Studies on the effect of functional food component on metabolic syndrome in genetic animal model of life style-related disease -Effect of processed soymilk and fermented rice bran-

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論文題目	Studies on the effect of functional food component on metabolic syndrome in genetic animal model of life style-related disease -Effect of processed soymilk and fermented rice bran-（遺伝的生活習慣病発症モデル動物における機能性食品成分がメタボリックシンドロームに与える影響の解析－とくに加工豆乳と発酵米糖の影響について－）
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Abstract for Ph.D. Dissertation

Studies on the effect of functional food component on metabolic syndrome in genetic animal model of life style-related disease

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Introduction

Life style-related diseases are the highest cause of death globally and cardiovascular disease is one of them that directly related to high blood pressure. Metabolic syndrome is a condition of cardiovascular risk factors like as hypertension, hyperglycemia, insulin resistance, excessive visceral fat storage, dyslipidemia, oxidative stress and chronic inflammation. The prevalence of metabolic syndrome and its impact on public health have increased attention in recent years. Certain dietary components and many plant based functional foods help to prevent or moderate metabolic syndrome by assisting the body homeostasis mechanism. Processed foods with antihypertensive effect such as soybean, banana, Chinese celery, grape fruit, olive oil, olive leaf, pumpkin, green tea and seaweed are effective against metabolic syndrome. Functional food is getting new momentum in response to maintain better health condition in both developed and developing countries. The term “Functional Food” originated in Japan 1980s and migrated to EU and United States. In general, functional food is a modified food that claims to improve health or well-being by providing benefit beyond that of the traditional nutrients contribution. Recent studies developed a concept of functional food as a convenient and inexpensive remedy to prevent chronic health problems. Recently functional food defines as natural or processed foods that contain biologically-active compounds, an effective nontoxic amount, and provide a clinically proven and documented health benefit for the prevention, management, or treatment of life style-related diseases. Regarding this point of view, many plant based food supplements reinforced the list of functional foods. Previously, our laboratory found that proteinase-treated soymilk was better than original soymilk to inhibit the angiotensin I-converting enzyme (ACE) in vitro and dietary rice bran fraction improved hypertension, glucose and lipid metabolisms in stroke prone spontaneously hypertensive rat (SHRSP).

In the present study, two types of functional food candidates, processed (proteinase-treated) soymilk and fermented rice bran, that were produced from original soymilk and rice bran, respectively, were investigated whether processed soymilk and fermented rice bran were effective in the prevention or improvement of metabolic syndrome or effective against cardiovascular risk factors in genetic animal model of life style-related diseases.

Chapter 1

Effect of processed soymilk on cardiovascular risk factors in genetic animal model of spontaneously hypertensive rat (SHR).

Metabolic syndrome is now considered one of the world's leading public health problem. Bioactive peptides deriving from milk proteins may play an important role in the prevention and treatment of metabolic syndrome via several mechanisms. Processed soymilk (PSM) was produced from original soymilk by treatment with a commercially available proteinase PROTIN SD-NY 10 (0.05% w/w) to generate inhibitory peptides against the angiotensin I-converting enzyme (ACE). Eight novel ACE inhibitory peptides and higher ACE inhibitory activity of PSM were determined in cell free assay (*Tomatsu et.al. 2013).* In order to determine whether the in vitro effect of PSM corresponds to a decrease in blood pressure in vivo, present experiment was conducted to investigate PSM's potential antihypertensive effect on spontaneously hypertensive rats (SHR). First, single oral administration at doses of 0.016, 0.167 and 0.583 ml/2ml/kg body weight was done to investigate which dose would be effective against blood pressure. In Figure 1, PSM at doses of 0.167 and 0.583 ml/2ml/kg body weight significantly decreased systolic blood pressure in SHR compared to control (non-processed soymilk). Repeated administration of PSM once a day for consecutive 3 weeks at doses of 0.167 and 0.583 ml/2ml/kg body weight significantly reduced systolic, diastolic and mean blood pressure in SHR compared to control (Figure 2). After repeated PSM administration, its inhibitory activity against serum ACE was significantly higher compared to the control (Figure 3 A). Serum levels of the angiotensin II (product of ACE) was significantly lower than control (Figure 3 B). Furthermore, PSM administration increased serum nitric oxide level but no effect on thoracic aorta (Figure 3 C and D). Serum glucose, liver lipid profiles and serum activities of aspartate aminotransferase and alanine aminotransferase were similar in PSM groups compared to control (Figure 4). Food intake, water intake, body weight and heart rate were unchanged (Table 1). Finally a tetrapeptide (FFYY) that identified as an active peptide (40 μg/mL) in PSM (*Shimakage et.al.2013*) was investigated its antihypertensive effect on SHR. Single oral administration of synthetic FFYY significantly reduced both systolic and diastolic blood pressure at a dose of 80

μg/kg body weight compared to control (captopril as a positive control 7 mg/kg body weight) group (Figure 5). Thus, PSM may be considered as a functional food that can ameliorate cardiovascular risk factors related to hypertension without causing adverse side effects.

