Journal of Functional Foods 78 (2021) 104356

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Preventive effect of fermented brown rice and rice bran on spontaneous type 1 diabetes in NOD female mice

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ARTICLE INFO

Keywords:
Fermented brown rice
Insulitis
NOD mouse
Pdx1
Type 1 diabetes

ABSTRACT

Consumption of brown rice and rice bran fermented with *Aspergillus oryzae* (FBRA) suppresses spontaneously occurring diabetes in female NOD mouse. While control diet-fed mice showed glucosuria and hyperglycemia at around 20 week of age and the ratio reached to 57% at 30 weeks of age, the ratio did not increase in the 0.5% FBRA-containing diet-fed group. The FBRA-fed group at 30 weeks of age kept higher ratio of intact islets and showed significantly lower insulitis score compared to the control diet group, with dose-dependency from 0.25% to 0.5% dietary concentration of FBRA. 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). These results indicate that the suppressive effects of dietary administration of 0.5% FBRA in delaying the spontaneous onset of diabetes in NOD mice is probably achieved by maintaining the number of intact islets.

1. Introduction

Type 1 diabetes is an autoimmune disease caused by immune cell targeting of pancreatic islets. Th1-mediating T cell populations have an important role in the onset of insulitis and destruction of islet β cells (Kahaly & Hansen, 2016; Paschou, Paradoupoulou-Marketou, Chrousos, & Kanaka-Gantenbein, 2018). Immune cell crosstalk in type 1 diabetes and released cytokines IFNγ, IL-1β, and TNFα also induce the production of reactive oxygen species (ROS) by β cells, and ROS have the potential to mediate apoptosis (Lehuen, Diana, Zaccone, & Cooke, 2010). Proinflammatory cytokine-mediated free radical generation in the pancreatic islets of rats has been reported (Tabatabaie, Vasquez-Weldon, Moore, & Kotake, 2003). Chronic pancreatic inflammation induces death of islet β cells and depletes insulin secretion, resulting in onset of diabetes. While pancreas-specific autoimmune responses can damage islet β cells, the mammalian pancreas possesses a regeneration potential to maintain its important function in blood glucose control. Regeneration of β cells has been reported in adult rodents with partial pancreatectomy, tissue injury and insulin resistance (Cano et al., 2008; Nir, Melton, & Dor, 2007; Tokoro, Tezel, Nagasaka, Kaneko, & Nakao, 2003; Yi, Park, & Melton, 2013). Pancreatic and duodenal homeobox 1 (Pdx1) and related molecules, forkhead box O1 (Foxo1), regenerating islet-derived 2 (Reg2), programmed cell death 4 (Pdcd4), are involved in islet functions and the fate of injured islet cells. Pdx1 and Foxo1 have an important role in pancreatic development and β cell functional regulation (Inagaki, Tajiri, Tate, Kunimura, & Morohashi, 2012; Liu et al., 2007; Meng et al., 2009). Regeneration gene families are expressed during the process of whole islet neogenesis and β cell regeneration in the pancreas, and a positive correlation between *Reg2* expression and a reduction of insulitis has been reported (Hill et al., 2013; Huszarik et al., 2010). *Pdcd4*, up-regulated during apoptosis, is a tumor suppressor gene and a potential target for anticancer therapies (Lankat-Buttgereit & Gőke, 2009). It was suggested that Pdcd4 may have a role in differentiation and disease, such as diabetes and inflammation. Its deficiency in diabetic model mice has been reported to confer resistance to diabetes (Ruan et al., 2011).

Non-obese diabetic (NOD) mice develop spontaneous autoimmune diabetes that shares many features with human type 1 diabetes (Gagnerault, Luan, Lotton, & Lepault, 2002). They demonstrated that pancreatic lymph nodes are required for the priming of autoreactive T cells in NOD mice around 3 week of age. The first signs of nondestructive insulitis appeared at 3–4 weeks of age, and a disequilibrium between regulatory and effector T cells occurred at around 12 weeks of age,

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https://doi.org/10.1016/j.jff.2021.104356
Received 13 April 2020; Received in revised form 28 December 2020; Accepted 29 December 2020
Available online 16 January 2021

resulting in β cell destruction and diabetes onset. Cyclophosphamide treatment has been known to disturb the immune system and promote the onset of type 1 diabetes in NOD mice. However, a spontaneous onset model in NOD female mice is often used to examine the effects of food components or probiotics (Babu, Liu, & Gilbert, 2013; Mishra et al., 2019).

In addition to genetic and immunologic factors, environmental factors such as infection, diet, and microbiota can also contribute to the pathogenesis of type 1 diabetes (Mishra et al., 2019; Paschou et al., 2018). Certain food components and antioxidants have been reported to show anti-diabetic effects in model mice and in cultured cells (Babu et al., 2013; Kaneto et al., 1999). Such polyphenols have worked through improved viability or decreased apoptosis of β cells in the pancreas (Babu et al., 2013; Zhang & Liu, 2011; Zhang, Zhen, Maechler, & Liu, 2013).

