*Original Article*

# The effect of 24-week continuous intake of quercetin-rich onion on age-related cognitive decline in healthy elderly people: a randomized, double-blind, placebo-controlled, parallel-group comparative clinical trial

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**Quercetin, a type of flavonoid, is believed to reduce age-related cognitive decline. To elucidate its potential function, we carried out a randomized, double-blind, placebo-controlled, parallel-group comparative clinical trial involving 24-week continuous intake of quercetin-rich onion compared to quercetin-free onion as a placebo. Seventy healthy Japanese individuals (aged 60 to 79 years old) were enrolled in this study. We examined the effect of quercetin-rich onion (the active test food) on cognitive function using the Mini-Mental State Examination, Cognitive Assessment for Dementia iPad version, and Neuropsychiatric Inventory Nursing Home version. The Mini-Mental State Examination scores were significantly improved in the active test food group (daily quercetin intake, 50 mg as aglycone equivalent) compared to the placebo food group after 24 weeks. On the Cognitive Assessment for Dementia iPad version for emotional function evaluation, we found that the scores of the active test food group were significantly improved, suggesting that quercetin prevents cognitive decline by improving depressive symptoms and elevating motivation. On the Neuropsychiatric Inventory Nursing Home version, we found significant effects on reducing the burden on study partners. Taking all the data together, we concluded that 24-week continuous intake of quercetin-rich onion reduces age-related cognitive decline, possibly by improving emotional conditions. Clinical trial register and their clinical registration number: This study was registered with UMIN (approval number UMIN000036276, 5 April 2019).**

***Key Words*: quercetin, onion, clinical trial, cognitive function, MMSE, CADi2, NPI-NH**

Cognitive decline caused by neurodegeneration due to advancing age is a serious health problem worldwide. According to a report from the World Health Organization, dementia is a rapidly growing public health problem affecting around 50 million people around the world.(1) There are nearly 10 million new cases every year, and this figure is set to triple by 2050. Dementia is a major cause of disability and dependency among aged people and can devastate the lives of affected individuals, their caregivers, and their families. In Japan, a survey by the Ministry of Health, Labour, and Welfare estimated that there were 4.62 million elderly people with dementia as of 2012. In addition, approximately 8 million people, including people with mild cognitive impairment (MCI), will develop dementia or possible dementia. Dementia not only causes deterioration of the patient's quality of life, but also places an extensive mental and financial burden on caregivers, such as family members. Although many therapeutic agents for dementia are currently under investigation, no fundamental curative method has been established. In recent years, a number of medical and health reports have been published on the effects of functional foods such as docosahexaenoic acid (DHA),(2,3) quercetin,(4) plasmalogen,(5) and astaxantin-tocotrienol combination on cognitive function.(6) These food compounds are expected to help prevent dementia, but further nutritional and clinical investigations are warranted.

Polyphenols, one of the major classes of plant food components, are suggested to prevent chronic diseases and cognitive decline.(7,8) Among the variety of polyphenol compounds, quercetin (3,3',4',5,6-pentahydroxyflavone) is a type of flavonoid that is ubiquitously present in onions, tea, green vegetables, and fruits. The average quercetin glycoside content of several yellow onion varieties is 36.1 mg/100 g fresh weight.(9) Green tea and black tea contain 4.3 and 5.2 mg of quercetin glycosides per 100 ml infusion, respectively.(10) A major function of quercetin is its antioxidant capacity.(11,12) In addition, habitual dietary intake of quercetin has been shown to suppress abdominal and hepatic fat accumulation. For example, hepatic steatosis in mice induced by a Western diet rich in fat and sucrose was reduced by quercetin, probably by decreasing oxidative stress, increasing the expression of peroxisome proliferator-activated receptor α (PPARα), and subsequently improving the expression of genes such as steatosis-related PPARγ and sterol regulatory element-binding protein 1 (SREBP1c).(13) Quercetin was suggested to suppress the accumulation and activation of macrophages and other immune cells and the resulting chronic inflammation and systemic insulin resistance in Western diet induced obese mice.(14) It is of interest that dietary flavonoids target injured arteries with activated macrophages in humans, probably to prevent atherosclerosis.(15) Moreover, it should be noted that foods containing multiple

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antioxidants such as quercetin accelerated energy metabolism during rest and exercise,[16] suggesting antioxidants in foods boost metabolic benefits in obesity.