Chapter 2

Effect of fermented rice bran on metabolic syndrome in genetic animal model of stroke prone spontaneously hypertensive rat (SHRSP).

Disease fighting properties of rice bran has advanced the development of rice bran for human use as a functional food and dietary supplements. To be more applicable of rice bran against metabolic syndrome, several techniques including fermentation have been used in biotechnological applications to enhance nutrition. Several studies have shown that the rice bran, fermented with *Saccharomyces cerevisiae*, *Aspergillus oryzae*, *and Issatchenkia orientalis,* has health beneficial effect. However, few studies to date have examined the effect of fermented rice bran (FRB) on the metabolic syndrome in SHRSP. In this study, the metabolic improvement effects of rice bran, fermented with *Aspergillus kawachii* and *Lactobacillus*, have been investigated in genetic animal model of metabolic syndrome. The fermented rice bran (FRB) was produced from rice bran (mixture of rice bran and rice powder 2:1 is called Non-FRB) by conventional fermentation process using *Aspergillus kawachii* (0.1% w/w) and *lactobacillus* (0.01% w/w) treatment. We investigated FRB's potential effect on metabolic syndrome using stroke prone spontaneously hypertensive rat (SHRSP). Single oral administration of FRB and Non-FRB at a dose of 2 g/kg body weight significantly decreased systolic blood pressure in SHRSP compared to control. Single oral administration also reduced serum glucose, insulin and hepatic glucose-6-phosphatase, catalytic subunit (G6PC) mRNA expression (Figure 6). Chronic supplementation for 4 weeks of FRB and Non-FRB (Table 2, experimental diet) significantly reduced food intake, body weight and epididymal fat mass (Figure 7). Chronic supplementation also reduced blood pressure elevation in SHRSP, and FRB effectively increased serum ACE inhibitory activity compared to the control group (Figure 8). FRB supplementation reduced plasma levels of glucose and insulin (Figure 9). FRB supplementation reduced OGTT, ITT

challenged glucose and insulin level, and their each area under the curve and insulin resistant HOMA-IR index (Figure 10). Furthermore, FRB supplementation improved serum adiponectin and leptin sensitivity (Figure 11). Liver lipid profiles were also significantly improved in the FRB experimental group (Figure 12). In the liver, glucose metabolism related hepatic gene expressions were suppressed to maintain glucose homeostasis in FRB treatment group. Fat metabolism related gene expressions (fatty acid synthase, stearyl-CoA desaturase and acetyl-CoA carboxylase) were significantly down-regulated by FRB treatment (Figure 13). Moreover, nuclear transcription factors liver X receptor (LXR), sterol regulatory element binding protein-1(SREBP-1c) and carbohydrate response element binding protein-alpha (ChREBPα) were also down- regulated by FRB supplementation (Figure 14). In conclusion, FRB may function as an effective functional food against hypertension and mitigate metabolic syndrome by regulating glucose, lipid, fat homeostasis, and improve serum adiponectin level in SHRSP. Thus, FRB was much more effective to reduce metabolic syndrome than non-fermentation component.

Conclusion

PSM that produce bioactive peptides, can play an important role in the prevention and treatment of metabolic syndrome and its complications. The bioactive peptides of processed soymilk can activate a wide range of mechanisms including ACE inhibition and nitric oxide production for the prevention of hypertension. Thus the proteinase treated soymilk was useful to develop effective functional foods against metabolic syndrome. Furthermore, FRB was effective to reduce metabolic syndrome than non-fermentation component, so fermentation process by *Aspergilus kawachii* and *Lactobacillus* was useful to develop effective functional foods from food staff. New finding of this study is that the diatary consumption of PSM and FRB negatively regulated cardiovascular risk factors those are main component of metabolic syndrome in genetic animal model of hypertensive rat. PSM effectively reduced cardiovascular risk factors in SHR, and FRB improved hypertension, insulin resistance and hepatic glucose, lipid, and fat metabolism in SHRSP. Thus, PSM and FRB can be used as nonpharmacologic therapy component against metabolic syndrome which cause better nutrition, ideal body weight maintainence and body homeostasis mechanisms by dietary supplementation. So, PSM and FRB

are regarded as beneficial functional foods for the prevention or treatment of life style-related disease.