Brown rice and rice bran fermented by *Aspergillus oryzae* (FBRA) is a processed food that is rich in partially digested fiber, rice bran-derived phytic acid, and plant polyphenols. Its anti-inflammatory effects have been reported in several animal disease models (Kataoka et al., 2008; Onuma et al., 2015; Phutthaphadoong et al., 2010). Here, we show the suppressive effects of dietary administration of FBRA against spontaneous onset of type1 diabetes in NOD female mice. We also examined its effect on the expression of Pdx1 and related molecules, which are known to effect islet cell, viability, death, and regeneration.

2. Materials and methods

2.1. Food material examined in this study

FBRA was provided by Genmai Koso Co. Ltd. Composition of FBRA analyzed by Japan Food Research Laboratories (Tokyo, Japan) was shown in Supplementary Table 1. During fermentation of brown rice and rice bran with Aspergillus oryzae, increase of polyamines, phenolic acids, and ergothioneine have been demonstrated by LC/ESI-MS/MS (Horie et al., 2019, 2020; Ogawa et al., 2017).

2.2. Animal experiments

NOD/ShiJcl (female, 4-week-old mice) and NOD/ShiJic-*scid* Jcl (female, 4-week-old mice) were purchased from CLEA Japan, Inc. (Tokyo, Japan). Mice were housed in plastic cages in a room environmentally controlled at a temperature of 23 °C ± 2 °C and a 12-h light/12-h dark cycle, and they were adapted to laboratory conditions with free access to a basal diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). All experiments were in accordance with guidelines of the Tokushima University Ethics Committee for the care and use of laboratory animals (approval No. 13046).

After a 1-week acclimation, mice were divided into a non-treated group and a FBRA-treated group. Mice in the FBRA-fed group were fed a 0.25−5% (w/w) FBRA-containing basal diet throughout the experimental period. We selected the dietary concentration based on our preliminary result and other animal research using 5% FBRA-containing diet without harmful effect. Body weight was recorded once a week. Glucosuria was weekly monitored with test paper (Terumo Co., Ltd., Tokyo, Japan). Onset of diabetes was confirmed in glucosuria-positive mice by measuring glucose levels in the blood from the tail vein with Glucose PILOT (Iwai Chemicals Co. Ltd., Tokyo, Japan). Mice showing 250 mg/dL or higher blood glucose were diagnosed as diabetic as described by Lian et al. (2012). At the end of the experimental periods, mice were euthanized and the pancreas was resected and HE-stained to compare insulitis levels among the groups. Spleen and pancreatic lymph node were used for flow cytometric analysis of lymphocyte populations, if necessary.

To better understand the inhibitory mechanisms of dietary FBRA in type 1 diabetes, adoptive transfer experiment were done using a splenic T cell fraction from donor NOD mice. Donor NOD mice and recipient NOD-*scid* mice were fed with an MF control diet or 0.5% FBRA-diet from 5 to 19 weeks of age. Splenocytes were collected from 5 mice in each donor group, pooled, and the $CD19^+$ B cell fraction was removed with anti-mouse CD19 antibody and BioMag® goat anti rat IgG beads (QIAGEN). The prepared T cell fraction (>95% $7AAD^-B220^-$ cells) was transferred intraperitoneally into age-matched NOD-*scid* mice (1×10^7 cells/0.2 mL PBS/mouse) (Supplemental Fig. 1). A T cell fraction from donor NOD mice maintained with control diet was transferred to NOD-*scid* mice fed with control diet (NOD → SCID) or to NOD-*scid* mice fed with 0.5% FBRA-diet (NOD → SCID + FBRA). A T cell fraction from donor NOD mice maintained with 0.5% FBRA diet was transferred to NOD-*scid* mice fed with control diet (NOD + FBRA → SCID). The recipient mice were maintained with the same diet as before the cell transfer for 4 weeks. At the end of experiment, mice were euthanized and the pancreas was resected and HE-stained to compare insulitis levels among the groups.

2.3. Histological analysis of insulitis

Resected pancreases were immediately fixed in 4% paraformaldehyde-phosphate buffer for at least 24 h, and sequentially dehydrated in 70%, 80%, 90%, 95% ethanol, and xylene, and then embedded with paraffin. From the paraffin block, 3 sections per each sample were rehydrated and HE- stained to observe pancreatic islets. The insulitis level of NOD mice was assessed based on the level of lymphocyte infiltration. The islets were graded scores 0, 1, 2, 3 or 4 as described by Serreze et al. (1998), and the insulitis score of each mouse was calculated as follows: accumulated score of observed islets/number of observed islets. Mice with glucosuria, which had already become diabetic, were euthanized before the end of the experimental period and they were graded as score 5.

