Review article

Brown rice-specific γ-oryzanol-based novel approach toward lifestyle-related dysfunction of brain and impaired glucose metabolism

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Abstract

For the purpose of controlling type 2 diabetes and metabolic syndrome, the improvement of lifestyle including dietary behavior, physical exercise and health literacy is critical. In addition to the amelioration of excess in total food intake and less of physical fitness, complexed factors related with brain dysfunction such as the dependence on animal fat should be comprehensively considered. The authors' laboratory has conducted the research on brown rice (rice bran)-derived γ-oryzanol in mouse experiments, cultured cells and human clinical trials. Making mice excessively eat animal fat, and resultantly evoking the endoplasmic reticulum (ER) stress in the brain hypothalamus induces robust preference for animal fat intake. Conversely, γ-oryzanol decreases ER stress in the hypothalamic area and alleviates animal fat dependence. In mouse experiments, γ-oryzanol improves dysfunction of pancreatic islet associated with gluco-lipotoxicity and resultant augmentation of endoplasmic reticulum (ER) stress, increases the glucose-stimulated insulin secretion (GSIS) from the pancreatic β cells and decreases excess glucagon secretion from the pancreatic α cells. In the crossover clinical trial targeting middle-aged patients with metabolic syndrome, it was clarified that the intake of γ-oryzanol-rich fermented beverage also decreases the preference for animal fat-rich diet, ameliorates the unbalance of gut microbiota, chronic constipation and skin problems in humans. As the underlying molecular mechanisms whereby γ- oryzanol improves the dysfunction of pancreatic islet, the following two processes are mainly involved: 1) the mechanism that, as a molecular chaperone, γ-oryzanol alleviates ER stress exaggerated in the pancreatic β cells in the condition of type 2 diabetes, and 2) the mechanism that γ-oryzanol inhibits the dopamine type 2 receptor (D2R) signaling in pancreatic islets, that is augmented by animal fat intake, at both transcriptional and translational levels. Our novel finding that the excess intake of animal fat increases the local D2R signaling in pancreatic islets reinforces the notion that unbalanced diet can induce type 2 diabetes in humans. For effectively preventing and improving type 2 diabetes and obesity, establishment of brand-new medicine using functional components contained specifically and abundantly in brown rice (rice bran) may strongly attract academic and bio-industrial interests.

KEY WORDS: γ-oryzanol, rice bran, ER stress, preference for animal fat, dopamine type 2 receptor (D2R)

1. Recent progress of molecular nutritional science surrounding functional components in rice bran

Brown rice (rice bran) is highlighted as a natural complete diet including abundantly a variety of vitamins, minerals, anti-oxidizing substances, trace elements, dietary fibers and functional components. Noticeably, the Chinese character of "bran" includes a part that means "health". On the other hand, the Chinese character connecting "rice" and "white" means "nothing remains" (*Fig. 1*).

From the result of the crossover interventional trial using brown rice targeting male patients with metabolic syndrome living in Okinawa Prefecture conducted by the authors' research team, beneficial effects on dysfunction of vascular endothelium, liver steatosis, body weight reduction, and furthermore, on the dependence to animal fat-rich diets such as junk foods and fast foods were verified [1].

From a line of the authors' research in mice, a mechanism of the vicious cycle was clarified. Endoplasmic reticulum (ER) stress in the brain hypothalamus, the center of appetite and feeding behavior, is aggravated by chronic and excessive intake of animal fat, which in turn further increases the preference for animal fat, resulting in the addiction to

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animal fat [2]. Of note, among various kinds of natural foods, γ-oryzanol, which is contained specifically and abundantly in rice bran (brown rice), functions as a kind of molecular chaperone and decreases ER stress in hypothalamus, and resultantly mitigates the strong dependence to animal fat in mice (*Fig. 2*) [2].

Furthermore, it was verified that γ-oryzanol improves dysfunction of pancreatic islet that is associated with gluco-lipotoxicity, and contributes to enhance glucose-stimulated insulin secretion (GSIS) and also to inhibit the excessive secretion of glucagon [3,4]. A line of authors' research clarified that such a metabolically-beneficial effect is involved in the following two molecular mechanisms:l) γ-oryzanol functions as a molecular chaperon and alleviates the ER stress in β cells, which is increased in pancreatic islets in the condition of type 2 diabetes (*Fig. 3*) [3], and 2) γ-orizanol inhibits the dopamine type 2 receptor (D2R) signaling in pancreatic islets, which is augmented by animal fat-rich diets at both transcription and translation levels (*Fig. 4*).

It is well documented that augmented signaling of D2R in pancreatic islets inhibits GSIS and resultantly increases blood glucose levels in humans and rodents. However, detailed molecular mechanisms have not been elucidated. In this context, the authors' finding that excess intake of animal fat increases the D2R signaling locally in pancreatic islets would be epoch-making [4], exemplifying a clinical example that unbalanced diet could induce type 2 diabetes in humans.