It is also of interest that quercetin extracted from plants had neuroprotective effects against oxidative stress in cell cultures.[17] In an animal study on the effect of quercetin on cognitive function, the phenolic compound improved cognitive impairment linked to spatial learning and memory in streptozotocin-induced diabetic rats.[18] Quercetin may prevent cognitive decline through the molecular pathway involving the activation of AMP-activated protein kinase leading to PP2C expression, observed in high cholesterol induced neurotoxicity in mice.[19] Moreover, quercetin improved memory in aged mice and delayed their memory decline by activating GADD34 in the early stages of Alzheimer's in mice.[20] Recently, series on the effect of quercetin on the prevention of cognitive dysfunction have been reported. *In vitro*, quercetin increased the survival rate of a PC12 Alzheimer's disease cell model.[21] *In vivo*, on the other hand, plasma exosome loaded with quercetin improved the cognitive function of Alzheimer model mice,[22] and quercetin alleviated the cognitive impairment of aged ICR mice induced by advanced glycation end products.[23]

We previously conducted a randomized, placebo-controlled, double-blind, parallel-group comparison study evaluating the effect of continuous intake of quercetin-rich onions 'Quel Gold' and 'Sarasara Gold' in 50 elderly subjects.[4] The study showed that continuous intake of quercetin-rich onion powder failed to improve cognitive function as evaluated by Mini-Mental State Examination (MMSE) and cognitive impairment rating scale scores, but an improvement of MMSE scores was observed in a younger group in a subclass analysis. The previous report was the first and only study demonstrating the effectiveness of quercetin-rich onion in cognitive improvement, and no other follow-up studies have been available to date. Accordingly, we needed to confirm the repeatability of the favorable function of quercetin-rich 'Sarasara Gold' onion in cognitive function, together with additional clinical parameters.

To elucidate the effect of quercetin-rich onions on age-related cognitive decline, we conducted a new clinical trial, a randomized, placebo-controlled, double-blind parallel group comparative study of the effect on cognitive function of continuous 24-week intake of quercetin-rich onions. This study included 70 Japanese men and women between the ages of 60 and 80 years as subjects.

## Materials and Methods

**Study subjects.** Members of the general public who were registrants of clinical trials managed by the Health Information Science Research Center, Hokkaido Information University, were notified of the study outline. We confirmed each applicant's willingness to receive an explanation of the test, and we obtained informed consent. Applicants recruited from the general public were required to register as potential study subjects for the clinical trial. Among them, appropriate subjects were selected according to the following inclusion and exclusion criteria.

The inclusion criteria permitted entry into the study for (1) subjects who fully understood the meaning, content, and purpose of this clinical trial and provided informed consent to participate; (2) Japanese men and women aged 60 to 80 years; (3) subjects who could undergo cognitive function tests and fill in diaries and questionnaires themselves; and (4) individuals as study partners who lived with the subjects in the same household and could evaluate their daily activities.

