Mini Review

Physiological Multifunctions of Rice Proteins of Endosperm and Bran

Motoni KADOWAKI^{1,2}, Masatoshi KUBOTA² and Reiko WATANABE³¹Faculty of Agriculture, Niigata University, Niigata 950–2181, Japan²Faculty of Engineering, Niigata Institute of Technology, Kashiwazaki, Niigata 945–1195, Japan³Department of Health and Nutrition, University of Niigata Prefecture, Niigata 950–8680, Japan

Summary Although it is considered a staple food, rice intake is under serious debate for its physiological usefulness, especially for diabetic patients, because of starch content. However, rice protein, the second major component of rice, has gained attention recently for its newly-discovered functions, which were previously unknown. Rice protein, a plant protein, shows multiple beneficial functions on lipid metabolism and diabetes and its complications, nephropathy, fatty liver and osteoporosis. Rice proteins of endosperm and bran, an ingredient of white rice and an unused product of brown rice, respectively, are valuable components for human health.

Key Words rice endosperm protein, rice bran protein, lipid metabolism, diabetes, nephropathy, fatty liver, osteoporosis

Introduction

Rice, one of the most important cereals in the world, is a major plant source of both energy and protein for human nutrition. Rice is a staple food for most Asian peoples and is important for life and survival. Research on rice has long focused mainly on starch because of rice's content of this major component. In recent years, because of the prevalence of diabetes, the usefulness of rice, especially white rice, has been questioned for human health (1–3). The same argument is raised between refined grains and health in the US (4). However, as historical trends show that there were far fewer diabetic patients in the 1950s and 60s when people consumed much more rice than today, we could postulate paradoxically that rice consumption may have a beneficial effect on diabetes.

Brown rice is considered healthier than white rice because most functional compounds such as phytochemicals and polyphenols are located mostly in rice bran. Therefore, do the people who eat white rice, over 90% of Japan's population, gain no benefit from eating it? This review addresses this question by investigating a “neglected” component, rice protein.

Rice protein is the second component of white rice; it is a third protein source of daily protein intake following meat and fish, and the primary protein source in plant foods in Japan (5). Somehow, it has been almost neglected despite its health benefits. However, the time has come for understanding the quality of rice protein. This is especially true for rapidly-aging societies including Japan in which preventing frailty and sarcopenia to extend healthy life expectancy is urgent. High protein intake and its quality may be associated with low frailty or sarcopenia rates in older people (6, 7). Thus, it

is worth considering the quality of rice protein and discovering its physiological functions.

Unfortunately, rice endosperm protein (REP) is not commercially available on the market. However, recently, a simple, mass preparative procedure for REP was developed using an alkali extraction method. This isolate is characterized by >90% crude protein. Rice bran protein (RBP) has been obtained using a similar method. By using either of these protein isolates, researchers investigated rice protein's physiological functions. In this review, after a summary of endosperm and bran rice proteins, rice protein's functions on lipid metabolism related to cholesterol and triglycerides and diabetes and its complications (nephropathy, fatty liver and osteoporosis) are described.

Rice Endosperm Protein and Rice Bran Protein

The term “rice protein” is confusing because it is found in endosperm and in bran. In some cases, rice protein is prepared from endosperm, bran or the whole grain. However, the composition is not necessarily the same among preparations. Recently commercially available rice protein is obtained from whole rice grain. However, in this review, we describe the functions of white rice REP, which is most consumed by humans, and of RBP from a by-product of rice milling separately. Rice protein from brown rice is a mixture of both.

Rice endosperm protein

REP is the isolate prepared from white rice and occupies 6–8% of white rice. It comprises a relatively small number of proteins, which are mainly storage proteins: glutelin (alkali-soluble, 60–65%), globulin (salt-soluble, 10–15%), albumin (water-soluble, 10–15%), prolamin (ethanol-soluble, 20–25%) (8), as classically defined by Osborne (9). However, the composition varies depending on cultivars. For example, the cultivar *Shunyo* has

E-mail: kadowaki@niit.ac.jp

low glutelin and high prolamin content (1/3-fold and 3-fold those of *Koshihikari*, respectively) (10) and is termed a low protein rice, which implies that it has little “digestible” protein. *Shunyo* is used with kidney disease patients.

REP is isolated mainly by two methods (11), the alkali extraction method using NaOH (AE-REP) and the starch-degraded method in which starch is degraded by a heat resistant α -amylase and removed (SD-REP). Kumagai et al. reported the mass preparative procedure by alkali extraction (12). The amino acid composition of AE-REP is poor in sulfur containing amino acids, i.e., cysteine and methionine, compared with rice flour and SD-REP because of the alkali treatment. The general feature is 1/2 lower in lysine, but 2.5 times higher in arginine compared with casein, one of the most typical animal proteins.

