



Instant White Rice with Pigmented Giant Embryonic Rice Improves Glucose Metabolism and Inhibits Oxidative Stress in High-Fat Diet-Fed Mice

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Received: August 11, 2015; Accepted: December 24, 2015

Abstract: The effects of instant cooked rice made from a combination of white rice and pigmented giant embryonic Keunnnunjami rice, in comparison with those of instant regular white or brown rice and instant non-pigmented giant embryonic brown rice, on the glucose metabolism and antioxidant defense status in high-fat diet-fed mice were investigated. 56 male C57BL/6N mice were randomly divided into 7 dietary groups: normal control, high fat (23 %, HF), and HF supplemented with normal white (HF + NW) or brown rice (HF + NB), non-pigmented giant embryonic rice (HF + GB), and white rice with 8 % Keunnnunjami (HF + KJ8) and 18 % Keunnnunjami (HF + KJ18). After 7 weeks, HF mice showed marked increases in blood glucose (156 mg/dL), plasma insulin (12.1 mg/mL), and lipid peroxidation, and a significant decrease in hepatic glycogen (14.2 mg/g) relative to the control group ($p < 0.05$). However, addition of instant NB, GB, KJ8, and KJ18 rice suppressed this high-fat diet-induced hyperglycemia and oxidative stress through altering glucose-regulating enzymes (glucokinase, glucose-6-phosphatase, and phosphoenolpyruvate carboxykinase) and activation of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase, and paraoxonase). Compared with HF mice, HF + KJ8 and HF + KJ18 groups exhibited significantly lower glucose (139–141 mg/dL), insulin (10.6–10.9 mg/mL), and lipid peroxidation and higher glycogen (15.3–16.4 mg/g) ($p < 0.05$). The hypoglycemic and antioxidant effects of instant KJ8 and KJ18 rice were generally comparable to those of instant NB and GB rice. These findings illustrate that instant rice made from white rice with 8 % Keunnnunjami rice may be useful as a functional food with therapeutic potential against hyperglycemia and oxidative damage.

Keywords: Instant rice, pigmented giant embryonic rice, Keunnnunjami, hyperglycemia, antioxidants

Introduction

Giant embryonic rice, mutant rice with an enlarged embryo, has been shown to possess higher concentrations of nutrients, such as proteins, lipids, amino acids, vitamins, and minerals, and higher antioxidant activity than ordinary normal embryo rice [1, 2]. It contains high amounts of the bioactive compounds gamma-oryzanol and gamma-aminobutyric acid [2]. Recently, an instant cooked rice made from giant embryonic brown rice has been reported to decrease body weight gain and body fat, to reduce cholesterol, triglyceride, and glucose levels, and to enhance the antioxidant defense system in mice under high-fat diet conditions, and its hypolipidemic, hypoglycemic, and anti-

oxidative effects were higher than those of instant rice made from giant embryonic white rice and normal embryo brown and white rice [3, 4]. The demand for instant rice is increasing among rice consumers with modern busy lifestyles due to the quick and easy preparation of instant rice. Although a wide array of instant cooked rice products, such as white, brown, organic, and whole-grain, are already available, food researchers and scientists are continuously developing instant rice with enhanced palatability and nutritional quality.

Pigmented rice varieties are known for their colored bran and high antioxidant capacity. Keunnnunjami is a new blackish-purple pigmented rice with a giant embryo. It is rich in cyanidin-3-glucoside, an anthocyanin compound

with strong antioxidant activity [5], and has greater hypolipidemic and anti-obesity effects than ordinary brown rice [6]. While studies have shown that pigmented and non-pigmented brown rice have more nutrients and greater physiological effects than white rice, consumers still generally prefer white rice because of the hard texture of brown rice. In Korea, an instant cooked rice made from 92 % white rice and 8 % pigmented brown rice, which has a better eating quality than instant rice made from 100 % brown rice, is commercially available. It would be interesting to know whether an instant rice prepared from a combination of white rice and pigmented rice with a giant embryo such as Keunnnunjami would have functional activities comparable to instant rice made from pure non-pigmented giant embryonic brown rice or normal ordinary brown rice. With the increasing prevalence of metabolic diseases, there is growing public interest in food products with improved nutritional qualities and enhanced pharmacological properties.

Characterized by hyperglycemia, diabetes is one of the leading causes of death worldwide. An estimated 382 million people had been reported to have diabetes in 2013, and the number was projected to rise to 592 million by 2035 [7]. With the rising costs of medical health care, food products with hypoglycemic and antioxidative properties may be useful in the prevention and management of hyperglycemia. This study was conducted to determine the comparative effects of instant cooked rice made from a combination of white rice and pigmented giant embryonic brown rice Keunnnunjami and those made from normal brown rice and non-pigmented giant embryonic brown rice on the blood glucose level and antioxidative defense system in mice on a high-fat diet. The hepatic enzymes associated with glucose metabolism and antioxidant enzymes were also analyzed.

