Rice Cakes Containing Dietary Fiber Supplemented with or without Artemisia Annua and Gynura Procumbens Merr. Alleviated the Risk Factors of Metabolic Syndrome

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Department of Food and Nutrition, College of Human Ecology, Yonsei University, Seoul 03722, Korea

We investigated whether the consumption of Korean rice cakes enriched with dietary fiber with or without polyphenol rich plants might decrease the risk factors of metabolic syndrome (MetS). Rice cakes were manufactured using fructooligosaccharides, resistant starch, and psyllium as sources of dietary fibers with and without polyphenol rich Artemisia annua and Gynura procumbens Merr. (RC+FP and RC+F, respectively), and prepared in three forms (songpyeon, seolgidduk, and chaldduk). Ninety subjects with at least one MetS risk factor were recruited for 6 weeks of dietary intervention. Sixty subjects were finally included for the analysis. Compared to the initial values, RC+FP group had decreased levels of fasting blood glucose (FBG), HOMA–IR and blood pressure after 6 weeks, whereas RC+F group didn’t have significant changes in them. Regarding the improvement of individual MetS risk factors, RC+FP group showed significant reduction in FBG and blood pressures but RC+F group only had reduction in systolic blood pressure. After the intervention, a reduction in the number of MetS risk factors was greater in the RC+FP group than in the RC+F group. In conclusion, Dietary fiber enriched rice cakes with or without polyphenols decreased the number and/or the levels of MetS risk factors. Polyphenol rich plant components may provide additional health benefits in controlling FBG and blood pressure.

Key Words: Metabolic syndrome, Risk factors, Rice cakes, Polyphenol rich plants, Dietary fibers

Intruduction

Metabolic syndrome (MetS) is characterized by the constellation of obesity, dyslipidemia, hypertension, high fasting blood glucose levels, and high blood pressure which are all independently related to cardiovascular risk factors and/or type 2 diabetes [1]. MetS has become a growing concern in developed and developing countries such as Korea [2]. Because Westernized diets are positively associated with the increasing prevalence of MetS, changes in dietary patterns and lifestyles with an increase in exercise are strongly recommended.

Increasing evidences suggest that fibers and/or polyphenols may ameliorate MetS. Low glycemic index diets characterized by low refined carbohydrates and low processed foods are considered to be effective in improving dyslipidemia [3]. Dietary fibers such as fructooligosaccharide, resistant starch, and psyllium husk were especially shown to improve dyslipidemia [4–6]. Furthermore, synergistic favorable effects on blood lipid profiles
in subjects with MetS were observed when low glycemic index diets were supplemented in combination with phytochemicals [7]. For instance, diets rich with high fibers and polyphenols found in spices were shown to lessen the increases in postprandial glucose and triglyceride (TG) levels [8,9]. There have been efforts to deliver functional ingredients in an easy accessible form of food such as bread [10,11]. However, rice cakes which are common in Asian diet have not been tried as a convenient food medium to deliver functional food ingredients.

Here for the first time we aimed to investigate whether rice cake supplemented with dietary fibers with or without polyphenol rich plants could be a healthy food item to promote beneficial effects in the prevention and treatment of the risk factors of MetS in Koreans. Rice cakes were developed to contain fibers (resistant starch, fructooligosaccharides, and psyllium) with or without Gynura procumbens Merr. and Artemisia annua as rich sources of polyphenols. Consumption of rice cakes for six weeks were compared in those with at least one risk factor for MetS before and after the intervention. Two kinds of rice cakes were manufactured: rice cake containing dietary fibers (RC+F), the fiber rich rice cakes; and rice cake containing dietary fibers, Artemisia annua and Gynura procumbens Merr. (RC+FP), the fiber and polyphenol rich rice cakes. We further varied cooking style for RC+F and RC+FP rice cakes, which were made in three forms (songpyeon, seolgidduk and chaldduk). The effects of 6 weeks’ dietary intervention of either RC+F or RC+FP was evaluated in terms of MetS risk factors, serum lipids and oxidative stress markers.