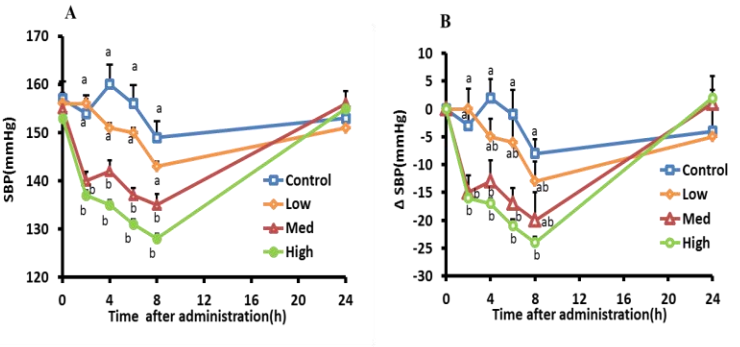


Figure 1. Effect of processed soymilk (PSM) on blood pressure (BP) by single oral administration in SHR. (A) Systolic BP (SBP) at three different doses of PSM and (B) Change in SBP from initial BP at the same doses. Values are expressed as the mean ± S.E.M., n = 6. Different letters represents significant differences (P < 0.05). Control, non-processed soymilk; Low, low-dosage PSM 0.016mL/2mL; Med, medium-dosage PSM 0.167mL/2mL; High, high-dosage PSM 0.583mL/2mL.

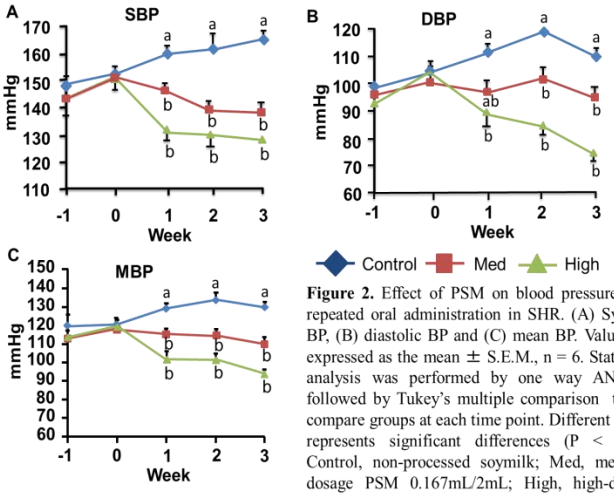


Figure 2. Effect of PSM on blood pressure after repeated oral administration in SHR. (A) Systolic BP, (B) diastolic BP and (C) mean BP. Values are expressed as the mean ± S.E.M., n = 6. Statistical analysis was performed by one way ANOVA, followed by Tukey's multiple comparison test to compare groups at each time point. Different letters represents significant differences (P < 0.05). Control, non-processed soymilk; Med, medium-dosage PSM 0.167mL/2mL; High, high-dosage PSM 0.583mL/2mL.

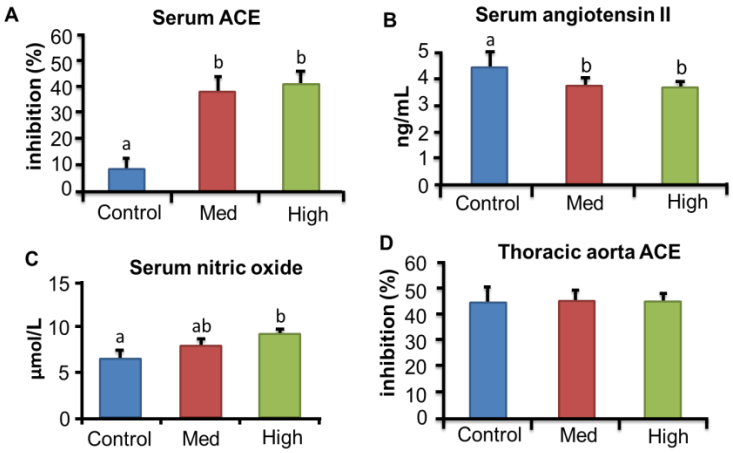


Figure 3. Effect of PSM on serum and thoracic aorta. (A) Serum ACE inhibition, (B) serum angiotensin II level (C) serum nitric oxide level and (D) ACE inhibition in thoracic aorta. Values are expressed as the mean ± S.E.M., n = 6. Statistical analysis was performed by one way ANOVA, followed by Tukey's multiple comparison test. Different letters represents significant differences (P < 0.05). Control, non-processed soymilk; Med, medium-dosage PSM 0.167mL/2mL; High, high-dosage PSM 0.583mL/2mL.

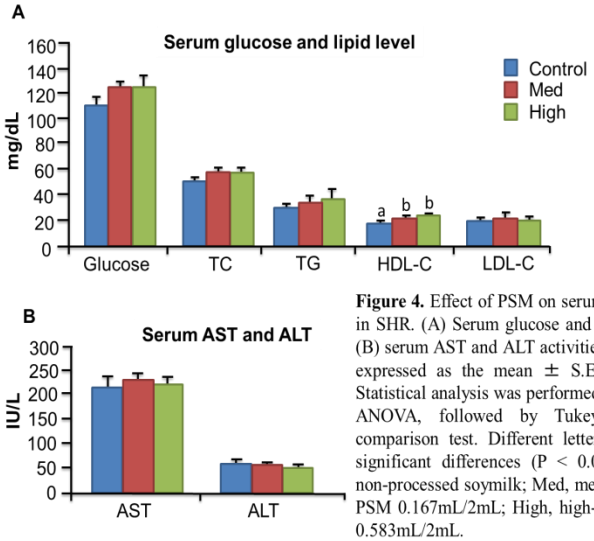
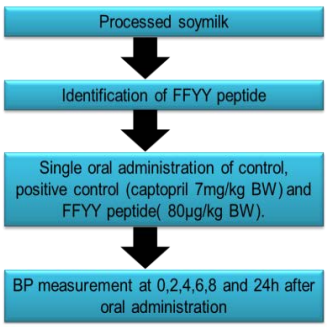