Within REP, prolamin is known for its indigestibility as it is excreted as fecal protein particles (13). However, prolamin in REP showed altered digestibility following alkali treatment and cooking (14, 15). The alkali extraction procedure keeps prolamin as digestible as that in raw rice and improves the bioavailability of whole REP compared with cooked rice (12, 16). Surprisingly, REP's protein efficiency ratio using alkali treatment was equivalent to that of casein. This was recently confirmed by Liu et al. (17), who found that cooking induced disulfide bond cross-linking and decreased prolamin digestibility. After heat treatment like cooking, prolamin may still be indigestible; therefore, it can be regarded as a kind of resistant protein. Any functional components other than protein in the REP fraction remain to be confirmed. However, interestingly, the REP fraction may include phytochemicals such as lysoglycerophospholipids, fatty acids, and fatty acid-DPHEHP esters bound to REP, which may have antitumor activities (18).

Rice bran protein

Rice bran is a by-product of the rice milling process that is derived from the outer layer of rice grain. RBP occupies about 12% of rice bran and is prepared using the alkali extraction method from defatted rice bran, which is essentially the same conventional method as that for REP (19). RBP composition is more varied than that of REP because it can be contaminated with REP depending on the degree of milling, and depending on researchers' and suppliers' purification methods. However, RBP's amino acid composition is generally 2 or 3 times higher in glycine, alanine and arginine, and 2/3 lower in lysine, glutamine/glutamate and tyrosine, and 1/2 lower in proline compared with casein (Kubota M, Manuscripts in preparation).

Protein fraction distribution differs among rice varieties. Abediyi et al. (20) reported an RBP composition of 37% albumin, 31% globulin, 2% prolamin and 27% glutelin using a Japanese rice variety. Fabian and Ju (21) summarized the more detailed classification of RBP. Recently, the proteomic approach was applied to RBP analysis (22), in which 43 proteins were identified and classified as signaling/regulation proteins, proteins with enzymatic activity, storage proteins, transfer and struc-

tural proteins.

Over several decades of rice bran use, extraction methods have been devised for RBP because it cannot be solubilized. For example, the use of endopeptidases increased protein recovery. Hydrolysates of RBP produced by partial proteolysis enhanced functionalities such as protein solubility, foam capacity, foam stability and emulsification for food processing (23, 24). Additionally, compared with soy protein isolates that often include phytochemicals like isoflavones, RBP from defatted rice bran does not include low molecular phytochemicals such as neutral sugar or feruloyl compounds, which are usually included in rice bran (Kubota M, Manuscript in preparation).

Improvement of Lipid Metabolism

Although phytochemicals such as γ -oryzanol, tocopherols and polyphenols from brown rice have hypocholesterolemic and antiatherogenic effects (25), the most-consumed rice by humans is white rice, so the effects of rice protein, a component of white rice, are reviewed.

Effect on plasma cholesterol and atherosclerosis

Studies on plant protein's physiological functions have been mostly performed on soy protein. There have been only limited studies with rice protein. Morita et al. first reported the hypocholesterolemic effect of REP and soy protein, the mechanism of which is through dietary low methionine contents rather than fecal bile acid excretion (11, 26). Ten years later, we reported that REP from *Koshihikari* lowered cholesterol and triglycerides in the plasma and liver compared with casein in adult Wistar rats. Another rice cultivar, *Shunyo*, which is lower in glutelin and higher in prolamin than *Koshihikari*, showed the same effect as *Koshihikari*, which suggests that prolamin's digestibility does not contribute to this effect. In growing rats, however, the effect of REP was only observed in liver tissue, not in the plasma, which indicates that the response of lipid metabolism to REP may vary at different life stages (27). As the mechanism of REP's cholesterol-lowering effect, increased fecal steroid excretion has often been observed, which may be ascribed to prolamin's property as resistant protein. However, considering that prolamin of AE-REP is digestible (14), there may be another mechanism for this cholesterol lowering effect. Additionally, not only the cholesterol metabolism's digestive step but also the hepatic step may be important for the REP's effect. In fact, by using isolated perfused liver, REP showed a lipid-lowering effect by inhibiting cholesterol and triglyceride hepatic secretion via VLDL into circulation, which decreased plasma lipids (28). Therefore, one possible mechanism is through gene expression of the hepatic cholesterol metabolism, especially leading to bile acid synthesis.

