Chapter

Fermented Brown Rice as a Functional Food

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Abstract

Brown rice, especially in a part of rice bran, contains many kinds of nutrients and biologically active components such as plant polyphenols and phytic acid, but is hard to eat. "Brown rice and rice bran fermented with *Aspergillus oryzae* (FBRA)" is a processed food that is easier for daily intake, commercially available, and rich in eating experience. During the fermentation process, dietary fibers is partially digested, and free vitamins and phenolic compounds have increased. These fermentation products are utilized for quality control to manage FBRA production. Recently, plant-derived polyphenols have shown anti-oxidative activity and biological function in various disease models. We and other research groups used raw powder FBRA to examine its biological activity through pathological and/or molecular biological analysis. Dietary administration of FBRA showed anti-tumorigenic effects in chemically induced tumors in rodents. Anti-inflammatory effects have been observed in DSS-induced colitis in rat and inflammation-mediated rodent tumor models. I will give an outline of the characteristic of FBRA, and then introduce our recently published work about "Preventive effect of FBRA on spontaneous type 1 diabetes in NOD female mice", including how to estimate the *in vivo* effect of dietary FBRA, its possible mechanisms and the limit of this study.

Keywords: Brown rice, Rice bran, Fermentation with *Aspergillus oryzae*, Animal disease model, Anti-inflammatory effect, Type 1 diabetes

1. Introduction

Lifestyle-related diseases, obesity, metabolic syndrome and cancer are now a global health problem, and the countermeasures are a global issue. Changes of our dietary habits such as increasing intake of refined sugar and polished rice largely account for the high prevalence of non-communicable chronic diseases, indicating the importance of minor food ingredients [1]. Then, improvement of the dietary habits is probably one of the best ways to decrease the risk of such diseases. Brown rice is a traditional food in Japan, and contains ordinary nutrients and many kinds of minor nutrients such as vitamins and minerals. It also contains biologically active components such as plant polyphenols and phytic acid, especially in its bran [1, 2]. Certain components in brown rice such as γ-oryzanol, have been demonstrated to show inhibitory effects on obesity and diabetes including the detailed mechanisms as described below. We should understand the benefits of such minor but biologically active ingredients, and incorporate it to our usual diet. Traditional foods are often produced by utilizing microorganisms-mediated fermentation. As well as minor bioactive components, fermented foods are attracting attentions as

health-promoting functional foods. It is possible that new bioactive components produced during fermentation may promote human health.

In this chapter, I will first introduce biological activities of brown rice or its components. Next, I will mention about fermented brown rice, its components and biological activities, including our recent research result about "Brown rice and rice bran fermented with *Aspergillus oryzae* (FBRA)". FBRA is a processed food, rich in partially digested fiber, rice bran-derived phytic acid, and free phenolic compounds. We and other research groups have examined various biological activities of FBRA and reported its anti-tumorigenic, anti-inflammatory, and other effects in cultured cells and/or animal disease models. I will give an outline of the characteristic of this processed brown rice, and then I will introduce our recent work about "Preventive effect of FBRA on spontaneous type 1 diabetes in NOD female mice". How to estimate the *in vivo* effect of dietary FBRA on type 1 diabetes model, its possible mechanisms and the limit of this study will be described.

2. Constituents and biological activity of brown rice before and after *Aspergillus oryzae*-mediated fermentation

2.1 Nutrients and non-nutrient components in brown rice

Brown rice generally contains many kinds of nutrients, carbohydrates, lipids, proteins, and micronutrients such as minerals and vitamins, which are essential for our life [1]. As compared to white rice, it contains higher amount of lipids, potassium, phosphorus, ferrous, manganese, alpha-tocopherol, vitamin B1, vitamin B2, niacin, Vitamin B6, folic acid, pantothenic acid, and dietary fiber. Non-nutrient components such as plant polyphenols and phytic acid are also important components in brown rice, especially rich in a part of rice bran [1, 2]. These phytochemicals carry antioxidative activity [1–4], protect plants themselves from environmental oxidative stress, and are distributed in free, soluble-conjugated, and bound forms in the endosperm and bran/embryo fractions of the whole rice grain [4]. Beneficial biological functions of brown rice or rice bran have been shown in cultured cells and animal disease models by using whole rice, extracts with various solvents, or identified biologically active components as described in Section 2.2.