Material and Methods

Subjects

Participants whose ages were between 20 and 65 were voluntarily recruited. Informed consent was obtained from all participants for being included in the study. Flow diagram was presented in Figure 1. A total of 104 subjects were examined for inclusion criteria, having at least one MetS risk factor. According to the 2005 International Diabetes Foundation, the risk
factors for MetS include the following: waist circumferences 90 cm or higher for men and 80 cm or higher for women, TG levels 150 mg/dL or higher, HDL-cholesterol less than 40 mg/dL for men and 50 mg/dL for women, blood pressure 130/85 mmHg or higher, and fasting glucose 100 mg/dL or higher [12]. MetS is the condition which meet more than three risk factors [12]. Height, body weight and body fat composition were measured using a bioimpedance analyzer (Inbody 370, Biospace, Seoul, Korea). Blood pressure was checked on the left arm using an automatic blood pressure monitor (FT-500R PLUS, Jawon Medical, Gyeongsan, Korea) at Yonsei University health service center. The waist circumference was measured with a measuring tape around the mid-section between the margin of the last rib and the iliac crest by trained personnel. Exclusion criteria included the following: 1) heavy alcohol/drug consumption; and 2) pregnancy, breastfeeding, or intention to become pregnant during the time of the study. Finally, 90 subjects were selected for a randomized, double-blind clinical trial and divided into two groups, RC+F and RC+FP. During the six weeks, 10 subjects dropped out and 20 subjects were excluded for the analysis in order to have comparable initial characteristics of the subjects between RC+F and RC+FP groups. The number of subjects for each component of risk factor in RC+F and RC+FP groups is presented in Supplemental Table 1. Only those who finished 6 weeks’ intervention were included for the analysis. Blood was collected at the beginning and the end of the study, respectively. Serum and plasma were separated by centrifugation at 3,000 rpm for 10 minutes. Aliquots were then frozen at -80°C until analysis. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The protocol was approved by the Institutional Review Board of Yonsei University (trial registration number, 1040917-201311-HRBR-121-03).

**Manufacturing rice cakes and providing to subjects**

Table 1 showed the composition of food ingredients used to manufacture the rice cakes. Psyllium (0.51% w/w), fructooligosaccharide (10.11% w/w), and resistant starch (5.28% w/w) were used for both RC+F and RC+FP. Gynura procumbens

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>RC+F</th>
<th>RC+FP</th>
<th>Total, g(%)</th>
<th>RC+F</th>
<th>RC+FP</th>
<th>Total, g(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Songpyeon, g</td>
<td>6.9</td>
<td>6.9</td>
<td>11.2 (12.6)</td>
<td>6.9</td>
<td>6.9</td>
<td>11.2 (12.6)</td>
</tr>
<tr>
<td>Seolgidduk, g</td>
<td>2.4</td>
<td>2.4</td>
<td>9.5 (10.7)</td>
<td>2.4</td>
<td>2.4</td>
<td>9.5 (10.7)</td>
</tr>
<tr>
<td>Chaldduk, g</td>
<td>2.0</td>
<td>1.2</td>
<td>3.2 (3.6)</td>
<td>1.2</td>
<td>1.2</td>
<td>3.2 (3.6)</td>
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<tr>
<td>Songpyeon, g</td>
<td>6.9</td>
<td>6.9</td>
<td>11.2 (12.6)</td>
<td>6.9</td>
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<td>11.2 (12.6)</td>
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<tr>
<td>Seolgidduk, g</td>
<td>2.4</td>
<td>2.4</td>
<td>9.5 (10.7)</td>
<td>2.4</td>
<td>2.4</td>
<td>9.5 (10.7)</td>
</tr>
<tr>
<td>Chaldduk, g</td>
<td>2.0</td>
<td>1.2</td>
<td>3.2 (3.6)</td>
<td>1.2</td>
<td>1.2</td>
<td>3.2 (3.6)</td>
</tr>
</tbody>
</table>

RC+F: rice cake containing dietary fibers, RC+FP: rice cake containing dietary fibers and Artemisia annua and Gynura procumbens Merr.

