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Role of Rice Protein in Prevention for Life-Style Related Diseases: Results from Experimental Animals

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INTRODUCTION

Rice is the main grain in Asian Countries and is a staple food for many countries. Investigators have evaluated the nutritional quality of rice with special reference to rice protein. Generally, the protein content of rice is too low (6-10% among varieties) to be adequate for growing children, when a protein content of 20-25 calories % has been recommended. Furthermore, rice protein contains lower content of lysine, one of the essential amino acids, than do animal proteins. Many efforts have been made to produce high-protein and high-lysine rice by breeding and genetic manipulation.

Research on the physiological effects of rice protein has been limited. Experimental animals fed purified diets containing vegetable proteins, especially soybean proteins are hypocholesterolemic when compared with animals on diets containing animal proteins. For accurate information about rice protein, it is necessary to prepare purified specimens of rice protein isolate (RPI), because the presence of a large amount of carbohydrate may affect interpretation of results. Simultaneously, it is necessary to examine a role of whole rice-grain consumption in health benefit, because people consume rice as it is.

In this symposium, I would like to review the nutritional quality of RPI and its effects on lipid metabolism in rats and on atherosclerosis development in apolipoprotein (apo) E-deficient mice, an animal model for human atherosclerosis. Then, the effects of rice flour consumption on risk factors for life-style related diseases will be described. Finally, I would like to briefly touch upon the tendency of the research in Japan which is going to raise healthy worth of rice by genetic modifications and drug-induced mutations.

I. Effect of Rice Protein Isolate on Lipid Metabolism and Atherosclerosis

Several reports had described methods for preparation of high protein rice flour, but purities of rice protein were less than 40%. Morita and Kiriya (J. Food Sci. 1993) developed a method for mass production of highly purified RPI and analyzed its chemical composition. In addition, they examined the nutritional quality of RPI and its effect on lipid metabolism in rats.

Preparation of RPI Rice flour, 70-35% milling fraction of *Oryza sativa* L. Japonica (cv. Todoroki) was digested with 10 liters of distilled water containing 60 ml of a heat stable α -amylase (Termamyl 120L) at 97°C for 2 h, and the residue was washed with boiling water followed by ethanol.

Composition of RPI The general composition of RPI was compared with that of the rice flour. The protein content of RPI was 10 times higher than that of the original flours. Total dietary fiber and ash were concentrated in the RPI. In contrast, the carbohydrate content was sharply reduced in RPI.

SDS-PAGE The SDS-PAGE patterns of protein extracts from RPI and rice flour were compared by Morita and Kiriyama. The major subfraction of glutelin and prolamin were observed for RPI as well as rice flour, indicating no change of the protein composition during RPI production.

Amino acid composition The amino acid composition of RPI was compared with that of casein and SPI. Lysine content (2.3g/100g) of RPI was much lower than those of casein (7.5g/100g) and soy protein isolate (SPI; 6.1g/100g), while sulfur-amino acid content of RPI (Met + Cys; 4.0g/100g) was higher than those of casein (3.1g/100g) and SPI (2.6g/100g). Furthermore, please note that the contents of Arg differ between animal and plant proteins (3.5g/100g, 8.1g/100g and 7.4g/100g for casein, RPI and SPI, respectively).

Lipid composition RPI contains a small amount of lipids. According to Moritya & Kiriyama, the RPI preparation contained 42.5% triacylglycerol, 24.9% phospholipids and 2.6% sterols. The major phospholipids was lysophosphatidylcholine, which are composed of 76.6% palmitic, 8.3% linoleic and 2.6% α -linolenic acid.

Nutritive values Morita & Kiriyama determined true digestibilities and biological values of RPI in rats fed 10% RPI and 10% casein diets. Both digestibility and biological values of RPI were less than that of casein. It is recommended that 0.9-1.0% lysine in the diet is required for maximum growth in weaning rats.

Hypocholesterolemic action Morita et al (J. Nutr. 1997) fed rats on cholesterol-free purified AIN-76 diets containing casein, RPI, potato protein or SPI. Each protein was fed at 250g/kg diet for 14 d. Growth rates of rats were the same in all groups. Serum total cholesterol concentrations were lower in rats fed the plant proteins than in those fed casein. In a separate experiment in rats fed diets containing amino acid mixtures simulating the RPI, potato protein and SPI diets, serum total cholesterol concentration was lower than in rats fed simulated casein. From these results, it is suggested that plant proteins lower serum cholesterol concentration in a similar manner.

Subsequently, we have determined whether dietary plant proteins such as RPI and SPI compared with casein could afford beneficial effects on atherosclerosis development in apo E-deficient mice (Br. J. Nutr. 2003). In experiment 1, male and female mice were fed on a AIN-93G purified diet containing 200g/kg casein, SPI or RPI for 9 week.

Lesion determination Morphometric determination of atherosclerosis was performed by the following two methods: an *en face* method that evaluates the aortic surface covered by lesion and cross-sectional lesion area in aortic roots.

Lesion and diet The *en face* lesion area in the aorta and the lesion size in the aortic root in male and female mice fed the casein-based diet tended to be greater than those in the SPI or RPI groups. Two way ANOVA revealed no main effect of gender on these lesions. Accordingly, the results were summarized as shown, and it turns out that both *en face* lesion area and the lesion size in the casein group was also significantly greater than in the SPI and the RPI groups.