2.2 Biological activity of components in brown rice

Brown rice and rice bran, and several known constituents in brown rice have been reported to show beneficial functions against diseased conditions such as tumor and life style-related diseases. Rice bran extract from pigmented rice, containing phenolic compounds and antioxidant activity, showed antiproliferative properties against the human and mammalian cancer cell lines [4–6]. Antigenotoxic activity of rice bran was shown in *Salmonella* mutation assay and sister chromatid exchange assay [6–8]. Antitumor effects through a modification of immunity were also reported [9, 10]. Henderson et al. [11] reviewed chemopreventive properties of dietary rice bran in *in vivo* and *in vitro* studies, and highlighted the effective components and their mechanism of action from *in vitro* studies with various cancer cell lines. They classified chemopreventive components with literature-supported activity to two items: 1) components in rice bran oil including fatty acids, tocopherol, flavonoids, γ-oryzanol, and other phenolic compounds; 2) components in defatted rice bran including polysaccharide, phytic acid, and dietary fiber. These components in rice bran contributed to chemopreventive effects on various stages

of carcinogenesis through anti-oxidative action, anti-proliferative/pro-apoptotic action, mucosal protection, and immune modulation [11].

Obesity and obesity-related diseases is another worldwide problematic issue of human health. As well as chemopreventive effects on multistage carcinogenesis, brown rice could show multifactorial functions against these diseased state. Certain food components such as phenolic compounds and antioxidants have been reported to have anti-diabetic effects in cultured cells and in model mice [12–14]. Such polyphenols have often worked to improve viability of β cells or decreased the apoptosis through modification of gene expression in the pancreas [13–15]. Pre-germinated brown rice prevented high fat diet induced hyperlipidemia [16] and showed hypocholesterolemic action [17]. A rice bran oil diet was reported to improve lipid abnormalities and hyperinsulinemic responses in type 2 diabetes model animal [18]. Acylated steryl β-glucosides in pre-germinated brown rice diet reduced oxidative stress in streptozotocin-induced diabetes [19]. Among bran-specific phenolic compounds, γ-oryzanol has been well demonstrated to be protective against diabetes and obesity [20–22]. It protected pancreatic islet β cells by directly ameliorating ER stress-induced β cells dysfunction [20, 21], and also functioned as an epigenetic controller in the brain reward system [20, 22]. Moreover, it enhances adipocyte differentiation and glucose uptake in insulin-resistant cells through cell signaling pathway [23]. Stress-induced and animal fat ingestion-induced hypoadiponectinemia have been ameliorated by γ-oryzanol and γ-aminobutyric acid [24, 25]. Sakai et al. [26] reported the importance of Glutathione peroxidase 4 (GPx4) against oxidative stress in the pathologies of vascular diseases such as athelosclerosis and diabetes, and suggested that vitamin E rich food such as brown rice, can compensate for GPx4 loss by protecting cells against lipid peroxidation.

