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A randomized, double-blind, placebo-controlled study evaluating the effects of quercetin-rich onions on cognitive function in elderly subjects

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ABSTRACT

Background: Quercetin, a phenolic compound, exhibits various functional effects that include anti-oxidant, anti-dyslipidemic, and anti-dysglycemic activities, in addition to beneficial effects on cognitive function. We evaluated the effects of a powder made from quercetin-rich onions (‘Quergold’ and ‘Sarasara-gold’) on cognitive function.

Methods: In this randomized, double-blind, placebo-controlled study, we randomized 50 adults (25 males and 25 females, aged 65–84 years) and made them consume products made from quercetin-rich (active test food group) or quercetin-free (placebo food group) onions. Cognitive function, hematological, and biological examinations were performed at the beginning (week 0) of the study and at weeks 12 and 24 after the start of the study.

Results: There were no differences in the Mini-Mental State Examination (MMSE) and cognitive impairment rating scale scores between the two groups. However, in younger subjects, the MMSE scores were significantly higher in the active test food group than in the placebo food group at week 24 ($p = 0.019$).

Conclusion: These results suggest that the ingestion of quercetin-rich onions improves cognitive function and reduce cognitive decline in elderly people.

Clinical trial registration: UMIN000015940

Keywords: clinical trial; cognitive function; mild cognitive impairment; Mini Mental, State Examination; onion; quercetin

BACKGROUND

The World Health Organization has reported that the number of people with dementia is increasing due to the aging global population [1]. The research group of the Ministry of Health, Labour, and Welfare of Japan has estimated that there are 4.62 million people with dementia and 4 million people with mild cognitive impairment (MCI) in Japan. Moreover, a previous collaborative cohort study presented age-related decline in cognitive test results [2]. Dementia deteriorates the quality of life of patients, and providing care for patients with dementia has an enormous mental and economic burden on caregivers. Effective therapies for dementia are limited. Therefore, strategies for the inhibition of cognitive decline, including diet modifications, have become extremely important. Previous studies have identified functional foods for the inhibition of cognitive decline and improving cognitive impairment. Docosahexaenoic acid (DHA), which is an n-3 long-chain polyunsaturated fatty acid most commonly found in oily fish, improves cognitive functions such as memory retention [3, 4]. Resveratrol, which is a polyphenol found in grapes, enhances sirtuin-

1 (SIRT-1) activity, which has neuroprotective effects [5]. Ginkgo biloba extracts improve short-term memory by reducing free radical production in the prefrontal cortex [6].

Quercetin (3,3′,4′,5,6-pentahydroxyflavone) is a flavone that is present in high concentrations in various vegetables and fruits [7]. The biological activities of quercetin have been demonstrated in various experimental models. It has been identified in several studies as a potent anti-oxidant [8-10]. Additionally, the dietary intake of quercetin reduces serum cholesterol levels and liver fat accumulation [11, 12]. Recent studies have suggested that quercetin has the potential to affect cognitive function. Hayakawa *et al*. (2015) revealed that quercetin may improve memory in aged mice and delay memory deterioration at early stage Alzheimer's disease (AD) in AD model mice [13]. This mechanism activates growth arrest and a DNA damage-inducible protein (GADD34), which inhibits the phosphorylation of eukaryotic translation initiation factor 2α, in the brain [13]. Other studies have reported that quercetin activates SIRT-1 [14, 15]. Therefore, the intake of quercetin may exert neuroprotective effects similar to resveratrol. Epidemiological studies have suggested that insulin resistance is related to AD onset [16], while quercetin improves insulin resistance [17]. The oral administration of quercetin resolves cognitive impairment induced by hyperglycemia and hyperlipidemia in vivo [18, 19]. These findings suggested that the consumption of quercetin-rich onions improves cognitive function directly and indirectly by suppressing risk factors for dementia such as hyperglycemia.

Onions (*Allium cepa* L.) usually contain quercetin, which exhibits various physiological activities including anti-oxidant [20], anti-hypertensive [21], anti-hyperglycemic [22, 23], anti-dyslipidemic [24, 25], and anti-coagulant [26] activities. An investigation of the estimated quercetin intake in Hokkaido, Japan, revealed that onions represent a major food source of quercetin throughout the year [27]. The onion cultivars 'Quergold' and 'Sarasara-gold' were developed via selective breeding to develop onions containing higher amounts of quercetin. One 'Quergold' onion bulb (approximately 140 g) contains 105 mg of quercetin aglycone, and one 'Sarasara-gold' onion bulb (approximately 200 g) contains 115 mg of quercetin aglycone. Furthermore, characteristics of 'Quergold' and 'Sarasara-gold' are suitable for cooking. Therefore, it is easy to include them in the daily diet. These facts illustrate that we can efficiently obtain quercetin from 'Quergold' and 'Sarasara-gold' onions from daily meals. Thus, we can expect further improvements in cognitive function.

A few previous studies have reported the effects of quercetin on cognitive function [13, 18, 19]. Additionally, a previous clinical trial has focused on its effect on cognitive function in patients

with early stage AD [28]. It is important to confirm the effect of quercetin-rich onions, which are present in the usual diets of elderly. Therefore, this 24-week, randomized, double-blind, placebo-controlled trial was conducted to determine the effects of consuming quercetin-rich onions on cognitive function in elderly subjects.