*Rice-cake with stuffing; †Steamed rice-cake; ‡Glutinous rice cake.*
Merr. (2.72% w/w) and Artemisia annua (2.90% w/w) were only added to RC+FP. The RC+F and RC+FP were manufactured in three forms: songpyeon, seolgidduk, and chaldduk. RC+F and RC+FP were sent to the study subjects via an express delivery in vacuum packed and frozen condition. The daily portion of RC+F or RC+FP was individually packaged, which was 100 g and approximately 200-250 kcal. In every week, RC+F and RC+FP were to be consumed in the form of songpyeon three times, seolgidduk twice and chaldduk twice. The average daily intake of rice cake was equivalent to a total of 42.9 g of songpyeon, 28.6 g of seolgidduk, and 28.6 g of chaldduk. The manufacturing process of seolgidduk, chaldduk, and songpyeon was described in the supplemental materials and methods.

**Nutritional analysis for rice cakes**

Protein, fat, dietary fiber, and moisture contents were analyzed by the methods according to the food standard code: the Kjedahl method for protein, the chloroform-methanol extraction method for fat, the total dietary fiber content method for dietary fiber, and the drying method by heating under normal pressure for moisture content [13]. Energy (kcal), sodium, and carbohydrate contents were analyzed using a Standard food composition table [14].

**Analysis of dietary intake**

Study subjects were educated on how to record the food records and how to estimate measurements using a ruler, a spoon size and a small paper cup which was commonly found in public spaces for automated coffee machine. A food record booklet and a serving size booklet were provided prior to the participation at the beginning of the study. During the six weeks of the study period, food records at least for two days including one week day and one weekend were asked to be recorded every week. Food records were collected in photographed images taken by a cellular phone and were sent via a cell phone or email to us every week. Total at least 12 to 18 days’ food records per each subject were analyzed for dietary intake with the use of the Computer Aided Nutritional Analysis Program (CAN pro4.0, the Korean Nutrition Society, Seoul, Korea). Dietary between intake in the two groups was presented in Supplemental Table 2. Even if there was a modest increased

<table>
<thead>
<tr>
<th>Table 2. Characteristics of study subjects</th>
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<tr>
<td></td>
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<tr>
<td><strong>RC+F</strong></td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>Gender, N</td>
</tr>
<tr>
<td>Age</td>
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<tr>
<td>BMI, kg/m²</td>
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<td>The number of risk factors, N</td>
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<tr>
<td>Component of risk factors</td>
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<td>WC, cm</td>
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<tr>
<td>Male</td>
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<tr>
<td>Female</td>
</tr>
<tr>
<td>TG, mg/dL</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
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<tr>
<td>Male</td>
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<tr>
<td>Female</td>
</tr>
<tr>
<td>FBG, mg/dL</td>
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<tr>
<td>BP</td>
</tr>
<tr>
<td>SBP, mmHg</td>
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<tr>
<td>DBP, mmHg</td>
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</tbody>
</table>

Mean ± S.D.; p-values derived from \( \chi^2 \) (gender) and independent t-test.

tendency of dietary fiber intake in RC+FP compared to RC+F, there were no significant differences in total calorie intake and dietary intake of protein, carbohydrate, lipid and dietary fibers between the two groups (Supplemental Table 2).

Biochemical analysis
Plasma levels of fasting glucose, Total-cholesterol (Total-C), HDL-cholesterol (HDL-C), and TG were analyzed using an automated blood chemistry analyzer (DRI-CHEM 4000i, Fujifilm, Tokyo, Japan). LDL and very low density lipoprotein (VLDL)-cholesterol levels were calculated using the Friedewald formula. LDL-C = Total-C - HDL-C - TG/5 and VLDL-C = TG/5. The atherogenic index (AI) was calculated using Haglund’s assay [15]. AI = (Total-C – HDL-C) / HDL-C. Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated, where HOMA-IR = fasting insulin (mU/L) × fasting blood glucose (mg/dL)/405 [16].

