the fat ratio of the diet significantly affects the level of obesity rather than that of the total amount of energy intake (22,23). In addition, high fat diets are known to lead to increase body weight (24). This study introduces obesity by applying high fat diets with 42 kcal fat %. Thus, as shown in Table 2, the weight and rate of weight increase in the early stage of the test for each group were similar, but the high fat diet group (HD) increased in the abdominal fat compared to the normal diet group (ND). In addition, the high fat diet + germinated brown rice water extract applied group (HD + BE) presented a low level as the ND group. These results are similar to the results of the recent study of Ha and Kim (25). This study reported that the weight and body fat ratio in a woman who takes brown rice-based raw foods significantly decreased, and the food especially facilitated the decrease in the abdominal fat. In addition, in the case of the single application of GABA (0.5 g/kg) to the mice that intakes the normal diet, the abdominal fat/BW (%) significantly decreased (26). These results may suggest that the GABA in germinated brown rice facilitates the decrease in the

abdominal fat of the mouse, while the extract of germinated brown rice was applied. In addition, the amount of caloric intake in the HD group was higher than the ND group. This means that mice attempted to maintain the homeostasis in the body themselves. There were no significant differences in the energy efficiency among the groups.

Serum lipid levels

Adiposity located centrally in the abdominal region is distinctly associated with hyperlipidemia, compared with generalized distributions of body fat, and is also associated with lipoprotein abnormalities characterized by elevated VLDL and LDL concentrations. Thus, we measured the level of the total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol in order to investigate the lipid change in the blood. As a result, the concentration of the triglyceride and total cholesterol of the HD group presented a higher value than that of the ND group. This was due to the intake of a high fat diet, and the concentration of the triglyceride and total cholesterol in the serum decreased as the level of the ND group by applying the germinated brown rice water extract (Fig. 1). There are some similar reports that the brown rice added to a diet decreased the concentration of the serum triglyceride and cholesterol. In addition, this is expected to prevent cardiovascular diseases (27). Serum LDL cholesterol concentrations are generally considered to be a good indicator of abnormal lipoprotein metabolism, and are directly correlated with risk for coronary heart disease and atherosclerosis (28,29). In this study, the water extracts of germinated brown rice decreased serum LDL cholesterol level, which was increased with the high fat diet. Also, the water extracts of germinated brown rice increased serum HDL cholesterol level, which was decreased with the high fat diet (Fig. 2). Based on these results, it is evident that the changed serum lipid level due to the intake of a high fat diet can be improved by applying the germinated brown rice water extract.

Table 2. Weight gain, feed consumption and feed efficiency ratio

	ND ³⁾	HD ⁴⁾	HD + BE ⁵⁾
Initial body weight (g)	25.83 \pm 1.78 ¹⁾	25.20 \pm 2.25	26.47 \pm 1.71
Weight gain (g)	4.81 \pm 1.29	4.71 \pm 1.90	4.62 \pm 1.91
Abdominal fat (%)	1.95 \pm 0.63 ^{b2)}	2.90 \pm 0.64 ^a	1.86 \pm 0.88 ^b
Feed consumption (g/d)	3.04 \pm 0.15 ^a	2.35 \pm 0.34 ^b	2.27 \pm 0.35 ^b
Feed efficiency ratio	1.63 \pm 0.42	2.13 \pm 0.81	2.26 \pm 0.62

¹⁾Values are means (n=6) \pm SD.

²⁾Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p < 0.05$.

³⁾Normal diet.

⁴⁾High fat diet.

⁵⁾Brown rice water extract.