METHODS

Study subjects

We recruited 87 volunteers who were either healthy or exhibited MCI. We included aging subjects to examine the effects of quercetin-rich onions on the inhibition of cognitive decline. Additionally, we selected subjects who had a study partner, such as a family member, to support them by also consuming the test food, to maintain a diary and to manage their physical condition. We screened all subjects and excluded individuals 1) receiving medications for dementia, AD, psychiatric disorders, or cerebrovascular diseases; 2) receiving hormone therapy; 3) with a history of psychiatric disorders, cerebrovascular diseases, or gastrointestinal disorders; 4) with severe acute or chronic diseases; 5) who underwent surgery; or 6) with a severe allergic reaction to food, particularly to onions. We determined the existence of any psychiatric diagnoses through interviews with the subjects and their study partners and obtained the medical histories and records of the subjects. We identified 60 eligible subjects (31 males and 29 females, aged 65–84 years) and randomly assigned them to the active test (quercetin-rich onion) or placebo food (quercetin-free onion) groups. Finally, adjustments for age, gender, and cognitive impairment rating scale scores were made. The randomization sequence was created using a permuted block randomization design stratified by age, gender, and cognitive impairment rating scale scores. A third-party data center allocated each subject to the relevant group according to the randomization sequence, thereby ensuring that each group was well balanced. The third-party data center concealed and securely maintained the allocation information, including the subjects' personal data. The information was disclosed only after the laboratory data were collected and analyzed and the statistical analysis method was finalized.

Study design

The clinical study was conducted as a double-blind, placebo-controlled trial. The schedule of this clinical study is shown in Figure 1. We performed the Mini-Mental State Examination (MMSE) and cognitive impairment rating scale assessments at weeks 0 (baseline), 12, and 24 after the start

of quercetin-rich and quercetin-poor onion therapy. At all three time points, a medical interview was conducted along with an assessment of vital signs, hematological and biological variables, and body composition. Additionally, the study partners completed the Japanese version of the Neuropsychiatric Inventory Questionnaire (NPI-Q-J). For the study, we asked subjects to consume 10 g per day of either the active onion powder containing 60 mg of quercetin or a placebo onion powder not containing quercetin. This intake was determined 1) in consideration of a previous trial [28], which estimated the daily quercetin intake in Hokkaido, Japan, to be approximately 16 mg/day (ranging from 0.5 to 56.8 mg/day) [27] and 2) in consideration of the amount that the subjects could easily ingest each day during the clinical trial (approximately 83 g of raw onions/day). During the course of the study, the subjects were asked to not change their daily activities including food consumption, medications, and exercise routines. However, they were asked to limit their intake of quercetin-containing food, such as other onions or teas, beginning 1 week before the start of the study (as a washout period to reduce the effect of dietary quercetin) until the end of the study. Primary outcomes were MMSE and cognitive impairment rating scale scores. Secondary outcomes included lipid metabolism [total cholesterol (TC) levels, high-density lipoprotein cholesterol (HDL-C) levels, low-density lipoprotein cholesterol (LDL-C) levels, and triglyceride (TG) levels], glucose metabolism [blood glucose (BG) levels and hemoglobin A1c (HbA1c) levels], oxidative parameters [oxidized LDL (ox-LDL) levels], and NPI-Q-J scores. The subjects underwent all examinations at the Health Information Science Center at Hokkaido Information University.

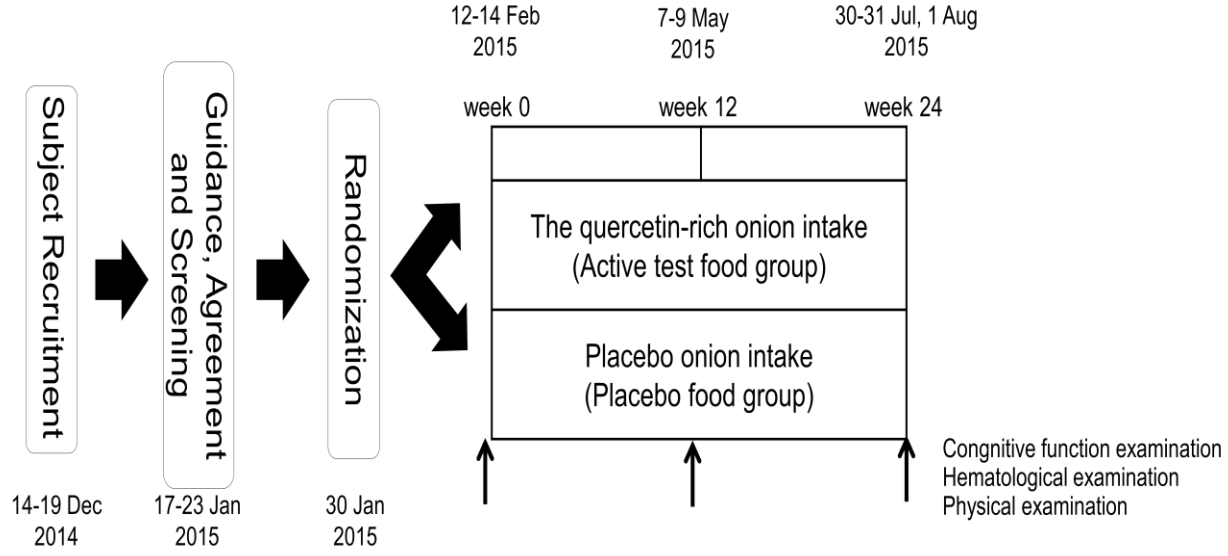

Figure 1. Schedule of the clinical study.

