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Effectiveness of rutin-rich Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) ‘Manten-Kirari’ in body weight reduction related to its antioxidant properties: A randomised, double-blind, placebo-controlled study

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ABSTRACT

Rutin, a phenolic compound, has antioxidant, anti-dyslipidaemic, and body weight-reducing effects. We evaluated the anti-arteriosclerotic, antioxidant, and body weight-reducing effects of rutin-rich Tartary buckwheat. We randomly divided 144 adult subjects into an active test food group consuming products containing rutin-rich Tartary buckwheat and a placebo food group. Body composition measurements and haematological and urine tests were performed at weeks 0, 4, 8, and 12, and at 3 weeks after termination. Atherosclerosis index and ox-LDL did not significantly differ between the groups. However, TBARS levels, BW and BMI in the active test food group were significantly lower than those in the placebo group at week 8 ($p = 0.027$, $p = 0.030$, respectively). BFP in the active test food group at week 4 ($p = 0.038$) was lower than that in the placebo group. Thus, rutin-rich Tartary buckwheat intake may be effective for body weight due to its antioxidant properties.

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Abbreviations: ALT, alanine aminotransferase; AI, atherosclerosis-index; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BFP, body fat percentage; BMI, body mass index; BUN, blood urea nitrogen; BW, body weight; Cr, creatinine; DBP, diastolic blood pressure; EIA, enzyme immunoassay; γ -GTP, gamma glutamyl transpeptidase; Hb, haemoglobin; HDL-C, high density lipoprotein cholesterol; Ht, haematocrit; LDH, lactate dehydrogenase; LDL-C, low density lipoprotein cholesterol; Ox-LDL, oxidised LDL; RCT, randomised controlled trial; TBARS, thiobarbituric acid reactive substance; TC, total cholesterol; TG, triacylglycerol; Plt, platelet count; RBC, red blood cells; SBP, systolic blood pressure; UAC, uric acid; WBC, white blood cells; 8-OHdG, urinary 8-hydroxy-2'-deoxyguanosine

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1. Introduction

Rutin is a flavonoid of the flavonol type, which is commonly found in plants (Al-Dhabi, Arasu, Park, & Park, 2015; Tranchimand, Brouant, & Iacazio, 2010). Rutin shows antioxidant effects via scavenging of radiation-induced free radicals (Carrasco-Pozo, Mizgier, Speisky, & Gotteland, 2012; Patil, Rao, Somashekarappa, & Rajashekhar, 2014). In addition, it has several pharmacological functions such as anti-inflammatory, anti-diabetic, and blood capillary strengthening properties (Chua, 2013; Griffith, Couch, & Lindauer, 1944; Shanno, 1946). Kamalakkannan and Prince (2006) reported that oral administration of rutin decreased blood glucose levels and increased insulin secretion in streptozotocin-induced diabetic rats. Other reports suggested that oral administration of rutin significantly decreased the levels of lipids in plasma and tissues in streptozotocin-induced diabetic rats (Stanely Mainzen Prince & Kannan, 2006). In addition, rutin has cardioprotective effects (Annapurna, Reddy, Akondi, & Rao, 2009), which are related to its ability to inhibit platelet aggregation (Pace-Asciak, Hahn, Diamandis, Soleas, & Goldberg, 1995). Although it has been reported that rutin has several pharmacological effects, its exact mechanism and metabolism were not fully elucidated.

Buckwheat is recognised as a functional food and a good source of nutritionally valuable amino acids (Jiang et al., 2007), dietary fibres (Bonafaccia, Marocchini, & Kreft, 2003), and minerals such as zinc and copper (Ikeda & Yamashita, 1994). In particular, Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) contains approximately 100-fold higher amounts of rutin in its seeds compared to common buckwheat (Fabjan et al., 2003; Morishita, Yamaguchi, & Degi, 2007). In a double-blind clinical trial, 2-week intake of Tartary buckwheat cookies with high rutin content (360 mg/day) decreased levels of total cholesterol (TC) and myeloperoxidase, an antioxidant marker, as compared to Tartary buckwheat cookies with low rutin content (17 mg/day) (Wieslander et al., 2011). This finding suggests that rutin-rich Tartary buckwheat can display beneficial functions, including anti-atherosclerotic and antioxidant effects. However, Tartary buckwheat contains a high level of rutinase, which hydrolyses rutin (Suzuki, Honda, Funatsuki, & Nakatsuka, 2002; Yasuda, Masaki, & Kashiwagi, 1992; Yasuda & Nakagawa, 1994). Thus, rutin in Tartary buckwheat is hydrolysed in a few minutes upon addition of water. Hydrolysis of rutin may diminish its beneficial functions and give a bitter taste. These facts have limited the use of Tartary buckwheat in food products.

A new variety of rutin-rich Tartary buckwheat 'Manten-Kirari' containing only trace amounts of rutinase has been developed by the NARO Hokkaido Agricultural Research Center (Suzuki et al., 2014). Therefore, most of rutin remains unhydrolysed, and products developed from 'Manten-Kirari', show high hydrophilic antioxidant capacity (H-ORAC) (Ishiguro, Morishita, Ashizawa, Suzuki, & Noda, 2016). These facts suggest that consumption of Manten-Kirari can provide rutin in sufficient amounts to perform its biological functions and at the same time, avoiding the bitter taste.