Materials and methods

Materials

Five kinds of instant cooked rice (Hatbahn), normal white (NW) rice, normal brown (NB) rice, non-pigmented giant embryonic brown (GB) rice, and white rice with 8 % (KJ8) and 18 % (KJ18) pigmented Keunnnunjami rice were provided by CJ CheilJedang Corp. (Seoul, Korea). NW and NB rice are from the same cultivar, Hwacheong, and the GB rice is the giant embryo mutant of Hwacheong rice. The Keunnnunjami rice, on the other hand, was developed from a three-way cross between Heugjinjubyeo, Suwon 425, and EM76 cultivars. The instant rice samples were freeze-dried and ground into powder using a grinder (HMF-3250S, Hanil Electronics, Seoul, Korea). Their proximate compositions were determined following the methods of AOAC [8], and the results are shown in Table I. The total phenolic content and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS)-radical scavenging activity of the samples, which were analyzed according to the methods previously described [9], were in the order: KJ18 > KJ8 > GB > NB > NW (Table I). The chemicals ethanol, potassium phosphate buffer, ketamine-HCl, trichloroacetic acid, and thiobarbituric acid were obtained from Merck KGaA (Darmstadt, Germany). All other chemicals used were purchased from Sigma-Aldrich, Inc. (Steinheim, Germany).

Animals and diets

4-week-old male C57BL/6N mice ($n = 56$), weighing approximately 12 g each, were purchased from Orient Inc. (Seoul, Korea). The animals were individually housed in a

Table I. Proximate composition, total phenolic content, and free radical scavenging activity of instant cooked rice.

Parameter	NW	NB	GB	KJ8	KJ18
Proximate composition (% dry basis)					
Protein	6.9	8.1	7.8	7.5	7.7
Fat	0.9	1.4	2.5	1.1	1.2
Ash	0.8	1.3	1.4	0.4	0.6
Carbohydrate	88.5	81.7	82.4	86.6	85.7
Dietary fiber	2.9	7.4	5.8	4.4	4.8
Total phenolic content (mg/100 g)	26.6 ± 0.6 ^a	45.2 ± 1.8 ^b	156.3 ± 6.9 ^c	172.7 ± 3.9 ^d	191.5 ± 5.2 ^e
ABTS scavenging activity (μg AAeq/g)	23.3 ± 1.3 ^a	68.3 ± 1.4 ^b	81.0 ± 3.2 ^c	234.1 ± 5.6 ^d	300.3 ± 5.0 ^e

Values (mean ± SD) in the same row not sharing a common superscript are significantly different at $p < 0.05$ ($n = 3$). NW, instant normal white rice; NB, instant normal brown rice; GB, instant giant embryonic brown rice; KJ8, instant white rice with 8 % Keunnnunjami rice; KJ18, instant white rice with 18 % Keunnnunjami rice; ABTS, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt; AAeq; ascorbic acid equivalent.

stainless steel cage in a room maintained at $25 \pm 2^\circ\text{C}$ with 50% relative humidity and a 12/12-h light/dark cycle. Mice were fed with a pelletized chow diet for 2 weeks upon arrival and then were randomly divided into seven dietary groups ($n = 8$). The first and second groups were fed with normal control (NC) and high-fat (23%, HF) diets, respectively. The other five groups were fed with HF diets supplemented with instant cooked rice powder: normal white rice (HF + NW), normal brown rice (HF + NB), non-pigmented giant embryonic brown rice (HF + GB), and white rice with 8% Keunnnunjami (HF + KJ8) or 18% Keunnnunjami (HF + KJ18) rice. The composition of the experimental diets (Table II) was based on the AIN-76 semisynthetic diet [10]. The mice were fed daily for 7 weeks and

allowed free access to food and water. At the end of the experimental period, the mice were anaesthetized with ketamine-HCl following a 12-h fast. Blood samples were drawn from the inferior vena cava into a heparin-coated tube and centrifuged at 1,000 g for 15 min at 4°C to obtain plasma and erythrocytes. Plasma and the buffy coat were removed after centrifugation and erythrocytes were washed with physiological saline, followed by hemolysis with distilled water. Hemoglobin concentration was analyzed using a commercial assay kit (Asan Pharmaceutical, Seoul, Korea). Liver and adipose tissues (epididymal, perirenal, and inguinal) were removed, rinsed with physiological saline, weighed, and stored at -70°C until analysis. The current study protocol was approved by the Ethics Com-

Table II. Composition of experimental diet.