Preparation of test food

The quercetin-rich onion cultivars 'Quergold' and 'Sarasara-gold' used for the active onion powder in this trial were cultivated in Hokkaido, Japan. White onions did not have detectable levels of quercetin (Table 1) and were used for the placebo powder. The active and placebo powders were manufactured in compliance with the Food Sanitation Act (Ministry of Health, Labour and Welfare of Japan) by Okamoto Plant Breeding Co., Ltd. (Hokkaido, Japan). The manufacturing process of onion powder was the following: peeled onions were soaked in hypochlorous acid solution for 20 min, thoroughly rinsed with water, cut into 2-mm wide pieces, dried at 45°C for 30 h, sterilized at 60°C for 120 min, and finally powderized. The active onion powder contained 'Quergold' and 'Sarasara-gold' at a ratio of 7:9, and the amount of quercetin contained in the active test food was adjusted accordingly. The analytical results of the nutrient composition of the active test and placebo foods used in this study are summarized in Table 1. The active test and placebo foods were identical in appearance.

Table 1. Nutrient composition of active test food and placebo food (powder 10 g/day)

	Active test food	Placebo food
Calories (kcal)	38.2	37.5
Water (g)	—	—
Proteins (g)	1.52	0.90
Lipids (g)	0.12	0.13
Carbohydrates (g)	7.8	8.1
Ash (g)	—	—
Sodium (mg)	—	—
Quercetin glycoside (mg)	60	—

Cognitive function examinations

Cognitive function examinations (MMSE, cognitive impairment rating scale, and NPI-Q-J) were performed at the beginning of the study and at weeks 12 and 24 after the start of therapy. The MMSE is a cognitive status test and it has been used most commonly to screen for MCI and dementia [29]. A well-trained nurse administered the MMSE to each subject. This questionnaire consists of 11 questions to evaluate registration, attention and calculation, recall, language, and the ability to follow simple commands and orientation. The maximum score on the test is 30 points.

The cognitive impairment rating scale consists of four examinations: executive function [Yamaguchi Kanji-Symbol Substitution Test (YKSST)] [30], memory recall, immediate memory, and short-term memory. In the YKSST, the patient is presented with combinations of kanji characters and symbols. Each subject was then given 2 min to fill in the lower blank boxes with the correct symbols matching the kanji characters written in the upper boxes. The perfect score on the YKSST is 75 points. For the memory recall test, subjects were required to write the names of as many vegetables as they could recall within one min. For the short-term memory test, the subject was first asked to memorize 10 words and to write them down immediately (immediate memory), then approximately 10 min later, the subject had to recall the same words to write them again (short-term memory). An evaluator with training on the cognitive impairment rating scale administered the questionnaires to all participants.

The Neuropsychiatric Inventory (NPI) is widely used in clinical research to evaluate behavioral and psychological symptoms of patients with dementia (BPSDs) and their response to treatment [31]. The NPI-Q-J measures 10 categories of behavioral disturbances: delusions, hallucination, excitation, depression/dysphoria, anxiety, euphoria, indifference, disinhibition, irritability, and abnormal behavior. The study partner completed the NPI-Q-J questionnaires regarding the 'Severity of subject's symptoms' [from 0 (no symptoms) to 3 points (serious symptoms)] and the 'Burden on study partner' [from 0 (no burden) to 3 points (serious burden)].

Physical, hematological, and biological examinations

Blood samples were taken for testing at the baseline and at weeks 12 and 24 after the start of the study. In addition to a medical interview by a medical doctor, each subject's body composition (body weight, body mass index, and body fat ratio) and blood pressure (BP) were measured. General blood tests were performed to measure anti-oxidant marker (ox-LDL) levels, lipid (TG, TC, HDL-C, and LDL-C) levels, glucose metabolism (BG and HbA1c), complete blood counts (CBCs) [white blood cell (WBC) count, red blood cells (RBC) count, hemoglobin (Hb) level, hematocrit (Ht), and platelet (Plt) count), liver function [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (γ-GTP), alkaline phosphatase, and lactate dehydrogenase (LDH) levels), kidney function [blood urea nitrogen (BUN), creatinine (CRE), and uric acid (UA) levels], albumin (Alb) levels, and total protein (TP) levels. The Sapporo Clinical Laboratory Inc. (Sapporo, Japan) performed all blood tests. Each subject's body composition and BP was measured using Body Composition Analyzer DC-320 (Tanita Corp,

Tokyo, Japan) and Automatic Blood Pressure Monitor HEM-7080IC (Omron Colin Co., Ltd., Tokyo, Japan), respectively.

Ethics committee

All subjects and their study partners provided written informed consent prior to undergoing any test related to this study. The study protocol was approved by the Ethics Committee of Hokkaido Information University and was in conformity to the Helsinki Declaration (approval date: Dec 1, 2014; approval number: 2014-19). This study was registered with UMIN (approval number: UMIN000015940).

Sample size

The sample size was statistically determined to obtain a power of 80% with an alpha value of 0.05. In order to demonstrate an effect in the MMSE at 24 weeks after the start of the study, which was postulated to be a 1.00 increase with a standard deviation of 6.00, a sample size of 48 (24 in the active test food group and 24 in the placebo food group) was required. Assuming a 20% loss during follow-up, 60 subjects were selected.

Statistical analysis

Values are presented in the tables as means ± standard deviations. The Mann–Whitney U test was used to evaluate changes in cognitive function. Student's t-test was used to analyze differences in the physical, hematological, and biological parameters between the active test and placebo food groups at each evaluation point. Additionally, to clarify the effects of age on the MMSE and cognitive impairment rating scale scores, we divided the subjects into two subgroups with respect to the median age: younger subject group (subjects <72 years old; 11 subjects in the active test food group and 13 in the placebo food group) and older subject group (subjects ≥72 years old; 14 subjects in the active test food group and 12 in the placebo food group). Statistical analyses were performed using SPSS Statistics 19 (IBM, Armonk, NY, USA). A p-value of <0.05 was considered to be significant, while a p-value of <0.10 was defined as a marginal difference.