2.3 Biologically active components in *Aspergillus oryzae*-mediated fermentation products of brown rice

Many kinds of biologically active compounds in rice bran have a potential to improve diseased condition and maintain human health, while rice bran itself is not easy for daily intake. However, plant-derived solid substances are often decomposed and utilized by various environmental lives. *Aspergillus* fungi are industrially important for food fermentation and production of biological/bioactive compounds, and *Aspergillus oryzae* is the most common mold for the fermentation of soybeans, rice, grains, and potatoes [27]. *A. oryzae* has also been used for Japanese traditional food, miso, sake, say sauce. During the fermentation process, *A. oryzae* produces amylase, protease, and β-glucosidase, and changes rice constituents to amino acids, fatty acids, organic acids, sugar and sugar alcohol, nucleotides, and various secondary metabolites [28]. Antioxidants and phenolic acid time-dependently increased during the fermentation [28, 29]. Production of natural iron chelator deferriferrichrysin from *A. oryzae* has been reported as a candidate of novel food-grade antioxidant [30]. *A. oryzae*-derived protease preparation showed beneficial effects on colonic environment in high-fat diet fed rats [31]. Neutral polysaccharide produced in fermented Korean brown rice vinegar was reported to have immunostimulatory activity [32]. *A. oryzae*-mediated fermentation of other grains wheat germ or sorghum also produced anti-adipogenic activity in cultured adipocytes [33] and anti-inflammatory effect in atherosclerotic mice model [34].

"Brown rice and rice bran fermented with *Aspergillus oryzae* (FBRA)" described in the following subsection is a processed food with *A. oryzae*-mediated fermentation (**Figure 1**). General constituents of raw powder FBRA used for our studies is shown in **Table 1** (excerpted from Kataoka et al. [36]). After the fermentation process, FBRA becomes to be taken more easily than the original material bran. Dried fermented

<Fermentation procedure>

Rice bran, misted with water
+
Steeped brown rice (1/10 of rice bran)
↓
First steaming, 100°C, 40min
Second steaming, 100°C, 70min in drum-type fermenter
↓
Innoculating with *Aspergillus oryzae*
Standing fermentation, approx. 12h
↓
Fermentation with stirring in rotating fermenter, approx. 20h at 37~43°C
↓
dried by heated air, 50°C, approx. 8h
↓
Fermented product

Figure 1.
Raw powder of FBRA. Upper panel shows photo of brown rice and rice bran as starting material, and raw powder of FBRA as fermented product (kindly provided by Dr. Hideyuki Nemoto, Koken Co., Ltd., Japan). Lower panel illustrates the fermentation procedure previously described in Horie et al [35].

product is packaged and is commercially available and has been accumulating eating experiences. While eating quality of FBRA is not directly estimated by comparing with cooked brown rice, 36 healthy adult participants in our clinical study could consume 21.0 g/day FBRA for 2 weeks without dropout [37]. Dietary fibers are partially digested, and free vitamins and phenolic compounds have increased in FBRA [38, 39]. Increase of polyamines, phenolic acids, and ergothioneine have been demonstrated by LC/ESI-MS/MS [35, 40, 41]. Polyamines such as putrescine and spermidine are essential for cell growth, proliferation and tissue regeneration, but the expression of polyamine synthetic enzymes have been declining with aging [35]. Ergothioneine is an amino acid derivative which has a strong antioxidant activity as a scavenger of reactive oxygen species, and also has the potential to prevent central neurological disorders [40]. Interestingly, Takusagawa et al. [42] recently reported that *A. oryzae* can synthesize ergothioneine from histidine contained in the food material such as rice.

Analysis items	**content**
Macronutrients	(/100 g of FBRA)
Moisture	2.6 g
Protein	16.3 g
Fat	22.1 g
Ash	11.7 g
Carbohydrate	47.3 g
Saccharides	23.4 g
Dietary fiber	23.9 g
Energy	406 Kcal
Minerals	(/100 g of FBRA)
Sodium	15.2 mg
(equivalent to NaCl)	(0.0386 g)
Phosphorous	2660 mg
Iron	7.20 mg
Calcium	429 mg
Potassium	2090 mg
Magnesium	1070 mg
Copper	0.69 mg
Zinc	5.39 mg
Manganese	23.3 mg
Selenium	9 μg
Vitamins	(/100 g of FBRA)
Thiamine (Vitamin B1)	2.10 mg
Riboflavin (Vitamin B2)	0.79 mg
Vitamin B6	3.79 mg
Vitamin B12	0.03 μg
Vitamin E (α-tocopherol)	9.4 mg
Vitamin K1 (Phylloquinone)	28 μg
Folic acid	190 μg
Pantothenic acid	6.86 mg
Biotin	48.6 μg
Niacin	66.0 mg
(Niacin as Nicotinic acid)	62.3 mg
(Tryptophan)	222 mg
Superoxide elimination activity	1.0×10^3 units/g
Phytic acid	7.64 g / 100 g
Enzyme activity	(units/g)
Amylase	3700
Acid protease	350
Neutral protease	470
Alkaline protease	160
Lipase	640