Dopamine receptor signal plays a critical role in homeostatic control in a variety of organs (*Fig. 5*). As a result of the authors' research in mice, novel functions of γ-oryzanol in both correcting brain reward system through the genomic modification (epigenome) of D2R and ameliorating the imbalance of gut microbiota have been clarified. It should be highlighted that brown rice (rice bran) with which we Japanese have been familiar since ancient times contains abundantly substances against metabolic syndrome. In this context, establishment of brand-new medicine using functional components contained specifically and abundantly in brown rice (rice bran) may attract academic and bio-industrial interests
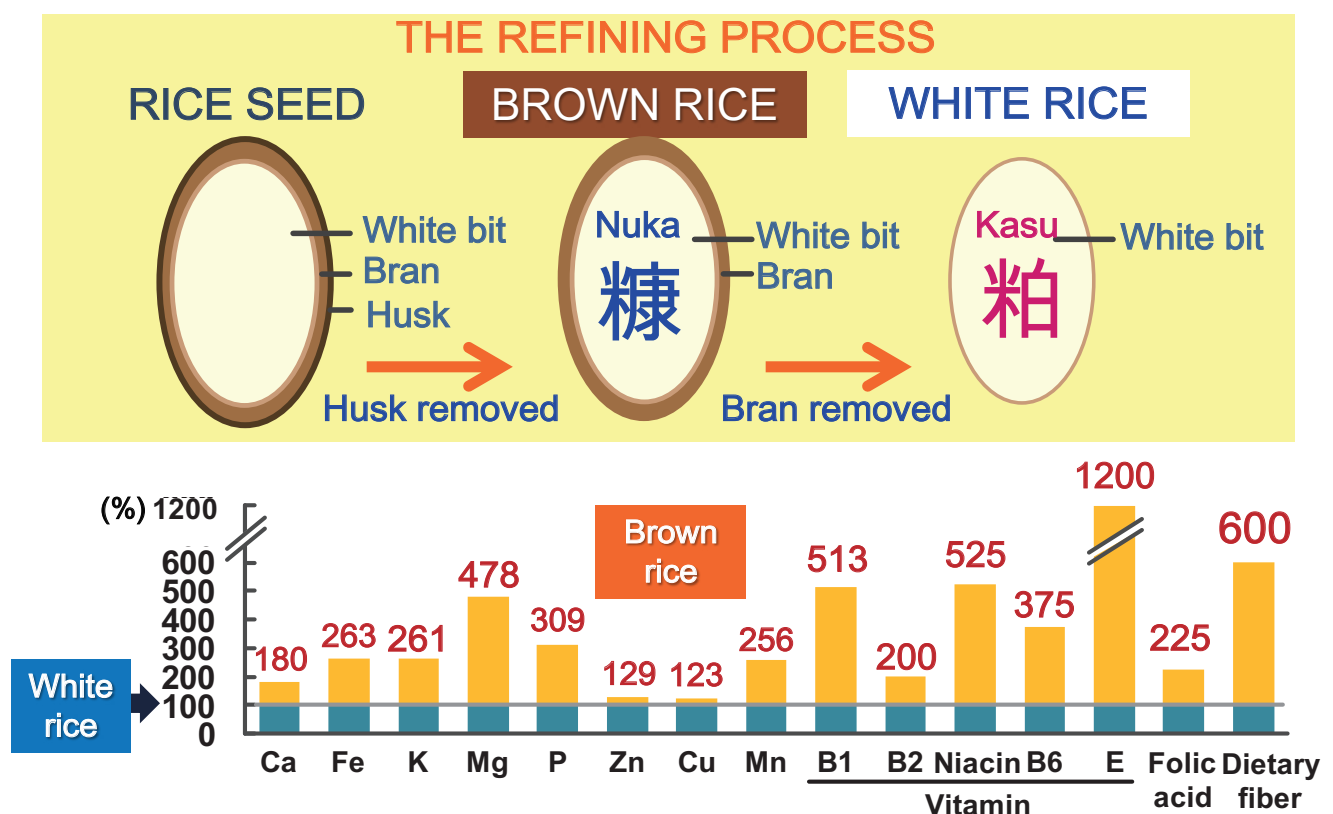

Fig. 1. Nutritional comparison between brown rice and white rice.
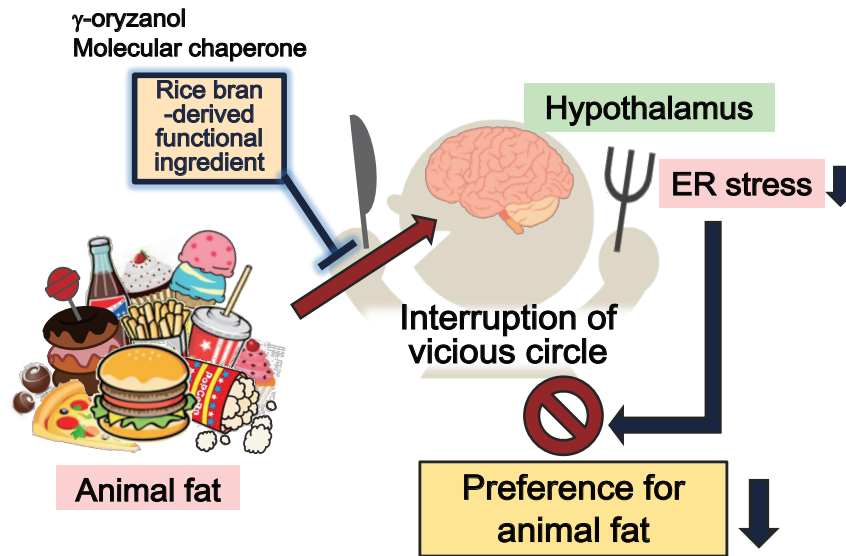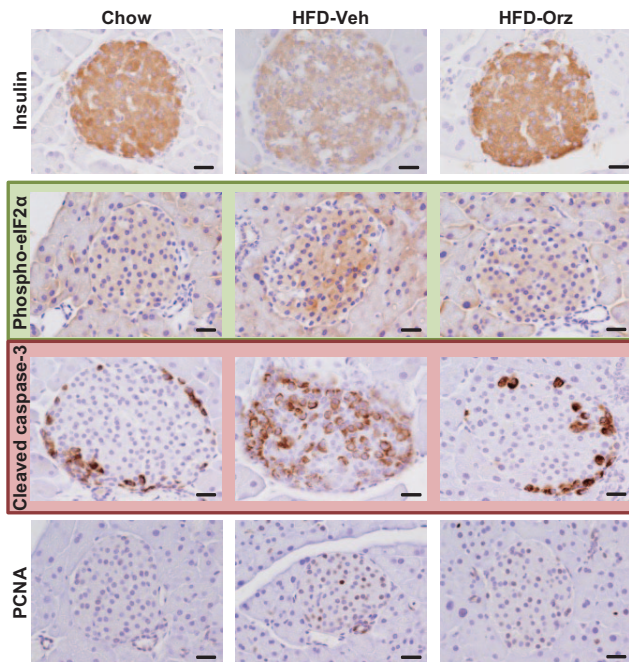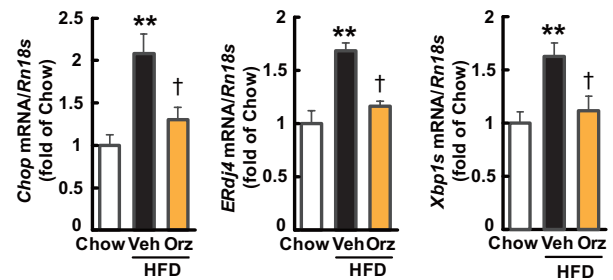Brown rice has bran, the nutrient-rich outer layer.