Yang's group (29) investigated the strong correlation between the risk of hypercholesterolemia and oxidative stress. REP improved oxidative stress through regulating glutathione metabolism and attenuating oxidative damage to lipids and proteins, which resulted in a hypocholesterolemic action. Moreover, REP stimulated

the endogenous antioxidant potential through the Nrf2 pathway (30) to prevent ROS generation and induce Phase II antioxidants/detoxication enzymes (31, 32). It may be possible that, within the components in REP, glutelin might play a dominant role in inducing an antioxidant response to rice protein compared with prolamin (33).

It is interesting to see the effect of REP on atherosclerosis development in apoE deficient mice. Ni et al. reported that REP lowered atherosclerotic lesion formation in the aorta and in the aortic root in apoE deficient mice (34). Burris et al. also reported that REP attenuates atherosclerosis initiation by upregulating key antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, to inhibit oxidative stress (35). REP suppressed oxidized LDL generation without changes in LDL. Furthermore, Tong et al. found that α -globulin, a salt soluble polypeptide of REP, is an active component for suppressing atherosclerosis progression (36). However, in these studies with apoE deficient mice, REP had no effect on serum cholesterol levels (34–36). This is surprising because hypercholesterolemia seems to be an established risk factor for atherosclerosis. Thus, in these mice, the mechanism that inhibits atherosclerosis progression may not be lowered serum cholesterol levels. This segregation of REP's effect on serum cholesterol level and atherosclerotic lesion in different experimental animals remains to be solved.

Effect on plasma triglycerides and obesity

Compared with the hypocholesterolemic effect, the effect on plasma triglycerides and obesity by rice protein is not so reproducible. Results on plasma triglycerides are less reproducible and show a large variation. Therefore, there is less evidence for the effect on triglycerides and obesity (37, 38). Yang et al. reported that REP improved plasma lipid levels and adiposity and body weight in Wistar rats by upregulating lipolysis (fatty acid synthase, glucose 6-phosphate dehydrogenase, and malate dehydrogenase) and downregulating lipogenesis (lipoprotein lipase and hepatic lipase) (37). In hamsters with high-fat diets, REP, brown rice protein, and their hydrolysates lowered body weight gain and hepatic lipid content. They stimulated hepatic gene expressions, *PPAR α* , *ACOX1*, *CPT1* for lipid oxidation, and *CYP7A1* for bile acid synthesis, and *CYP51* for cholesterol synthesis (38).

We tested the effect of REP and RBP on the body weight and adipose tissue mass (epididymal tissue) of C57BL/6J mice with high-fat diets. RBP significantly suppressed their body weight and adipose tissue weight (Kubota M. Manuscript in preparation). In this case, REP showed a similar tendency to RBP, but its suppression was not significant. However, REP α -globulin had no effect on plasma triglyceride levels in SD rats (36), and the result that other fractions than α -globulin had a strong antiobesity effect was obtained in our laboratory.

Because rice protein has a higher arginine content than casein, REP's and RBP's mechanism may be due to arginine. In fact, dietary supplementation with arginine,

the physiologic precursor of nitric oxide (NO), reduced white fat mass in Zucker Diabetic Fatty (ZDF) rats by enhancing NO synthesis, lipolysis and glucose and octanoate oxidation (39). Moreover, in diet-induced obese rats, arginine supplementation shifted nutrient partitioning to promote muscle over fat gain and improved the metabolic profile and reduced white body fat (40).

Amelioration of Diabetes, Its Nephropathy and Osteoporosis

Diabetes is the most prevalent non-communicable disease in the world. Whether white rice consumption is associated with a risk of diabetes remains disputed (1–3). Therefore, it might be difficult to agree with the results that rice protein, a component of rice, has a beneficial effect on diabetes. However, at least in animal studies, rice protein has positive effects on diabetes and its complications including chronic renal disease, as described below.

Effect on glucose metabolism through intestinal GLP-1 secretion

Rice protein controls glucose metabolism by secreting gut hormone to promote insulin secretion. Glucagon like peptide-1 (GLP-1), one of the incretins, is an antidiabetic gut hormone released from L cell in the lower small intestine by luminal nutrient stimulation. Ishikawa et al. (41) observed that REP, RBP and their peptides stimulated the GLP-1 release from cultured enteroendocrine cell line GLUTag into the medium and increased its release and enhanced the ratio of active/total GLP-1 in the blood from ligated ileal loops of anesthetized SD rats. These peptides also suppressed dipeptidyl peptidase IV activity, which shortens the half-life of plasma GLP-1 by immediately degrading the peptide. This GLP-1 secretion finally improves glucose tolerance by enhancing insulin secretion. Protein hydrolysate fractions from REP—globulin, albumin, prolamin and glutelin—were examined, all of which exhibited glycemic response, but globulin had the highest effect. As another protein stimulant, corn zein hydrolysate has a distinct effect on GLP-1 secretion (42, 43).