Component (%)	Dietary group						
	NC	HF	HF + NW	HF + NB	HF + GB	HF + KJ8	HF + KJ18
Corn starch	15.0	8.48	4.88	7.58	7.30	5.65	6.01
Sucrose	50.0	20.1	–	–	–	–	–
Dextrose	–	11.6	–	–	–	–	–
Casein	20.0	23.3	19.5	18.1	19.2	19.3	19.2
Soybean oil	5.00	2.91	2.91	2.91	2.91	2.91	2.91
Lard	–	20.7	20.3	20.1	19.7	20.2	20.2
Cellulose	5.00	5.83	5.35	4.34	3.94	4.92	4.66
AIN-76 vitamin mix	1.00	1.17	1.17	1.17	1.17	1.17	1.17
AIN-76 mineral mix	3.50	5.24	5.24	5.24	5.24	5.24	5.24
L-cysteine	0.30	0.35	0.35	0.35	0.35	0.35	0.35
Choline bitartrate	0.20	0.23	0.23	0.23	0.23	0.23	0.23
NW	–	–	40.0	–	–	–	–
NB	–	–	–	40.0	–	–	–
GB	–	–	–	–	40.0	–	–
KJ8	–	–	–	–	–	40.0	–
KJ18	–	–	–	–	–	–	40.0
Total (%)	100	100	100	100	100	100	100
Total protein	20.0	23.3	19.5	18.1	19.2	19.3	19.2
Total carbohydrate	70.0	46.1	50.2	51.9	51.2	50.6	50.7
Total fat	10.0	23.6	23.2	23.0	22.6	23.2	23.1
Calories (kcal/g)	385	467	467	470	469	468	469

NC, normal control diet; HF, high-fat diet; HF + NW, high-fat diet + instant normal white rice; HF + NB, high-fat diet + instant normal brown rice; HF + GB, high-fat diet + instant giant embryonic brown rice; HF + KJ8, high-fat diet + instant white rice with 8% Keunnnunjami rice; HF + KJ18, high-fat diet + instant white rice with 18% Keunnnunjami rice.

mittee of Kyungpook National University for animal studies. A schematic diagram of the experimental design is presented in Figure 1.

Blood glucose analysis

Blood glucose level was measured using Accu-Chek Active Blood Glucose Test Strips (Roche Diagnostics GmbH, Mannheim, Germany). Blood samples were drawn from the tail vein of the mice at 3-week intervals for 6 weeks.

Measurement of hepatic glycogen and plasma insulin levels

The hepatic glycogen concentration was determined according to the method of Seifter et al. [11]. Briefly, a liver aliquot (100 mg) was mixed with 30 % KOH, heated at

100 °C for 30 min, 1.5 mL ethanol (95%) added, and kept overnight at 4 °C. The pellet was mixed with 4 mL distilled water and the mixture (500 mL) was then added with 0.2% anthrone (in 95% H₂SO₄). Absorbance was measured at 620 nm and the results were calculated based on a standard calibration curve for glucose. Plasma insulin concentration was measured using enzyme-linked immunosorbent assay kits (TMB Mouse Insulin ELISA kit, Sibayagi, Japan).

Analysis of lipid peroxidation

Plasma and erythrocyte thiobarbituric acid reactive substances (TBARS) were determined based on the method of Ohkawa et al. [12]. To 50 µL of plasma or red blood cells trichloroacetic acid (5%, v/v) and 0.06 M thiobarbituric acid was added, incubated at 80 °C for 90 min. The mixture was cooled to RT and centrifuged at 2,000 g for 25 min. Absorbance of the resulting supernatant was measured at