RESULTS

Subject dropouts, exclusions and characteristics

During the trial, 10 subjects withdrew due to personal reasons (dropout of study partner, n = 3; non-participation in examination, n = 2; and difficulty in continuing the trial, n = 5). Consequently,

50 subjects completed this trial (25 subjects in each group). Although some subjects were taking drugs, including anti-allergics, anti-hyperuricemics, and anti-osteoporotics, they did not change their drugs during the study period. Additionally, the number of subjects taking these drugs was not different between the active test and placebo food groups. Moreover, there was one smoker among our subjects. This subject smoked five cigarettes daily and did not change the number of cigarettes smoked per day during the study period. Therefore, we determined that drugs and nicotine/smoking may have not influenced outcome levels on the MSSE. No subject was excluded from the analysis of the "per protocol set." The flow diagram of the study is shown in Figure 2. The mean age, height, body weight, body mass index, body fat ratio, years of education, and cognitive impairment rating scale score for each group are presented in Table 2. These data did not significantly differ between the active test and placebo food groups, confirming the appropriate allocation of subjects in the two groups.

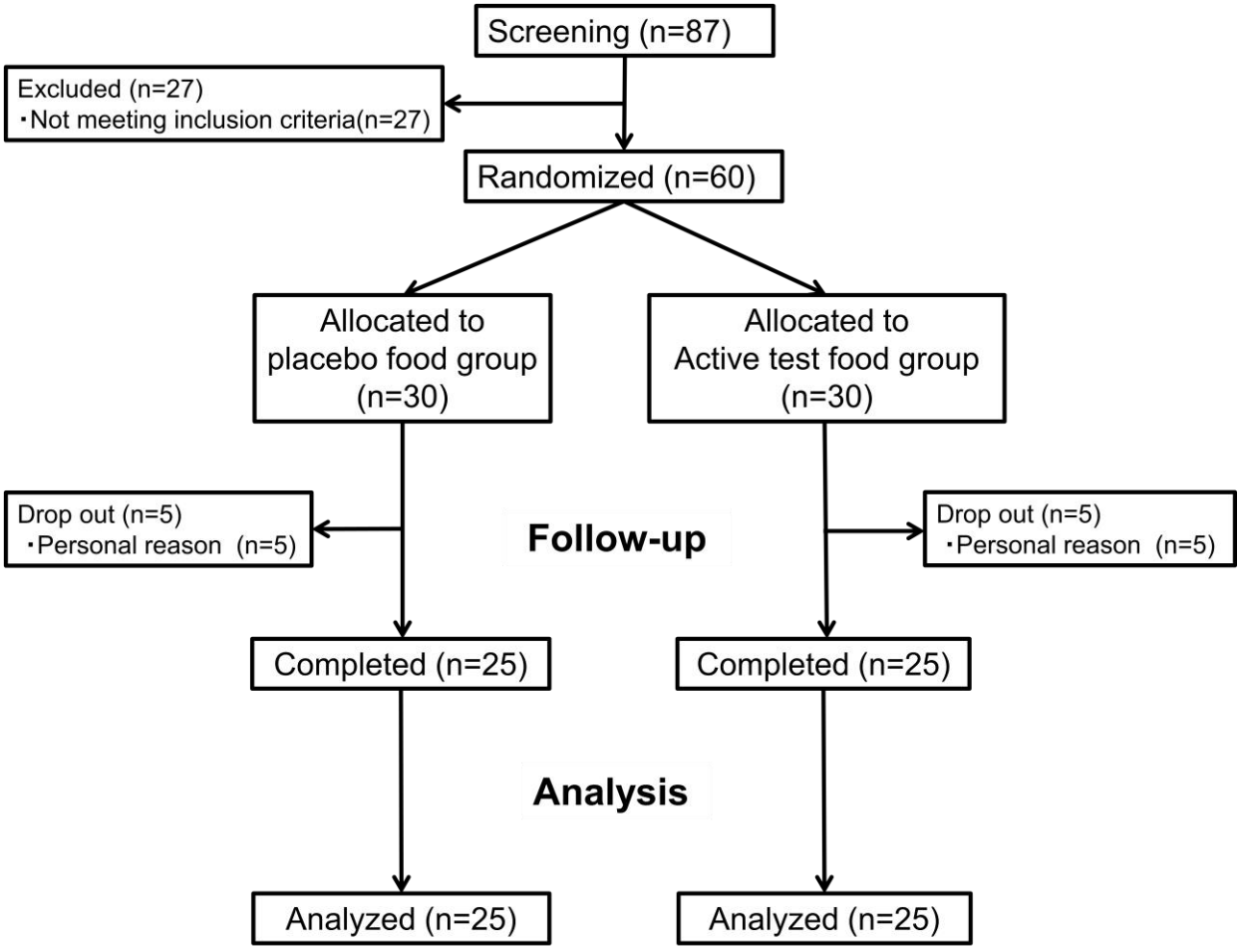

Figure 2. Flow diagram of the study.

Table 2. Characteristics of the subjects in the active test and placebo food groups at the screening test.