Effects on diabetic nephropathy in animals and humans

Type 2 diabetes mellitus (T2DM) is associated with various complications of microvascular diseases including diabetic neuropathy, diabetic retinopathy and diabetic nephropathy. Two types of T2DM exist, obese and non-obese types. The quality of dietary protein to diabetic nephropathy has been reported using soy protein (44–47). The effect of rice protein has also recently been reported in experimental models of T2DM, Goto-Kakizaki (GK) rats and ZDF rats. In non-obese, spontaneous T2DM model GK rats, which are a diabetic model for Asian patients, REP was investigated (48). When they were fed with diets including 30% sucrose—known to be deleterious to diabetic rodents—compared with casein diet for 10 wk under the equal feed intakes, they showed no improvement in blood glucose levels, but significantly ameliorated kidney damage such as urinary albumin excretion and mesangial matrix, an index of

glomerular tissue damage. In obese, spontaneous T2DM model ZDF rats, which have a defect in the leptin receptor and are a genetically obese, a diabetic model for Caucasian patients, the experiments were performed for 8 wk (49). Symptoms of diabetes, glucose homeostasis, fatty liver and nephropathy were much more severe than those in GK rats. When they were fed regular diets with 20% protein under the equal feed intakes, both REP and RBP markedly suppressed hemoglobin A1c and lipid accumulation in the liver, which improved glucose homeostasis and fatty liver. The effect of RBP on fatty liver was remarkable: hepatic lipids declined to normal levels. Additionally, it significantly lowered urinary albumin excretion and mesangial matrix score, which ameliorated renal failure progression. In ZDF rats, RBP always exhibited superior effects on symptoms. Recently, similar findings were reported in *db/db* mice that RBP hydrolysates ameliorate diabetic nephropathy progression by lowering blood glucose levels, suppressing pro-inflammatory and profibrotic protein expression and enhancing the antioxidant system function (50).

During diabetic nephropathy in the conservative phase patients, dietary protein reduction is recommended to protect nephropathy progression, although no clear evidence of the effect of protein restriction on chronic kidney disease (CKD) patients has been established (51–53). However, dietary interventions to improve nutritional states are urgently necessary in patients with end stage of renal disease when maintenance hemodialysis (MHD) treatment is introduced. A low-protein intake is associated with poor survival (54). Thus, dietary protein should be increased in these patients. Additionally, hyperphosphatemia is a common problem in patients with advanced CKD and may lead to renal osteodystrophy and arteriosclerosis stimulation with higher mortality (55). In such cases, reducing dietary phosphate may be critically important. Fortunately, REP is characterized by >80% protein, with lower amounts of phosphorus compared with soy (230 vs. 790 mg/100 g) (48). By using this unique property of rice protein, Hosojima et al. investigated the usefulness of REP for MHD patients who consumed protein without increased phosphorus and suggested REP may become an excellent source of dietary protein supplement for MHD patients (56). REP was palatable for most patients and no patients dropped out during the 4 wk study. Food with a low phosphorus to protein ratio may be most appropriate for CKD patients. Choosing the appropriate type of protein may delay diabetic nephropathy progression without the risks associated with protein restriction. As a plant protein source, REP is comparable to soy protein, and might be more effective from the perspective of its low P content and an increased palatability for patients in Asian countries.

Effect on osteoporosis in diabetes

Diabetes mellitus adversely affects the skeleton and is associated with an increased risk of osteoporosis and fragility fractures recognized as skeletal complications (57, 58). Because the plasma osteocalcin level was improved by both rice proteins during the experi-

ment with ZDF rats, the effect of rice proteins on bone metabolism was investigated in our laboratory (59, 60). In ZDF rats, parathyroid hormone and fibroblast growth factor 23, which regulates blood phosphorus, increased according to the condition and bone strength decreased despite no change in bone mineral density. REP and RBP restored bone maximum load and stiffness and also improved bone microstructures such as cortical bone thickness, trabecular bone thickness and trabecular number of ZDF rats. Both proteins were effective, but RBP was always stronger, which implies the contamination of unknown compounds in RBP.

Interestingly, dietary protein from bonito fish improves T2DM-induced bone frailty in GK rats (61). Additionally, the effects of L-arginine and soy protein were reported in streptozotocin-induced diabetic rats (62). However, L-arginine, the precursor of NO, did not prevent bone loss in postmenopausal women (63). The mechanism of rice proteins on osteoporosis in T2DM should be solved.