Listed items were analyzed by Japan Food Research Laboratories (Tokyo, Japan).
(excerpeted from Kataoka K et al. [36], Journal of Functional Foods. 2021; 78: 104356, supplemental ***Table 1****)*

Table 1.
Ingredient of "Brown rice and rice bran fermented with Aspergillus oryzae *(FBRA)".*

Bioactive components produced through *A. oryzae*-mediated fermentation could bring us more beneficial effects and will contribute to preventing or ameliorating various diseased conditions. On the other hand, we should clarify what elements are effective and how the elements works against diseased conditions, including optimal dose and adverse effects. In manufacturers, management of fermentation process with using adequate control compounds described by Lee et al. [28] is essential to keep the products quality.

2.4 Biological activity of raw powder FBRA in *in vitro* and *in vivo* disease models

In parallel with the above component analysis, many research groups have been conducting *in vitro* and *in vivo* studies to estimate the functions of FBRA. A raw powder FBRA used in our study is provided by the manufacturer (Genmai Koso Co. Ltd., Sapporo, Japan). In *in vitro* studies, FBRA extract induced apoptosis of tumor cells by activating mitochondrial pathway in human colorectal tumor cells [43] and via death receptor pathway in human lymphoblastic leukemia cells [44]. These results are consistent with the previous results with those of nonfermented brown rice, while the results should be carefully interpreted because of direct addition of an aqueous extract to cultured cell lines.

Antitumorigenic *in vivo* effects of dietary administration of FBRA have been examined at 5 or 10% dietary concentration. In chemically induced tumorigenesis models, preventive effects of FBRA were demonstrated in colon [45], liver [46], esophagus [47], urinary bladder [48], oral cavity [49], stomach [50], lung [51], pancreas [52]. Preventive effects were also demonstrated in prostate carcinogenesis in TRAP rats [53] and spontaneous lymphomagenesis in AKR/NSlc female mice [54]. Sakurai et al. [55] reported inhibitory effect of oral FBRA on metastasis of colon tumor cells to the liver through a mechanism leading to a Th1-dominant immune state and activation of macrophages via anti-oxidative properties. Chemoprevention mechanisms associated with dietary brown rice components have been reviewed by Henderson et al. [11]. They depicted that rice bran constituents act through anti-oxidative protection against free radicals in initiation stage, anti-proliferative/pro-apoptotic action on malignant cells, modulation of immunity and inflammation in the early or late stage, and mucosal protection through altered microbiota and intestinal environment, and that complex mixture of rice bran-derived bioactive compounds cooperatively suppress many stages of carcinogenic process. Antitumorigenic components in FBRA might be basically the same as those in brown rice and rice bran, while fermentation process possibly influence the activity of FBRA.

Anti-inflammatory effects of FBRA have been observed against the development of hereditary hepatitis in Long-Evans Cinnamon rats [56], DSS-induced colitis in rats [57], and inflammation-related tumor models [58, 59]. Phutthaphadoong et al. [58] presented that DSS-induced inflammation promoted the colorectal carcinogenesis in $Apc^{Min/+}$ mice, but the increased severity was ameliorated by FBRA feeding. Onuma et al. [59] have demonstrated that FBRA prevents inflammation-related carcinogenesis in mice through inhibition of inflammatory cell infiltration.