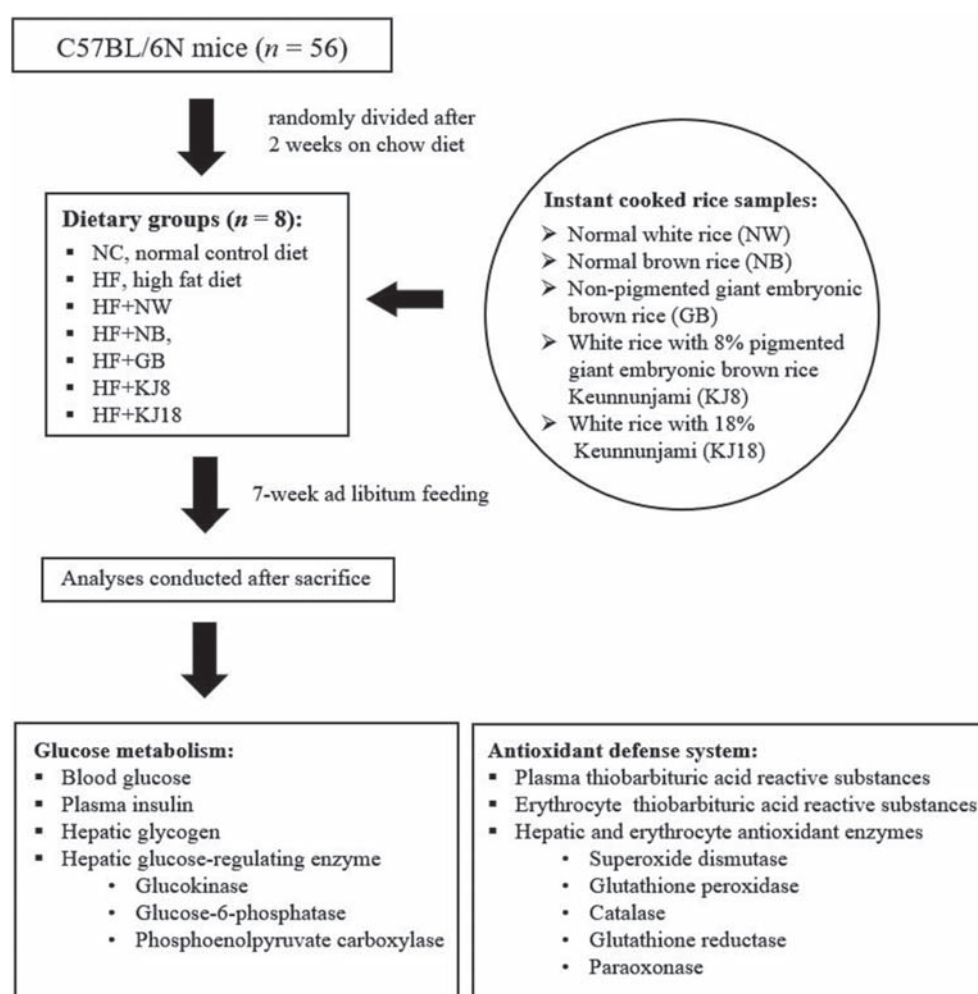


Figure 1. Schematic diagram of the experimental design.

535 nm. A malondialdehyde solution was used as standard in a 5-point calibration curve and results were expressed as nmol/mL or g Hb.

Determination of hepatic glucose-regulating enzymes and hepatic and erythrocyte antioxidative enzymes activities

The hepatic enzyme source was prepared according to the method of Hulcher and Oleson [13]. The liver (0.3 g) was homogenized in buffer solution (0.1 M triethanolamine, 0.2 M EDTA, 0.002 M dithiothreitol) and centrifuged at 1,000 g for 15 min at 4 °C. The supernatant was centrifuged at 10,000 g for 15 min at 4 °C, and the resulting precipitate served as the mitochondrial fraction, while the supernatant was further centrifuged at 105,000 g for 1 h at 4 °C. The resulting supernatant and precipitate served as the cytosol and microsome fractions, respectively. The protein content was measured using Bradford protein assay [14].

For the hepatic glucose-regulating enzyme analysis, the activity of glucokinase (GK) was measured following the method of Davidson and Arion [15]. The reaction mixture was incubated at 37 °C for 10 min and the change in absorbance at 340 nm was recorded. Glucose-6-phosphatase (G6pase) activity was determined using the method of Alegre et al. [16]. The reaction mixture was incubated at 37 °C for 4 min and the change in absorbance at 340 nm was measured. The activity of phosphoenolpyruvate carboxykinase (PEPCK) was analyzed according to the method of Bente and Lardy [17]. Absorbance of the reaction mixture was measured at 340 nm at 25 °C. Activities of GK, G6pase, and PEPCK were expressed as nmol/min/mg protein.

For the analysis of hepatic and erythrocyte antioxidant enzymes, superoxide dismutase (SOD) activity was spectrophotometrically measured using the method of Marklund and Marklund [18]. SOD was detected based on its ability to inhibit superoxide-mediated reduction. The reaction mixture was incubated at 25 °C for 10 min and absorbance was measured at 420 nm. Glutathione peroxidase (GPx) activity was analyzed according to the method of Paglia and Valentine [19]. The assay mixture was incubated at 25 °C for 5 min and absorbance was measured at 340 nm. A molar extinction coefficient of 6.22/mM/cm was used to calculate the activity, which was expressed as nmol or μ mol oxidized NADPH/min/mg protein or Hb. Catalase (CAT) activity was determined based on the method of Aebi [20]. The disappearance of hydrogen peroxide in the reaction mixture was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041/mM/cm was used to determine the CAT activity, which was expressed as nmol or μ mol decreased H_2O_2 /min/mg protein or Hb. Glutathione reductase (GR)

activity was measured based on the method of Mize and Langdon [21]. Absorbance of the assay mixture was measured at 340 nm and activity was expressed as nmol or μ mol oxidized NADPH/min/mg protein or Hb. Paraonase (PON) activity was determined using the method described by Mackness et al. [22]. Absorbance of the reaction mixture was measured at 412 nm at 25 °C. A molar extinction coefficient of 17100/M/cm was used to calculate PON activity.

Statistical analysis

All data are presented as the mean $\pm$ standard error (SE). Data were evaluated by one-way ANOVA using a Statistical Package for Social Sciences software program version 19.0 (SPSS Inc., Chicago, IL, USA) and the differences between the means were assessed using Tukey's test. The normality of distribution and equality of variance were measured using Kolmogorov-Smirnov and Levene's tests, respectively. Statistical significance was considered at $p < 0.05$.

Results

Body weight gain

Total body weight gain, adipose tissue weight, and feed intake significantly increased in the HF group relative to the control group (Table III). On the other hand, mice fed

Table III. Body weight gain, feed intake, and adipose tissue weight in mice fed with high-fat diet supplemented with instant cooked rice.