2.4. Flow cytometry

Lymphocytes were obtained from the spleen and pancreatic lymph nodes. They were stained with fluorochrome-conjugated antibodies to CD4, CD8, B220 (eBioscience). To stain intracellular IFNγ, isolated cells were stimulated with 250 ng/mL PMA (Sigma-Aldrich) and 1

μg/mL ionomycin (Sigma-Aldrich) for 5 h in the presence of monensin. 7-aminoactinomycin D (7AAD) (Sigma-Aldrich) was used to exclude dead cells. Stained cells were analyzed with a FACS Canto II (BD Bioscience) and data were analyzed using FlowJo software (Tree Star).

2.5. Expression of Pdx1 and related molecules

To investigate how FBRA inhibited the spontaneous onset of diabetes, pancreases were removed at 19 or 22 weeks of age from NOD mice that had been fed either 0.5% FBRA-containing food or a control-diet. A part of the pancreas was immediately preserved in RNAlater for RNA preparation according to the manufacturer's instruction, and the remaining part was sectioned as described above and used for immunohistochemical analysis of the expression of Pdx1, insulin II or Pdcd4. Pancreatic sections were incubated with anti-PDX1 antibody (1:2000; ab47267), anti-insulin antibody (1:2000; ab63820), anti-PDCD4 antibody (1:1000; HPA001032) with biotinylated anti-rabbit IgG as the secondary antibody, using a Histofine SAB-PO(R) kit (NICHIREI BIOSCIENCE INC.).

Pancreatic RNA was extracted with RNeasy Mini kit, its quality was confirmed with the presence of ribosomal RNA bands in denatured agarose gel electrophoresis, and was reverse-transcribed to cDNA using a Prime Script™ RT reagent kit (TaKaRa). First-strand cDNA synthesis was followed by PCR to detect the expression of *Pdx1*, *Foxo1*, *Reg2*, *Ins2* (*insulin II*), *Pdcd4*, and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*). The sequences of the PCR primers are shown in supplemental Table 1. The reaction products were separated on 1.5% agarose gel and stained with ethidium bromide. Levels of mRNA of *Pdx1*, *Foxo1*, and

Pdcd4 were further quantified by real time PCR (SYBR® Premix Ex Taq™ II) and analyzed by the ΔΔCt method using *Gapdh* as a reference gene. The sequences of the real time PCR primers are shown in supplemental Table 2. Conditions for RT-PCR and RT-qPCR were as described previously (Abe et al, 2012; Klelnert et al, 2016; Chen et al., 2016; Yoshimura et al., 2013).