Enzyme-linked immunosorbent assay (ELISA)
Insulin (Human insulin ELISA kit, Sunredbio, Shanghai, China), malondialdehyde (MDA) (Lipid peroxidation assay kit, Biovision, Milpitas, CA, USA), and 8-hydroxy deoxyguanosine (8-OHdG) Quantitation (Human 8-OHdG ELISA kit, Mybiosource, California, CA, USA) were analyzed using commercial kits according to the manufacturers’ instructions. Blood urea nitrogen (BUN), creatinine, aspartate transaminase (AST) and alanine transaminase (ALT) were analyzed using Modular analytics (PE, ROCHE, Germany).

Statistical analysis
Statistical analysis was performed using SPSS ver. 21.0 (SPSS Inc., IL, USA). All data was presented as mean ± standard deviation (SD). p < 0.05 was considered to be statistically significant. Data which didn’t follow normalization was log transformed. Independent t-test was used to compare the general characteristics between RC+F and RC+FP groups. A comparison of the net change values between the two groups was performed by analysis of covariance with adjustment for baseline values.

Results
General characteristics of study subjects and nutritional information of rice cakes
Total 90 subjects were enrolled at the beginning of the study. But 10 people dropped out for personal reasons (5 in RC+F and 5 in RC+FP) and 10 subjects per each group were additionally excluded in order to have statistically similar sex ratios between the two groups. Finally 30 subjects per each group successfully completed the study (Figure 1).

Both RC+F and RC+FP had the mixture of fructooligosaccharide, resistant starch, and psyllium (Table 1); however, Artemisia annua and Gynura procumbens were added only to RC+FP, resulting in higher total polyphenol contents compared to RC+F (Table 1 and Figure 2). The average daily portion of RC+F or RC+FP contained 243.6 kcal and 222.4 kcal, 49.4 g and 43.7 g of carbohydrate, 5.3 g and 5.0 g of protein, 2.5 g and 2.6 g of fat, 6.5 g and 7.0 g of fibers, and 39.4 g and 89.2 g of total polyphenols, respectively.

The mean age for a study population was 35.0 ± 11.1 years. 61.7 % of the subjects were male and 38.3% were female. The comparison of general characteristics and mean values of MetS risk factors in RC+F and RC+FP groups were presented in Table 2. The average ages in RC+FP group (n = 30) was significantly higher than those in RC+F group (n = 30). However, the average values of BMI, waist circumference, TG, HDL-C, fasting blood glucose, and systolic, and diastolic blood pressures were not significantly different between the two groups.

Anthropometric indicators and blood pressure levels after six weeks of dietary intake of RC+F and RC+FP
Compared to the initial values, body weight, BMI and waist circumferences were not significantly changed in both groups after 6 weeks (Figure 3). However, blood pressures in both systolic and diastolic blood pressures were significantly decreased only in RC+FP group (Figure 3). These data indicated that
RC+FP might have a blood pressure lowering effect.

Changes in insulin resistance index, blood lipid profiles and oxidative stress markers in RC+F and RC+FP

Only in RC+FP group, serum levels of FBG and HOMA-IR were decreased at the end of the study compared to the beginning (Figure 4A and 4B). Serum levels of TG, Total-C, LDL-C, MDA and 8-OHdG, and AI were not significantly changed after 6 weeks compared to the initials in both groups (Figure 4C-H). Consumptions of RC+F and RC+FP did not induce the changes in BUN, AST, and ALT (data not shown). Our findings indicated that RC+FP appeared to improve FBG levels and HOMA-IR.

Changes in the number of MetS risk factors after six weeks’ consumption of RC+F and RC+FP

After 6 weeks, the proportions of the subjects with at least one MetS risk factor were decreased to 70.0% in RC+F and 60.0% in RC+FP groups (Figure 5). The number of subjects with MetS risk factor of two or higher was also decreased from 76.6% to 43.3% in RC+F group and from 76.7 % to 26.6% in RC+FP group. Subsequently, the percentage of subjects without a MetS risk factor increased in both groups (30% in RC+F and 40% in RC+FP) (Fig. 5A and 5B). Next, we examined whether those who had a specific MetS risk factor had improvement in values of their corresponding risk factor after 6 weeks’ intervention. RC+FP group had decreased the levels of FBG and systolic and diastolic blood pressures whereas RC+F group had reduced systolic blood pressure (Table 3). These results suggested that RC+FP was more effective in reducing the risk of MetS compared to RC+F.