Figure 4. Effect of PSM on serum parameters in SHR. (A) Serum glucose and lipid profile, (B) serum AST and ALT activities. Values are expressed as the mean ± S.E.M., n = 6. Statistical analysis was performed by one way ANOVA, followed by Tukey's multiple comparison test. Different letters represents significant differences (P < 0.05). Control, non-processed soymilk; Med, medium-dosage PSM 0.167mL/2mL; High, high-dosage PSM 0.583mL/2mL.

Table 1. Effect of chronic administration of processed soymilk on Serum, liver, urine and growth parameters. Values are expressed as the mean ± S.E.M., n = 6. Control, non-processed soymilk; Med, medium-dosage PSM 0.167mL/2mL; High, high-dosage PSM 0.583mL/2mL.

Serum parameters	**Control**	**Med**	**High**
Total protein (g/dL)	6.40±0.19	6.62±0.14	6.85±0.07
Albumin (g/dL)	4.32±0.11	4.40±0.08	4.55±0.06
BUN (mg/dL)	20.97±1.05	22.00±0.78	23.03±0.73
Creatinine (mg/dL)	0.23±0.01	0.23±0.01	0.23±0.01
LDH (IU/L)	1322.67±132.55	1302.33±136.41	1235.33±170.25
Liver parameters			
Total lipid (mg/g tissue)	39.93±1.26	44.70±3.66	33.81±0.98
TG (mg/g tissue)	6.22±0.45	4.28±0.73	6.80±1.29
TC (mg/g tissue)	3.31±0.25	2.17±0.42	3.31±0.64
Urine parameters			
Creatinine (mg/day)	7.31±1.18	9.13±1.11	8.27±0.53
Nitric oxide (μmol/day)	2.20±0.53	3.58±0.84	2.75±0.48
8-OHdG (ng/day)	1.52±0.06	1.65±0.15	1.63±0.17
Growth parameters			
Body weight (g)	301±1.42	305±3.70	304±5.93
Food intake (g/day)	23±0.75	22±0.62	21±0.60
Water intake (mL/day)	35±0.49	38±0.74	36±0.61
Heart rate (beats/min)	390±13.30	394±8.54	376±16.30



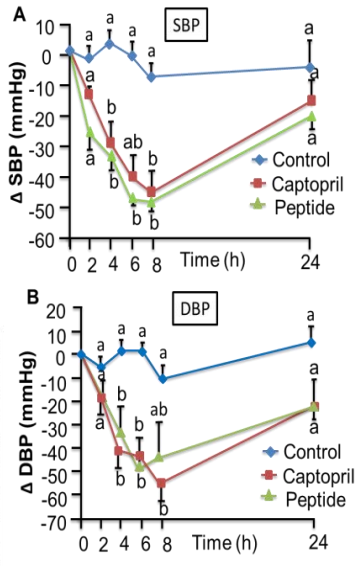


Figure 5. Identification of active peptide from PSM and its hypotensive effect after single oral administration on SHR. (A) change in SBP, (B) change in DBP. Data are expressed as the mean ± S.E.M., n = 6. Statistical analysis was performed by one way ANOVA, followed by Tukey's multiple comparison test to compare groups at each time point. Different letters represents significant differences (P < 0.05). Captopril, 7mg/kg body weight; Peptide FFYY, 80μg/kg body weight.

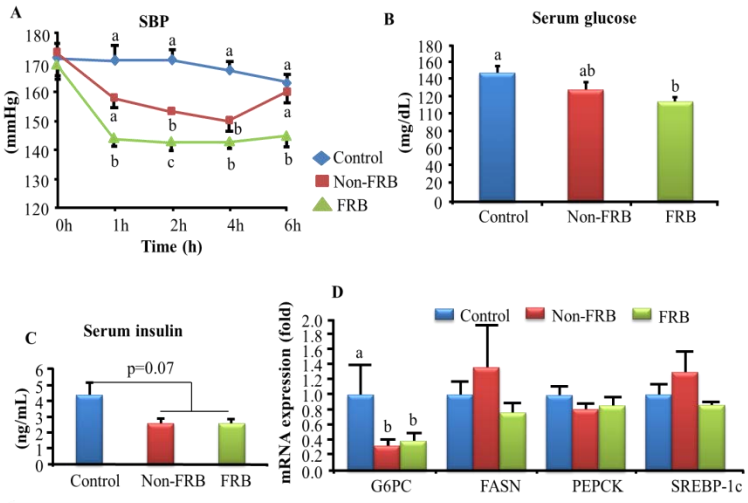


Figure 6 Effect of FRB (2g/kg body weight) on blood pressure, serum parameters and gene expressions after single oral administration. (A) Systolic blood pressure. (B) serum glucose after 2h oral administration. (C) serum insulin level. (D) mRNA expression after 2h oral administration. All date are represented as mean ± S.E.M. n=4. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison tests. Different letters indicate significant difference ($p < 0.05$). Control; Water, Non-FRB; mixture of rice bran and rice powder 2:1, FRB; Fermented rice bran.