2.6. Statistical analysis

Comparisons were performed using the Mann-Whitney's *U* test

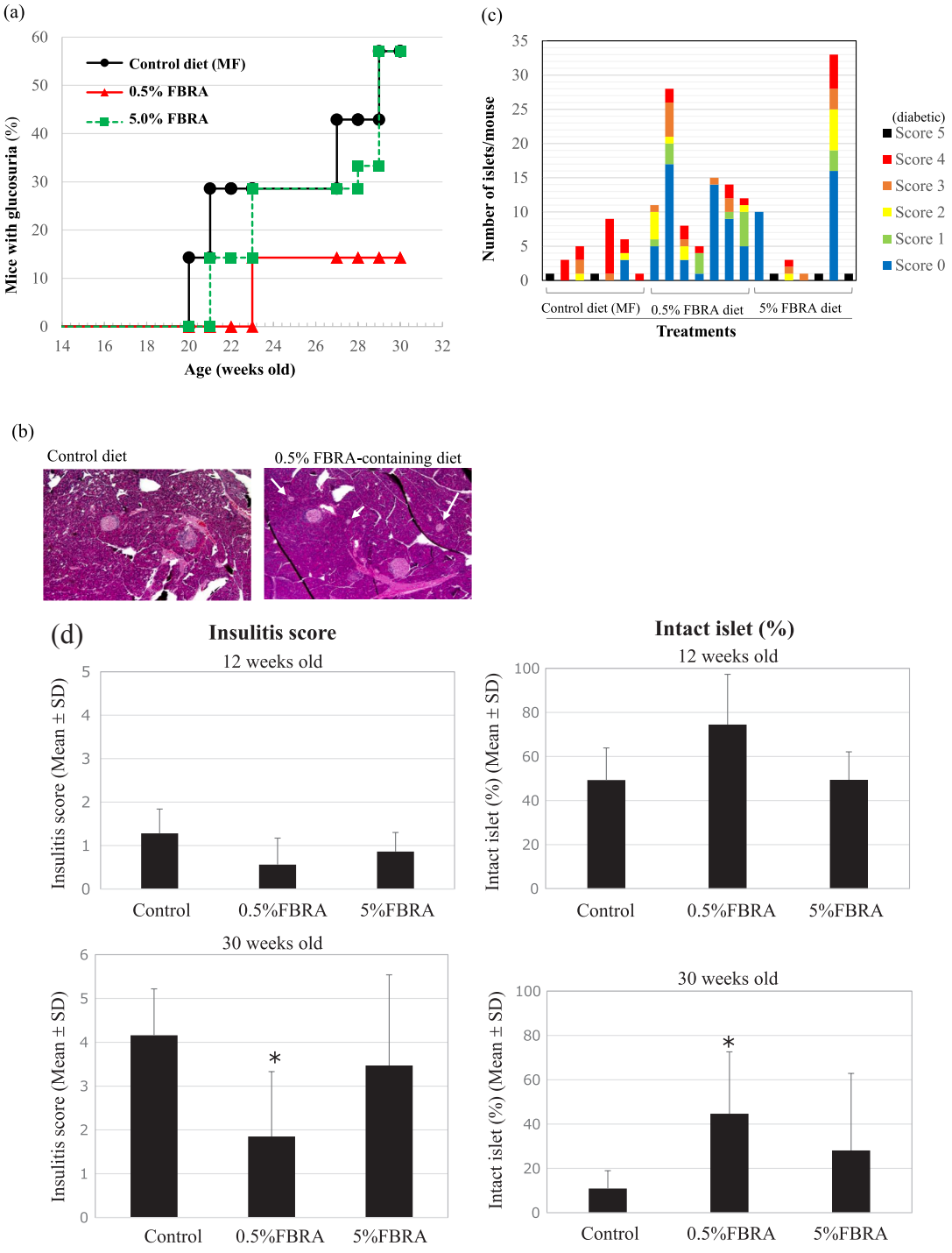

Fig. 1. Effects of FBRA feeding on the spontaneous onset of diabetes and insulitis in NOD female mice. (a) Appearance of glucosuria in NOD mice fed with a control-diet, or a diet containing 0.5% or 5% FBRA. Diabetic mice were detected with test paper and confirmed with higher blood glucose levels. (b) Representative insulitis in HE-stained pancreatic section of NOD mice fed control diet or 0.5% FBRA-containing diet (HE stain, ×50). At the age of 30 weeks, pancreases were resected from all of the remaining mice and the paraffin- embedded sections were HE-stained for analysis of insulitis. Small intact islets often observed in 0.5% FBRA-fed mice are shown with white arrows. (c) Number of islets with scores 0–4 in individual mice. Insulitis was evaluated by the extent of lymphocyte infiltration as observed with HE-stained sections. Mice that were diagnosed as diabetic before the end of the experimental period were assigned a score of 5. (d) Insulitis score and percentage of intact islets in NOD mice fed with control diet or FBRA-containing diet at 12 weeks of age (n = 3) or 30 weeks of age (n = 7). Asterisk means a statistical difference between the two groups (Mann-Whitney's *U* test, $P < 0.05$).

between two groups or the Kruskal-Walis test in multiple groups. Differences in onset rates of diabetes between control diet and FBRA-containing diet group were analyzed by the Kaplan-Meier log-rank test. A p value < 0.05 was considered to be statistically significant.

3. Results

3.1. Suppressive effect of dietary FBRA in spontaneously occurring diabetes in NOD mice

A spontaneously occurring diabetes model in NOD female mice was used to examine the effects of dietary FBRA. Body weight diagrams are shown in supplementary Fig. 2. The onset of diabetes was determined by assessing glucosuria and confirmed by measuring blood glucose levels. The first diabetic mice in the control diet group appeared at an age of 20 weeks and the ratio reached to 57% at 30 weeks of age (Fig. 1a). In the 0.5% FBRA-fed group, the spontaneous onset of diabetes started at 23 weeks of age, but the ratio of diabetic mice did not increase. Since insulitis has been known to occur in the pancreas prior to diabetes onset, we compared the levels of insulitis using HE-stained pancreatic tissue sections. Fig. 1b shows representative insulitis in mice maintained on the control diet or 0.5% FBRA at the end of the experiment. While lymphocyte-infiltrated pancreatic islets were observed in both panels, small intact islets were frequently found in the pancreatic tissues of 0.5% FBRA-fed mice. The insulitis score was still low at an age of 12 weeks, but at 30 weeks of age, the ratio of intact islets decreased to 10% of total examined islets in the control diet-fed mouse (Fig. 1c, d). However, the 0.5% FBRA-containing diet-fed group had a significantly higher ratio of intact islets and showed significantly lower insulitis scores compared to the control diet group at 30 weeks of age (Mann-Whitney test, $p < 0.05$). 5% FBRA-feeding showed no significant effect on either the appearance time of glucosuria or the severity of insulitis.