Discussion

Here, we demonstrated that rice cakes containing dietary fibers and polyphenol rich plant powders decreased the risk factors of MetS. RC+FP group significantly improved FBG levels, HOMA-IR and blood pressure, whereas RC+F group had reduced blood pressure (Table 3). These results suggested that RC+FP was more effective in reducing the risk of MetS compared to RC+F.
Rice Cakes with Fiber and Polyphenols Alleviate Risk of MetS

Dietary fibers were commonly supplemented in our rice cakes to increase daily fiber intake. RC+F and RC+FP contained 6.5 g and 7.0 g per 100 g of dietary fiber, respectively. According to the Korean Dietary Recommended Intake, 25 g for men and 20 g for women are recommended as the adequate daily intake of dietary fiber in adults who are older than 12 years [17]. However, the actual daily dietary fiber intake among Koreans was reported to be much lower at 6.4–7.2 g/d [18]. Thus, the additional dietary supply appears to be necessary for even healthy adults. The amount of dietary fiber contained in the daily portion of RC+F and RC+FP did additionally provide in between 26% and 35% of the daily requirements, which might have been sufficient to elicit the health-benefits in lowering the risk factors of MetS.

![Figure 4](image_url)

**Figure 4.** Blood glucose, insulin resistance index, blood lipids and antioxidant markers in RC+F and RC+FP before and after the intervention. Fasting blood glucose levels (A), HOMA-IR (B), blood TG levels (C), Total-C (D), LDL-C (E), AI (F), MDA (G) and 8-OHdG (H). Data are presented as mean ± SD. Asterisk indicates significant difference between the values in 0 week and those in 6 weeks in RC+F or RC+FP by independent t-test.


![Figure 5](image_url)

**Figure 5.** The number of affected subjects in metabolic syndrome factors in RC+F group (A) and RC+FP group (B) before and after the six weeks’ intervention. RC+F: rice cake containing dietary fibers, RC+FP: rice cake containing dietary fibers and Artemisia annua and Gynura procumbens Merr.

might appear to be more effective in improving MetS risk factors than RC+F.
Dietary fiber intake was reported to be associated with lowering blood pressure in several meta-analysis studies [19,20]. In consistent with our observations with RC+F and RC+FP, the rice cakes containing dietary fibers reduced systolic blood pressure in those subjects with the risk of hypertension according to the criteria of MetS. In particular, RC+FP consumption demonstrated far greater effects on blood pressures by inducing the reduction in both systolic and diastolic blood pressure. This could be due to the presence of Gynura procumbens Merr., which were not present in RC+F. Gynura procumbens Merr. was reported to decrease blood pressure [21]. For instance, the extracts of Gynura procumbens Merr. lowered the blood pressure in an animal model for hypertension [22]. Thus, the polyphenol rich Gynura procumbens Merr. might have provided additional effects on blood pressure.

Previous studies demonstrated that fructooligosaccharide and resistant starch were successful in lowering the glycemic index when provided as ingredients in bread along with whole rye grain [10]. Yamada et al. reported that an intake of bread containing 6 g of resistant starch lowers postprandial levels of insulin and blood glucose in those whose fasting blood glucose levels are in the range of 100-140 mg/dL [11]. Psyllium from the husk of the Plantago genus was also shown to reduce blood glucose and/or cholesterol levels [23,24]. However, the blood glucose-lowering effect was not observed in RC+F but seen in the RC+FP group in our study. These may indicate that the amount of dietary fibers provided in the rice cakes might not be enough to exert dietary fiber-induced blood glucose lowering effects in our study subjects. In addition, this hypoglycemic effects might be caused by the polyphenol rich plant powders. The functional effects of Gynura procumbens Merr. were reported to include anti-diabetes probably via increasing glucose uptake into the tissues [25,26]. Artemisia annua was also considered to have hypoglycemic effects [27,28] as well as antioxidant [29] and anti-inflammatory effect [30]. These polyphenol rich plant powders in RC+FP might contribute to the hypoglycemic effects in our study subjects.