To investigate whether consumption of rutin-rich Tartary buckwheat could reduce arteriosclerosis, display antioxidant effects, and change body composition, we conducted this double-blind, placebo-controlled study.

2. Material and methods

2.1. Preparation of rutin-rich Tartary buckwheat

A variety of Tartary buckwheat, 'Manten-Kirari', cultivated in Hokkaido, Japan, was used for preparation of the active test food in this trial. Hard wheat flour prepared from 'Yumechikara' was used for formulation of the placebo food. The active test food was manufactured and packed under strict quality control at the plant of Kobayashi Shokuhin Co., Ltd. (Hokkaido, Japan) in compliance with the Food Sanitation Act (Ministry of Health, Labour, and Welfare of Japan). The manufacturing process of buckwheat noodles included the following (in that order): mixing of the raw materials (Active test foods: 'Manten-Kirari' buckwheat flour 50%, 'Yumechikara' wheat flour 47%, wheat albumin 3%; placebo foods: 'Yumechikara' wheat flour 97% and wheat albumin 3%), addition of some water, preparation of primary noodle dough, pressing to prepare noodle sheets, cutting, casing, and drying. The manufacturing process of cookies included the following (in that order): mixing of the raw materials (Active test cookies: 'Manten-Kirari' buckwheat flour 50.4%, beet sugar 17.2%, egg 25.9%, butter 6.2% and salt 0.3%; Placebo cookies: 'Yumechikara' wheat flour 50.4%, beet sugar 17.2%, egg 25.9%, butter 6.2% and salt 0.3%), shaping with cookie cutter, and baking in oven. Although the rutinase activity in 'Manten-Kirari' is lower than those in other varieties (the rutinase activity, 'Manten-Kirari': other varieties = 1:1000), 'Manten-Kirari' contains trace amounts of rutinase. Therefore, the rutin in the dough gradually hydrolysed upon addition of water. The degree of rutin hydrolysis increases with the increase in the dough water concentration, temperature and ratio of Tartary buckwheat flour in the dough (Suzuki, Morishita, Takigawa, Noda, & Ishiguro, 2015b). For example, more than 90% of rutin remained in 'Manten-Kirari' whereas the majority of rutin was hydrolysed in other varieties within 30 minutes after addition of water. To reduce rutin hydrolysis, it is very important to shorten the processing time from water addition to drying. In this study, casing was completed within 40 minutes after water addition, and raw noodles were immediately dried to decrease the water concentration. As a result, we obtained rutin-rich noodles and cookies. Nutrition facts regarding the active test food and placebo food used in this study are provided in Table 1. Rutin concentration in the test food and placebo food was measured using HPLC. Briefly, 1.0 g of rutin-containing sample was extracted with a mixture of 7.2 mL of methanol and 1.8 mL of 0.1% phosphoric acid at 80 °C for 2 hours (Suzuki et al., 2002). After extraction, the sample was centrifuged at 5000 g for 10 minutes, and the resultant supernatant was filtered through a 0.45-mm filter and assayed using HPLC. HPLC was performed using a Cadenza CD-C18 column (Imtakt, Japan) at a flow rate of 0.2 mL/min. The elution gradient program was set at 0–20 min with isocratic flow conditions at solvent A [acetonitrile–water–TFA (7.5:92.2:7.0.3)]: solvent B [acetonitrile–water–TFA (55:44.7:0.3)] as 63:37. The chromatogram was visualised at 360 nm. According to the study design, subjects should take 500 mg of rutin every day from the active test food. However, since about 20% of rutin would be lost during the boiling process, therefore, we adjusted the content of rutin in

Table 1 – Nutrition facts pertaining to the rutin-rich Tartary buckwheat (Manten-Kirari) foods and the placebo (Yumechikara) foods.

	Active test food prepared from rutin-rich Tartary buckwheat ('Manten-Kirari')		Placebo food prepared from hard wheat flour ('Yumechikara')	
	Noodles (80g dry weight)	Cookies (50 g)	Noodles (80 g dry weight)	Cookies (50 g)
Calories (kcal)	280	190	280	207
Water (g)	9.7	8.2	9.4	4.3
Proteins (g)	12.7	5.8	13.0	7.1
Lipids (g)	2.1	5.6	1.3	5.4
Carbohydrates (g)	51.4	28.4	53.1	31.9
Ash (g)	–	–	–	–
Sodium (mg)	434	97	382	107
Rutin (mg)	619.8	321.1	0	0

Analysis methods: Calories were calculated by formula: proteins (g/100g) × 4 kcal/g + lipids (g/100g) × 9 kcal/g + carbohydrates (g/100g) × 4 kcal/g + fibre (g/100g) × 2 kcal/g; Water, atmospheric heat drying method; protein, Kjeldahl method; lipid, acid digestion; carbohydrates were calculated by formula: 100 – (water + protein + lipid + ash + fiber); ash, direct ashing method; sodium, atomic absorption analysis method.

the dry noodles at more than 500 mg. The active test food and the placebo food were identical in appearance. Previous reports suggested that a dose of 5000 mg flour/kg bodyweight was the No Observed Adverse Effect Level (NOAEL) determined by *in vivo* acute and subacute toxicology studies (Suzuki, Morishita, Noda, & Ishiguro, 2015a).