The exclusion criteria prohibited the following from participating in the study: (1) subjects with an MMSE score of 23 or less (Dementia Disease Guideline 2017, The Japanese Neurological Society); (2) subjects who were treated for dementia, Alzheimer's disease, mental illness, or cerebrovascular disease; (3) subjects with a history or suspicion of mental illness or cerebrovascular disease; (4) subjects suffering from serious cerebrovascular disease, heart disease, liver disease, renal disease, digestive system disease, infectious disease, etc.; (5) subjects who had a history of gastrointestinal surgery, such as gastrectomy, gastrointestinal suture, or intestinal resection; (6) subjects with chronic blood pressure (BP) problems or who showed chronic medical problems by anthropometry and blood tests; (7) subjects with severe anemia; (8) pre- and post-menopausal women with marked symptoms in their mental and physical conditions; (9) subjects who may have allergic reactions to drugs or foods (especially onions); (10) subjects who have engaged in long-term use of specific medications, health foods, or supplements (DHA, EPA, *Ginkgo biloba* extract, γ-aminobutyric acid, glycine, plasmalogen, etc.) that may affect cognitive function; and (11) heavy smokers, alcohol addicts, or subjects with an irregular lifestyle.

A consent form approved by the Bioethics Committee of Hokkaido Information University was provided to the subjects and a detailed explanation of this clinical trial was given in writing, and written informed consent was then obtained. Subjects agreed to maintain their diet and exercise habits during the study at the same levels as before the study, and to avoid strenuous exercise and drinking the day before the test.

We assessed any signs of psychiatric problems through medical interviews with the subjects and their study partners, and then obtained the medical histories and records of the subjects (Fig. 1). We identified 70 eligible subjects (30 men and 40 women, aged 60–79 years) and randomly assigned them to the active test food (quercetin-rich onion) or placebo food (quercetin-free onion) group.

Adjustments for age, gender, and MMSE score were then completed. The randomization sequence was created using a permuted block randomization design, stratified by age, gender, and MMSE score. A third-party data center allocated each subject to the relevant group according to stratification factors of gender, age structure, and MMSE score, thereby ensuring that each group was well balanced. The block size was 24 [gender (male/female), age (60s and 70s), and MMSE score (24–29)]. 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 fixed, and the data were then subjected to statistical analysis.

**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 on the MMSE at 24 weeks after the start of the study, which was postulated to be a 1.00 increase with a SD of 1.3, a sample size of 56 (28 in the active food group and 28 in the placebo food group) was required. Assuming a 20% loss during follow-up, 70 subjects were selected.

**Study design.** The clinical study was conducted as a double-blind, randomized, placebo-controlled trial. The schedule of this clinical study is shown in Fig. 1 and Supplemental Table 1*. Seventy qualified subjects participated in this study. The washout period was set as 1 week before the intake of either test food was started. We performed multiple cognitive function tests [MMSE, Cognitive Assessment for Dementia iPad version (CADi2), a verbal recall test, and Neuropsychiatric Inventory Nursing Home version (NPI-NH)] at week 0 (baseline), 12, and 24 after the start of the active test food or placebo food intake. 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 understood the Japanese version of NPI-NH and provided informed consent. We set the primary outcome as the MMSE score.

To control the confounding source of quercetin in the diet,

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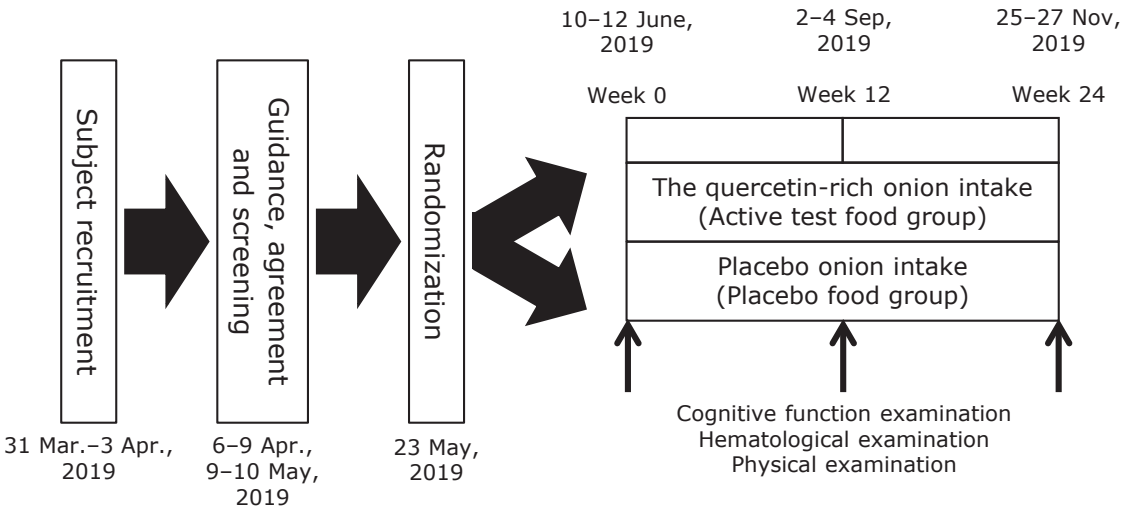