Dietary fibers have been also implicated to ameliorate dyslipidemia [4-6]. Resistant starch lowered LDL-C levels [31] and Total-C [32]. Hamsters that were fed high cholesterol diets with resistant starch had lower Total-C levels than those fed with diets not containing resistant starch [32]. On the other hand, 8 g of fructooligosaccharides lowered Total-C and LDL-C levels in Japanese type 2 diabetes [33]. However, RC+F and RC+FP didn’t show any significant change in our study populations.

Because of proposed antioxidant roles by polyphenols, we further investigated the levels of lipid peroxidation products such as MDA, protein carbonyl, and 8-OHdG before and after the rice cake consumption for 6 weeks. Neither of these oxidative parameters was significantly affected by RC+FP, suggesting that polyphenols in RC+FP might have lowered FBG and blood pressure independent of its antioxidant effects.

In this study, there are limitations. Because the study sub-

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**Table 3. Changes in the value of each component of MetS risk factors in those who had corresponding risk factor in RC+F and RC+FP**

<table>
<thead>
<tr>
<th>Type of risk factors</th>
<th>RC+F</th>
<th></th>
<th></th>
<th>RC+FP</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>Before</td>
<td>After</td>
<td>p</td>
<td>N</td>
<td>Before</td>
</tr>
<tr>
<td>WC, cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>97.3 ± 8.4</td>
<td>94.5 ± 10.0</td>
<td>0.416</td>
<td>11</td>
<td>96.5 ± 5.9</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>84.1 ± 3.9</td>
<td>82.8 ± 4.4</td>
<td>0.630</td>
<td>13</td>
<td>85.4 ± 4.4</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>10</td>
<td>193.9 ± 30.8</td>
<td>205.1 ± 135.2</td>
<td>0.801</td>
<td>12</td>
<td>228.7 ± 104.8</td>
</tr>
<tr>
<td>FBG, mg/dL</td>
<td>21</td>
<td>131.0 ± 44.5</td>
<td>108.2 ± 43.3</td>
<td>0.101</td>
<td>22</td>
<td>114.1 ± 17.1</td>
</tr>
<tr>
<td>BP, mmHg</td>
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<tr>
<td>SBP</td>
<td>17</td>
<td>140.7 ± 6.5</td>
<td>134.9 ± 8.8*</td>
<td>0.037</td>
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<td>142.5 ± 11.7</td>
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<td>DBP</td>
<td>7</td>
<td>89.7 ± 7.8</td>
<td>83.3 ± 5.3</td>
<td>0.096</td>
<td>6</td>
<td>89.7 ± 5.5</td>
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<tr>
<td>HDL-C, mg/dL</td>
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<tr>
<td>Male</td>
<td>2</td>
<td>38.5 ± 0.7</td>
<td>48.5 ± 0.7</td>
<td>0.544</td>
<td>-</td>
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<tr>
<td>Female</td>
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<td>-</td>
<td>-</td>
<td></td>
<td>3</td>
<td>44.0 ± 4.4</td>
</tr>
</tbody>
</table>

Mean ± S.D., p-value by independent t-test; *p < 0.05; †p < 0.01.
Rice Cakes with Fiber and Polyphenols Alleviate Risk of MetS

subjects were not housed in a controlled setting, their diet during the day may not be similar between RC+F and RC+FP. In addition, dietary intake analysis was not performed periodically until the end of the study. Thus, there is a chance to have impact of changes in the diets during the study period on our experimental results.

In conclusion, for the first time we demonstrated that RC+F and RC+FP; rice cakes containing a mixture of dietary fibers, resistant starch, fructooligosaccharides, and psyllium with or without Gynura procumbens Merr. and Artemisia annua decreased the levels of MetS risk factors, especially FBG and/or blood pressure. Considering the rising populations with MetS in developed and developing Asian countries, rice cakes supplemented with dietary fibers as well as polyphenol rich plant powders may be another choice for functional foods to ameliorate the risk factors of MetS.

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Conflict of Interest
The authors state no conflict of interests.

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