The optimal concentration of FBRA in the diet was determined by comparing the insulitis scores obtained at 16 weeks of age among the 0%, 0.25%, 0.5%, and 1% FBRA-fed groups ($n = 3$) (Fig. 2a). The average insulitis score decreased in a dose-dependent manner and was lowest at 0.5% FBRA, but increased at 1% addition. The ratio of intact islets (%) was also highest at 0.5% dietary concentration. The delayed onset of diabetes in the 0.5% FBRA-fed group was confirmed with an increased number of mice ($n = 15$) (Fig. 2b). At 24 weeks of age, the percentage of diabetic mice was significantly lower in the 0.5% FBRA-fed group as compared to the control diet-fed group ($p = 0.01$, log rank test). These results indicate that dietary administration of FBRA could suppress the spontaneous onset of type 1 diabetes in NOD female

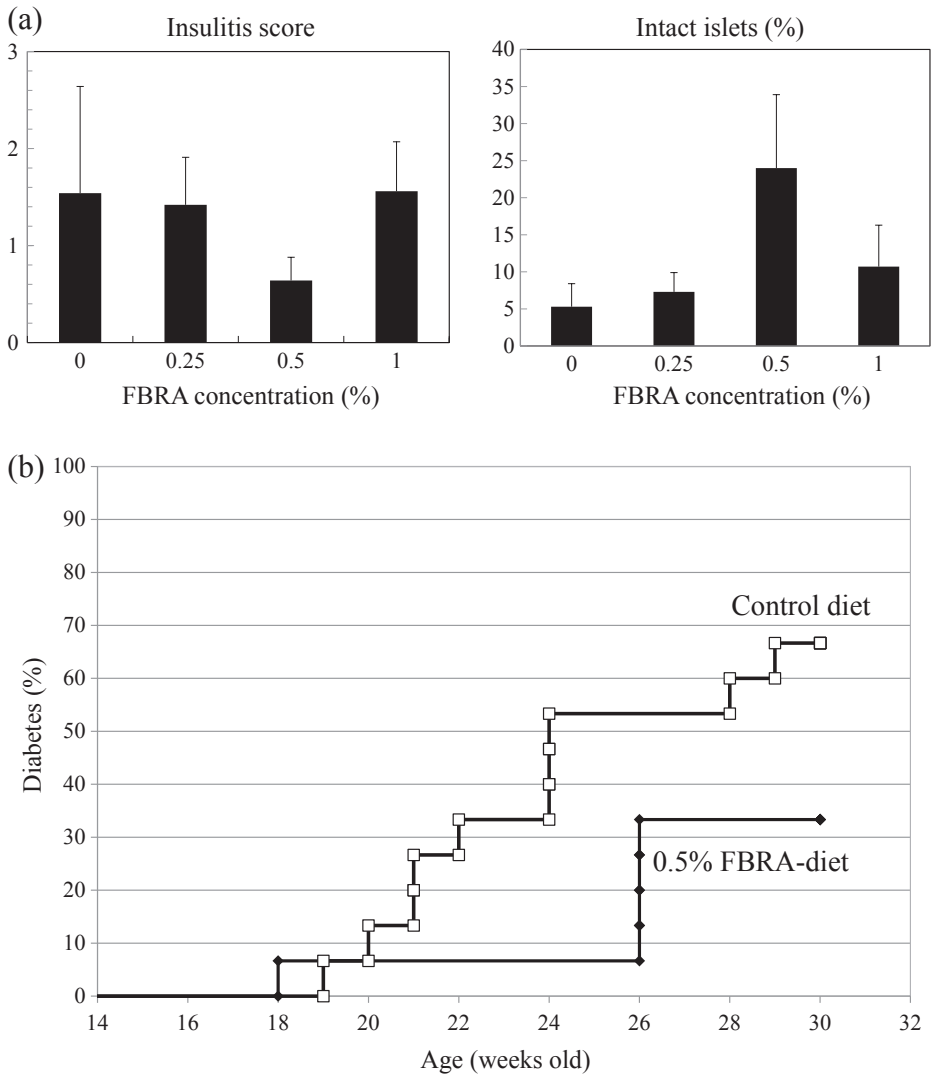

Fig. 2. (a) Concentration dependent effect of dietary FBRA on the suppression of insulitis in NOD female mice. Mice were fed a 0–1.0% FBRA-containing diet until 16 weeks of age ($n = 3$/group), and insulitis was evaluated as described in Fig. 1. (b) Delayed appearance of glucosuria in NOD mice fed with 0.5% FBRA-containing diet ($n = 15$/group).

mice, with 0.5% addition being the optimal concentration.

T lymphocyte populations were collected from spleens and pancreatic lymph nodes of NOD mice at 12 weeks of age (Fig. 1c, d) and analyzed by flow cytometry. No significant difference in the percentages and numbers of $CD4^+$ and $CD4^+IFNg^+$ T cells was observed between control diet-fed and 0.5% FBRA-fed mice at that time (n = 3, Supplemental Fig. 3).

