Long-Term Ultra-High Hydrostatic Pressurized Brown Rice Intake Prevents Bone Mineral Density Decline in Elderly Japanese Individuals

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Summary Bone embrittlement with aging, namely osteoporosis, is characterized by low bone mass and deterioration of bone tissue, and can lead to increased risk of fracture. The development of functional foods that can prevent geriatric diseases is in progress. Our focus was on brown rice because of its properties. An interventional study using of ultra-high hydrostatic pressurized brown rice (UHHPBR) for human has not yet been conducted. In this study, we investigated whether long-term dietary intake of UHHPBR prevents aging-related decline of bone mineral density in elderly Japanese individuals. Elderly participants (n=40; mean 73.1 y) in Iinan-cho, Shimane, Japan, were randomly divided into two groups. The UHHPBR-intake group (n=20) consumed 100 g of UHHPBR and 100 g of white rice (WR) per day for 12 mo, while the WR-intake group (n=20) consumed 200 g of WR per day. Pre- and 12-mo post-intervention, bone mineral density was evaluated by quantitative ultrasound. After 12 mo of intervention, the UHHPBR group’s bone mineral density was significantly higher than the WR group’s bone mineral density. Moreover, chronic intake of UHHPBR had no adverse side effects on participants. Long-term oral UHHPBR intake may have beneficial effects on bone mineral density decline and may attenuate osteoporosis in the elderly.

Key Words ultra-high hydrostatic pressurizing brown rice, bone mineral density, elderly people, osteoporosis, aging

As the world’s population ages, the number of patients with geriatric diseases such as osteoporosis and neurodegenerative disorders are increasing (1, 2). Meanwhile, the economic and social burden of geriatric disease is also expected to increase (3). As such, the development of functional foods that can prevent geriatric diseases is in progress and is currently more feasible than radical therapy pharmaceuticals (4). Numerous familiar foods contain bioactive substances, and thus are applicable as treatments and preventative means for geriatric diseases (5–9). We have been investigating functional foods that may exert beneficial effects for the elderly and that fit a Japanese style diet. Our focus was on rice, a Japanese staple food, and brown rice in particular. When compared with white rice (WR), brown rice, containing the hull, germ, and bran layer, has an abundance of nutrients, vitamins, minerals, polyphenols, amino acids, and dietary fibers. Brown rice also contains beneficial components for the treatment of geriatric diseases including γ-aminobutyric acid (GABA) and ferulic acid. However, a part of people believe that brown rice is difficult to eat on a daily basis because it is difficult to cook and its texture and smell are undesirable when compared to WR.

An ultra-high hydrostatic pressure (UHHP) system is a non-thermal food processing technique (10). In recent years, a UHHP system that can apply 600 MPa water pressure has been developed (11). Brown rice processed with a UHHP system, termed ultra-high hydrostatic pressurized brown rice (UHHPBR), has solved various problems that relate to brown rice. UHHPBR is easy to cook because of its high-water absorbance, has less odor than standard brown rice, and good texture, and thus is easier to eat compared to regular brown rice (11). UHHP also has a powerful bactericidal effect and improves food storage life. The number of common bacteria in UHHPBR is significantly lower than that in regular brown rice. For instance, regular brown rice has previously tested positive for Escherichia coli, whereas UHHPBR did not (11). UHHP also lowers the allergen content and maintains food quality by enzyme inactivation. Furthermore, UHHP treatment physically disrupts cells and connective tissue and chemically denatures proteins, starch, and lipids. Unlike heat treatment, UHHP treatment does not disrupt covalent bonds, and thus improves food digestion and absorption without nutrient loss (11, 12). Considering all of the aforementioned favorable characteristics of UHHPBR, testing the effects...
of chronic UHHPBR intake would be an easy decision. However, such an interventional study for human has not yet been conducted. In this study, we investigate the feasibility of UHHPBR for the prevention of osteoporosis through investigating the effects of long-term UHHPBR intake on bone mineral density and plasma bone metabolism markers in elderly Japanese subjects.

**Materials and Methods**

Participants. Elderly residents of Iinan-cho, Shimane prefecture, Japan, (Mean age, 73.1: n=40) participated in this study. Prior to intervention (baseline), lifestyle, dietary habits, medication history, physical measurements, blood collection, and bone mineral density were investigated. Exclusion criteria for participation in this study were as follows: 1) Eating brown rice every day; 2) Taking supplements or medicines for the treatment of bone disease or for the prevention of osteoporosis.

This study was carried out in accordance with the principles of the Preparing for Submission Declaration of Helsinki and Good Clinical Practice. The protocol was approved by the Ethics Committee of Shimane University (approval numbers 1940, 2504), and all participants gave written informed consent before participation.