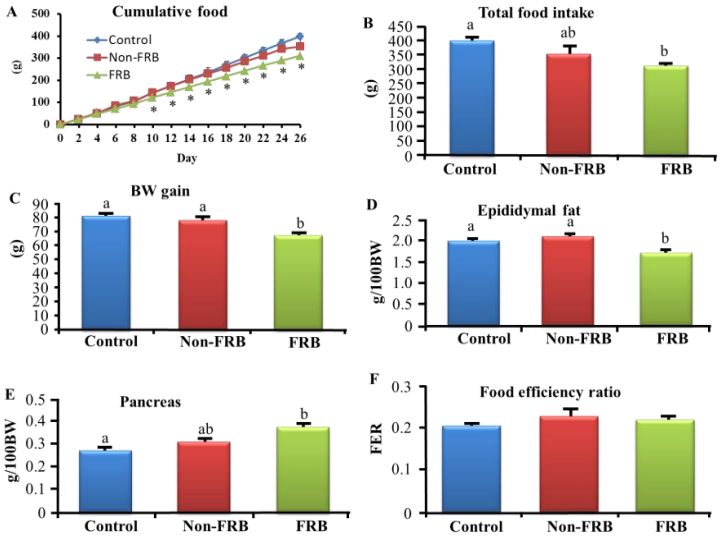


Figure 7 Effect of FRB on growth parameters. (A) Cumulative food intake, (B) Total food intake, (C) Final body weight gain, (D) Epididymal fat weight, (E) pancreas weight and (F) Food efficiency ratio (gram final body weight gain per gram diet that consumed over experimental period). All date are represented as mean ± S.E.M. n=6. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison tests. *$p < 0.05$ significant difference from the control. Different letters indicate significant difference ($p < 0.05$). Control; AIN93M diet, Non-FRB; mixture of rice bran and rice powder 2:1, FRB; Fermented rice bran.

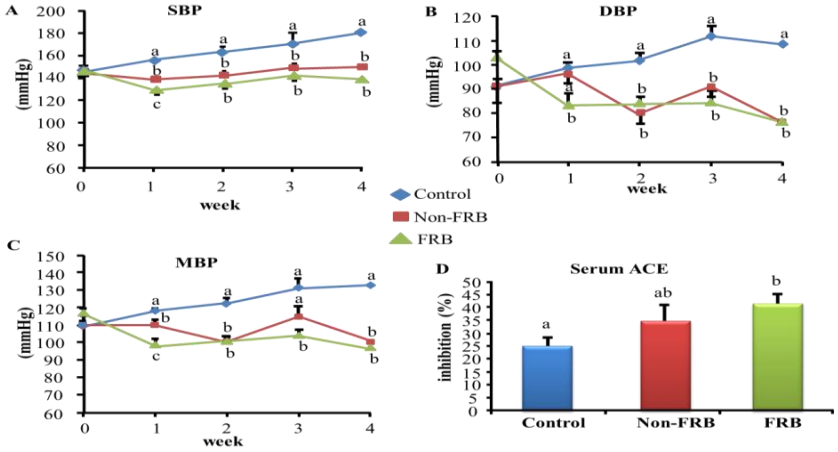


Figure 8. Effect of FRB on blood pressure and serum ACE inhibition after chronic supplementation. (A) Systolic blood pressure (B) Diastolic blood pressure (C) Mean blood pressure and (D) Serum ACE inhibitory activity. All date are represented as mean ± S.E.M. n=6. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison tests. Different letters indicate significant difference ($p < 0.05$). Control; AIN93M diet, Non-FRB; mixture of rice bran and rice powder 2:1, FRB; Fermented rice bran.