3.2. Effect of dietary FBRA on insulitis in adoptive transfer experiment

We next examined whether or not dietary FBRA inhibited a step in the activation of islet-targeting T lymphocytes. T cell fractions from NOD mice (NOD) that were fed with/without 0.5% FBRA were prepared and transferred into NOD *scid* mice (SCID) fed with/without 0.5% FBRA. Four weeks after the adoptive transfer, insulitis levels in recipient mice were assessed. Compared to the control diet-fed group (NOD → SCID), 0.5% FBRA-feeding to donor mice (NOD + FBRA → SCID) tended to increase the mean insulitis score and the percentage of islets with a score of 3 or 4 (Fig. 3, Table 1), while the difference was not significant probably due to small number of mice and large interindividual variation (Kruskal-Walis test, p = 0.099 for both insulitis score and the percentage of islets with a score of 3 or 4). On the other hand, recipient mice fed with 0.5% FBRA (NOD → SCID + FBRA) retained more intact islets and the mean insulitis scores tended to be lower.

These results suggest that FBRA might maintain intact islets in the pancreas, rather than inhibiting islet-specific T lymphocyte activation.

3.3. Effects of FBRA on the expression of Pdx1 and related molecules

In 0.5%FBRA-fed mice, small but intact islets were frequently observed in pancreatic tissue sections and the number of intact islets were increased but severely infiltrated islets did not decrease. Then, we examined the influence of FBRA on the expression of Pdx1 and related molecules that were involved in the function, proliferation/regeneration and survival of islet β cells. Whole pancreases were removed from 19- and 22- week-old NOD female mice that has been fed either a control diet (n = 5) or a 0.5% FBRA-diet (n = 5). They were used to compare the expression of *Pdx1, Foxo1, Reg2, and Pdcd4*. RT-PCR and RT-qPCR showed similar mRNA levels (Fig. 4a, b), suggesting similar levels of gene expression. Immuno-histochemical analysis (Fig. 4c) showed similar levels of insulin, Pdcd4 and Pdx1 in pancreatic sections.

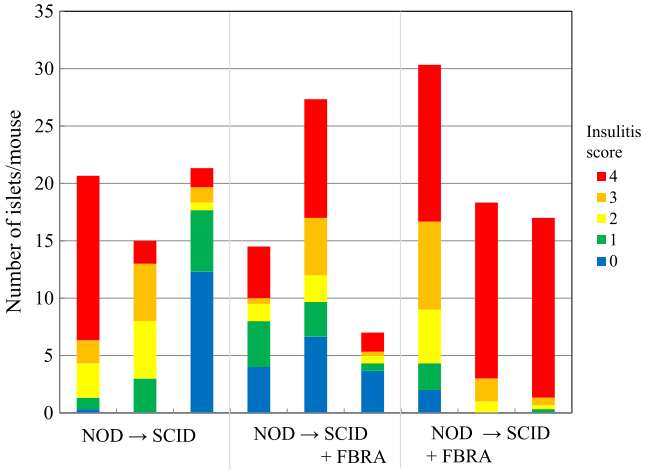

Fig. 3. Effects of 0.5% FBRA feeding on insulitis in NOD SCID female mice after adoptive transfer of a splenic T cell fraction. A total T cell fraction from donor NOD mice maintained with or without 0.5% FBRA treatment was transferred to NOD SCID mice with or without 0.5% FBRA. Four weeks after the adoptive transfer, insulitis level was evaluated on HE-stained sections. The number of islets with scores 0–4 in individual recipient mice is shown.

Table 1
Effects of 0.5% FBRA feeding on insulitis in NOD *scid* female mice after adoptive transfer of a splenic T cell fraction.

	NOD → SCID	NOD → SCID + FBRA	NOD + FBRA → SCID
Insulitis score	2.2 ± 1.3	1.85 ± 0.48	3.5 ± 0.5
Intact islets (%)	19.8 ± 32.9	34.8 ± 15.3	2.2 ± 3.8
Islets with score 3 or 4 (%)	46.6 ± 32.5	39.7 ± 14.5	87.0 ± 14.5

A T cell fraction from donor NOD mice (NOD) with or without 0.5% FBRA treatment was transferred to NOD *scid* mice (SCID) with or without 0.5% FBRA. Four weeks after the adoptive transfer, insulitis level was assessed based on the level of lymphocyte infiltration observed in HE-stained sections. The insulitis score was calculated as described in Materials and Methods. Values are mean ± SD.

However, the distribution of Pdx1 tended to be greater in the nuclei in the 0.5% FBRA-fed group compared to the control diet- fed group.

4. Discussion

FBRA contains anti-oxidative components such as phytic acid and plant polyphenols, and it has shown anti-inflammatory effects in some animal disease models (Kataoka et al., 2008; Onuma et al., 2015; Phutthaphadoong et al., 2010). Here, we examined its suppressive effects on autoimmune-mediated diabetes by using spontaneously occurring diabetes and insulitis in NOD female mice.