Group	Body weight gain (g)	Adipose tissue weight (g)	Total feed intake (kcal/kg body weight)
NC	10.91 $\pm$ 0.22 ^a	6.58 $\pm$ 0.26 ^a	386.2 $\pm$ 6.0 ^a
HF	24.00 $\pm$ 0.34 ^e	10.53 $\pm$ 0.38 ^d	494.9 $\pm$ 8.3 ^c
HF + NW	21.82 $\pm$ 0.70 ^d	10.23 $\pm$ 0.21 ^d	488.3 $\pm$ 6.2 ^c
HF + NB	17.82 $\pm$ 0.56 ^c	9.74 $\pm$ 0.14 ^b	469.9 $\pm$ 7.8 ^b
HF + GB	15.41 $\pm$ 0.48 ^b	9.61 $\pm$ 0.19 ^b	473.1 $\pm$ 6.0 ^b
HF + KJ8	18.23 $\pm$ 0.44 ^c	9.69 $\pm$ 0.22 ^b	468.1 $\pm$ 8.5 ^b
HF + KJ18	17.61 $\pm$ 0.48 ^c	10.07 $\pm$ 0.41 ^c	462.8 $\pm$ 8.4 ^b

Values are means $\pm$ SE ($n = 8$). Means in the same column not sharing a common superscript are significantly different at $p < 0.05$. NC, normal control diet; HF, high-fat diet; HF + NW, high-fat diet + instant normal white rice; HF + NB, high-fat diet + instant normal brown rice; HF + GB, high-fat diet + instant giant embryonic brown rice; HF + KJ8; high-fat diet + instant white rice with 8% Keunnnunjami rice; HF + KJ18, high-fat diet + instant white rice with 18% Keunnnunjami rice.

with NB, GB, KJ8, and KJ18 showed considerably lower body weight and amount of body fat than the HF mice, suggesting that these instant rice samples could inhibit the high-fat diet-induced weight gain in mice.

Blood glucose, plasma insulin, and hepatic glycogen levels

HF and NC mice showed the highest and lowest blood glucose levels, respectively (Figure 2). All instant rice-fed groups exhibited considerably lower glucose level than HF mice. No significant difference was found between the HF + GB and the HF + KJ18 groups. The HF and HF + NW

mice showed the highest plasma insulin level and lowest hepatic glycogen level among the animal groups (Table IV). The insulin level was lowest in HF + KJ18 mice, followed by the HF + NB and NC groups. The glycogen level, on the other hand, was highest in NC, HF + GB, and HF + KJ18 groups.

Hepatic glucose-regulating enzyme activities

The enzyme GK activity significantly decreased, while the G6pase and PEPCK activities increased in the HF the group relative to the NC group (Table IV). On the other

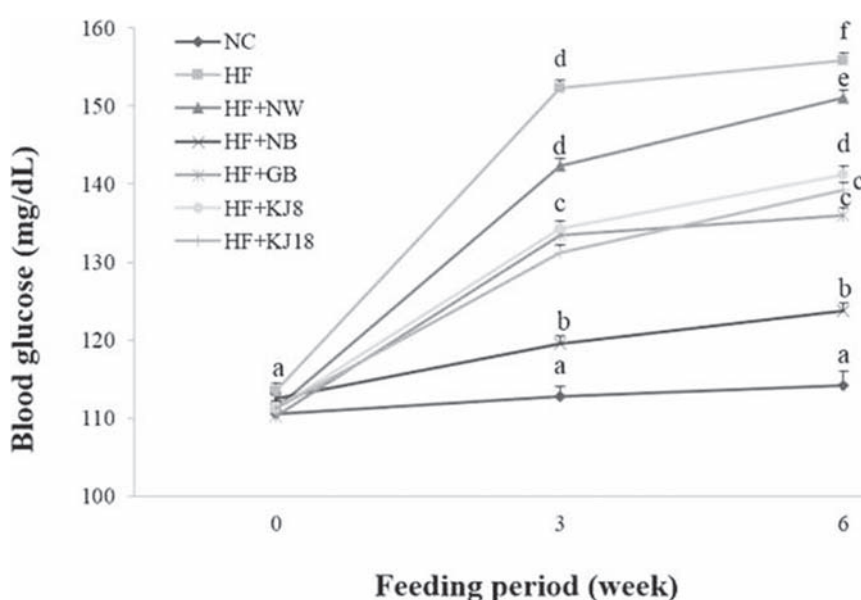


Figure 2. Effect of diet supplementation of instant cooked rice on blood glucose levels in high-fat diet-fed mice. Means not sharing a common superscript are significantly different at $p < 0.05$. Bars represent standard errors ($n = 8$). NC, normal control diet; HF, high-fat diet; HF + NW, high-fat diet + instant normal white rice; HF + NB, high-fat diet + instant normal brown rice; HF + GB, high-fat diet + instant giant embryonic brown rice; HF + KJ8, high-fat diet + instant white rice with 8% Keunnnunjami rice; HF + KJ18, high-fat diet + instant white rice with 18% Keunnnunjami rice.

Table IV. Plasma insulin and hepatic glycogen concentrations and hepatic glucose-regulating enzyme activity in mice fed with high-fat diet supplemented with instant cooked rice.