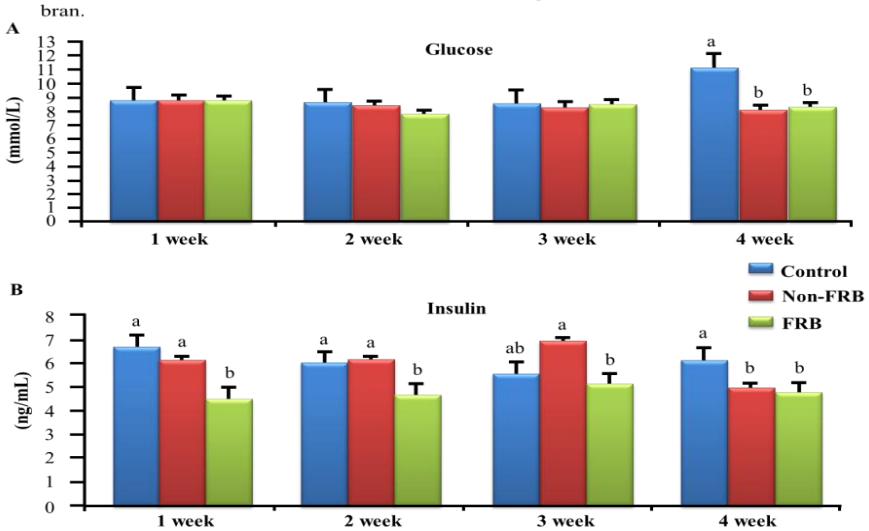


Figure 9. Effect of FRB on plasma glucose and insulin levels during chronic supplementation. (A) Plasma glucose level, (B) Plasma insulin levels. All date are represented as mean ± S.E.M. n=6. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison tests. Different letters indicate significant difference ($p < 0.05$). Control; AIN93M diet, Non-FRB; mixture of rice bran and rice powder 2:1, FRB; Fermented rice bran.

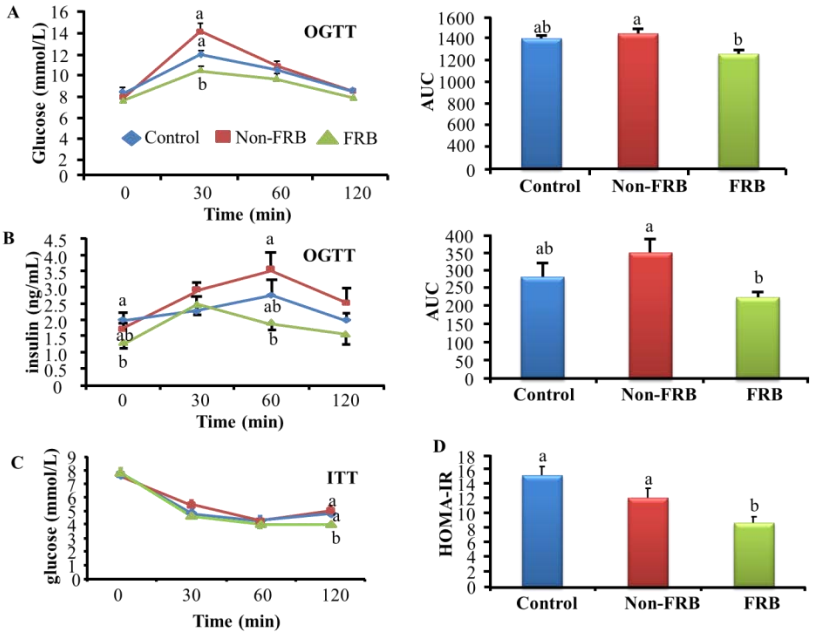


Figure 10. Effect of FRB on OGTT and ITT after 3 weeks supplementation. (A) Oral glucose tolerance test OGTT (1.8g/kg body weight) glucose (B) OGTT insulin (C) Insulin tolerance test ITT (0.6U/kg body weight) glucose and (D) HOMA-IR. All date are represented as mean ± S.E.M. n=6. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison tests. Different letters indicate significant difference ($p< 0.05$). Control; AIN93M diet, Non-FRB; mixture of rice bran and rice powder 2:1, FRB; Fermented rice bran.

Table 2 Composition of experimental diet. Basically AIN93M formula.

Ingredient (g/kg)	Control	Non-FRB	FRB
Cornstarch	602.65	589.65	589.65
Casein	140.5	133	133
Sucrose	95	95	95
Cellulose	58.5	47.5	47.5
Soybean oil	43	38	38
Mineral mixture	38.25	33.25	33.25
Vitamin mixture	9.5	9.5	9.5
Choline bitartrate	2.37	2.375	2.375
L-cystine	1.71	1.71	1.71
Tert-butylhydroquinone	0.0076	0.0076	0.0076
Test material	8.5 (water)	50	50
Total	1000	1000	1000