Dietary administration of 0.5% FBRA significantly delayed the appearance of diabetes in mice, and significantly lowered the level of insulitis (Figs. 1 and 2). Lymphocyte infiltration into pancreatic islets was observed at the age of 12 weeks. However, the 0.5% FBRA-fed group frequently had small intact islets and showed a significantly higher ratio of intact islets, resulting in significantly lower insulitis score at 30 weeks of age compared to the control diet-fed group. In adoptive transfer experiments (Fig. 3, Table 1), the number and ratio of intact islets also tended to increase only when recipient mice were fed with the 0.5% FBRA- diet. Recipient mice who received a T cell fraction from 0.5% FBRA-fed NOD mice, could not maintain the ratio of intact islets and rather increased the ratio of severely damaged islets. 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. In autoimmune-mediated insulitis, IFNγ released from activated T cells has an important role as a trigger of inflammation and β-cell dysfunction (Kahaly & Hansen, 2016; Mishra et al., 2019; Pondugala, Sasikala, Guduru, Rebala, & Nageshwar, 2015). However, 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 (n = 3, Supplemental Fig. 1). This is consistent with the above results in adoptive transfer experiments. These results suggest that FBRA or its components have a suppressive effect on type 1 diabetes at 0.5% dietary concentration through maintaining a sufficient number of intact islets in NOD mice.

The suppressive effect of FBRA on type 1 diabetes was observed at lower concentrations, but not at higher doses (1% and 5%). FBRA is processed from brown rice and rice bran by fermenting with *Aspergillus oryzae*, during which polyphenols increase (Tanaka et al., 2017). Various polyphenols possess beneficial effects on diabetes *in vivo* and *in vitro* through enhanced β cell viability and proliferation (Babu et al., 2013). These actions may explain the suppressive activity of FBRA. On the other hand, we previously reported that 5% dietary FBRA could increase resident *Lactobacillus* species in mouse intestine (Kataoka et al., 2007). Some *Lactobacillus* species have been shown to mitigate type 1 diabetes through a decrease of proinflammatory cytokine production, oxidative stress, or changes in the intestinal environment (Matsuzaki