Characteristic	Active test food group	Placebo food group	*p*
Subjects, *n*	25	25	–
Males, *n* (%)	13 (52%)	12 (48%)	1.000
Age, years	71.52 ± 4.67	71.96 ± 5.05	0.750
Height, cm	160.00 ± 9.23	158.16 ± 8.38	0.465
Body weight, kg	60.29 ± 11.02	58.68 ± 10.57	0.600
Body fat ratio, %	27.56 ± 7.37	26.67 ± 4.94	0.619
Body mass index, kg/m^2	23.45 ± 3.11	23.29 ± 2.39	0.839
Education period, years	12.88 ± 2.39	11.92 ± 2.33	0.156
Immediate memory, score	4.96 ± 1.27	5.00 ± 1.12	0.833
Executive function, score	49.28 ± 10.72	47.96 ± 9.56	0.985
Memory recall, score	10.08 ± 2.02	10.96 ± 2.79	0.111
Short term memory, score	6.76 ± 1.30	6.72 ± 1.86	0.851
Intake rate, %	96.87 ± 4.16	98.49 ± 2.59	0.104

Values are shown as the mean ± standard deviation. Analyses were performed using Student's *t*-test for age, height, body weight, body fat ratio and body mass index, the chi-squared test for gender and Mann–Whitney's U test for the cognitive impairment rating scale and intake rate. *n* = number of subjects.

Effect of quercetin-rich onions on the MMSE and cognitive impairment rating scale scores

First, we evaluated the effect of quercetin-rich onions on cognitive function using the MMSE (Figure 3 and Table 3). There were no significant differences between the active test and placebo food groups regarding changes in the MMSE scores from the baseline to the other time points (Figure 3A). Although there were no significant differences between the active test and placebo food groups in the older subject group (Figure 3C), in the younger subject group there was a significant difference between the active test and placebo food groups in the change in the MMSE scores from baseline to week 24 (placebo: −0.31 ± 2.10, test: 1.64 ± 2.11, p = 0.019) (Figure 3B). We also examined the effect of quercetin-rich onions on the cognitive impairment rating scale scores (Figure 4 and Table 3). The values did not significantly differ between the active test and placebo food groups as a result of total analysis and subgroup analysis.

Effect of quercetin-rich onions on the NPI-Q-J scores

To confirm the effect of quercetin-rich onions on study partner parameters, we evaluated changes in the two NPI-Q-J indices: "Severity of subject's symptoms" and "Burden on study partner."

Consumption of the active test food marginally decreased "Severity of subject's symptom" at weeks 12 (change from baseline to week 12: placebo, 0.36 ± 2.38; test, −1.04 ± 2.56; p = 0.067) and 24 (placebo, −0.04 ± 1.49; test, −0.80 ± 1.76; p = 0.060) after the start of the study (Table 3). Moreover, compared to the placebo food group, "Burden on study partner" decreased in the active test food group at week 24 (change from baseline to week 24: placebo, 0.08 ± 1.91; test, −0.84 ± 2.27; p = 0.093) after the start of the study (Table 3).

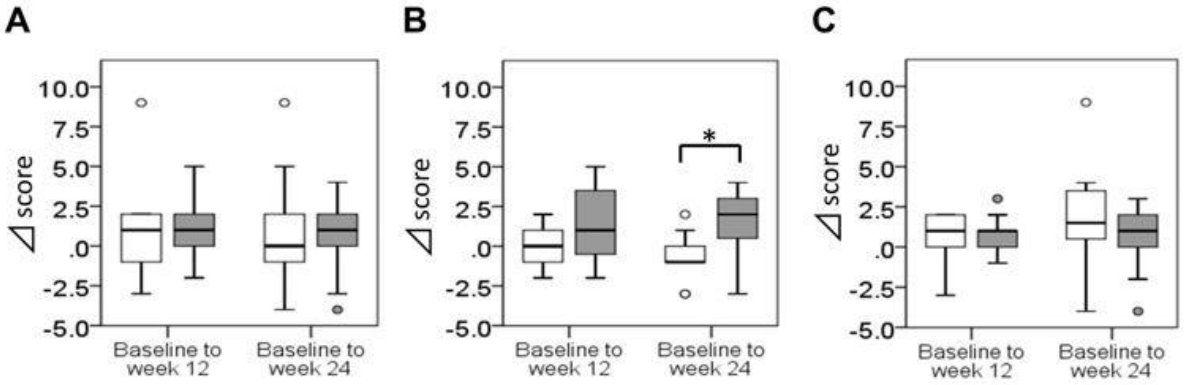

Figure 3. Changes in Mini-Mental State Examination (MMSE) scores at baseline and at each time point.

(A) MMSE score in all subjects. (B) MMSE score in subjects less than 72 years old. (C) MMSE score in subjects 72 years old or older. White boxplot: placebo food group. Grey boxplot: active test food group. *Statistically significant, p < 0.05. #Marginally significant, p < 0.10.

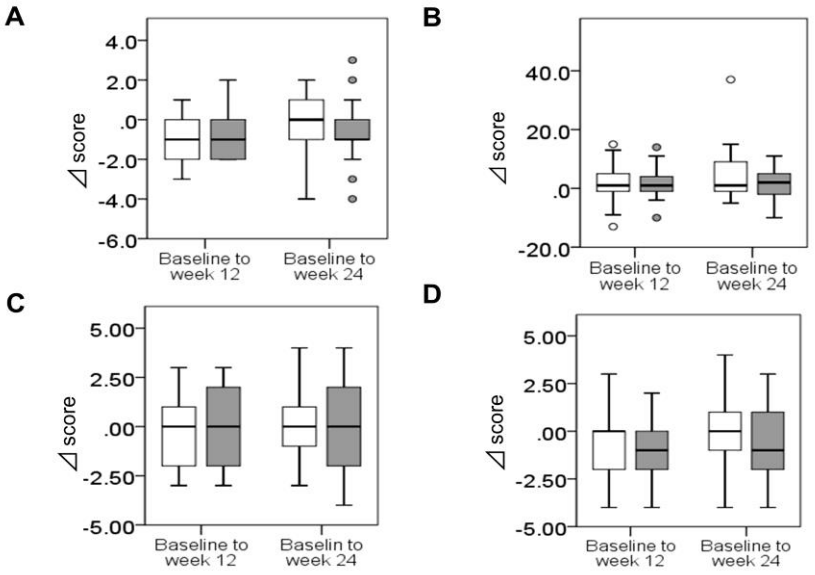

Figure 4. Changes in the cognitive impairment rating scale scores at baseline and at each time point. (A) Immediate memory. (B) Executive function. (C) Memory recall. (D) Short term memory. White boxplot: placebo food group. Grey boxplot: active test food group. *Statistically significant, p < 0.05. #Marginally significant, p < 0.1