2.2. Study subjects

We recruited 231 volunteers, of whom 230 provided written informed consents to participate in this clinical study. Finally, we selected 149 subjects (42 males and 107 females aged 30–69 years; atherosclerosis-index (AI), 2.25 ± 0.65) through a screening test, excluding the following: individuals with a recent history of gastrointestinal disorders; pregnancy; severe acute or chronic diseases; surgery; severe allergic reaction to food, particularly buckwheat and wheat, and/or current use of any medications including anti-hyperlipidaemic medications. These

149 eligible subjects were randomly assigned to either the active test food ($n = 74$) or the placebo food group ($n = 75$), with adjustments for age, sex, and AI. The randomised allocation sequence was created using a permuted-block randomisation design stratified by age, gender, and AI, where the block size was a multiple of two. Each subject was allocated by a third-party data centre according to the random allocation sequence into a relevant group. The third-party data centre concealed the allocation information, including the subjects' personal data, and kept them secure. This information was disclosed only after the laboratory and analytical data were fixed, and the method of statistical analysis was finalised.

2.3. Study design

The clinical study was conducted as a double-blind, placebo-controlled trial. The time schedule for the study is shown in Fig. 1. We performed body composition measurements, including body weight (BW), body mass index (BMI), and body fat percentage (BFP) analyses, at weeks 0 (baseline), 4, 8, and 12 after the start of rutin ingestion, and 3 weeks after the end of rutin ingestion. At all five time points, a medical interview was conducted along with a check of the vital signs and haematological and urine tests. We asked the subjects to take 80 g (dry weight) of the active test noodles or placebo noodles per day at any time of the day and cook them using any cooking method they liked. When the subjects could not cook the noodles, they were allowed to consume cookies instead for up to 2 days per week. During the course of this study, subjects were asked to not change their daily activities, including food consumption, medications, and exercises. The primary outcomes were AI and oxidised LDL (ox-LDL) levels. The secondary outcomes were the thiobarbituric acid reactive substance (TBARS), urinary 8-hydroxy-2'-deoxyguanosine (urinary 8-OHdG), TC, high-density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) levels, BW, BFP, and BMI.

2.4. Physical and haematological examinations

Blood samples were collected at the following time points: baseline, at weeks 4, 8, and 12 after the start of rutin ingestion and at 3 weeks after the end of rutin ingestion. In addition to a medical interview, each subject's body composition (BW, BMI, and BFP) and blood pressure (BP) were measured. Subjects fasted for 12 hours before blood collection. General blood tests were

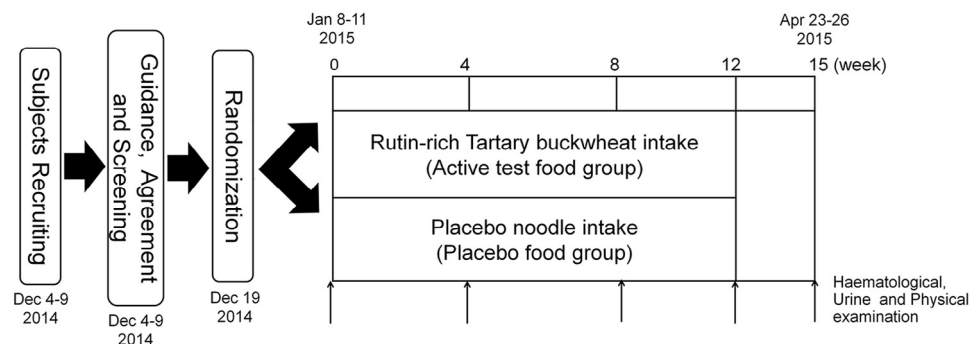


Fig. 1 – Time course of this clinical study.

performed antioxidant markers (ox-LDL and TBARS), lipid profile (AI, TG, TC, HDL-C, and LDL-C), complete blood count [CBC; white blood cells (WBC), red blood cells (RBC), haemoglobin content (Hb), haematocrit value (Ht), and platelet count (Plt)], liver function [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ -GTP), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)], and kidney function [blood urea nitrogen (BUN), creatinine (Cr), and uric acid (UAC)], and urine tests including 8-OHdG levels were measured.

Haematological tests were performed at Sapporo Clinical Laboratory, Inc. (Sapporo, Japan). Ox-LDL and TBARS were measured using ox-LDL ELISA kit (Sekisui Medical Co., Ltd., Tokyo, Japan) and TBARS assay kit (Cayman Chemical, Michigan, USA). TG, TC, HDL-C, and LDL-C were measured by free glycerol method, cholesterol oxidase method, selective inhibition method and selective solubilisation method. AI was calculated in LDL-C/HDL-C. WBC, RBC, Hb, Ht, and Plt were measured by flow cytometry method, electrical resistivity measurement, SLS-Hb method and electrical resistivity measurement. AST, ALT, γ -GTP, ALP, and LDH were measured by Japan Society of Clinical Chemistry (JSCC) reference method. BUN, Cr, and UAC were measured by urease-GLDH method, enzyme assay and uricase-POD method. 8-OHdG was measured by New 8-OHdG Check Elisa (Japan Institute for the Control of Aging, NIKKEN SEIL. CO., Ltd., Shizuoka, Japan) and corrected for creatinine concentrations. Each subject's body composition and BP were measured using a Body Composition Analyzer DC-320 (Tanita Corp, Tokyo, Japan) and an Automatic Blood Pressure Monitor HEM-7080IC (Omron Colin Co., Ltd., Tokyo, Japan).