Conclusion

These functions of rice protein described above can be beneficial as a supplement and should be evaluated to determine whether they are efficacious even when ingested as whole rice, whether white rice or brown. This understanding will add new knowledge on the functional properties of plant proteins together with soy protein. In addition to the above topics, other functions such as immunological properties as a hypoallergenic protein and prolamin's unique feature as a resistant protein in the gastrointestinal environment will also be clarified. Furthermore, clinical studies are required for evaluating these new intriguing functions.

Disclosure of State of COI

Dr Motoni Kadowaki reports receiving grants from Kameda Seika Co. Ltd. The other authors report no conflicts of interest relevant to the content of this review.

Acknowledgments

Our findings on REP and RBP are all attributed to favorable and continuous supplies from Kameda Seika Co. Ltd. and Tsuno Food Industrial Co. Ltd., respectively. We thank both companies for their generosity.

REFERENCES

- 1) Hu EA, Pan A, Malik V, Sun Q. 2012. White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review. *Brit Med J* **344**: e1454.
- 2) Soriguer F, Colomo N, Oliveira G, Garcia-Fuentes E, Esteve I, Ruiz de Adana MS, Morcillo S, Porras N, Valdes S, Rojo-Martinez G. 2013. White rice consumption and risk of type 2 diabetes. *Clin Nutr* **32**: 481–484.
- 3) Lee SJ, Lee SY, Sung SA, Chin HJ, Lee SW. 2017. Low rice intake is associated with proteinuria in participants of Korea National Health and Nutrition Examination Survey. *PLoS One* **12**: e0170198.
- 4) Gaesser GA. 2019. Perspective: Refined grains and health: Genuine risk, or guilt by association? *Adv Nutr* **10**: doi.org/10.1093/advances/nmy104.
- 5) Ministry of Health, Labour and Welfare. 2017. National