Group	Insulin (mg/mL)	Glycogen (mg/g liver)	Hepatic enzyme activity (nmol/min/mg protein)		
			Glucokinase	Glucose-6-phosphatase	Phosphoenolpyruvate carboxykinase
NC	10.89 ± 0.03 ^{ab}	16.48 ± 0.16 ^b	1.13 ± 0.04 ^d	84.51 ± 9.79 ^{ab}	0.91 ± 0.08 ^d
HF	12.06 ± 0.16 ^d	14.20 ± 0.08 ^a	0.50 ± 0.07 ^a	107.4 ± 18.0 ^b	1.11 ± 0.06 ^e
HF + NW	12.02 ± 0.10 ^d	14.02 ± 0.03 ^a	0.68 ± 0.02 ^b	99.36 ± 8.54 ^b	0.92 ± 0.04 ^d
HF + NB	10.71 ± 0.03 ^{ab}	15.72 ± 0.71 ^{ab}	0.99 ± 0.05 ^{cd}	79.86 ± 4.80 ^{ab}	0.74 ± 0.04 ^c
HF + GB	11.51 ± 0.08 ^c	17.53 ± 0.10 ^b	0.86 ± 0.04 ^c	85.53 ± 1.19 ^{ab}	0.81 ± 0.10 ^c
HF + KJ8	10.94 ± 0.04 ^b	15.30 ± 0.78 ^{ab}	0.96 ± 0.08 ^{cd}	56.72 ± 3.22 ^a	0.68 ± 0.05 ^b
HF + KJ18	10.61 ± 0.02 ^a	16.36 ± 0.12 ^b	1.00 ± 0.06 ^{cd}	71.90 ± 2.57 ^{ab}	0.40 ± 0.04 ^a

Values are means ± SE ($n = 8$). Means in the same column not sharing a common superscript are significantly different at $p < 0.05$. NC, normal control diet; HF, high-fat diet; HF + NW, high-fat diet + instant normal white rice; HF + NB, high-fat diet + instant normal brown rice; HF + GB, high-fat diet + instant giant embryonic brown rice; HF + KJ8, high-fat diet + instant white rice with 8% Keunnnunjami rice; HF + KJ18, high-fat diet + instant white rice with 18% Keunnnunjami rice.

Table V. Plasma and erythrocyte thiobarbituric acid reactive substances levels and antioxidant enzyme activity in mice fed with high-fat diet supplemented with instant cooked rice.

Parameter	NC	HF	HF + NW	HF + NB	HF + GB	HF + KJ8	HF + KJ18
Thiobarbituric acid reactive substances level							
Plasma (nmol/mL)	0.86 ± 0.02 ^b	1.62 ± 0.06 ^d	1.06 ± 0.06 ^c	0.92 ± 0.03 ^b	0.87 ± 0.04 ^b	0.55 ± 0.08 ^a	0.71 ± 0.08 ^{ab}
Erythrocyte (nmol/g Hb)	4.89 ± 0.09 ^c	5.85 ± 0.19 ^d	5.48 ± 0.14 ^d	4.83 ± 0.22 ^c	2.91 ± 0.11 ^a	4.14 ± 0.07 ^b	2.87 ± 0.05 ^a
Hepatic enzyme activity (nmol/min/mg protein)							
Superoxide dismutase	7.52 ± 0.47 ^{cd}	3.39 ± 0.66 ^a	4.89 ± 0.22 ^b	6.49 ± 0.67 ^c	6.91 ± 0.29 ^c	6.08 ± 0.18 ^c	7.90 ± 0.55 ^d
Glutathione peroxidase	0.22 ± 0.01 ^a	0.20 ± 0.03 ^a	0.20 ± 0.02 ^a	0.20 ± 0.01 ^a	0.25 ± 0.01 ^c	0.22 ± 0.01 ^b	0.27 ± 0.00 ^d
Catalase	0.12 ± 0.01 ^d	0.08 ± 0.00 ^a	0.09 ± 0.00 ^b	0.12 ± 0.01 ^d	0.12 ± 0.01 ^d	0.10 ± 0.00 ^c	0.12 ± 0.00 ^d
Glutathione reductase	5.30 ± 0.14 ^a	5.15 ± 0.18 ^a	5.22 ± 0.18 ^a	6.99 ± 0.37 ^b	5.82 ± 0.26 ^a	5.22 ± 0.05 ^a	7.09 ± 0.07 ^b
Paraoxonase	0.17 ± 0.04 ^b	0.09 ± 0.01 ^a	0.10 ± 0.01 ^a	0.10 ± 0.03 ^a	0.12 ± 0.02 ^a	0.18 ± 0.01 ^b	0.20 ± 0.03 ^b
Erythrocyte enzyme activity (μmol/min/mg Hb)							
Superoxide dismutase	16.52 ± 1.20 ^a	14.98 ± 0.60 ^a	14.50 ± 0.20 ^a	16.72 ± 1.16 ^a	15.75 ± 1.02 ^a	14.10 ± 0.82 ^a	18.08 ± 1.09 ^b
Glutathione peroxidase	0.12 ± 0.01 ^b	0.08 ± 0.01 ^a	0.08 ± 0.01 ^a	0.09 ± 0.00 ^a	0.13 ± 0.00 ^c	0.12 ± 0.00 ^b	0.14 ± 0.01 ^c
Catalase	0.13 ± 0.01 ^a	0.11 ± 0.01 ^a	0.11 ± 0.01 ^a	0.12 ± 0.01 ^a	0.12 ± 0.01 ^a	0.11 ± 0.01 ^a	0.13 ± 0.01 ^a
Glutathione reductase	0.12 ± 0.01 ^c	0.04 ± 0.01 ^a	0.07 ± 0.01 ^b	0.14 ± 0.01 ^c	0.12 ± 0.02 ^c	0.08 ± 0.01 ^b	0.26 ± 0.02 ^d