Non-FRB, mixture of rice bran and rice powder 2:1; FRB, fermented rice bran

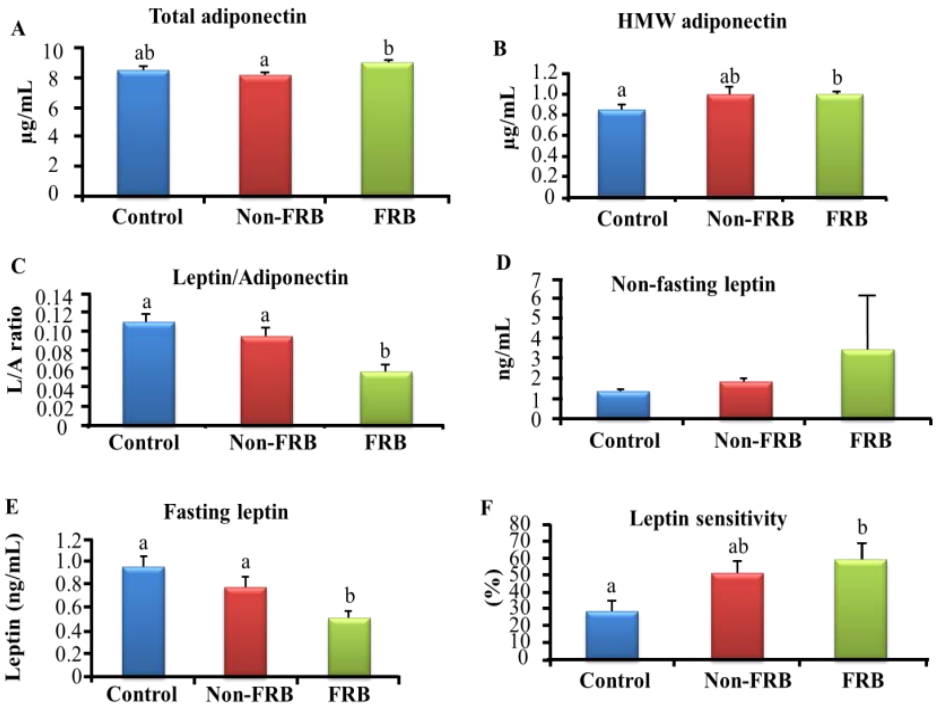
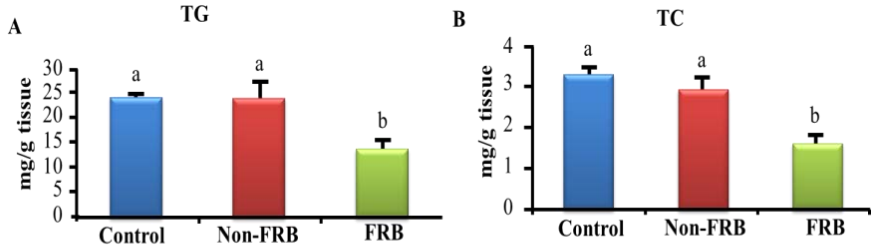


Figure 11. Effect of FRB on serum adiponectin and leptin level. (A) Serum total adiponectin (B) high molecular weight adiponectin (C) leptin/adiponectin ratio (D) non-fasting leptin (E) fasting leptin level (16 h) (F) leptin sensitivity calculated by (non fasting leptin - fasting leptin/non fasting leptinX100). All date are represented as mean ± S.E.M. n=6. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison tests. Different letters indicate significant difference ($p< 0.05$). Control; AIN93M diet, Non-FRB; mixture of rice bran and rice powder 2:1, FRB; Fermented rice bran.



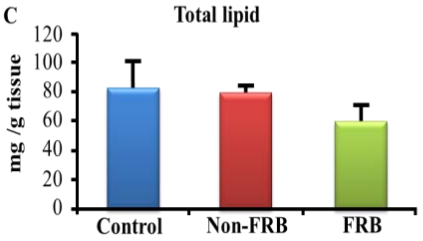


Figure 12. Effect of FRB on liver lipid. (A) Liver triacylglyceride (B) liver total cholesterol and (C) liver total lipid. All date are represented as mean ± S.E.M. n=6. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison tests. Different letters indicate significant difference ($p < 0.05$). Control; AIN93M, Non-FRB; mixture of rice bran and rice powder 2:1, FRB; Fermented rice bran.

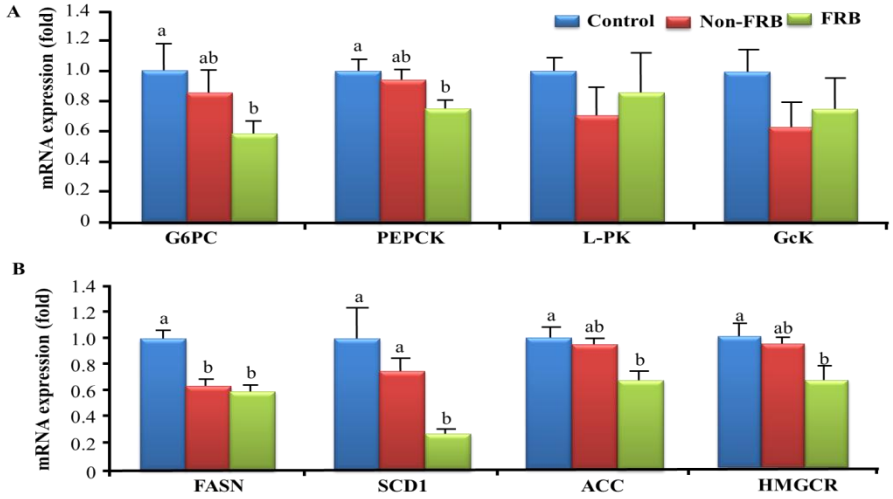


Figure 13. Effect of FRB on glucose and fat metabolism gene expressions. (A) Glucose metabolism related hepatic mRNA expression (B) fat metabolism related hepatic mRNA. All date are represented as mean ± S.E.M. n=6. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison tests. Different letters indicate significant difference ($p < 0.05$). Control; AIN93M, Non-FRB; mixture of rice bran and rice powder 2:1, FRB; Fermented rice bran.