**Fig. 1.** Schedule of clinical study.

subjects were restricted from consuming onions and tea (green, black, and oolong) during this study.

**Preparation of test foods.** The quercetin-rich 'Sarasara Gold' onion used in this study is an F1 obtained from parental crosses by selecting breeding materials based on their quercetin-rich characteristics. One 'Sarasara Gold' onion bulb (approximately 200 g) contains a significant amount of quercetin (an average of 200 mg of glycosides). 'Sarasara Gold' was cultivated in Hokkaido, Japan. White onions did not have detectable levels of quercetin and were used as placebo quercetin-free onions. The active and placebo test food onion powders were manufactured by Okamoto Plant Breeding Co., Ltd. (Kuriyama, Japan) in compliance with the Food Sanitation Act (Ministry of Health, Labour and Welfare).

The following process was used to manufacture the onion powder: peeled onions were soaked in hypochlorous acid solution (200 ppm concentration) 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 'Sarasara Gold' and the amount of quercetin contained in the active food was adjusted accordingly. The analytical results of the nutrient composition of the active and placebo foods used in this study are summarized in Table 1. The active and placebo foods were identical in appearance.

Concerning food safety, the daily intake of air-dried powder of the active food, 11 g, corresponded to about 120 g of raw onion (about half of a medium-sized onion), which is a safe intake amount. The amount of quercetin, a functional ingredient of this active food (aglycone equivalent), was about 50 mg, and this was comparable to 72 mg of quercetin (aglycone equivalent) contained in products approved as foods for specified health uses. Thus, the intake was considered to be safe. In addition, since the estimated daily intake of quercetin is approximately 50 to 100 mg per day, the amount of quercetin in this active food was within the range of the normal daily intake.

**Table 1.** Nutrient composition of test food per day

| Nutrient | Unit | Placebo | Quercetin-rich onion |
|---|---|---|---|
| Calories | kcal | 42.5 | 42.9 |
| Water | g | 0.4 | 0 |
| Proteins | g | 0.7 | 1.0 |
| Lipids | g | 0.3 | 0.2 |
| Carbohydrates | g | 9.1 | 9.3 |
| Sodium | mg | 14.9 | 2.5 |
| Quercetin aglycone | mg | 0 | 50 |

Subjects ingested 11 g of test food daily for 24 weeks. We did not restrict intake times or cooking methods.

**Examination of cognitive function.** Cognitive function examinations, including MMSE, CADi2 including the verbal recall test, and NPI-NH, were performed at the beginning of the study and at weeks 12 and 24 after the start of therapy. 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.

**MMSE.** The MMSE is a cognitive status test that has been used most commonly to screen for MCI and dementia.[24] A well-trained nurse administered the MMSE to each subject. The questionnaire consists of 11 questions used to evaluate registration, attention and calculation, recall, language, and the ability to follow simple commands and space orientation. The maximum score on the test is 30 points.