Fig. 2. Action of γ-oryzanol on the central nervous system.

γ-Oryzanol attenuates the preference for animal fat-rich diet by decreasing the elevated ER stress in hypothalamus. ER, endoplasmic reticulum.
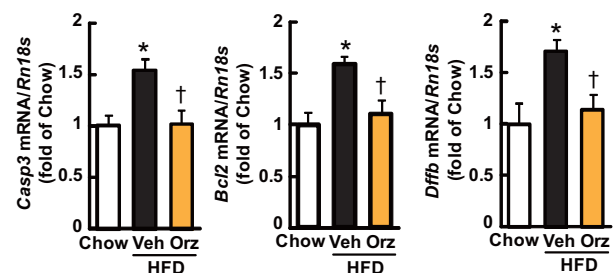

Fig. 3. Animal fat-rich diet-induced ER stress and apoptosis in pancreatic β cells in mice. Impact of γ-oryzanol on dysfunction of β cells.

a) Immunostaining images. **b)** mRNA expression for ER stress-related genes. **c)** mRNA expression for apoptosis-related genes. Chow, normal diet group, HFD-Veh, high fat diet- and vehicle-treated group; HFD-Orz, high fat diet- and γ-oryzanol-treated group. Levels for mRNA are shown for *Chop*, *ERdj4*, *Xbp1s*, *Casp3*, *Bcl2* and *Dffb* in the hypothalamus, detected by quantitative real-time PCR and normalized with RN18S. High-fat diet induces ER stress, while γ-oryzanol ameliorates ER stress-induced β-cell dysfunction and subsequent apoptosis. Data are expressed as mean ± SEM (n = 6 in each group), *$p < 0.05$, **$p < 0.01$ vs. Chow, † $p < 0.05$ vs. HFD-Veh. Phospho-eIF2, phosphorylation of the eukaryotic initiation factor 2 (eIF2) α subunit; PCNA, proliferating cell nuclear antigen; *Chop*, Chop is a C/EBP homologous protein, also known as growth arrest- and DNA damage-inducible gene 153 (GADD153); *ERdj4*, ERdj4 is a soluble ER DnaJ family protein that interacts with ER-associated degradation machinery; *Xbp1s*, Xbp1 is a transcription factor that regulates the expression of genes in the cellular stress response; *Casp-3*, caspase-3, sequential activation of caspases plays a central role in apoptosis; *Bcl2*, the BCL-2 families determine the commitment of cells to apoptosis; *Dffb*, Dffb triggers both DNA fragmentation and chromatin condensation during apoptosis. Data are quoted from References 2, 3.
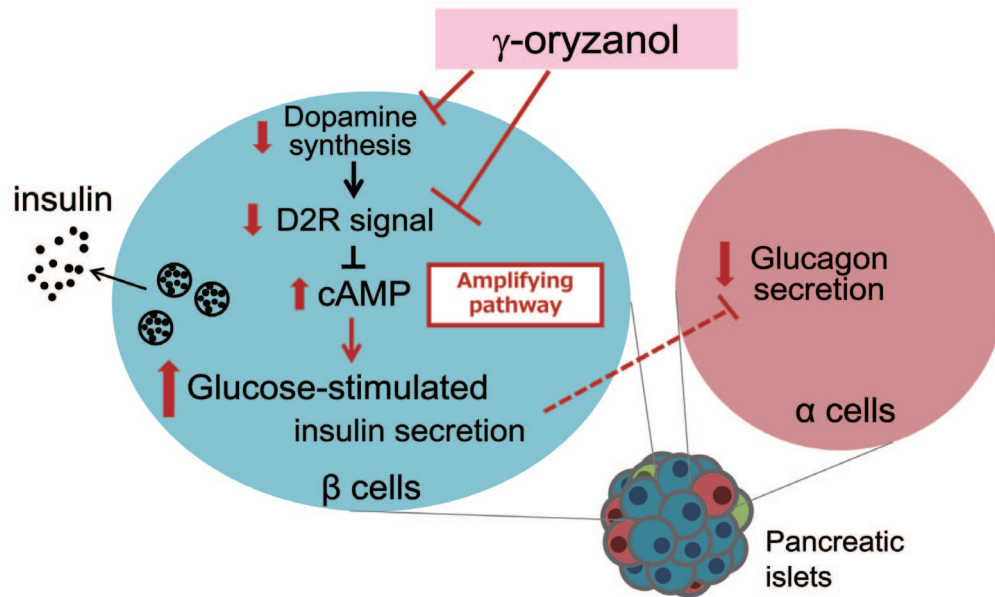
Fig. 4. **Schematic representation of γ-oryzanol action in pancreatic islets.**