- Health and Nutrition Survey Japan, 2017.
- 6) Kobayashi S, Asakura K, Suga H, Sasaki S. 2013. High protein intake is associated with low prevalence of frailty among old Japanese women: a multicenter cross-sectional study. *Nutr J* **12**: 164.
 - 7) Ishikawa-Takata K, Takimoto H. 2018. Current protein and amino acid intakes among Japanese people: Analysis of the 2012 National Health and Nutrition Survey. *Geriatr Gerontol Int* **18**: 723–731.
 - 8) Ogawa M, Kumamaru T, Satoh H, Iwata N, Kasai Z, Tanaka K. 1987. Purification of protein body-I on rice seed and its polypeptide composition. *Plant Cell Physiol* **28**: 1517–1527.
 - 9) Osborne TB. 1924. The Vegetable Proteins, 2nd ed. Longmans, Green and Co., London.
 - 10) Iida S, Amano E, Nishio T. 1993. A rice (*Oryza sativa* L.) mutant having a low content of glutelin and a high content of prolamine. *Theor Appl Genet* **87**: 374–378.
 - 11) Morita T, Oh-hashii A, Kasaoka S, Ikai M, Kiriyaama S. 1996. Rice protein isolates produced by the two different methods lower serum cholesterol concentration in rats compared with casein. *J Sci Food Agric* **71**: 415–424.
 - 12) Kumagai T, Kawamura H, Fuse T, Watanabe T, Saito Y, Masumura T, Watanabe R, Kadowaki M. 2006. Production of rice protein by alkaline extraction improves its digestibility. *J Nutr Sci Vitaminol* **52**: 467–472.
 - 13) Tanaka Y, Hayashida S, Hongo M. 1975. The relationship of the feces protein particles to rice protein bodies. *Agr Biol Chem* **39**: 515–518.
 - 14) Kubota M, Saito Y, Masumura T, Kumagai T, Watanabe R, Fujimura S, Kadowaki M. 2010. Improvement in the in vivo digestibility of rice protein by alkali extraction is due to structural changes in prolamin/protein body-I particle. *Biosci Biotechnol Biochem* **74**: 614–619.
 - 15) Kubota M, Saito Y, Masumura T, Watanabe R, Fujimura S, Kadowaki M. In vivo digestibility of rice prolamin/protein body-I particle is decreased by cooking. 2014. *J Nutr Sci Vitaminol* **60**: 300–304.
 - 16) Kumagai T, Watanabe R, Saito M, Watanabe T, Kubota M, Kadowaki M. 2009. Superiority of alkali-extracted rice protein in bioavailability to starch degraded rice protein comes from digestion of prolamin in growing rats. *J Nutr Sci Vitaminol* **55**: 170–177.
 - 17) Liu K, Zheng J, Chen F. 2019. Effect of domestic cooking on rice protein digestibility. *Food Sci Nutr* **7**: 608–616.
 - 18) Yu SG, Fang NB, Li QL, Zhang JX, Luo HF, Ronis M, Badger TM. 2006. In vitro actions on human cancer cells and the liquid chromatography-mass spectrometry/mass spectrometry fingerprint of phytochemicals in rice protein isolate. *J Agric Food Chem* **54**: 4482–4492.
 - 19) Ali R, Shih FF, Riaz MN. 2010. Processing and functionality of rice bran proteins and peptides. In: Bioactive Proteins and Peptides as Functional Foods and Nutraceuticals (Mine Y, Li-Chan E, Jiang B, eds), p 233–246. Blackwell Publishing Ltd. and Institute of Food Technologists, Ames, Iowa.
 - 20) Adebisi AP, Adebisi AO, Hasegawa Y, Ogawa T, Muramoto K. 2009. Isolation and characterization of protein fractions from deoiled rice bran. *Eur Food Res Technol* **288**: 391–401.
 - 21) Fabian C, Ju YH. 2011. A review on rice bran protein: Its properties and extraction methods. *Crit Rev Food Sci Nutr* **51**: 816–827.