Test rice. UHHPBR and WR were obtained from Elise Co., Ltd. (Iinan-cho, Shimane, Japan). Briefly, UHHPBR was prepared from brown rice by exposing it to water at a hydrostatic pressure of 600 MPa for 5 s using a hydrostatic pressurizer (11). UHHPBR and WR were all cultivated in Iinan-cho, Shimane, Japan, and produced from the same variety of rice. WR and UHHPBR nutrients were measured at Shimane Environment & Health Public Corporation (Shimane, Japan) and Shimane Institute for Industrial Technology (Shimane, Japan), and are summarized in Table 1.

Study design. Participants were randomly divided to two groups. The UHHPBR-intake group (n=20, male 8; female 12) consumed 100 g of UHHPBR and 100 g of WR per day for 12 mo. The WR-intake group (n=20, male 10; female 10) consumed 200 g of WR per day. The method of cooking and ingesting UHHPBR and/or WR was freely selected by the participants.

Body composition assessment and self-administered questionnaire. At baseline and month 12, body area ratio (BAR) was measured by quantitative ultrasound, i.e., the speed of sound (SOS) of the right calcaneus, measured by ultrasound bone densitometer (Venous-a, Nihon Kohden, Tokyo, Japan). BAR values were measured with this instrument significantly correlate with bone mineral density of the lumbar vertebrae (r=0.77, p<0.01) and/or the calcaneus (r=0.83, p<0.01) by dual-energy x-ray absorptiometry (14), respectively. Serum bone metabolism markers, namely tartrate-resistant acid phosphatase-5b (TRACP-5b) and bone alkaline phosphatase (BAP), were measured with a chemiluminescent enzyme immunoassay and an enzyme-linked immunosorbent assay, respectively. Serum calcium (Ca) and phosphate (Pi) concentrations were evaluated with an ion-selective electrode method.

Statistical analysis. Results are expressed as mean ± SEM. Differences between WR-intake and UHHPBR-intake groups were analyzed using a Student’s t-test or Mann-Whitney U test. All statistical analyses were carried out with SPSS version 18.0 (IBM-SPSS Japan, Tokyo, Japan). All hypothetical tests were two sided, and p < 0.05 was considered significant.

**Results**

Health status at study enrollment. Since there were significant age differences between the WR-intake and
UHHPBR-intake groups at baseline, age was considered a confounding factor during analysis. No significant difference was observed between the WR-intake and UHHPBR-intake groups in their height, body weight, fat mass, BMI, degree of obesity, waist circumference, or blood pressure before the study (Table 2). Similarly, no significant difference in blood biochemical variables, aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transpeptidase (γ-GTP), albumin (ALB), total cholesterol (T-cho), triglyceride (TG), blood urea nitrogen (BUN), creatinine (CRE), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), glomerular filtration rate (GFR), blood sugar or HbA1c level at baseline was observed between groups (data not shown).

**Participant adherence.** Study participants showed high adherence. The consumption rate of each test rice provided during the intervention was 95 ± 3%, and there was no significant difference in test rice consumption between groups. Side effects disturbing participants’ daily lives such as allergic reactions, palpitation, and irritated stomach were not observed by the consumption of UHHPBR.

**Body constitution and blood biochemical findings.** No significant difference in height, body weight, body fat mass, muscle mass, BMI, degree of obesity, waist circumference, or blood pressure was observed between groups at month 12 (Table 3). Similarly, no significant difference in blood biochemical parameters, blood sugar, or HbA1c was observed between groups (Table 3). Furthermore, the change from baseline to month

### Table 2. Characteristics of study participants before intervention

|                          | WR          | UHHPBR     | p value |
|--------------------------|-------------|------------|---------|
| Sex (male/female)        | 20 (8/12)   | 20 (10/10) |         |
| Age (y)                  | 74.9±1.1    | 71.3±1.2   | 0.034   |
| Height (cm)              | 153.3±1.7   | 158.5±2.5  | 0.093   |
| Body weight (kg)         | 53.7±1.9    | 60.7±3.2   | 0.070   |
| BMI (kg/m²)              | 22.8±0.6    | 23.9±0.7   | 0.177   |
| SBP (mmHg)               | 142.5±6.6   | 141.8±3.0  | 0.944   |
| DBP (mmHg)               | 77.4±3.6    | 78.7±2.1   | 0.722   |
| Body fat (%)             | 27.4±1.7    | 28.9±2.1   | 0.177   |
| Degree of obesity (%)    | 3.4±2.6     | 8.9±3.0    | 0.172   |
| Waist circumference (cm) | 86.1±1.7    | 87.1±2.1   | 0.721   |

WR, white rice-intake group; UHHPBR, ultra-high hydrostatic pressurized brown rice-intake group; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. Data presented as mean±SEM.