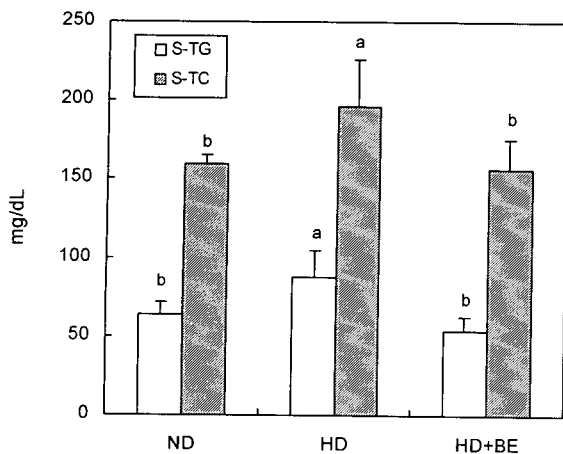


Fig. 1. Effect of brown rice water extract on serum triglyceride and total cholesterol. Values are means ($n=6$) \pm SD. a, b: Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p < 0.05$. S, serum; TG, triglyceride; TC, total cholesterol; ND, normal diet; HD, high fat diet; BE, brown rice water extract.

Serum HDL cholesterol/total cholesterol ratio

The ratio of the HDL cholesterol in the total cholesterol, which is one of the indications of cardiovascular disease, in the germinated brown rice water extract applied group presented a higher level than that of the HD group (Fig. 3). Therefore, the administration of germinated brown rice water extract reversed the deleterious effects of high fat diet on serum lipids. The HDL cholesterol is a type of fat protein, which plays a role in the reverse transmission of the cholesterol in peripheral tissues to the liver. Venter et al. (30) reported that the HDL cholesterol particle contributes to the activation of

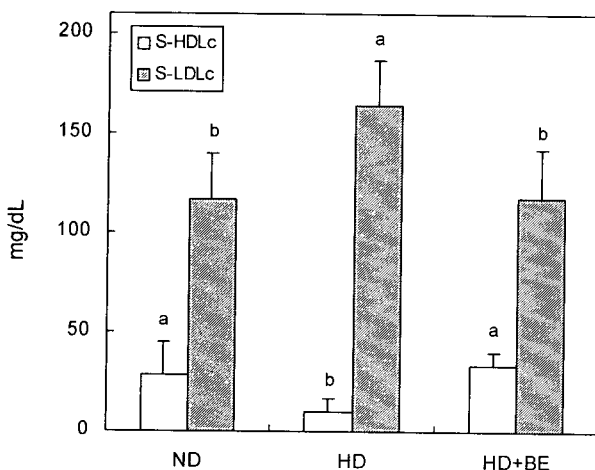


Fig. 2. Effect of brown rice water extract on serum HDL cholesterol and LDL cholesterol. Values are means ($n=6$) \pm SD. a, b: Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p < 0.05$. S, serum; HDLc, HDL cholesterol; LDLc, LDL cholesterol; ND, normal diet; HD, high fat diet; BE, brown rice water extract.

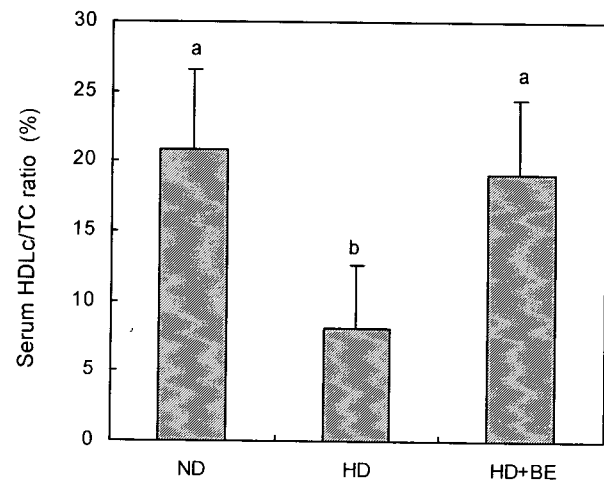


Fig. 3. Effect of brown rice water extract on serum HDL cholesterol/total cholesterol ratio. Values are means ($n=6$) \pm SD. a, b: Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p < 0.05$. HDLc, HDL cholesterol; TC, total cholesterol; ND, normal diet; HD, high fat diet; BE, brown rice water extract.

the lecithin:cholesterol acetyltransferase (LCAT) to perform an esterification of the free cholesterol in the HDL cholesterol. Subsequently, it can facilitate an anti-arteriosclerosis operation by suppressing the introduction of cholesterol into cells. Based on these results, it is possible to conduct serious studies on the relationship between the intake of grains and cardiovascular disease in the future.