Besides decreasing ER stress as a molecular chaperone, γ-oryzanol also improves animal fat-rich diet-induced dysfunction of pancreatic islet by suppressing dopamine D2 receptor (D2R) signalling. GSIS is augmented via the cAMP/PKA pathway (amplifying pathway) in pancreatic β cells, while D2R signaling is known to inhibit cAMP/PKA pathway. Furthermore, γ-oryzanol ameliorates exaggerated secretion of glucagon from pancreatic α-cells under animal fat-rich diet. D2R, dopamine D2 receptor; GSIS , glucose-stimulated insulin secretion; PKA, protein kinase A. The scheme is quoted from Reference 4 .
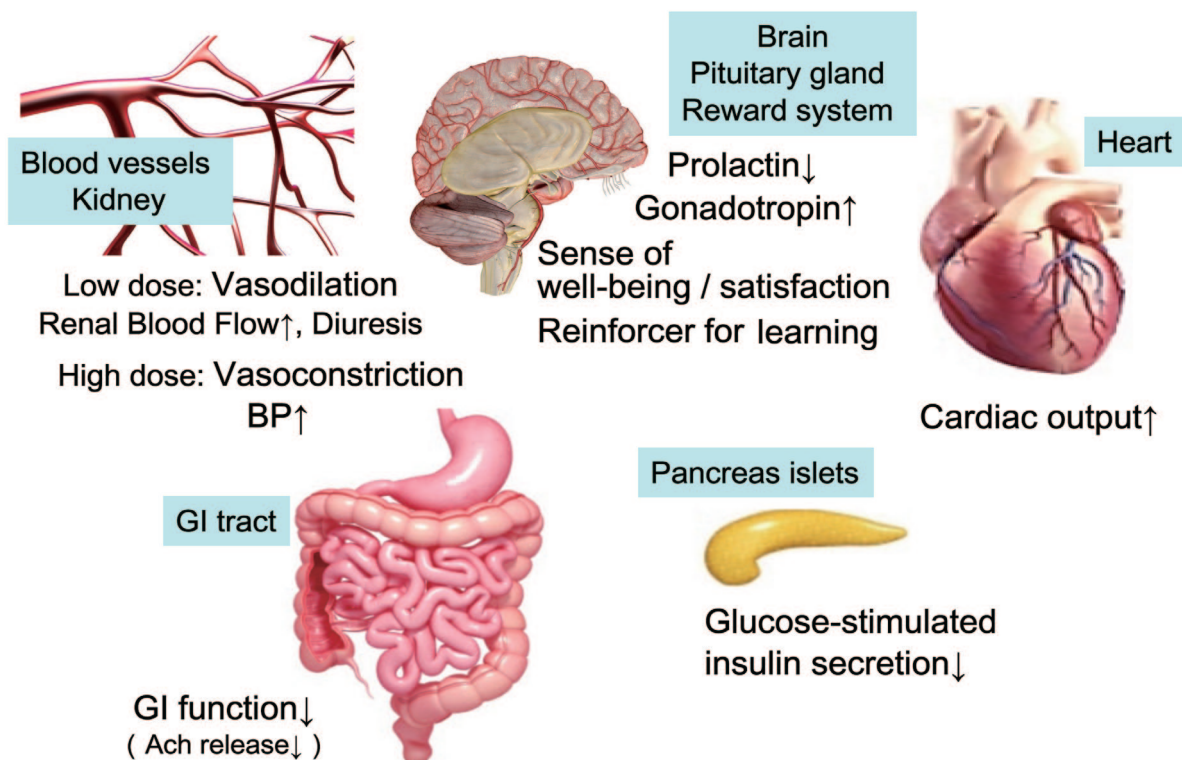

Fig. 5 **Schematic Illustration of a wide variety of actions of dopamine throughout the body.**

2. Chronic inflammation and dysfunction of brain commonly occurred in subjects with obesity

Excess intake of animal fat does attenuate the action of leptin to the brain, a hormone derived from adipocytes (also known as leptin resistance), leading to difficulties in body weight reduction. In the brain hypothalamus, the arcuate nucleus is a main control tower for appetite regulation through the input of a variety of hormones and autonomic nerve system (regulatory system of metabolic hunger). Importantly, the excess intake of animal fat provokes the inflammation and cellular stress such as ER stress and oxidative stress in the hypothalamus, paralyzing the regulatory system of metabolic hunger and creating a condition where the brain cannot correctly judge the calorie intake necessary for the body. It is as if the brain were hacking.

Noticeably, when mice are fed animal fat-rich foods, activated microglia infiltrates the hypothalamus within a short period of time. Subsequently, hypothalamic damage and leukocyte migration aggravated, resulting in the condition of chronic inflammation [5]. On the other hand, it is interesting to note that microglial inflammation occurred in the hypothalamus of mice associated with excess animal fat intake is drastically improved by having morbidly obese mice exercise on a regular basis [6].

The similarity between the dependence on animal fat and that on narcotics, nicotine and alcohol is of great importance. Among various cases of dependence, the mechanism whereby the intake of stimulants increases by degrees seems similar to that the recognition threshold of the brain reward system gradually increases and the brain cannot obtain the same satisfaction and pleasure as before. In obese mice on a high animal fat and high sugar diet, the level that the brain reward system can respond to animal fat gradually rises, reminiscent to the cases in dependence on cocaine and heroin. It is therefore suggested that the mice fall into a vicious cycle where no matter how much it eats, it cannot be satisfied. Notably, in rat experiments using narcotics, nicotine and alcohol, when these addictive substances were forcibly blocked, the dependence in the brain rapidly disappear within three days. However, the dependence on animal fat was not improved even two weeks after the animal fat diet was blocked. It is absolutely a shocking result that animal fat is more addictive than narcotics [7].