2.5. Ethics committee

All subjects provided written informed consent prior to undergoing any of the tests related to this study. The study protocol was approved by the Ethics Committee of Hokkaido Information University in conformity with the Helsinki Declaration (No. 2014-17, date of approval: 27 October 2014). This study was registered in UMIN (No. UMIN000015682).

2.6. Sample size

The sample size was statistically determined to obtain a power of 80% with an alpha error of 0.05. In order to demonstrate the postulated change in AI at week 12 (0.36 reduction with a standard deviation of 0.70), a sample size of 120 (60 in the test group and 60 in the placebo group) was required. Assuming a 20% loss to follow-up, 149 subjects were included.

2.7. Statistical analysis

Mean and standard deviation were calculated for each group. Changes in the subject values were analysed using repeated measures ANOVA between the groups. In addition, changes in the subject values were analysed using Student's *t*-test to compare the mean of the active test food group and placebo food group at each evaluation point. Statistical analyses were performed using SPSS Statistics 19 (IBM, Armonk, NY, USA). $p < 0.05$ was considered as significant, and $p < 0.10$ was considered as marginally different.

3. Results

3.1. Dropouts, exclusions, and characteristics of the subjects

During the trial, 4 subjects dropped out for personal reasons ($n = 4$). As a result, 145 subjects completed this trial, 73 in the active test food group and 72 in the placebo group. One person in the placebo group was excluded from the analysis because of low ingestion rate ($< 80\%$). As a result, 144 persons (73 in the active test food group and 71 in the placebo group) were included in the final analysis. The study flow diagram is shown in Fig. 2. Mean age, height, BW, BMI, BFP, AI, and LDL-C for each group are presented in Table 2. No significant differences existed between the active test food and the placebo food groups, showing appropriate assignment of subjects into the two groups.

3.2. Effect of rutin-rich Tartary buckwheat on atherosclerosis-index and oxidised LDL

First, we evaluated the effect of rutin-rich Tartary buckwheat on AI and ox-LDL (Fig. 3 and Table 3). Table 3 shows that the interaction of group by time did not differ significantly between the groups. In addition, there was no significant difference between the active test food and the placebo food group in the change in AI levels from the baseline to evaluation points (Fig. 3A). Moreover, ox-LDL decreased at week 8 in the placebo food group compared to the active test food group (change in level from baseline to week 8, placebo: -6.70 ± 25.50 U/L, test: 2.88 ± 24.81 U/L, $p = 0.024$) (Fig. 3B).

3.3. Effect of rutin-rich Tartary buckwheat on oxidative stress markers

We also examined the effect of rutin-rich Tartary buckwheat on oxidative stress markers (Fig. 3 and Table 3). Urinary 8-OHdG did not differ between the groups (Fig. 3D). However, TBARS levels significantly decreased at week 8 in the active test food group (change in level from baseline to week 8, placebo: 0.86 ± 3.95 μ M, test: -0.56 ± 3.62 μ M, $p = 0.027$) (Fig. 3C).

3.4. Effect of rutin-rich Tartary buckwheat on lipid metabolism

We also evaluated the effect of rutin-rich Tartary buckwheat on lipid metabolism parameters. TC, LDL-C and HDL-C did not differ between the groups. The group \times time interaction gave marginally significant difference to TG levels ($p = 0.072$) (Table 3). However, there were no differences between the active test group and placebo group in the changes in TG levels from baseline to each evaluation points.

3.5. Effect of rutin-rich Tartary buckwheat on body composition

To determine the effect of rutin-rich Tartary buckwheat on body composition, we evaluated the changes in BW, BMI, and BFP. No significant differences in the interaction of group by time