### Table 3. Anthropometric and biochemical variables

|                          | Month 12 | Change amount (Δ) |
|--------------------------|----------|-------------------|
|                          | WR       | UHHPBR            | WR       | UHHPBR |
| Sex (male/female)        | 20 (8/12) | 20 (10/10)        | —        | —      |
| Age (y)                  | 74.9±1.1 | 71.3±1.2          | 0.6±0.5  | 0.7±0.6 | 0.779   |
| Height (cm)              | 153.3±1.7| 158.5±2.5         | 0.1±0.3  | −0.4±0.5| 0.094   |
| Body weight (kg)         | 53.7±1.9 | 60.7±3.2          | 1.7±3.9  | 4.7±4.6 | 0.924   |
| BMI (kg/m²)              | 22.8±0.6 | 23.9±0.7          | 0.8±2.2  | 2.9±2.1 | 0.864   |
| SBP (mmHg)               | 142.5±6.6| 141.8±3.0         | −0.1±0.2 | −0.2±0.2| 0.119   |
| DBP (mmHg)               | 77.4±3.6 | 78.7±2.1          | 1.0±0.3  | 1.0±0.8 | 0.621   |
| Body fat (%)             | 27.4±1.7 | 28.9±2.1          | −0.5±0.9 | −1.0±0.9| 0.092   |
| Waist circumference (cm) | 86.1±1.7 | 87.1±2.1          | 2.4±0.9  | −0.8±0.6| 0.421   |
| AST (IU/L)               | 26.4±1.7 | 27.9±1.5          | 1.5±1.4  | 0.7±1.0 | 0.779   |
| ALT (IU/L)               | 18.7±2.1 | 21.3±1.5          | 1.6±1.6  | 0.4±1.3 | 0.383   |
| γ-GTP (IU/L)             | 32.8±9.3 | 45.1±11.9         | 0.8±1.6  | 2.5±2.9 | 0.461   |
| ALB (g/dL)               | 4.2±0.1  | 4.2±0.1           | 0.1±0.1  | 0.1±0.0 | 0.620   |
| T-cho (mg/dL)            | 196.6±6.6| 197.5±5.4         | 4.3±6.0  | 5.9±2.8 | 1.000   |
| TG (mg/dL)               | 129.1±10.5| 144.1±19.8       | 4.5±11.0 | 23.6±15.2| 0.758   |
| BUN (mg/dL)              | 15.6±1.1  | 15.0±0.6          | −1.3±0.8 | −0.3±0.6| 0.289   |
| BS (mg/dL)               | 118.6±7.0 | 109±4.9          | 4.9±8.7  | 8.1±4.3 | 0.986   |
| CRE (mg/dL)              | 0.7±0.0  | 0.7±0.0           | 0.0±0.0  | 0.0±0.0 | 0.414   |
| HDL-C (mg/dL)            | 66.2±3.9  | 62±2.8           | 1.8±1.9  | 1.9±1.5 | 0.779   |
| LDL-C (mg/dL)            | 104.7±5.3 | 104±5.6          | 1.7±7.0  | −0.7±3.2| 0.947   |
| HbA1c (%)                | 5.9±0.1  | 6.0±0.1           | 0.2±0.4  | 0.1±0.1 | 0.121   |
| GFR (mL/min)             | 72.7±3.1 | 76±3.2           | 1.7±2.1  | 0.3±1.5 | 0.411   |

WR, white rice-intake group; UHHPBR, ultra-high hydrostatic pressurized brown rice-intake group; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AST, aspartate transaminase; ALT, alanine transaminase; γ-GTP, γ-glutamyltranspeptidase; ALB, albumin; T-cho, Total cholesterol; TG, triglyceride; BUN, blood urea nitrogen; CRE, creatinine; BS, blood sugar; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; HbA1c, hemoglobin A1c; GFR, glomerular filtration rate. Data are means±SEMs. Part of the data within this table are reprinted with permission from Oyo Yakuri (92(3): 69–73, 2017).
Ultra-High Hydrostatic Pressurized Brown Rice Improves Bone Mineral Density in the Elderly

Bone mineral density and serum bone metabolism marker levels. At baseline, there was no significant difference between the two groups in their calcaneus BAR, SOS, and %YAM (mean ± SEM). The calcaneus BAR value in the UHHPBR-intake group was significantly higher than that in the WR-intake group at month 12 (Fig. 1A, p < 0.05). Similarly, %YAM and SOS in the UHHPBR-intake group at month 12 was significantly higher than that in the WR-intake group although no significant difference in calcaneus bone width was observed between the groups (data not shown). The BAR change amount from baseline to month 12 showed significantly higher values in the UHHPBR-intake group than in the WR-intake group (Fig. 1B, p < 0.05). There were no significant differences in serum BAP and TRACP-5b levels between the WR-intake and UHHPBR-intake groups (data not shown). Concentrations of serum Ca and Pi in the UHHPBR-intake group at month 12 tended to be higher than those in the WR-intake group; however, this difference was not significant (data not shown).