3. Molecular mechanism for dependence on animal fat

When wild-type C57B6 mice are food-deprived for 48 hours and given both high carbohydrate food and high animal fat food placed side by side, they choose preferentially high carbohydrate food in order to avoid protracted hypoglycemia. On the other hand, in similar experiments using mice that were made obese by high animal fat foods, even though they show low level of blood glucose, they still choose high animal fat food. Such a phenomenon can be explained by the notion that mice became unable to mentally judge how many calories they need and which food they should choose under the chronic excess intake of animal fat. From a series of mouse experiments by the authors' research

group, it was clarified that exaggerated ER stress in the hypothalamus strongly impacts on the preference for animal fat food. Mice were allowed to choose between normal chow food and high animal fat food, and were also dosed with 4-phenyl butyric acid (4-PBA) that functions as a molecular chaperone. The proportion of choosing high animal fat foods by mice was significantly decreased and their obesity and hyperglycemia were concomitantly alleviated. These results raise a possibility that a vicious cycle is formed in which the excess intake of animal fat increases ER stress in the hypothalamus and the increased ER stress further strengthens the preference for animal fat [2].

4. γ-Oryzanol functions as an ER stress reliever in a variety of tissue

Okinawan people of senior age have been fond of eating brown rice. In the course of searching for the impact of food given on deteriorated eating behavior, the authors' research group focused on γ-oryzanol, a functional component specifically and abundantly contained in brown rice (rice bran). It was clarified by the a line of experiments that γ-oryzanol functions as a molecular chaperone that promotes protein folding, decreases ER stress increasing in the hypothalamus by the chronic excess intake of animal fat, thereby attenuating the dependence on animal fat and improves fuel dyshomeostasis and insulin resistance [2]. Brown rice is the fruit of the rice plant, richly contains a wide variety of nutritional components including anti-oxidant substances, dietary fibers, vitamins, minerals and lipids in an ideal balance. Brown rice is also characterized as a low GI (glycemic index) food inhibiting postprandial hyperglycemia.

Through a pilot clinical study implemented to target middle-aged male patients with metabolic syndrome living in Okinawa (BRown rice And Visceral Obesity and fuel dysmetabolism: BRAVO study) conducted by Tomishiro Central hospital and Second Department of Internal Medicine, University of the Ryukyus, beneficial effects on obesity, postprandial hyperglycemia and hyperinsulinemia, lever steatosis, fatty liver, endothelial dysfunction and preference for fatty junk foods were confirmed by substituting refined white rice as a staple food with brown rice for eight weeks [1].

The scientific name of rice is *Oryza Sativa*, and γ-oryzanol called "*komenoabura* (rice oil)" was firstly extracted from brown rice by Dr. Tsuchiya and Dr. Kaneko, Japanese researchers. Structually, γ-oryzanol is a ferulic acid ester compound of several kinds of triterpene alcohol or phytosterol [8]. From the authors' mouse experiments, it is clarified that a part of γ-oryzanol, when orally dosed, passes the blood-brain barrier, keeping its ester binding as it is, and is distributed in the brain in high concentrations [3]; γ-Oryzanol decreases ER stress in the hypothalamus and the genome modification effect in the brain reward system.

The authors conducted an experiment, giving mice normal chow diet and animal fat-rich diet at the same time and let them freely choose the diets to evaluate the preference for animal fat. It is well recognized that C57/B6 mice show a strong preference for animal fat as humans. Mice preferentially took animal fat-rich food and became obese. In an experimental paradigm that a part of the carbohydrates of both normal chow diet and animal fat-rich diet were substituted with brown rice powder or refined white rice

powder with the same calories, only the mice of the group in which carbohydrates were substituted with powered brown rice was significantly decreased the preference for animal fat diet (~20%). Consequently, the obesity and fuel dysmetabolism in mice were markedly improved (**Fig. 6**). The authors confirmed, using a HEK293 cell, that γ-oryzanol significantly suppresses the transcriptional activity in the ER stress responsive element that is induced by tunicamycin, and clarified that γ-oryzanol surely functions as a chaperone in a viable cell system. It should be noted that such an effect was not observed in ferulic acid *per se* as a single compound generated by the cleavage of ester binding in γ-oryzanol. Furthermore, experiments using a fetal mouse brain-derived nerve cell primary culture verified that γ-oryzanol also remarkably suppresses the gene expression of ER stress-related molecules induced by tunicamycin [2].

5. Functional relationship between dependence on animal fat and dysfunction of brain reward system

The signal of the brain reward system is conveyed by dopamine neurons. Importantly, the decrease of D2R activity in dorsal striatum is recognized in obese persons as cocaine abusers. In accordance with this notion, in clinical research using a functional MRI, the activation of striatum (increase in blood flow) after a meal was disappeared in obese people, indicating a decrease in the D2R signal of

brain reward system. It may be construed as that because they cannot appropriately accept the brain reward, they became unable to cut off the vicious cycle of overeating [9].

As molecular mechanisms on the decrease in the D2R signal of the brain reward system associated with eating habits of animal fat-rich foods, the involvement of genome modification associated with DNA hyper-methylation in the promoter region (CpG island) of D2R gene would be suggested. For example, as one of the reasons why visceral fat tends to accumulate in the excess of animal fat-rich diet, it is verified by the mouse experiment that the expression level of PPARγ, a master transcription factors that controls accumulation of subcutaneous fat depots, is decreasing on a high fat diet in mice. DNA hyper-methylation in the promoter region of PPARγ gene is involved in this cause [10]. It is therefore likely that there is a mechanism where chronic excess intake of animal fat inactivates various genes involved in the metabolic and endocrine homeostasis.