Modifying effects of FBRA feeding on intestinal environment was investigated in rats and healthy human adults. Dietary FBRA increased resident *Lactobacillus* species in rat [60]. In healthy adults, significant effect of FBRA intake was not detected, but no adverse phenomenon was found in this clinical study at the used dose [37].

2.5 Preventive effect of FBRA on spontaneous type 1 diabetes in NOD female mice

Based on the previous findings of anti-oxidative and anti-inflammatory effect *in vivo*, and the presence of antidiabetic components in FBRA, we planned to examine suppressive effects of FBRA on autoimmune-mediated type 1 diabetes. By using spontaneously occurring model in non-obese diabetic (NOD) female mice, we have recently reported that dietary administration of 0.5% FBRA delayed the spontaneous onset of diabetes [36]. How to estimate the *in vivo* effect, its possible mechanisms and the limit of this study are introduced below.

Diabetes in NOD mice shares many features with human type 1 diabetes [61]. In this model mice, autoreactive T cells are primed in the pancreatic lymph nodes and a disequilibrium between regulatory and effector T cells occurred at around 12 weeks of age triggers β cell destruction, resulting in diabetes onset [61]. Cyclophosphamide, an immune system disturbing agent, has often used to promote an onset of diabetes in immunological studies, whereas a spontaneous onset model is often used to examine effects of food-derived components or probiotics [13, 62]. While genetic and immunologic factors are important factors in the pathogenesis of type 1 diabetes, environmental factors such as diet and microbiota can also correlate to it [62, 63]. As mentioned above, certain rice bran components such as plant polyphenols and antioxidants have been shown to be anti-diabetic in mice models and in cultured cells. Those components have worked through improved viability or decreased apoptosis of β cells in the pancreas, or through regulating expression of related genes [12–15, 19–26].

Dietary concentration of FBRA was set to 0.5% based on daily food intake of NOD mice and our preliminary result, in which intragastric administration of FBRA at 0.33 mg/g body weight/day could delay an appearance of diabetes in NOD mice. Dose-dependent effect of FBRA was examined at the concentration of 0.25% – 1.0%. The highest concentration 5% was selected since the other animal studies have been using 5% or 10% FBRA containing diet with no harmful effect.

The criteria for diabetes onset and severity of insulitis in NOD mice were decided according to previous studies. Glucosuria was weekly monitored with test paper, and the onset of diabetes was further confirmed by measuring blood glucose levels as described by Lian et al. [64]. Mice showing 2.5 mg/ml or higher blood glucose were diagnosed as diabetic. To compare insulitis levels among the groups, pancreas was resected at the end of the experimental periods and HE-stained. Level of insulitis was assessed based on the level of lymphocyte infiltration, and the islets were graded scores 0, 1, 2, 3 or 4 as described by Serreze et al. [65]. The insulitis score of each mouse was calculated as follows: accumulated score of observed islets/number of observed islets.

Dietary administration of 0.5% FBRA significantly delayed the appearance of diabetes in mice and lowered the level of insulitis score. Glucosuria and hyperglycemia appeared at around 20 week of age and the ratio of diabetic mice increased age-dependently in control diet-fed mice, but the ratio did not increase in the 0.5% FBRA -fed group. The percentage of diabetic mice was significantly lower at 24 weeks of age as compared to the control group ($p = 0.01$, log rank test). On HE-stained sections of mice pancreas, lymphocyte infiltration into pancreatic islets has already been observed at the age of 12 weeks. However, the 0.5% FBRA-fed group frequently had small intact islets and the ratio of intact islets was significantly higher than that of control-diet-fed mice (**Figure 2**, excerpted from Kataoka et al. [36]). Insulitis score of FBRA-fed group was also significantly lower compared to the control diet group. From these results, the suppressive effects of dietary FBRA is probably achieved by maintaining the number of intact islets (**Figure 3**).