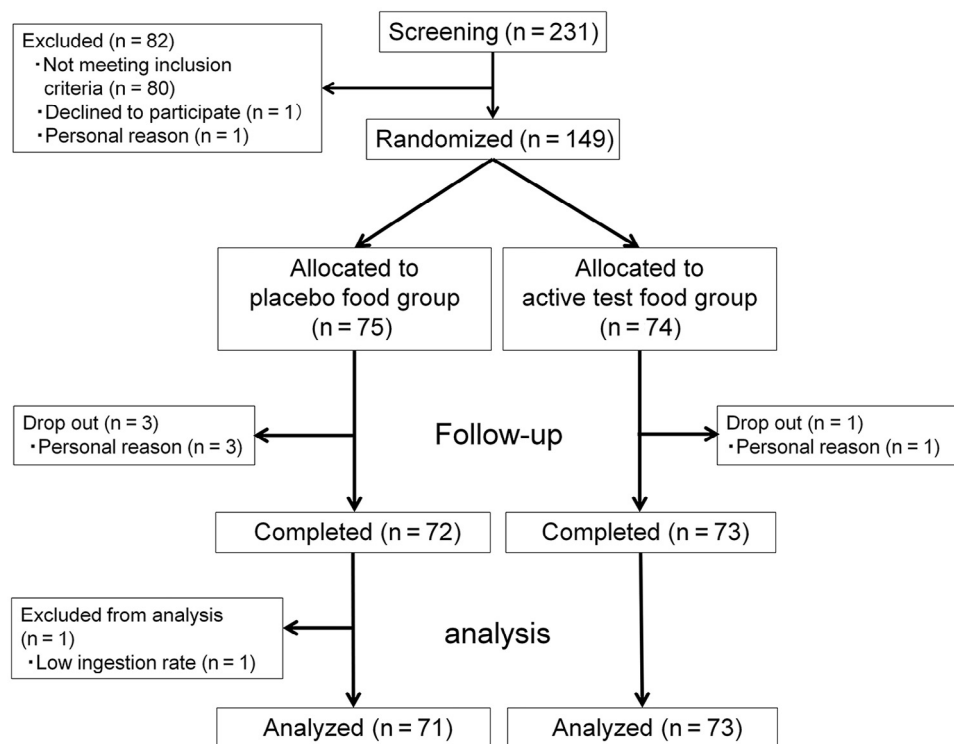


Fig. 2 – Flow diagram of the present study.

of BW, BMI, and BFP (Table 4) were observed between the groups. However, the ingestion of the active test food significantly decreased BW and BMI at week 8 (change level in BW from baseline to week 8, placebo: 0.02 ± 1.18 kg, test: -0.35 ± 0.82 kg, $p = 0.030$) (change level in BMI from baseline to week 8, placebo: 0.02 ± 0.44 kg/m², test: -0.14 ± 0.32 kg/m², $p = 0.016$) (Fig. 4A and C). Moreover, BFP significantly decreased in the active test food group compared to the placebo food group at week 4 (change level in BFP from baseline to week 4, placebo: $0.36 \pm 1.07\%$, test: $-0.30 \pm 2.39\%$, $p = 0.038$) (Fig. 4B).

Table 2 – Characteristics of the subjects in the active test food and the placebo food group.

Characteristic	Active test food group	Placebo food group	p-value
Subjects, n	73	71	–
Males, n (%)	21 (30.00%)	20 (31.25%)	0.937
Age, years	54.58 ± 9.06	53.66 ± 8.78	0.540
Height, cm	161.22 ± 7.74	159.27 ± 7.19	0.120
Body weight, kg	57.91 ± 10.9	56.73 ± 10.59	0.510
Body fat percentage, %	27.71 ± 6.73	27.76 ± 7.09	0.968
Body mass index, kg/m ²	22.18 ± 3.12	22.25 ± 3.30	0.886
Arteriosclerosis index	2.18 ± 0.57	2.32 ± 0.73	0.193
LDL cholesterol, mg/dL	153.21 ± 26.21	152.30 ± 27.42	0.839
Intake rate, %	98.90 ± 3.04	98.70 ± 2.99	0.386

Values shown are mean \pm standard deviation. Student's t-test was performed for age, height, body weight, body fat percentage, body mass index, atherosclerosis index and LDL cholesterol. Chi-square test was performed for gender and Mann-Whitney U test for intake rate. n = number of subjects.

3.6. Safety

We evaluated the CBC, liver and renal function, and BP after the ingestion of rutin-rich Tartary buckwheat products. Minimal changes were observed in the CBC parameters (WBC, RBC, Hb, Ht, and Plt), liver function (ALP, AST, ALT, LDH, and γ -GTP), renal function (BUN, creatinine, and UAC), and BP (Supplementary Table S1). Although few subjects showed adverse events (headache (n = 40), runny nose/nasal congestion (n = 18), fever (n = 18), pharyngeal pain (n = 18), cough (n = 17)), their symptoms were mild, and they recovered in a few days. Thus, the principal investigator judged that there were no adverse events related to the ingestion of the test food. These results suggested that the ingestion of rutin-rich Tartary buckwheat (Manten-Kirari) had no or minimal unfavourable effects even at a dose of 50 g/day (as buckwheat flour).

4. Discussion

The results of our randomised, double-blind, placebo-controlled, parallel-group trial confirmed the potential effects of rutin-rich Tartary buckwheat, 'Manten-Kirari' on lipid metabolism, antioxidation, and body composition. There were no significant differences in AI and ox-LDL levels, and in lipid metabolism parameters between the active test food and the placebo food groups. However, TBARS levels decreased in the test group. In addition, the ingestion of the active test food decreased BW, BMI and BFP.