Recently, the authors are verifying that γ-oryzanol acts on the brain reward system in obese mice fed animal fat-rich diet and changes "the brain that cannot be satisfied" to "the brain that can be satisfied," as an epigenome controller (manuscript under review). Although deciphering the human genome has been completed, the mechanism of the onset and advancement of obesity had been little explained by genomic variation. In this context, the genome modification that the accumulation of unhealthy lifestyle changes the reading patterns of genes, not *via* the structural anomaly of genes including single nucleotide polymorphism (SNP), holds the key for the breakthrough of the prevention and improvement of lifestyle diseases. Since 2011, the deciphering plan of all epigenome in humans has been advancing worldwide.
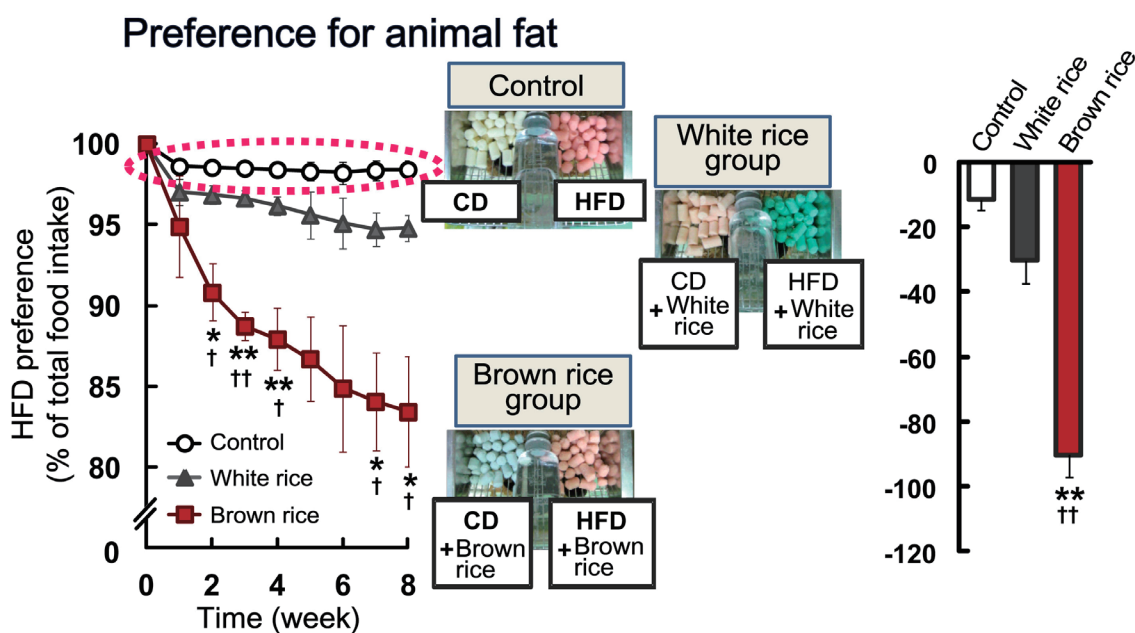

Fig. 6. Impact of brown rice on preference for animal fat in mice.

Preference for animal fat was evaluated in the two-food choice test in male C57BL/6J mice with ages of 8 ~ 16 weeks. Mice were allowed free access to CD and HFD in Control group; free access to CD + white rice and HFD + white rice in White rice group; free access to CD + brown rice and HFD + brown rice in Brown rice group. Data are expressed as mean ± SEM (n = 8 in each group). * $p < 0.05$, ** $p < 0.01$ vs. Control, † $p < 0.05$, †† $p < 0.01$ vs. White rice group. CD, control diet; HFD, high fat diet. Data are quoted from Reference 2.

6. Approach aiming at practical use and social implementation

The approach aiming at the practical use and social implementation of the achievements shown above is currently underway. A fermented brown rice beverage richly containing γ-oryzanol has been successfully developed by the cross-ministerial Strategic Innovation Promotion (SIP) Program, a Japanese national strategic project by Aizu Tenpo Jozo K.K., an industry, Japanese government and academic joint research with the High-tech Plaza of the Fukushima Prefecture, the Ministry of Agriculture, Forestry and Fisheries and Cabinet Office. Fortunately, its practical use (product sales) has already begun, and the product was commended with the Food Action Nippon Award (FAN) of the Ministry of Agriculture, Forestry and Fishery, Research Development New Technology Excellence Award 2015.

As a result of the crossover trial using fermented brown rice beverage richly containing γ-oryzanol for 40 adults with metabolic syndrome in Okinawa, improvement in obesity and metabolic syndrome, preference for animal fat, the balance of gut microbiota, constipation and skin problems were observed. Notably, in the case of those who did not habitually eat Japanese foods and those whose intestinal flora balance had been aggravated before the trial, the effect was significantly exaggerated (*Fig. 7*).

Furthermore, the authors have recently succeeded in developing a novel delivery system that remarkably increases the metabolically-beneficial effects of γ-oryzanol by use of nanoparticle capsule [11]. Mice show marked hyperglycemia under animal fat-rich food. On the other hand, when they are given γ-oryzanol simultaneously, their blood glucose level was evidently improved for a long period of time (*Fig. 8*). It is clarified that the effect of nanoparticle capsulated γ-oryzanol is increased by several hundred times [11].