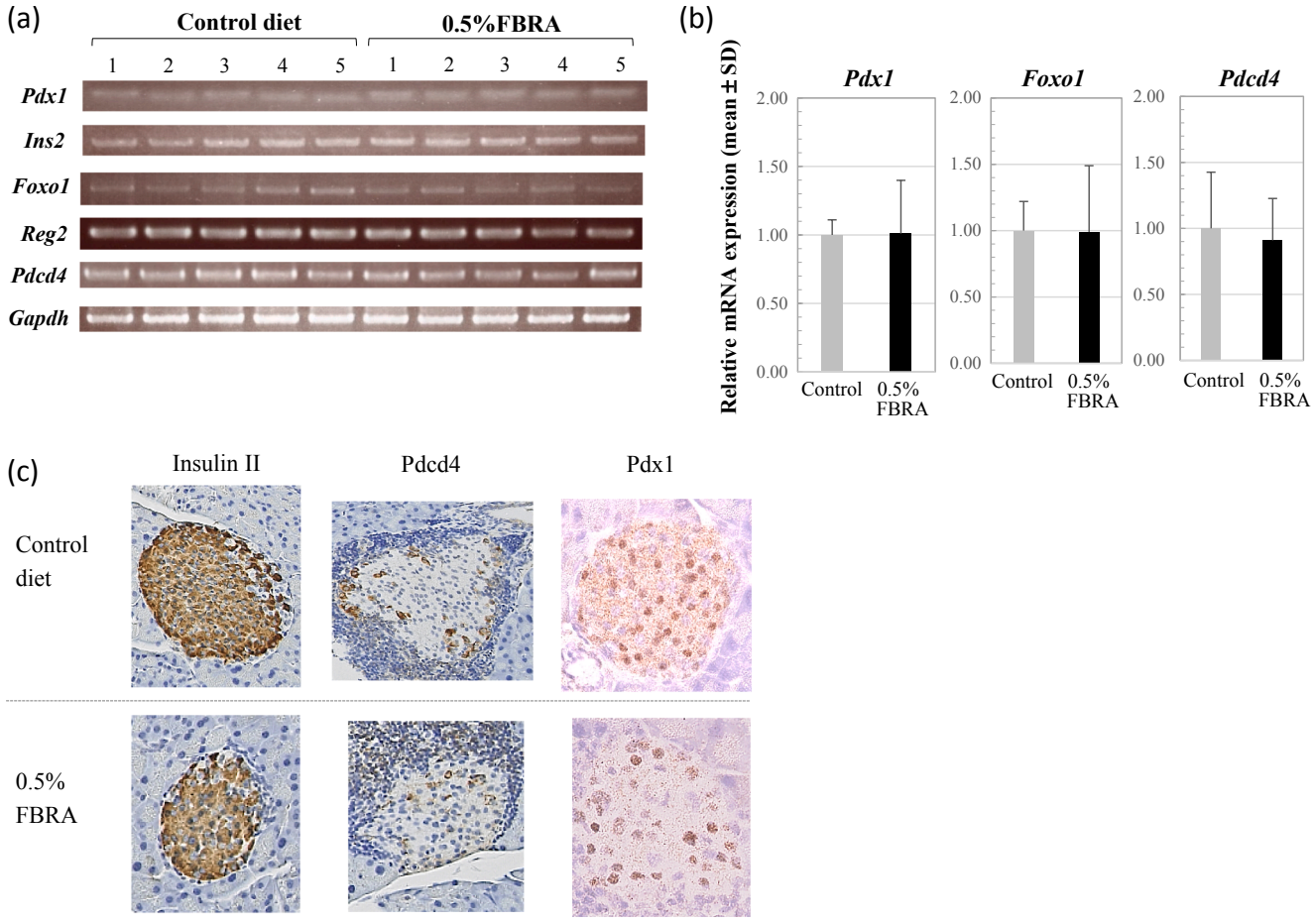

Fig. 4. Expression of pancreatic β-cell function-related genes in NOD female mice fed with a control diet or a 0.5% FBRA-containing diet. (a) RT-PCR targeting the indicated genes. Pancreatic RNA was extracted from 22-week-old mice, and it was reverse-transcribed to cDNA, followed by PCR to detect the expression of *Pdx1*, *Foxo1*, *Reg2*, *Ins2, Pdcd4,* and *Gapdh.* (b) Relative expression of *Pdx1, Foxo1,* and *Pdcd4* in pancreas. RT-qPCR by ΔΔCt method was done. (c) Expression of insulin II, Pdcd4, and Pdx1 in pancreatic islets. Pancreatic tissue was resected from 22-week-old mice, and immuno-histochemical detection was conducted.

et al., 1997; Valladares et al., 2010; Yadav et al., 2018). But, activation of Th1 immunity by *Lactobacillus* species has also been reported (Castanheira et al., 2007; Segawa et al., 2008; Wen et al., 2014). FBRA administration at higher concentrations might enhance Th1 immunity and reverse its beneficial effects on spontaneously occurring diabetes in NOD mice.

In adult rodents, the pancreas has regenerative potential for autoimmune- or other factor-mediated damage to of islet β cells (Tokoro et al., 2003; Cano et al., 2008; Nir et al., 2007; Yi et al., 2013). Pdx1 and related molecules Foxo1, Reg2, Pdcd4 have important roles in islet function and the fate of injured islet cells (Hill et al., 2013; Huszarik et al., 2010; Inagaki et al., 2012; Liu et al., 2007; Meng et al., 2009; Ruan et al., 2011). Pdx1 and Foxo1 are involved in pancreatic development and β cell functional regulation through changes in their intracellular translocation (Meng et al., 2009). The inflammatory cytokine IFNγ is shown to decrease nuclear localization of Pdx1 and trigger β cell dysfunction (Pondugala et al., 2015). On the other hand, polyphenolic compounds in food have recently been reported to have anti-diabetic actions via various mechanisms, including increased expression of pdx1 or restoration of nuclear localization of Pdx1 (Babu et al., 2013; Pondugala et al., 2015; Zhang et al., 2013). In our study, mRNA levels of *Pdx1* and related genes were similar in whole pancreases in 19- and 22-week-old mice in 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. Intracellular localization of Pdx1/Foxo1 and their phosphorylation level should be further examined at appropriate ages in NOD mice with/without 0.5% FBRA treatment.

In this study, we demonstrated a suppressive effect of FBRA on the spontaneous onset of type 1 diabetes in NOD mice. FBRA is processed food and no harmful phenomenon has been observed in healthy adults (Nemoto et al., 2011). For clinical application of FBRA, ameliorating effects after onset of type 1 diabetes should be examined, and suppressive mechanisms of FBRA including determination of active ingredients and its optimal dose should be clarified in future studies.

5. Ethics statement

Animal experiments were conducted with the approval of the institutional animal care and use committee of the Tokushima University (approval No. 13046), and all experiments were done in accordance with guidelines of the Tokushima University Ethics Committee for the care and use of laboratory animals.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: K. K. received financial support for this work from Genmai Koso Co. Ltd. H. N. is an employee of Koken Co., Ltd., a FBRA preparing factory, and involved in preparing this article in terms of study design; data collection and analysis under the supervision of K.Y. M.S. is an employee of Genmai Koso Co., Ltd., a provider of FBRA, but had no involvement in preparing this article in terms of study design; collection, analysis, and

interpretation of data; writing of the report; or the decision to submit the article for publication. A.K and K.Y. have no competing interests to declare in preparing this article.

Acknowledgements

This study was supported in part by JSPS KAKENHI Grant Number 15K00820 and financially supported by Genmai Koso Co., Ltd., Japan. This study was also technically supported by Support Center for Advanced Medical Sciences, Graduate School of Biomedical Sciences, Tokushima University.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104356.

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