Previous reports indicated that 22-day-continuous ingestion of rutin decreased TBARS levels in a dose-dependent

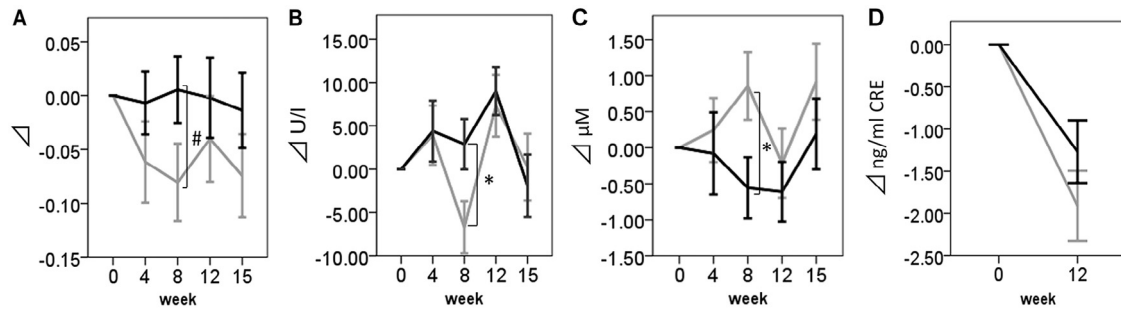


Fig. 3 – Changes in atherosclerosis index (AI) and antioxidant markers level from the baseline and each time point. Black bar: placebo, grey bar: rutin-rich Tartary buckwheat. Values are shown as mean ± standard error (SEM). (A) AI, and (B) oxidised LDL (ox-LDL), (C) thiobarbituric acid reactive substance level (TBARS) and (D) urinary 8-hydroxy-2'-deoxyguanosine level corrected for creatinine concentration (8-OHdG). *p < 0.05, #p < 0.10.

manner (from 10 mg/kg to 1000 mg/kg) in rats, although it affected neither the level of lipid metabolism parameters in serum and liver nor the level of steroid excretion into faeces (Nakamura, Ishimitsu, & Tonogai, 2000). In addition, 5-week-continuous ingestion of 100 mg/kg rutin improved diabetic neuropathy and decreased TBARS levels in diabetic rat models (Ola et al., 2015). It was suggested that the antioxidant mechanism of flavonoids, such as rutin, involves radical scavenging

activity (Carrasco-Pozo et al., 2012; Patil et al., 2014). Free radicals that are formed through the auto-oxidation of unsaturated lipids in plasma and membrane lipids, react with polyunsaturated fatty acids, and lead to lipid peroxidation (detected by TBARS) (Belguith-Hadriche et al., 2016). Our clinical study suggested that rutin-rich Tartary buckwheat had antioxidant effects. However, ox-LDL decreased at week 8 in the placebo food group compared to the active test food group. This may

Table 3 – Change in lipid metabolism parameters and oxidative stress markers, and body composition after ingestion of ‘Manten-Kirari’ or placebo food.

		Change in value at week 4	Change in value at week 8	Change in value at week 12	Change in value at week 15	p-value ^a
AI	Placebo	-0.06 ± 0.32	-0.08 ± 0.30	-0.04 ± 0.33	-0.07 ± 0.32	0.862
	Active	-0.01 ± 0.25	0.01 ± 0.26	0 ± 0.31	-0.01 ± 0.30	
	p-value ^b	0.251	0.071 [#]	0.486	0.244	
Ox-LDL (U/L)	Placebo	3.91 ± 28.64	-6.70 ± 25.50	7.31 ± 30.05	0.24 ± 32.35	0.144
	Active	4.38 ± 29.98	2.88 ± 24.81	8.99 ± 23.20	-1.92 ± 30.79	
	p-value ^b	0.924	0.024 [*]	0.71	0.682	
TBARS (μM)	Placebo	0.24 ± 3.71	0.86 ± 3.95	-0.22 ± 4.04	0.92 ± 4.46	0.288
	Active	-0.08 ± 4.86	-0.56 ± 3.62	-0.61 ± 3.48	0.19 ± 4.18	
	p-value ^b	0.655	0.027 [*]	0.533	0.315	
Urinary 8-OHdG	Placebo	-	-	-1.91 ± 3.50	-	-
	Active	-	-	-1.25 ± 3.10	-	
	p-value ^b	-	-	0.583	-	
TC (mg/dL)	Placebo	-9.30 ± 22.11	-8.94 ± 20.67	-9.45 ± 22.55	-3.20 ± 23.03	0.683
	Active	-5.95 ± 21.12	-4.58 ± 20.53	-5.11 ± 23.35	-2.52 ± 21.58	
	p-value ^b	0.355	0.205	0.262	0.856	
HDL-C (mg/dL)	Placebo	-1.23 ± 6.37	-2.08 ± 6.67	-1.20 ± 8.80	1.03 ± 8.43	0.415
	Active	-1.19 ± 7.24	-1.79 ± 7.55	-0.27 ± 8.47	-0.53 ± 7.53	
	p-value ^b	0.974	0.808	0.522	0.242	
LDL-C (mg/dL)	Placebo	-6.01 ± 20.47	-8.37 ± 19.55	-4.58 ± 21.42	-1.66 ± 19.61	0.216
	Active	-2.73 ± 18.91	-3.33 ± 18.71	-1.21 ± 19.87	-1.88 ± 19.35	
	p-value ^b	0.32	0.116	0.333	0.947	
TG (mg/dL)	Placebo	3.57 ± 37.57	20.11 ± 90.78	9.56 ± 48.58	0.10 ± 41.60	0.072 [#]
	Active	4.97 ± 33.47	8.59 ± 32.78	3.08 ± 35.16	10.32 ± 37.61	
	p-value ^a	0.877	0.295	0.438	0.211	

AI, atherosclerosis-index; Ox-LDL, oxidised LDL; TBARS, thiobarbituric acid reactive substance; 8-OHdG, urinary 8-hydroxy-2'-deoxyguanosine; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triacylglycerol. Values are expressed as mean ± standard division.