Values are means ± SE (*n* = 8). Means in the same row not sharing a common superscript are significantly different at *p* < 0.05. NC, normal control diet; HF, high-fat diet; HF + NW, high-fat diet + instant normal white rice; HF + NB, high-fat diet + instant normal brown rice; HF + GB, high-fat diet + instant giant embryonic brown rice; HF + KJ8, high-fat diet + instant white rice with 8 % Keunnnunjami rice; HF + KJ18, high-fat diet + instant white rice with 18 % Keunnnunjami rice. Hb: hemoglobin.

hand, the instant rice-fed groups showed significantly higher GK activity and lower G6pase and PECK activities than the HF mice. Among the instant rice-fed groups, HF + NW exhibited the lowest GK activity while, HF + KJ8 and HF + KJ18 showed the lowest G6pase and PEPCK activities, respectively.

Plasma and erythrocyte lipid peroxides

High-fat diet feeding resulted in the elevation of plasma and erythrocyte TBARS levels (Table V). However, addition of instant rice powder in the high-fat diet significantly decreased the TBARS levels. In particular, mice fed with instant KJ8 and KJ18 samples showed the lowest plasma TBARS level, while HF + GB and HF + KJ18 groups exhibited the lowest erythrocyte TBARS levels.

Hepatic and erythrocyte antioxidant enzyme activities

The activities of hepatic SOD, CAT, and PON enzymes and erythrocyte GPx and GR enzymes significantly de-

creased in HF mice relative to the control group (Table V). On the other hand, the activities of these enzymes were considerably higher in instant rice-fed groups compared with that of the HF mice. The HF + KJ18 mice generally exhibited the highest antioxidant enzyme activities among the animal groups.

Discussion

In the past years, food companies have developed and produced various kinds of instant cooked rice, from simple instant white rice to instant whole-grain and flavored rice. With the increasing popularity of instant cooked rice, there is a growing demand for instant rice that has higher nutritional values than instant white rice and has a better eating quality than instant brown rice. The present study investigated the effects of instant rice made from a combination of white rice and pigmented giant embryonic rice Keunnnunjami, compared to instant ordinary white and brown rice and instant non-pigmented giant embryonic rice, on the glucose metabolism and antioxidant defense status in mice under high-fat diet condi-

tions. Results showed that the high-fat diet markedly increased body weight gain, blood glucose and plasma insulin levels, and lipid peroxidation, and decreased the hepatic glycogen concentration in mice. However, diet supplementation of instant NB, GB, KJ8, and KJ18 rice samples inhibited this high-fat diet-induced hyperglycemic effect and oxidative stress. The instant NW rice, on the other hand, showed a marginal effect on the glucose metabolism and antioxidant defense status in mice. It has been previously reported that giant embryonic rice could reduce body weight, glucose level, and lipid peroxidation in diabetic rats [23]. A study conducted by Kang et al. [24] on functional rice also revealed a significant reduction in the blood glucose level and an enhancement of antioxidant defense status in mice fed with high-fat diet supplemented with giant embryonic rice. Dietary feeding of instant rice made from ordinary brown rice and giant embryonic brown rice has been recently shown to improve the glucose metabolism and to suppress oxidative stress in mice under high-fat diet conditions [4]. The hypoglycemic property of brown rice and giant embryonic brown rice is believed to be associated with their relatively high amount of dietary fibers, which could modulate glucose and lipid metabolisms due to its ability to form a viscous gel matrix in the gastrointestinal tract leading to reduced diffusion of nutrients for absorption [25, 26]. Moreover, giant embryonic brown rice contains a high amount of gamma-aminobutyric acid, which is known for its functional properties including hypolipidemic, hepatoprotective, antihypertensive, and anti-cancer properties [2, 3, 27].

Compared with instant NB and GB rice, instant KJ8 and KJ18 rice exhibited generally similar hypoglycemic and antioxidative effects. These instant rice samples contained 82 or 92 % white rice with only 8 or 18 % Keun-nun-jami. Previous studies have shown that pigmented rice and giant embryonic rice have greater biological activities and functional properties than regular white rice [3, 4, 28, 29]. Results of the present study also indicate that instant NW rice had the least influence on glucose metabolism, lipid peroxidation, and enzyme activities among the samples analyzed. Therefore, the strong hypoglycemic and antioxidative activities of instant KJ8 and KJ18 rice samples were likely due to the Keun-nun-jami rice and not the white rice. It is notable that the instant KJ8 and KJ18 had physiological activities similar to instant NB and GB rice despite the relatively small amounts of Keun-nun-jami they contained. Dietary feeding of Keun-nun-jami rice powder has been previously shown to suppress body weight gain and hyperlipidemia in high-fat diet-fed mice [6]. The relatively high total phenolic content and free radical scavenging activity of instant KJ8 and KJ18 rice compared with the other instant rice sam-