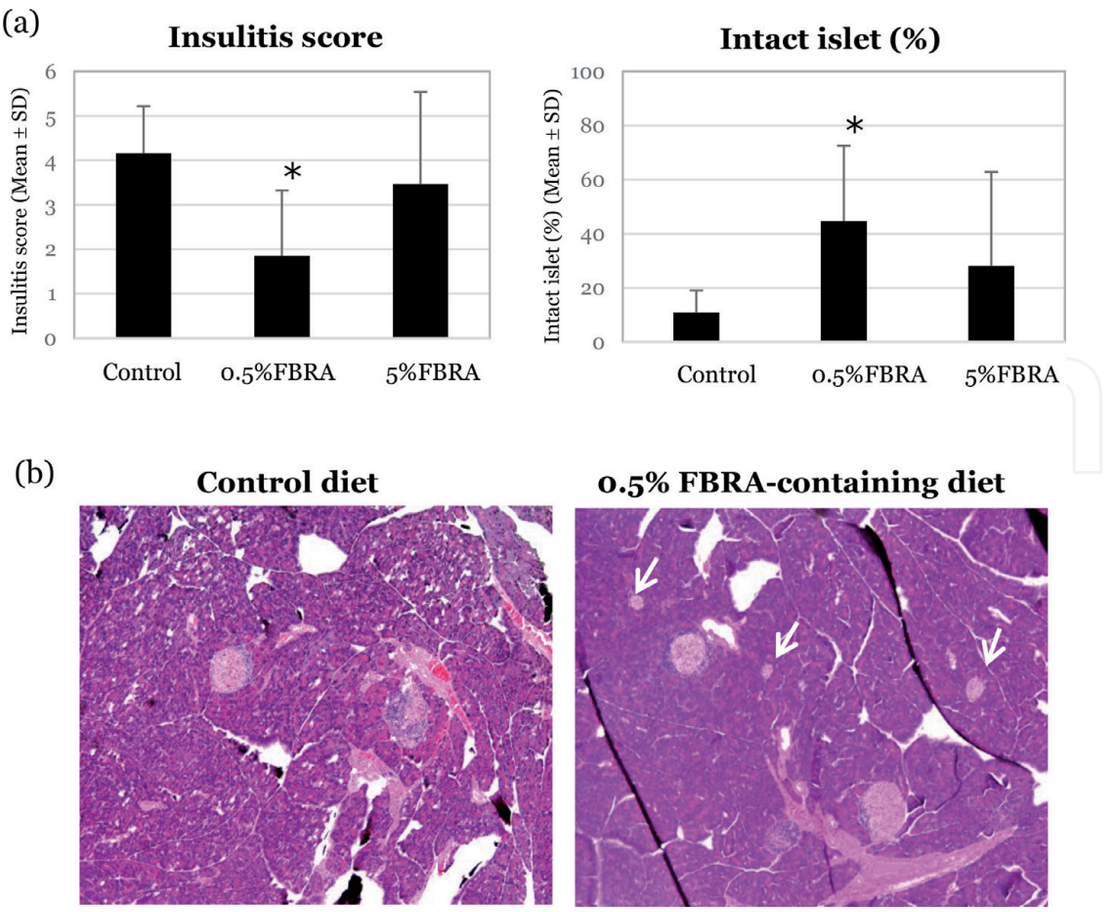

Figure 2.
Suppressive effect of FBRA on insulitis in NOD female mice. (a) Insulitis score and percentage of intact islets in NOD mice fed with control diet or FBRA-containing diet at 30 weeks of age (n = 7). Asterisk means a statistical difference between the two groups (Mann–Whitney's U test, P < 0.05). (b) Representative insulitis in HE-stained pancreatic section of NOD mice fed control diet or 0.5% FBRA-containing diet at the age of 30 weeks (HE stain, ×50). Small intact islets often observed in 0.5% FBRA-fed mice are shown with white arrows. (excerpeted from Kataoka K et al. [36], Journal of Functional Foods. 2021; 78: 104356, ***Figure 1****, with slight modification).*

Possible targets of dietary FBRA in this type 1 diabetes model include: 1) islet-specific T lymphocyte activation; 2) islet-targeting lymphocyte infiltration; 3) cytokine-mediated inflammation or ROS production; 4) regeneration of damaged islets or apoptotic cell death of damaged islets. An Inflammatory cytokine IFNγ, released from activated T cells, has an important role as a trigger of inflammation and β-cell dysfunction in autoimmune-mediated insulitis [62, 66, 67].