The chronic and excessive intake of animal fat results in impaired cognitive function and addiction to animal fat. The inflammation and ER stress in the hypothalamus as well as epigenome adjustment in the brain reward system are promising targets for preemptive medicine, preventive medicine and personalized precision medicine in lifestyle-related diseases. It is highly expected to achieve a novel strategy to modify the dietary habits through focusing on functional components derived from Japanese traditional foods (*Fig. 9*).

Conclusion

The excessive intake of animal fat and resultant dependence on animal fat complexly act on the onset and advance of type 2 diabetes and obesity syndrome. In the pancreatic islets, animal fat-rich diet increases the D2R signal in pancreatic β cells, decreases the glucose-stimulated insulin secretion and increases glucagon secretion from the pancreatic α cells. In the brain hypothalamus, animal fat evokes ER stress, increases the preference for animal fat and decreases the motivation of physical exercise. In the intestine, animal fat aggravates the balance of gut microbiota. On the other hand, in the hypothalamus, γ-oryzanol functions as a molecular chaperone and relieves ER stress, resulting in the considerable amelioration of preference for animal fat. In the pancreas, γ-oryzanol improves the glucose-stimulated secretion of insulin and decreases the secretion of glucagon as well as remedies the unbalance of gut microbiota. The authors hope the development of brand-new medical care utilizing the excellent functional components contained in brown rice (rice bran), with which we Japanese have been familiar since ancient times, for the prevention and improvement of type 2 diabetes and obesity syndrome.

Conflict of Interest

The authors state that the performance of the current study entailed no issues representing a conflict of interest.
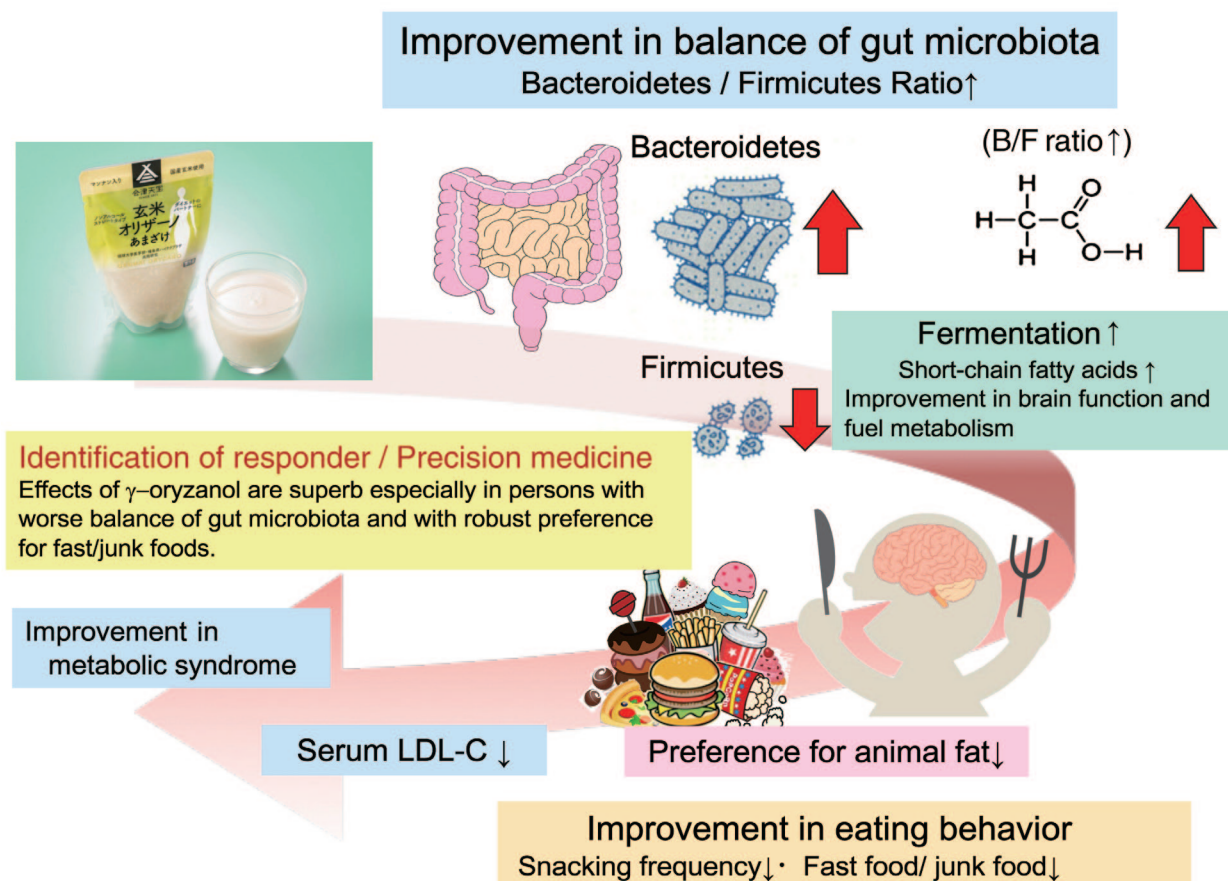
Fig. 7. Ongoing clinical trial : Does γ-oryzanol-rich fermentation drink "Genmai Oryzano" show metabolically-beneficial effects in humans with metabolic syndrome ?