Statistically significant, *p < 0.05, marginally significant, #p < 0.10.

^a Changes in subject values were analysed using repeated measures ANOVA between the groups.

^b Changes in subject values were analysed using Student's t-test to compare the mean of the active test food group and the placebo food group at each evaluation point.

Table 4 – Change in body composition after ingestion of ‘Manten-Kirari’ or placebo food.

		Change in value at week 4	Change in value at week 8	Change in value at week 12	Change in value at week 15	p-value ^a
BW (kg)	Placebo	-0.01 ± 0.90	0.02 ± 1.18	-0.03 ± 1.46	-0.15 ± 1.50	0.240
	Active	-0.17 ± 0.73	-0.35 ± 0.82	-0.32 ± 0.87	-0.39 ± 1.09	
	p-value ^b	0.244	0.030*	0.154	0.279	
BFP (%)	Placebo	0.36 ± 1.07	0.21 ± 1.15	0.29 ± 1.32	-0.10 ± 1.43	0.164
	Active	-0.30 ± 2.39	0.11 ± 1.10	0.04 ± 1.27	-0.36 ± 1.26	
	p-value ^b	0.038*	0.584	0.250	0.241	
BMI (kg/m ²)	Placebo	0.01 ± 0.34	0.02 ± 0.44	0 ± 0.55	-0.05 ± 0.57	0.286
	Active	-0.07 ± 0.27	-0.14 ± 0.32	-0.13 ± 0.33	-0.15 ± 0.41	
	p-value ^b	0.133	0.016*	0.098 [#]	0.233	

BW, body weight; BFP, body fat percentage; BMI, body mass index. Values are expressed as mean ± standard deviation.
 Statistically significant, *p < 0.05, marginally significant, #p < 0.10.
^a Changes in subject values were analysed using repeated measures ANOVA between the groups.
^b Changes in subject values were analysed using Student's t-test to compare the mean of the active test food group and the placebo food group at each evaluation point.

be attributed to a higher initial level of ox-LDL in the placebo food group compared to that in the active test food group (actual level of ox-LDL at week 0, test: 129.32 ± 33.18 U/L, placebo: 140.44 ± 37.01 U/L, p = 0.059). In addition, ox-LDL was positively correlated with serum LDL-C levels (Kondo et al., 2003). Since serum LDL-C decreased by a higher value in the placebo food group compared to the active test food group, the ox-LDL level decreased by a higher magnitude in the placebo group. Moreover, no significant difference was observed in the ox-LDL/LDL ratio between the two groups. The underlying cause is not clear; however, it is generally considered that the flavonoid glycoside rutin is hydrolysed by the intestinal microflora (Kühnau, 1976). Metabolites containing a vicinal hydroxyl structure such as quercetin, 3,4-dihydroxyphenylacetic acid (3,4-DHPAA) and 3,4-dihydroxytoluene (3,4-DHT) play important roles in the antioxidant effects of rutin (Chua, 2013). It is well known that Tartary buckwheat contains other functional compounds such as vitamins B1, B2, and B6 and proteins (Guo, Zhu, Zhang, & Yao, 2010); however, the interaction between rutin and these compounds was not fully investigated. Since the metabolic pathways of rutin in *Manten-Kirari* and that of refined rutin are different, it is assumed that their metabolites and effects may also be different. Therefore, further research is re-

quired on the antioxidant effects of ‘*Manten-Kirari*’ and rutin metabolites present in ‘*Manten-Kirari*’ *in vivo* and *in vitro* using purified rutin as a control.

No significant differences in lipid metabolism parameters such as TC, HDL-C, LDL-C, and TG existed between the active test food group and the placebo food group. However, the ingestion of the active test food significantly decreased BW and BMI at week 8. In addition, BFP decreased at week 4 in the active test food group compared to the placebo food group. A previous study has reported that the ingestion 110 mg quercetin for 12 weeks decreased visceral fat area in subjects whose BMI was between 25 kg/m² and 30 kg/m² (Egawa et al., 2012). The mechanism by which quercetin caused visceral fat area improvement was suggested as inhibition of the gene expression of peroxisome proliferator-activated receptor gamma (PPAR γ) and sterol regulatory element-binding protein 1c (SREBP-1c) (Eseberri, Miranda, Lasa, Churruga, & Portillo, 2015) and facilitation of gene expression of proteins involved in β -oxidation (Kobori, Masumoto, Akimoto, & Oike, 2011), as well as inhibition of the mitogen-activated protein kinase (MAPK) signalling factors extracellular signal-regulated kinase (ERK)1/2, Jun-N-terminal kinase (JNK), and p38 MAPK in adipocytes and macrophages (Seo, Lee, Hwang, Kim, & Lee, 2015a). Rutin caused improvement of BW and BFP in rats fed with a high-fat