 - 22) Ferrari F, Fumagalli M, Profumo A, Viglio S, Sala A, Dolcini L, Temporini C, Nicolis S, Merli D, Corana F, Casado B, Iadarola P. 2009. Deciphering the proteomic profile of rice (*Oryza sativa*) bran: A pilot study. *Electrophoresis* **30**: 4083–4094.
 - 23) Bandyopadhyay K, Misra G, Ghosh S. 2008. Preparation and characterization of protein hydrolysates from Indian defatted rice bran meal. *J Oleo Sci* **57**: 47–52.
 - 24) Hamada JS. 2008. Characterization and functional properties of rice bran proteins modified by commercial exopeptidases and endopeptidases. *J Food Sci* **65**: 305–310.
 - 25) Ravichanthiran K, Ma ZF, Zhang H, Cao Y, Wang CW, Muhammad S, Aglago EK, Zhang Y, Jin Y, Pan B. 2018. Phytochemical profile of brown rice and its nutrigenomic implications. *Antioxidants* **7**: 71.
 - 26) Morita T, Oh-hashii A, Takei K, Ikai M, Kasaoka S, Kiriyaama S. 1997. Cholesterol-lowering effects of soybean, potato and rice proteins depend on their low methionine contents in rats fed a cholesterol-free purified diet. *J Nutr* **127**: 470–477.
 - 27) Yang L, Kumagai T, Kawamura H, Watanabe T, Kubota M, Fujimura S, Watanabe R, Kadowaki M. 2007. Effect of rice proteins from two cultivars, *Koshihikari* and *Shunyo*, on cholesterol and triglyceride metabolism in growing and adult rats. *Biosci Biotechnol Biochem* **71**: 694–703.
 - 28) Yang L, Kadowaki M. 2009. Effects of rice proteins from two cultivars, *Koshihikari* and *Shunyo*, on hepatic cholesterol secretion by isolated perfused livers of rats fed cholesterol-enriched diets. *Ann Nutr Metab* **54**: 283–290.
 - 29) Yang L, Chen JH, Xu T, Zhou AS, Yang HK. 2012. Rice protein improves oxidative stress by regulating glutathione metabolism and attenuating oxidative damage to lipids and proteins in rats. *Life Sci* **91**: 389–394.
 - 30) Nguyen T, Nioi P, Pickett CB. 2009. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J Biol Chem* **284**: 13291–13295.
 - 31) Cai J, Yang L, He H, Xu T, Liu H, Wu Q, Ma Y, Liu Q, Nie M. 2014. Antioxidant capacity responsible for a hypocholesterolemia is independent of dietary cholesterol in adult rats fed rice protein. *Gene* **533**: 57–66.
 - 32) Li H, He H, Wang ZX, Cai J, Sun B, Wu Q, Zhang Y, Zhou GY, Yang L. 2016. Rice protein suppresses ROS generation and stimulates antioxidant gene expression via Nrf2 activation in adult rats. *Gene* **585**: 256–264.
 - 33) Wang ZX, Li H, Liang MC, Yang L. 2016. Glutelin and prolamin, different components of rice protein, exert differently in vitro antioxidant activities. *J Cereal Sci* **72**: 108–116.
 - 34) Ni W, Tsuda Y, Takashima S, Sato H, Sato M, Imaizumi K. 2003. Anti-atherogenic effect of soya and rice-protein isolate, compared with casein, in apolipoprotein E-deficient mice. *Br J Nutr* **90**: 13–20.
 - 35) Burris RL, Xie CH, Thamphi P, Wu X, Melnyk SB, Nagarajan S. 2010. Dietary rice protein isolate attenuates atherosclerosis in apo E-deficient mice by upregulating antioxidant enzymes. *Atherosclerosis* **212**: 107–115.
 - 36) Tong LT, Fujimoto Y, Shimizu N, Tsukino M, Akasaka T, Kato Y, Iwamoto W, Shiratake S, Imaizumi K, Sato M. 2012. Rice α -globulin decreases serum cholesterol concentrations in rats fed a hypercholesterolemic diet and ameliorates atherosclerotic lesions in apolipoprotein E-deficient mice. *Food Chem* **132**: 194–200.
 - 37) Yang L, Chen JH, Lv J, Wu Q, Xu T, Zhang H, Liu QH, Yang HK. 2012. Rice protein improves adiposity, body weight and reduces lipids level in rats through modifica-