ples may have partly contributed to their strong physiological effects. Polyphenols have been reported to reduce fasting glucose, body weight, glycemia, and oxidative stress in various in vivo and in vitro studies [30]. Consumption of polyphenol-rich food enhances antioxidant capacity and decreases oxidative stress through mechanisms involving radical scavenging activity and modulation of antioxidant enzymes [31]. Previous investigations also revealed that polyphenols, such as anthocyanins and phenolic acids, inhibit digestive enzymes, thus delaying carbohydrate digestion and reducing the rate of glucose release and absorption in the small intestine, resulting in the suppression of hyperglycemia [32]. According to Han et al. [5], Keun-nun-jami contains a high amount of cyanidin-3-glucoside, an anthocyanin compound with strong antioxidant properties, that has been found to ameliorate hyperglycemia in diabetic rats and mice [33, 34]. The instant KJ18-fed mice showed significantly lower blood glucose, erythrocyte TBARS, and insulin levels, and higher antioxidant enzymes activity than the KJ8-fed mice. However, the difference in the influence on glucose metabolism between these two rice samples was marginal. For instance, KJ18 decreased the glucose and insulin levels by only 1 and 3 %, respectively, relative to KJ8 rice, suggesting that an increase of Keun-nun-jami content by 10 % in the instant rice was not sufficient to dramatically affect its hypoglycemic properties. Further studies involving higher concentrations of Keun-nun-jami in instant rice are needed to determine whether increasing the concentration by more than 10 % will greatly enhance the glucose-lowering effect of the instant rice. Nevertheless, instant cooked rice made from white rice with 8 % Keun-nun-jami may be useful as functional food with hypoglycemic and antioxidative properties.

The increased activity of hepatic GK and decreased activities of hepatic G6pase and PEPCK in mice fed instant rice relative to that of the HF group are possibly associated with the reduced glucose levels and enhanced glycogenesis observed in these animals. GK, G6pase, and PEPCK are enzymes involved in glucose metabolism, wherein an increased GK activity has been related to increased glycogen production and decreased blood glucose levels, while increased activities of G6pase and PEPCK have been linked to elevated glucose production [35–37]. The increased activities of the antioxidant enzymes SOD, GPx, CAT, GR, and PON and the decreased TBARS levels found in mice fed instant rice suggest a marked improvement in the antioxidant defense status in these animals. The antioxidant enzymes are part of a highly complex antioxidant protection system that modulates the harmful effects of free radicals and controls oxidative damage. The SOD enzyme converts superoxide radicals into hydrogen peroxides, which in turn are degraded by GPx and CAT into non-toxic prod-

ucts, thereby protecting the cells from oxidative damage [38]. On the other hand, the GR enzyme converts oxidized glutathione into antioxidant-reduced glutathione, and the PON enzyme hydrolyzes biologically active oxidized phospholipids and degrades lipid hydroperoxides [39, 40]. Chung et al. [4] also reported a reduction in the TBARS levels and G6pase and PEPCK activities and an increase in GK, SOD, CAT, GR, and PON activities in mice fed a high-fat diet supplemented with instant brown rice and giant embryonic rice. Moreover, Lee et al. [23, 41] reported that giant embryonic rice powder significantly decreased the lipid peroxidation and increased the hepatic antioxidant enzymes activities in hypercholesterolemic and diabetic rats. Progression of hyperglycemia has been associated with oxidative stress resulting from increased generation of free radicals and an impaired antioxidant defense system [42]. Hence, functional foods with strong antioxidant potential could decrease the glucose level by protecting cells against free radicals under hyperglycemic conditions. The strong antioxidative properties of the instant rice samples, particularly NB, GB, KJ8, and KJ18, may have been partly responsible for the improvement of glucose metabolism in mice.

In conclusion, dietary feeding of instant cooked rice made from white rice with added pigmented giant embryonic rice Keunnunjami could improve the glucose metabolism and suppress oxidative stress in mice under high-fat diet conditions through modulation of hepatic glucose-regulating enzyme activities and activation of antioxidant enzymes. This instant white-Keunnunjami rice exhibited similar hypoglycemic and antioxidative effects to instant rice made from pure ordinary brown rice or non-pigmented giant embryonic brown rice. Results of the present study demonstrate that instant white rice with 8% Keunnunjami may be beneficial as a functional food with a therapeutic potential against high-fat diet-induced oxidative stress and metabolic disorders such as hyperglycemia and diabetes.

Acknowledgements

This work was carried out with the support of the Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ013140), Rural Development Administration, Republic of Korea. The authors' contributions to the work are as follows: S. I. Chung collected and analyzed the data and drafted the manuscript; C. W. Rico analyzed and interpreted the data and drafted the manuscript; S. C. Lee designed the study and critically revised the manuscript; M. Y. Kang designed the study, supervised the work and critically revised the manuscript. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

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