Discussion

The long-term intake of UHHPBR did not have significant influences on participants’ blood pressure, body fat mass, muscle mass, the degree of obesity, basal metabolism, or visceral fat mass (Table 3).

Furthermore, no significant between-group differences in the changing rates of liver and kidney functions or lipid and carbohydrate metabolism were observed before and after intervention (Table 3). Since, to our knowledge, the chronic intake of UHHPBR did not cause any adverse health effects in the participants. UHHPBR can be considered as a safe food to include in one’s daily diet.

Evaluation of the long-term intake of UHHPBR on bone mineral density showed that the BAR in the UHHPBR-intake group was significantly higher than that in the WR-intake group 12 mo after the start of intervention (Fig. 1A). Moreover, the BAR in the UHHPBR-intake group increased from baseline to month 12, whereas the BAR in the WR-intake group decreased during the same period (Fig. 1B). These results demonstrate that the long-term oral intake of UHHPBR improves bone mineral density in the elderly, and thus may act to prevent or attenuate osteoporosis. Osteoporosis in the elderly is categorized into two types; primary senile osteoporosis and postmenopausal osteoporosis. The amount of bone tissue in the skeleton can keep growing until around age 30. At that point, bones have reached their maximum strength and density, known as peak bone mass, which then decreases with age. Postmenopausal women are susceptible to primary osteoporosis, as osteoporosis is closely related to estrogen deficiency. Although osteoporosis is typically associated with women, it is also diagnosed in men, who account for an estimated one in five of people who have osteoporosis or low bone mineral density (15). Thus, it is important to increase the maximum bone density during juvenile to middle age in both men and women to prevent the osteoporosis (15).

In the case that bone density has not been maximized in adolescence, however, it is important to develop an ideal lifestyle habit that minimizes the rate of bone loss in the elderly population (16). One simple way to develop such a lifestyle is to modify environmental factors including daily nutrient intake. The UHHPBR used in this study has high water absorbance and is easy to cook. Also, a large number of functional ingredients such as polyphenols, lipids, and dietary fibers contained in UHHPBR remain at high levels (Table 1). UHHPBR also contains Ca and Pi, important elements for bone formation, and high concentrations of electrolytes such as magnesium, potassium, and iron. Moreover, UHHPBR contains vitamins B1, B6, and niacin, and abundant bioactive substances such as GABA, inositol, and ferulic acid (Table 1). The effects of UHHPBR for the improvement of bone mineral density shown in this study may be related to these nutrients. For example, it has been reported that ferulic acid increases blood estradiol and induces the activation of alkaline phosphatase in an ovariectomized rat, attenuating the decrease in bone mineral density (17). Numerous studies reported that ferulic acid has anti-oxidative effects and preventive effects on dementia, and thus its potentials for the treatment of geriatric disease is drawing a lot of attention (18–20). Moreover, UHHPBR contains a high amount of vitamin B6, which is known to be closely involved in the formation of collagen cross-links in bone tissue. Vitamin B6 deficiencies and a decrease in bone mineral density are highly correlated (21). GABA-enriched brown rice induced the
activation of osteoblast activity and promoted the formation of bone in ovariectomized rats (22). There is a possibility that other components contained in UHHPBR are related to the prevention of bone mineral density decline, therefore this mechanism must be investigated further.

Overall, this study demonstrates that the long-term oral intake of UHHPBR is effective for the prevention of bone mineral density decline in the elderly. Furthermore, this study showed that long-term UHHPBR intake did not cause any negative effects on overall health of the study participants. While further research is required to elucidate the mechanism of its action, UHHPBR consumption is expected to improve bone health and subsequent quality of life in the elderly by preventing osteoporosis and promoting the increase in bone mineral density.

Limitations. Some limitations of this study include its relatively small sample size, its localized sample population (only a cohort of Japanese individuals from one town), its lack of information on subject’s genetic background and its lack of follow up after the termination of the study.

Disclosure of State of COI

No conflicts of interest to be declared.

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