Liver lipid concentration

There were no significant differences in the concentration of the total cholesterol in the liver among the test groups (Fig. 4). The cholesterol in the blood lipid decreased by applying the germinated brown rice water

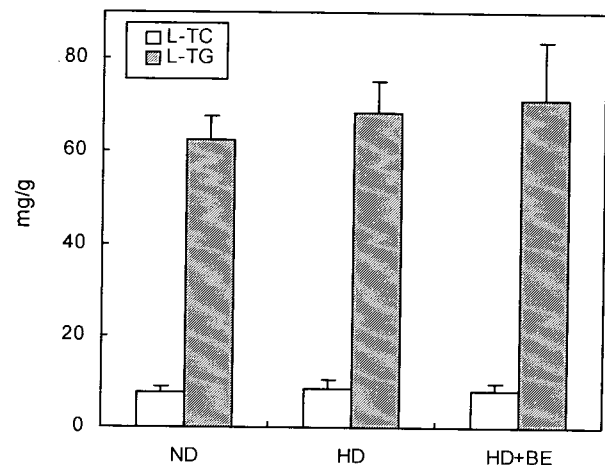


Fig. 4. Effect of brown rice water extract on liver lipid concentration. Values are means ($n=6$) \pm SD. L, Liver; TG, triglyceride; TC, total cholesterol; ND, normal diet; HD, high fat diet; BE, brown rice water extract.

extract, but the application did not affect the triglyceride fat and total cholesterol in the liver. It can be assumed that there were no changes in the concentration because all synthesized cholesterols in the liver were used.

This study presents a significantly improved effect for the parameter, which is related to the hyperlipidemia in mice, due to the high fat diet by applying germinated brown rice water extracts. There are some studies indicating that types of extracts and powders, which include much GABA, effectively act on the fat metabolism and improvement of the liver function (9,11) for the alcohol applied mice. In addition, the water extracts of germinated brown rice showed immune cell stimulating activities (12) and suppression effect of the multiplication of cancer cells (13). GABA levels in the water extracts of brown rice germinated in chitosan/glutamic acid and in glutamic acid were similarly high and both of the extracts were effective for the improvement of immunoregulatory action (12) and for the suppression of cancer cells multiplication (13). We also found that when chitosan was used in media for the germination of brown rice, fungal contamination was reduced markedly (31) and that when glutamic acid was used in the media, germinating brown rice efficiently reduced glutamic acid concentrations by converting it to GABA (32). These data suggest that the effects of the brown rice water extract on the serum lipid profiles of C57BL/6J mice are not directly related to the chitosan and/or glutamic acid used in media for the germination of brown rice, but possibly related to the components including GABA. The germinated brown rice extract and cabbage root powder that include much GABA decreased the LDL-cholesterol in the blood, which is caused by a chronic alcohol administration, as the normal level (9, 11). The brown rice water extract (BE) used in this study was prepared from the germinated brown rice and the BE contained 841 nmole of natural GABA per mL sample (11). This GABA concentration is relatively high compared to those of nongerminated brown rice (31) and other plant sources such as pharbitis seeds (10) at 136 and 125 nmoles per gram fresh weights, respectively. It was interesting that the germinated brown rice extract affected the suppression of obesity. However, it is difficult to define what elements affect the suppression of obesity. It is necessary to verify a new function of the GABA through serious and systematic studies on the anti obesity and suppression of the hyperlipidemia of the GABA and GABA included foods in the future. In addition, it is expected that a new functional food can be developed based on these studies.

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