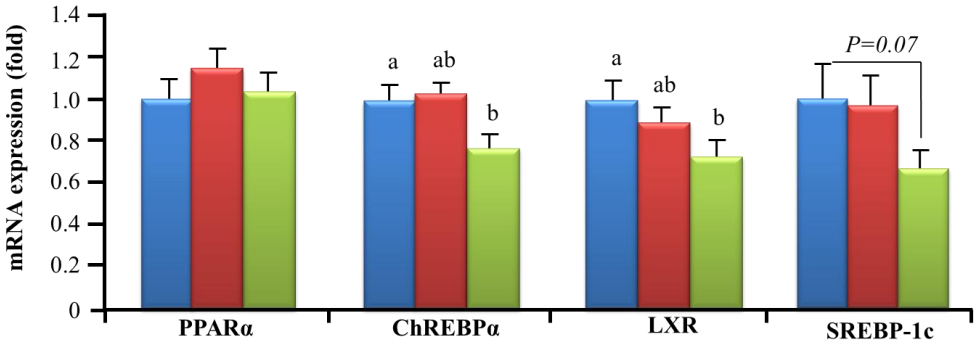


Figure 14. Effect of FRB on the mRNA level of nuclear transcription factor. All date are represented as mean ± S.E.M. n=6. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison tests. Different letters indicate significant difference ($p < 0.05$). Control; AIN93M, Non-FRB; mixture of rice bran and rice powder 2:1, FRB; Fermented rice bran. Peroxisome proliferator-activated receptor alpha (PPARα), Carbohydrate-response element-binding protein (ChREBPα), liver X receptor (LXR), Sterol regulatory element-binding protein 1(SREBP-1c).

論文審査の結果の要旨及び担当者

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学位論文題目	Studies on the effect of functional food component on metabolic syndrome in genetic animal model of life style-related disease -- Effect of processed soymilk and fermented rice bran -- （遺伝的生活習慣病発症モデル動物における機能性食品成分がメタボリックシンドロームに与える影響の解析 －　とくに加工豆乳と発酵米糖の影響について　－）

論　文　審　査　の　結　果　の　要　旨

高血圧・糖尿病などの生活習慣病は、世界的な問題となってきており、医療費軽減の目的から、とくに食品や食品成分による予防が重要となってきている。本研究では、高血圧・糖尿病のモデル動物となっている SHR（自然発症高血圧ラット）と SHRSP（脳卒中易発性高血圧自然発症ラット）を用いて、微生物プロテアーゼ加工処理した豆乳と発酵コメ糠を用いて種々の検討を行った。すなわち、一つ目は細菌（*Bacillus amyloliquefaciens*）由来プロテアーゼで豆乳を加工した加工豆乳、二つ目は共同研究者らが新規開発した *Aspergillus oryzae* と *Lactobacillus* を用いた風味改善発酵法による発酵コメ糠を用いて、生活習慣病抑制効果に関する研究を行った。

先ず、加工豆乳の効果について、in vivo に検討したところ、0.167 ml および 0.583 ml/2 ml/ Kg 体重の１回の経口投与によって、高血圧が抑制された。また、3 週間の慢性投与によっても血圧上昇が抑制された。種々の検討の結果、この作用機構は ACE（アンジオテンシン変換酵素）活性の抑制（実際に血漿アンジオテンシンⅡのレベルが有意に低下していた）と NO 産生の抑制によるものと推察された。この加工で生ずる FFYY などのテトラペプチドの高血圧抑制効果を調べたところ、降圧剤である captopril と同程度の強い高血圧抑制効果が確認された。

次に、発酵コメ糠の影響について検討したところ、この成分の 2 g/Kg 体重の単回経口投与で高血圧が抑制されたほかに、糖代謝も改善させることが分かった。さらに、４週間の慢性投与によっては、高血圧の抑制のほかに、脂肪組織重量の減少、ACE 活性の抑制、糖代謝を改善し、SHRSP のインスリン抵抗性の改善効果も認められた。この作用機構には、アディポネクティン分泌の改善やレプチン抵抗性の改善が関係していた。肝臓における脂質代謝・糖代謝の遺伝子発現レベルを調べた結果、FAS 等の脂質生合成系のダウンレギュレーションが確認されたほかに、LXR, SREBP-1c, ChREBPa などの調節因子がダウンレギュレーションされており、糖質代謝の大幅改善も認められた。

これらの新しい成果の前半部は、Journal of Functional Foods, 2015 に既に掲載されているほかに、後半部分は既に論文にまとめられており、投稿直前となっている。当該研究者は、他の研究論文も３報持っており、益々の発展が期待されている。

よって、審査委員一同は、本研究者に博士（農学）の学位を授与するに値するものと判定した。