Table 3. Cognitive function examination

		Week 0	Week 12	Week 24	⊿ week 12	⊿ week 24
MMSE in all subjects (score)	Placebo	27.16 ± 2.36	27.8 ± 1.68	27.92 ± 1.75	0.64 ± 2.36	0.76 ± 2.85
	Active	27.36 ± 1.96	28.4 ± 1.58	28.32 ± 1.65	1.04 ± 1.84	0.96 ± 2.01
	p	—	—	—	0.466	0.399
MMSE in younger subjects (score)	Placebo	28.23 ± 1.74	28.38 ± 1.80	27.92±1.71	0.15 ± 1.46	−0.31±2.10
	Active	27.36 ± 1.75	28.91 ± 1.22	29.00±1.26	1.55 ± 2.38	1.64 ± 2.11
	p	—	—	—	0.151	0.019*
MMSE in older subjects (score)	Placebo	26.00 ± 2.45	27.17 ± 1.34	27.92 ± 1.88	1.17 ± 3.04	1.92 ± 3.18
	Active	27.36 ± 2.17	28.00 ± 1.75	27.79 ± 1.76	0.64 ± 1.22	0.43 ± 1.83
	p	—	—	—	0.510	0.144
Immediate memory (score)	Placebo	5.12 ± 1.20	4.12 ± 0.97	4.76 ± 1.48	−1.00 ± 1.22	−0.36± 1.58
	Active	5.16 ± 1.25	4.44 ± 1.26	4.52 ± 1.29	−0.72 ± 1.31	−0.64± 1.55
	p	—	—	—	0.533	0.355
Executive function (score)	Placebo	50.12 ± 9.40	51.24± 10.47	53.12± 10.64	1.12 ± 7.09	3.00 ± 6.94
	Active	52.56± 11.71	54.48± 11.59	53.80± 11.83	1.92 ± 5.39	1.24 ± 5.62
	p	—	—	—	0.899	0.527
Memory recall (score)	Placebo	11.16 ± 2.41	10.96 ± 2.37	11.28 ± 1.93	−0.20 ± 1.94	0.12 ± 1.94
	Active	10.92 ± 2.16	10.76 ± 1.98	10.68 ± 1.57	−0.16 ± 1.99	−0.24±2.39
	p	—	—	—	0.945	0.754
Short term memory (score)	Placebo	5.96 ± 1.79	5.44 ± 2.36	5.96 ± 1.90	−0.52 ± 1.73	0 ± 1.71
	Active	6.52 ± 1.16	5.64 ± 1.80	5.80 ± 1.73	−0.88 ± 1.67	−0.72± 1.93
	p	—	—	—	0.560	0.193
NPI-Q-J Severity of subject's symptom (score)	Placebo	1.16 ± 2.25	1.52 ± 3.08	1.12 ± 2.40	0.36 ± 2.38	−0.04±1.49
	Active	2.56 ± 4.51	1.52 ± 2.52	1.76 ± 3.52	−1.04 ± 2.56	−0.80±1.76
	P	—	—	—	0.067#	0.060#
NPI-Q-J Burden on study partner (score)	Placebo	1.00 ± 2.31	1.36 ± 3.25	1.08 ± 2.12	0.36 ± 2.77	0.08 ± 1.91
	Active	2.92 ± 6.56	1.76 ± 3.62	2.08 ± 4.74	−1.16 ± 3.57	−0.84± 2.27
	P	—	—	—	0.382	0.093#

Values are shown as the mean ± standard deviation. Mann–Whitney's U test was performed to analyse changes in values from baseline to weeks 12 and 24. MMSE, Mini Mental State Examination; NPI-Q-J, Neuropsychiatric

Inventory-Questionnaire Japanese-language version; ⊿ week 12, change from baseline to weeks 12; ⊿ week 24, change from baseline to weeks 24. *Statistically significant, $p < 0.05$. #Marginally significant, $p < 0.10$.

Effect of quercetin-rich onions on lipid metabolism, glucose metabolism, and oxidative parameters

We evaluated the effect of quercetin-rich onions on lipid metabolism, glucose metabolism, and oxidative parameters and discovered no significant differences between the active test and placebo food groups (Table 4).