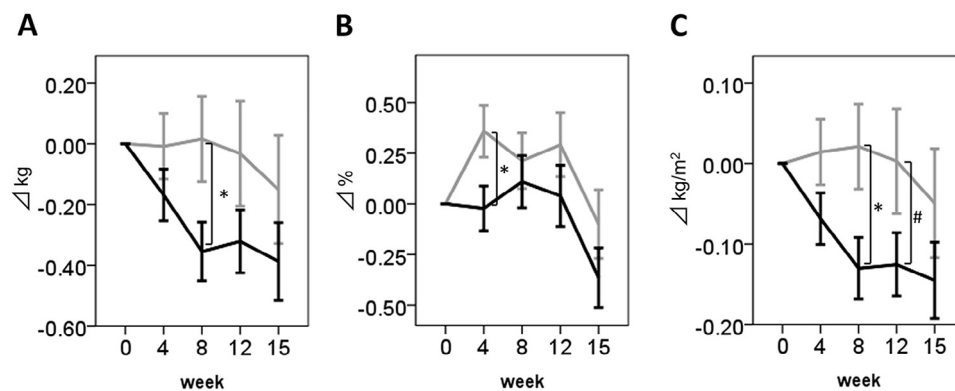


Fig. 4 – Changes in body composition from the baseline at each time point. Black bar: placebo; grey bar: rutin-rich Tartary buckwheat. Values are shown as mean ± standard error (SEM). (A) Body weight (BW), (B) body fat percentage (BFP), (C) body mass index (BMI). *p < 0.05, #p < 0.10.

diet through induction of gene expression related to and activation of 5' AMP-activated protein kinase (AMPK) in skeletal muscles and mitochondrial biosynthesis (Seo et al., 2015b). In addition, another report suggested that mRNA expressions such as PPAR γ and CCAAT/enhancer binding protein- α (C/EBP α) in 3T3-L1 cells were down regulated by rutin treatment (Choi, Park, Choi, & Lee, 2006). These facts suggested that improvement of BW and BFP due to the ingestion of 'Manten-Kirari' was related to the activation of AMPK and increase in the energy conversion, and would regulate the expression of adipogenic transcription factors in adipocyte. The effect of BW-reduction by the ingestion of 'Manten-Kirari' was limited in our clinical study. However, the mean of BMI in our subjects was 22.2 kg/m², and it was regarded as non-obese. Therefore, we expected that ingestion of 'Manten-Kirari' might more reduce BW and BFR in obese subjects. On the other hand, in the placebo food group, the change value in TBARS levels from baseline to week 8 significantly demonstrated positive correlation with the change value in BMI (Pearson's correlation: $r = 0.278$, $p = 0.019$); however, in the active test food group, the change value in TBARS levels did not show positive correlation with the change value in BMI (Pearson's correlation: $r = -0.036$, $p = 0.764$). These results suggested that 'Manten-Kirari' suppressed the increase in oxidative stress induced by the increase in BMI. Obesity contributed oxidative stress and inflammation (Garcés-Rimón et al., 2016; Keaney et al., 2003; Ramos, Shintani, Ikizler, & Himmelfarb, 2008). It was reported that there was a significant positive association between BMI or waist/hip ratio and urinary levels of 8-epi-prostaglandin F2 α (Keaney et al., 2003). The adipose tissue in obesity increased oxidative stress via the increase of NADPH oxidase, the enzyme production of reactive oxygen species (ROS), and the reduction of antioxidant enzyme, such as superoxide dismutase (SOD), glutathione peroxidase and catalase (Furukawa et al., 2004). Rutin treatment to high-fat diet-fed rats showed not only the weight reduction of body, liver organ and adipose tissue but also the reduction of TBARS levels (Hsu, Wu, Huang, & Yen, 2009). On the other hand, secretion defect of adipokine, such as tumor necrosis factor-1 (TNF- α), monocyte chemoattractant protein-1 (MCP-1), leptin and adiponectin, were observed in obesity (Freitas Lima et al., 2015). Rutin also inhibited the expression of leptin and then up-regulated the expression of adiponectin at the protein level in 3T3-L1 adipocytes (Hsu et al., 2009). Moreover, oxidative stress could be also related to adipokine imbalance (Décordé et al., 2009). Therefore, the inhibition of TNF- α secretion and the increase of adiponectin secretion and leptin sensitivity related to antioxidant effects may improve obesity through inhibition of inflammation, improvement of insulin resistance (Kadowaki et al., 2006; Ye, 2013), or correctness for imbalance between food intake and energy expenditure (Jung & Choi, 2014). These facts suggested that rutin might have directly or indirectly improved adipokine imbalance, and then reduced BW and BFR.

In conclusion, the results of this study revealed that rutin-rich Tartary buckwheat, 'Manten-Kirari', showed potential effects on decreasing BW, BFP, and oxidative stress. Although additional studies are still needed to elucidate the molecular mechanisms underlying these effects and to confirm the results of this study, the present study facilitates the development of new applications of processed foods using "Manten-kirari".

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Appendix: Supplementary material

Supplementary data to this article can be found online at [doi:10.1016/j.jff.2016.08.004](https://doi.org/10.1016/j.jff.2016.08.004).

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