However, in our experiment, the percentage and number of $CD4^+$ and $CD4^+$ $IFN\gamma^+$ T cells in the spleens and pancreatic lymph nodes at 12 weeks of age were not significantly different between control diet-fed and 0.5% FBRA-fed mice. Additionally, in adoptive transfer experiments, recipient mice who received a T cell fraction from spleen of 0.5% FBRA-treated NOD mice, could not keep the ratio of intact islets and rather increased the ratio of severely damaged islets, while the number and ratio of intact islets tended to increase in 0.5%FBRA-treated recipient mice who received a T cell fraction derived from control diet-fed donor mice. These results supported that FBRA or its components might suppress the onset of diabetes through keeping an enough number of intact islets in pancreas of NOD mice, not through inhibiting the step of islet-specific T lymphocyte activation.

Pancreas has been known to have regenerative potential for autoimmune- or other factor-mediated damage to islet β cells even in adult rodents [68–70]. Pdx1 and related molecules Foxo1, Reg2, Pdcd4 have important roles in islet function and the fate of injured islet cells [15, 71–73]. Pdx1 and Foxo1 are involved in pancreatic

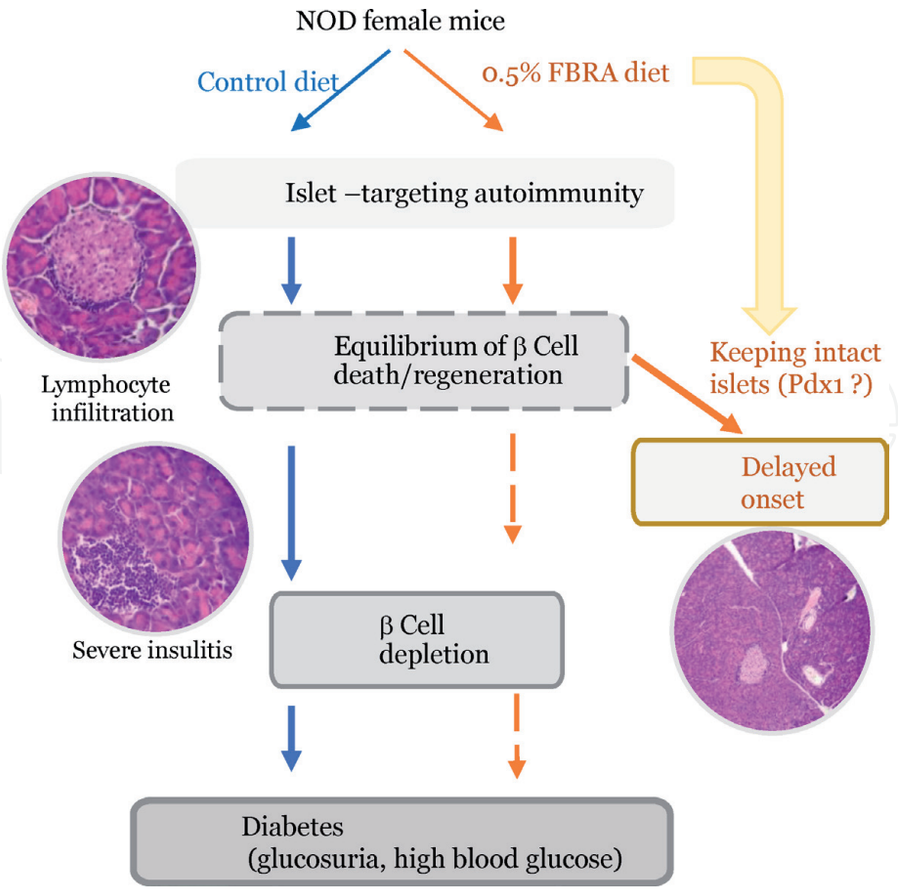

Figure 3.
Hypothetical schema of suppressive effect of FBRA on type 1 diabetes in NOD mice.