Table 4. Lipid metabolism, glucose metabolism and oxidative parameters

		Week 0	Week 12	Week 24	⊿ week 12	⊿ week 24
TC (mg/dl)	Placebo	221.36±38.38	221.20±39.94	220.80±40.75	−0.16 ± 21.07	−0.56 ± 30.05
	Active	219.56±41.73	222.68±42.53	215.12±40.00	3.12 ± 12.76	−4.44 ± 14.93
	p	—	—	—	0.509	0.566
HDL-C (mg/dl)	Placebo	66.68 ± 18.87	66.12 ± 18.65	64.68 ± 19.58	−0.56 ± 6.70	−2 ± 6.35
	Active	68.84 ± 15.40	67.44 ± 14.61	64.52 ± 13.46	−1.4 ± 7.23	−4.32 ± 6.57
	p	—	—	—	0.672	0.210
LDL-C (mg/dl)	Placebo	137.52±30.10	137.88±33.54	137.48±34.69	0.36 ± 17.44	−0.04 ± 26.02
	Active	136.04±36.30	136.48±34.42	131.72±35.35	0.44 ± 11.81	−4.32 ± 16.07
	p	—	—	—	0.985	0.487
TG (mg/dl)	Placebo	125.20±57.69	135.80±71.10	121.80±55.02	10.60 ± 35.56	−3.40 ± 39.88
	Active	110.68±41.49	129.28±52.07	119.72±42.91	18.60 ± 47.84	9.04 ± 40.97
	p	—	—	—	0.505	0.282
BG (mg/dl)	Placebo	104.00±19.19	96.8 ± 18.91	105.48±20.20	−7.2 ± 17.22	1.48 ± 12.45
	Active	110.44±25.71	100.52±18.22	102.92±15.20	−9.92 ± 16.80	−7.52 ± 20.24
	p	—	—	—	0.575	0.064#
HbA1c (%)	Placebo	5.63 ± 0.44	5.52 ± 0.48	5.61 ± 0.47	−0.10 ± 0.27	−0.02 ± 0.23
	Active	5.50 ± 0.55	5.49 ± 0.47	5.52 ± 0.44	−0.01 ± 0.25	0.01 ± 0.25
	p	—	—	—	0.215	0.645
ox-LDL (U/l)	Placebo	128.64±37.84	114.88±37.87	112.12±32.87	−13.76 ± 25.79	−16.52 ± 39.40
	Active	119.32±44.11	119.24±44.50	105.36±34.41	−0.08 ± 26.92	−13.96 ± 30.38
	p	—	—	—	0.073	0.798

Values are shown as the mean ± standard deviation. Student's *t*-test was performed to analyse changes in values from baseline to weeks 12 and 24. TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; BG, blood glucose; HbA1c, hemoglobin A1c; ox-LDL, oxidised LDL; ⊿ week 12, the change value from baseline to weeks 12; ⊿ week 24, the change value from baseline to weeks 24. *Statistically significant, $p < 0.05$. #Marginally significant, $p < 0.10$

Safety: We evaluated CBCs, liver and renal function, and BP after the ingestion of quercetin-rich onions. As shown in Table 5, minimal changes were observed in CBC parameters (WBC count, RBC count, Hb level, Ht, and Plt count), liver function (AST, ALT, γ-GTP, ALP, and LDH), renal function (BUN, CRE, and UA), Alb, TP, and BP. Although few subjects exhibited adverse events, they only had mild symptoms and recovered from these symptoms within a few days. Accordingly, the principal investigator judged that no adverse events were related to the ingestion of the test food. These results suggested that the ingestion of quercetin-rich onions ('Quergold' and 'Sarasara-gold') had no or minimal unfavorable effects, even at a dose of 83 g/day (raw-onion).

Table 5. Hematological and biochemical data

		Week 0	**Week 12**	**Week 24**
WBC (10^3/μl)	Placebo	5.44±1.24	5.71±1.05	5.89±1.38
	Active	5.71±1.22	5.90±1.08	5.62±1.02
RBC (10^4/μl)	Placebo	445.28±46.31	443.84±45.70	441.40±38.29
	Active	458.20±40.60	455.12±44.34	449.08±43.59
Hb (g/dl)	Placebo	13.86±1.47	13.61±1.29	13.65±1.20
	Active	14.30±1.03	14.04±1.28	13.87±1.18
Ht (%)	Placebo	41.74±4.18	41.91±3.83	41.29±3.23
	Active	42.70±2.83	42.75±3.56	41.68±3.51
Plt (10^4/μl)	Placebo	21.00±4.21	20.87±4.61	20.89±4.89
	Active	20.28±4.84	20.93±5.17	20.16±5.38
AST (U/l)	Placebo	26.52±7.04	25.76±6.69	26.20±7.53
	Active	24.24±4.19	23.88±3.40	22.88±3.92
ALT (U/l)	Placebo	21.44±9.73	19.64±7.98	20.12±9.11
	Active	20.92±7.93	21.40±7.19	19.84±6.90
γ-GTP (U/l)	Placebo	27.20±15.80	25.48±13.17	28.36±17.47
	Active	28.40±15.45	29.76±20.15	28.72±21.80

		Week 0	Week 12	Week 24
ALP (U/l)	Placebo	240.72±73.56	240.56±69.83	229.36±63.83
	Active	235.12±64.94	241.48±68.14	231.08±72.58
LDH (U/l)	Placebo	209.24±32.93	227.16±32.09	218.48±41.72
	Active	199.72±20.69	207.96±21.29	200.08±23.90
BUN (mg/dl)	Placebo	16.16±3.25	16.77±2.84	17.33±3.70
	Active	16.59±3.68	16.68±3.08	16.80±3.49
CRE (mg/dl)	Placebo	0.81±0.17	0.82±0.16	0.82±0.15
	Active	0.80±0.16	0.78±0.14	0.78±0.14
UA (mg/dl)	Placebo	5.11±1.52	5.08±1.38	5.13±1.53
	Active	4.98±1.09	4.94±1.31	4.94±1.10
Alb (g/dl)	Placebo	4.54±0.19	4.62±0.18	4.58±0.23
	Active	4.66±0.21	4.71±0.23	4.65±0.24
TP (g/dl)	Placebo	7.36±0.39	7.51±0.39	7.39±0.33
	Active	7.40±0.37	7.58±0.41	7.39±0.36
SBP (mmHg)	Placebo	137.76±19.99	131.20±17.23	129.36±21.14
	Active	138.32±20.75	133.16±19.29	128.84±15.96
DBP (mmHg)	Placebo	78.04±10.98	76.08±9.06	73.92±10.62
	Active	79.20±12.00	76.52±9.27	73.48±10.34

WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin, Ht, hematocrit; Plt, platelet count; AST, Aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; blood urea nitrogen, BUN; CRE, creatinine; UA, uric acid; SBP, systolic blood pressure; DBP, diastolic blood pressure. Values are mean ± standard division

DISCUSSION

Dementia has become a serious problem in aging societies such as Japan. Research on dementia therapy has been globally conducted. However, to date there are no therapeutics available to prevent or treat dementia. Recently, it has been recognized that lifestyle modifications, including exercise and diet, are important for preventing early stage dementia [32-34]. The results of our randomized, double-blind, placebo-controlled clinical trial identified the potential effects of quercetin-rich onions (‘Quergold’ and ‘Sarasara-gold’) on cognitive function. There were no significant differences between the active test and placebo food groups regarding changes in the

MMSE scores. However, among younger subjects the MMSE scores were significantly improved in the active test food group. These results suggest that quercetin-rich onions have potential beneficial effects on cognitive function.

Recently, Nakagawa *et al.* (2016) reported the results of a double-blind, placebo-controlled study on the effects of 'Quergold' onion powder (18 g for four weeks) on memory recall in patients with early stage AD. They used the revised Hasegawa Dementia Scale (HDS-R) for the evaluation and found a significant improvement in treated subjects compared to placebo subjects [28]. Although our clinical study did not uncover significant differences in memory recall, their results support the possibility of cognitive improvement induced by quercetin-rich onions. In the memory recall questionnaire of the HDS-R, the evaluator could provide hints if subjects could not immediately answer. However, the cognitive impairment rating scale used in our study did not provide hints. This difference in methodology might explain the differences in the results of the two studies.

It is unclear why the MMSE scores increased by treatment with quercetin-rich onions only in younger subjects. It has been reported that MMSE scores are related to the number of years of education [35]. In our clinical study, the number of years of education in the younger subject group exceeded that of the elderly subject group (younger subject group, 13.08 ± 2.24 years; elderly subject group, 11.77 ± 2.37 years; $p = 0.063$). These findings suggest that changes in cognitive functions in our elderly subjects with a low number of years of education cannot be detected by the MMSE. Alternatively, our results suggested that the intervention of dietary food might be required at an early stage to inhibit in cognitive decline. Additionally, our study period was shorter than those in other clinical trials that tested the effects of other foods (e.g., DHA and Ginkgo biloba) on cognitive function [36, 37]. Further investigations that take the number of years of education, in addition to alternative methods of cognitive function into consideration, are needed.

Quercetin has been shown to have anti-obesity [38], anti-diabetic [17], and anti-dyslipidemic [11, 12] effects. These diseases are known risk factors for dementia [39]. However, in our study measures of lipid metabolism, glucose metabolism, and oxidative parameters did not significantly differ between the placebo and active test food groups. Moreover, no relationship was observed between these parameters and changes in cognitive function values. Our hypothesis is that as these parameters were normal in most of our study subjects, the values were not affected by the ingestion of quercetin-rich onions. Further research will be necessary to elucidate the anti-dyslipidemic, anti-

dysglycemic, and anti-oxidant effects of quercetin-rich onions and the relationship of cognitive function with these parameters.

The NPI-Q-J score improved among subjects who consumed quercetin-rich onions. Notably, irritability was the most improved factor among the subquestions. BPSDs comprise a core set of symptoms in patients with dementia and can be observed before making a diagnosis of clinical dementia [40]. The development of BPSDs is associated with a faster cognitive decline, greater impairment in the activities of daily living, and lower quality of life for patients and caregivers [41]. In our clinical study, study partners answered "no symptoms (0 points)" to most questions because the study subjects were healthy or exhibited MCI. Although further research is needed, these results suggest that quercetin-rich onions improve BPSDs in patients with early stage dementia.

Our clinical trial included healthy or MCI subjects who were not receiving medications for dementia, AD, psychiatric disorders, or cerebrovascular diseases. In the future, we need to investigate the effects of quercetin-rich onions in different types of patients.

CONCLUSION

The results of our present study revealed that quercetin-rich onions had positive effects on cognitive function. Onions are components of the typical diets of elderly people, so we can provide new applications of quercetin-enriched food using quercetin-rich onions. The biological mechanism of these results should be elucidated to fully understand the effects of quercetin-rich onions. Although we demonstrated the effect of quercetin-rich onions on subjects of a particular age group, our results suggested that the intervention of dietary food is required at an early stage to improve cognitive function. Further studies of quercetin-rich onions will be needed to develop new treatment strategies for maintaining cognitive function.

List of Abbreviations: AD, Alzheimer's disease; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BG, blood glucose; BFR, body fat ratio; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; BW, body weight; CBC, complete blood count; CRE, creatinine; γ-GTP, gamma glutamyl transpeptidase; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; Ht, hematocrit; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; MCI, mild cognitive impairment; min, minute; MMSE, Mini Mental State Examination; NPI-Q-J, Neuropsychiatric Inventory-Questionnaire Japanese-language version; ox-LDL, oxidised LDL; Plt, platelet; RBC, red blood cell; TC, total cholesterol; TG, triglyceride; UA, uric acid; WBC, white blood cell.

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