Serum lipids, amino acids and NO Dietary protein had no significant effect on the serum cholesterol (Triacylglycerols does not show the result, either.). This result is in contrast to that for rat experiments as described before, probably because cholesterol levels differ. Reflecting the dietary content of Arg, the serum Arg concentration in male apo E-deficient mice was higher when plant proteins were given than when casein was consumed. The concentration of Met in the casein group was lower than that in the plant protein groups, although the dietary Met proportion in casein was approximately 2-fold as high as in the SPI. With respect to the serum NO₂ plus NO₃ concentrations, two-way ANOVA revealed protein effect, but there was no gender effect. Accordingly, results from both genders were summarized, that indicate that the serum NO metabolite concentrations in the casein group was significantly lower than in the SPI and RPI groups.

Role of NO in vasculature Endothelial NO synthase (NOS3) and inducible NO synthase (NOS2) can be found in the vasculature. NO relaxes smooth muscle, inhibits smooth muscle cell migration and proliferation, and decrease platelet adherence and aggregation. NO also inhibits vascular inflammation. Furthermore, atherosclerosis is increased in mice deficient in NOS2 or NOS3. L-Arg is the precursor of NO. Serum determination of NO metabolites such as NO₂ plus NO₃ has been suggested to be one of the useful indicators for systemic NO production rates from L-Arg in various physiological and pathophysiological conditions.

NO synthesis inhibitor To confirm such a role of NO synthesis in vivo, the mice were fed a diet containing casein and given water or water containing NOS inhibitor, N^w-nitro-L-argininemethylester (L-NAME). The inhibitor treatment, compared with no inhibitor, resulted in a prominent reduction in the concentration of serum NO metabolites and an increased lesion area in the aorta and lesion size in the aortic root. These results confirm that an efficient utilization of L-Arg for NO synthesis is a prerequisite for preventing an increase of the lesion formation in apo E-deficient mice.

Arg supplementation to casein diet It is interesting to investigate whether the content of Arg of casein and the plant proteins has contributed to the difference in progress of arteriosclerosis. For this purpose, Arg was supplemented to the casein-based diet; in this way, the Arg was equivalent to that in SPI-diet. Met was also supplemented to the SPI based diet. Supplementary Met to the SPI diet resulted in neither an elevation of the serum Met nor aggravation of the lesion formation. The Arg supplemented group, compared with the casein group, had increased concentrations of the serum Arg and the NO metabolites to an extent that was in the same range as that in the SPI-based diet-fed groups. However, in contrast to the SPI-based diet groups, the Arg-supplemented casein-based diet, compared with the casein group, had no significant prevention from the lesion size in the aortic root. These results indicate that the Arg supplementation may not be sufficient enough to lower the lesion formation in apo E-deficient mice. Alternatively, these results suggest that antiatherogenic potentials of SPI and RPI should be ascribed to not only an increased NO formation due to high Arg content in the plant proteins but also an unclarified factor belonging to the plant proteins.

Conclusion In summary, the plant proteins including rice protein lower serum cholesterol concentration. In addition, dietary RPI and SPI, in comparison with dietary casein, lead to reduced lesion formations that appeared to be preceded by an increase in the concentration of the serum Arg and NO metabolites. Dietary Arg supplementation results in an increased concentration of the serum NO metabolites, but this was not accompanied with the lowering of lesion formations. Therefore, antiatherogenic potentials of the plant proteins include not only an increase in the formation of NO from Arg but also an uncharacterized factor

intrinsic to the SPI and RPI.

2. Effect of Rice Flour on Risk Factors for Life-Style Related Diseases

Food consumption pattern The consumption pattern of the carbohydrates, rice, lipids and animal foods in the energy base changes over 1992 from 1955 in Japan. The consumption of carbohydrates and rice decreased during the period, and consumption of animal food and lipids increased. In 1992, Japanese had got energy from carbohydrate 60%, rice 35%, lipids 25% and animal foods 23%, respectively.

Composition of rice diet Based on the food consumption patterns in Japan, we have prepared a AIN-93G purified diet containing rice flour at 2 levels, high and low. The high level is almost equal to the amount of consumption of the present rice. Lipids were added on the low and high levels. That is, six meal groups were made. The control diet group does not include rice flour. It was a little that rice flour contributes to the lipid and protein in the rice-containing diet groups.

Protocol These diets were given to the mouse for 4 and 8 weeks. Blood and internal organs were extracted at the time of sacrifice.

Food intake and body weight The amount of food consumption was the same among the groups. The rice-containing diet fed mouse had the large amount of increases in weight, and it was reflected as an increase in weight of adipose tissues.

Effect on risk factors The addition to the diet of rice influenced the serum cholesterol, in particular HDL-cholesterol, and glucose concentration which are the risk factor of lifestyle-related diseases. By the diet which added rice on the low level, the level of the glucose of serum became the lowest. Such influence was not observed with the diet which added rice on the high level. Dietary lipid level influenced the serum total cholesterol concentration. However, there was no rice effect. In contrast, the increase in the HDL-cholesterol regarded as the addition to the diet of rice reducing the danger of arteriosclerosis was brought about. The addition to the diet of rice had no significant effect on the serum lower density lipoprotein-cholesterol (data not shown).

Conclusion Rice consumption resulted in an increased feed efficiency and an increased adipose tissue weight which might be caused by an increased utilization of serum insulin by peripheral tissues. An elevation of serum HDL-cholesterol is also considered to be one of the beneficial effects of rice consumption.

3. Tendency of the Research in Japan on Improving Health Benefit of Rice

To date most modifications to crop plants - such as decreasing pesticide and herbicide usage, enhancing yields and extending shelf life- have benefited producers. Concern over the impact on the safety of the food supply is substantially elevated in Japanese consumers. This research to modify genetically crop plants including rice has spread in the 2nd generation of which it complains to a consumer's profits. In National Institute of Agrobiological Sciences, for example, the rice which has an effect in the improvement of hay fever and lifestyle-related diseases, such as diabetes and high blood pressure, is under development using transgenics technology.