Our working hypothesis is as follows: Intake of "Genmai Oryzano" could improve inbalance of gut microbiota and could consequently elevate circulatiing levels of short-chain fatty acids, thus resulting in reduction of preference for animal fat in humans with metabolic syndrome. Intriguingly, effects of γ-oryzanol are superb especially in persons with worse balance of gut microbiota and with robust preference for fast/junk foods.
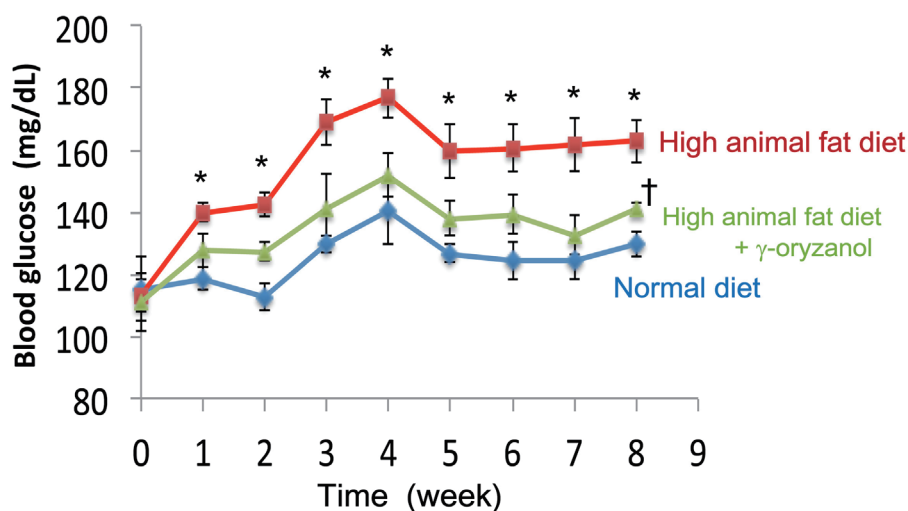

Fig. 8. Long term effect of γ-oryzanol on animal fat-rich diet-induced hyperglycemia in mice.

γ-Oryzanol ameliorates glucose dyshomeostasis for a long period of time in mice. Data are expressed as mean ± SEM, * $p < 0.05$ vs. normal diet, † $p < 0.05$ vs. high animal fat diet, n = 6. Related data are quoted from Reference 11.
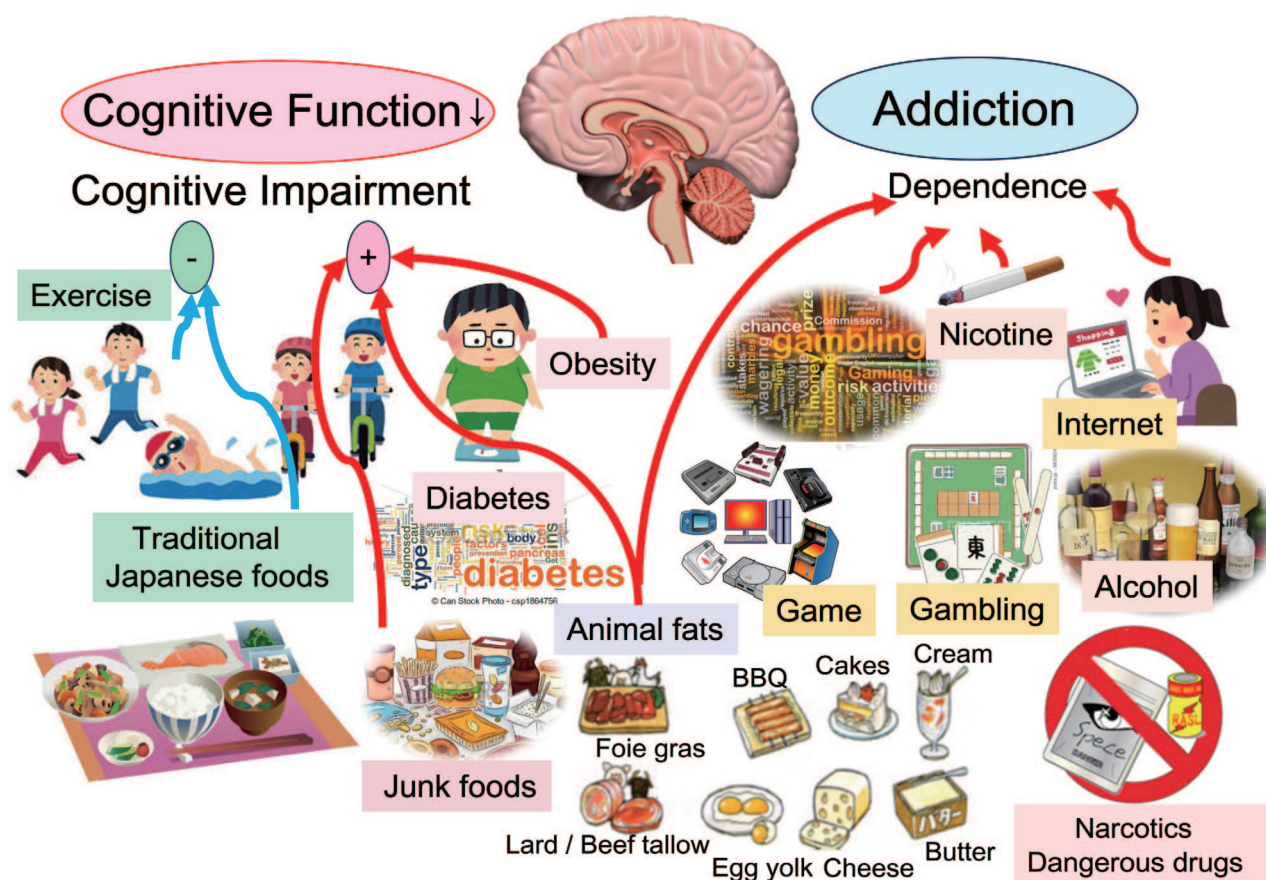
Fig. 9. **Dysfunction of brain, a key health issue in a superaging society in Japan.**
Chronic and excesive intake of animal fat apparently increases the risk for cognitive impairemnt and addiction to animal fat. Our research raises a possibility that γ-oryzanol is a promising candidate to defuse such a crisis.

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