development and β cell functional regulation through changes of their intracellular translocation [71]. The inflammatory cytokine IFNγ is reported to decrease nuclear localization of Pdx1 and to trigger β cell dysfunction [67]. Oppositely, phenolic compounds in plant-derived food have recently been reported to show anti-diabetic actions via various mechanisms, including increased expression of pdx1 or restoration of nuclear localization of Pdx1 [13, 15, 67].

Then, we examined effects of FBRA on expression of Pdx1 and related molecules. In our study, mRNA levels of *Pdx1* and related molecule genes were similar in whole pancreases of 19- and 22- week-old mice between the control diet-fed group and the 0.5% FBRA-fed group. However, immuno-histochemical analyses of pancreatic sections showed a tendency for more Pdx1 in the cell nuclei in the 0.5% FBRA-fed group. Then, intracellular localization of Pdx1/Foxo1 and their phosphorylation level at appropriate ages should be further examined in NOD mice with/without 0.5% FBRA treatment.

Our study showed that consumption of FBRA throughout the experimental period suppressed the spontaneously occurring diabetes in female NOD mouse with 0.5% dietary concentration as optimal. But several limitations are still present. At the first, ameliorating effects after onset of type 1 diabetes should be examined in animal model, because autoimmune-mediated diabetes suddenly occur and is generally found as glucosuria. The second is which component (s) and how the component(s) work against pathogenesis of type 1 diabetes. Although FBRA is commercially available processed food and no harmful phenomenon has not been observed in human including clinical trial Volunteers [37], to clarify suppressive mechanisms of FBRA including optimum dose of active component(s) is very important for using it as functional food. Finally, the suppressive effect of FBRA on type 1 diabetes was observed only at lower concentrations, but not at

higher concentrations (1% and 5%). At lower dietary concentration, anti-diabetic components in FBRA probably suppressed the development of diabetes through enhanced β cell viability and proliferation, but at higher dietary concentration, the other functional effect of FBRA might appear. For example, 5% dietary FBRA could increase resident *Lactobacillus* species in rat intestine [60], and certain *Lactobacillus* species has been reported to activate Th1 immunity [74–76].

3. Conclusions and perspectives

Brown rice and rice bran contains many kinds of biologically active components and their beneficial effects against diseased conditions have been demonstrated. "Brown rice and rice bran fermented with *Aspergillus oryzae* (FBRA)" is a processed food in which free vitamins and phenolic compounds have increased during *A. oryzae*-mediated fermentation. Dietary administration of FBRA showed anti-tumorigenic and anti-metastatic effects in various tumor model animals, and anti-inflammatory effects in rat hepatitis, rat colitis, and inflammation-mediated rodent tumor models. Based on these previous findings of FBRA, considering antidiabetic components in brown rice, we examined suppressive effects of FBRA on autoimmune-mediated type 1 diabetes. In non-obese diabetic (NOD) female mice, dietary administration of 0.5% FBRA delayed the spontaneous onset of diabetes and significantly reduced the insulitis score [36]. While relation of the rice variety and these anti-disease activities was not investigated, phytochemical profile is varied among different kinds of rice [2–4].

Brown rice and fermented brown rice showed very attractive beneficial functions in *in vitro* and *in vivo* studies. However, for clinical application of FBRA, its action mechanisms including determination of active ingredients and its optimal dose should be clarified in future studies.

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Conflict of interest

The author do not have any conflicts of interest regarding this manuscript.

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