- tion of triglyceride metabolism. *Lipids Health Dis* **11**: 24.
- 38) Zhang H, Bartley GE, Mitchell CR, Zhang H, Yokoyama W. 2011. Lower weight gain and hepatic lipid content in hamsters fed high fat diets supplemented with white rice protein, brown rice protein, soy protein, and their hydrolysates. *J Agric Food Chem* **59**: 10927–10933.
 - 39) Fu WJ, Haynes TE, Kohli R, Hu J, Shi W, Spencer TE, Carroll RJ, Meininger CJ, Wu G. 2005. Dietary L-arginine supplementation reduces fat mass in Zucker diabetic fatty rats. *J Nutr* **135**: 714–721.
 - 40) Jobgen W, Meininger CJ, Jobgen SC, Li P, Lee MJ, Smith SB, Spencer TE, Fried SK, Wu G. 2009. Dietary L-arginine supplementation reduces white fat gain and enhances skeletal muscle and brown fat masses in diet-induced obese rats. *J Nutr* **139**: 230–237.
 - 41) Ishikawa Y, Hira T, Inoue D, Harada Y, Hashimoto H, Fujii M, Kadowaki M, Hara H. 2015. Rice protein hydrolysates stimulate GLP-1 secretion, reduce GLP-1 degradation, and lower the glycemic response in rats. *Food Func* **6**: 2525–2534.
 - 42) Hira T, Mochida T, Miyashita K, Hara H. 2009. GLP-1 secretion is enhanced directly in the ileum but indirectly in the duodenum by a newly identified potent stimulator, zein hydrolysate, in rats. *Am J Physiol Gastro Liver Physiol* **297**: G663–G671.
 - 43) Higuchi N, Hira T, Yamada N, Hara H. 2013. Oral administration of corn zein hydrolysate stimulates GLP-1 and GIP secretion and improves glucose tolerance in male normal rats and Goto-Kakizaki rats. *Endocrinol* **154**: 3089–3098.
 - 44) Teixeira SR, Tappenden KA, Erdman JW Jr. 2003. Altering dietary protein type and quantity reduces urinary albumin excretion without affecting plasma glucose concentrations in BKS.cg-m +Lepr^{db}/+Lepr^{db} (db/db) mice. *J Nutr* **133**: 673–678.
 - 45) Trujillo J, Ramirez V, Perez J, Torre-Villalvazo I, Torres N, Tover AR, Munoz RM, Uribe N, Gamba G, Bobadilla NA. 2005. Renal protection by a soy diet in obese Zucker rats is associated with restoration of nitric oxide generation. *Am J Physiol Renal Physiol* **288**: F108–F116.
 - 46) Anderson JW, Blake JE, Turner J, Smith BM. 1998. Effects of soy protein on renal function and proteinuria in patients with type 2 diabetes. *Am J Clin Nutr* **68**: 1347S–1353S.
 - 47) Teixeira SR, Tappenden KA, Carson LA, Jones R, Prabhudesai M, Marshal WP, Erdman JW Jr. 2004. Isolated soy protein consumption reduces urinary albumin excretion and improves the serum lipid profile in men with type 2 diabetes mellitus and nephropathy. *J Nutr* **134**: 1874–1880.
 - 48) Kubota M, Watanabe R, Kabasawa H, Iino N, Saito A, Kumagai T, Fujimura S, Kadowaki M. 2013. Rice protein ameliorates the progression of diabetic nephropathy in Goto-Kakizaki rats with high-sucrose feeding. *Br J Nutr* **110**: 1211–1219.
 - 49) Kubota M, Watanabe R, Yamaguchi M, Hosojima M, Saito A, Fujii M, Fujimura S, Kadowaki M. 2016. Rice endosperm protein slows progression of fatty liver and diabetic nephropathy in Zucker diabetic fatty rats. *Br J Nutr* **116**: 1326–1335.
 - 50) Boonloh K, Lee ES, Kim HM, Kwon MH, Kim YM, Pan-nangpetch P, Kongyingyoes B, Kukonbviriyapan U, Thawornchinsombut S, Lee EY, Kukongviriyapan V, Chung CH. 2018. Rice bran protein hydrolysates attenuate diabetic nephropathy in diabetic animal model. *Eur J Nutr* **57**: 761–772.
 - 51) Robertson LM, Waugh N, Robertson A. 2007. Protein restriction for diabetic renal disease. *Cochrane Database Syst Rev* **4**: CD002181.
 - 52) Nezu U, Kamiyama H, Kondo Y, Sakuma M, Morimoto T, Ueda S. 2013. Effect of low-protein diet on kidney function in diabetic nephropathy: meta-analysis of randomized controlled trials. *BMJ Open* **3**: e002934.
 - 53) Otoda T, Kanasaki K, Koya D. 2014. Low-protein diet for diabetic nephropathy. *Curr Diab Rep* **14**: 523.
 - 54) Shinaberger CS, Kilpatrick RD, Regidor DL, McAllister CJ, Greenland S, Kopple JD, Kalantar-Zadeh K. 2006. Longitudinal associations between dietary protein intake and survival in hemodialysis patients. *Am J Kidney Dis* **48**: 37–49.
 - 55) Kalantar-Zadeh K, Gutekunst L, Mehrotra R, Kovesdy CP, Bross R, Shinaberger CS, Noori N, Hirschberg R, Benner D, Nissenson AR, Kopple JD. 2010. Understanding sources of dietary phosphorus in the treatment of patients with chronic kidney disease. *Clin J Am Soc Nephrol* **5**: 519–530.
 - 56) Hosojima M, Shimada H, Obi Y, Kuwahara S, Kaseda R, Kabasawa H, Kondo H, Fujii M, Watanabe R, Suzuki Y, Kadowaki M, Miyazaki S, Saito A. 2017. A randomized, double-blind, crossover pilot trial of rice endosperm protein supplementation in maintenance hemodialysis patients. *Sci Rep* **7**: 18003.
 - 57) Hofbauer LC, Brueck CC, Singh SK, Dobing H. 2007. Osteoporosis in patients with diabetes mellitus. *J Bone Miner Res* **22**: 1317–1328.
 - 58) Hamann C, Kirschner S, Gunther K-P, Hofbauer LC. 2012. Bone, sweet bone—osteoporotic fractures in diabetic mellitus. *Nature Rev Endocrinol* **8**: 297–305.
 - 59) Watanabe R, Kubota M, Sugaki S, Hosojima M, Saito A, Fujii M, Sasagawa I, Fujimura S, Kadowaki M. 2017. Beneficial effects of rice endosperm protein on chronic kidney disease-mineral and bone disorder (CKD-MBD) in Zucker diabetic fatty rats. 21th Intern Congr Nutr, Abs.218507, Buenos Aires, Argentina.
 - 60) Kubota M, Sugaki S, Komori E, Watanabe R, Hashimoto H, Harada Y, Hosojima M, Saito A, Aoyagi S, Fujimura S, Kadowaki M. 2018. Rice bran protein suppresses deterioration of bone microstructure and strength in obese type 2 diabetic rats. 3rd ISRGH, Abs.78, Kyoto, Japan.
 - 61) Ochiai M, Kuroda T, Gohtani S, Matsuo T. 2015. Dietary protein derived from dried bonito fish improves type-2 diabetes mellitus-induced bone frailty in Goto-Kakizaki rats. *J Food Sci* **80**: H848–H856.
 - 62) EL-Maraghy SA, Mehana NA. 2015. Modulatory effects of L-arginine and soy enriched diet on bone homeostasis abnormalities in streptozotocin-induced diabetic rats. *Chem Biol Inter* **229**: 9–16.
 - 63) Baecker N, Boese A, Schoenau E, Gerzer R, Heer M. 2005. L-Arginine, the natural precursor of NO, is not effective for preventing bone loss in postmenopausal women. *J Bone Miner Res* **20**: 471–479.