**CADi2.** Mass screening for dementia requires a short, simple test that does not require expert examiners. Cognitive function is sometimes affected by emotional functions, for which CADi2 (iPad version) has been used for brain checkups in patients with cognitive decline and in healthy subjects.[25–27] The CADi2 consists of cognitive function and emotional function sections [Self-Rating Depression Scale (SDS) and apathy scales]. While cognitive function is evaluated by the total score and response time, emotional function is assessed based on mood and motivation. A verbal recall test was also carried out to enhance the CADi2 test. In the verbal recall test, the subjects wrote down as many specifically categorized words as they could recall in 1 min.

**NPI-NH.** 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.[28] The NPI-NH measures 10 categories of behavioral disturbance: delusions, hallucinations, excitation, depression/dysphoria, anxiety, euphoria, indifference, disinhibition, irritability, and abnormal behavior. The study partner completed the NPI-NH questionnaires regarding "Severity of subject's symptoms" (from 0, no symptoms, to 3, serious

symptoms) and "Burden on study partner" (from 0, no burden, to 3, serious burden).

**Physical, hematological, and biological examinations.** Blood samples were taken for testing at 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 (BMI), and body fat percentage] and BP were measured. General blood tests were performed to measure lipids [triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol]; glucose metabolism [blood glucose (BG) and HbA1c]; complete blood count [CBC; white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), hematocrit (Ht), and platelets (Plt)]; liver function [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)]; kidney function (blood urea nitrogen (BUN), creatinine (CRE), and uric acid (UA)]; albumin (Alb); total protein (TP); and blood quercetin. The Sapporo Clinical Laboratory, Inc. (Sapporo, Japan), performed all blood tests. Body composition and BP were measured using a DC-320 Body Composition Analyzer (Tanita Corp, Tokyo, Japan) and an HEM-7080IC Automatic Blood Pressure Monitor (Omron Colin Co., Ltd., Tokyo, Japan), respectively.

**Serious adverse events.** If adverse events including death, life-threatening conditions, or hospitalization occurred, they were classified as serious adverse events. The investigator would immediately discontinue the subject's intake of the active food and take the necessary measures, regardless of whether the adverse event was causally related to the test foods.

**Ethics committee.** This study conformed to the Ethics Guideline for Medical Research on Humans (28 February 2017, Ministry of Education, Culture, Sports, Science and Technology, Partial Revision), and the Declaration of Helsinki (October 2013, Revision of World Medical Association Fortaleza General Assembly). Prior to study implementation, the feasibility of the clinical trials and the ethical and scientific validity were examined and approved by the Bioethics Committee of Hokkaido Information University. The test was conducted based on the test plan approved by the Ethics Review Committee (approval date: 26 February 2019, approval number: 2018-21). This study was registered with UMIN (approval number UMIN000036276, 5 April 2019).

**Statistical analysis.** Values are presented in the tables as means ± SD. Data normality was checked by visual inspection of the histograms. 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 and placebo food groups at each evaluation point. Statistical analyses were performed using SPSS Statistics 19 (IBM, Armonk, NY). A $p$ value of $<0.05$ was considered significant, while a $p$ value of $<0.10$ was defined as a marginal difference.

We planned this study with consideration of possible factors affecting cognition (see Study subjects, and Study design), but we could not perfectly exclude subjects who used medication regularly, such as antihypertensive drugs and therapeutic agents for dyslipidemia. We performed subclass analysis in order to rule out the possibility that the medicines used by the subjects may have affected the efficacy of the test food. In the subclass analysis, the subjects who used medicines regularly were excluded from the efficacy analysis population, and efficacy analysis of the test foods was performed. The definition of a subject on medicine was "a person who regularly took prescription drugs during the study period."

## Results

**Subject characteristics.** As a result of the screening, we selected 70 subjects eligible to participate in the clinical study. The details of enrollment show the change in the number of subjects during the trial period as a flowchart (Fig. 2). In brief, two subjects withdrew before starting the intake of test food for personal reasons. In the active food group, one subject with a food intake rate below 80% was excluded, and three subjects dropped out because of uterine cancer, fatty liver, and difficulty with food intake. On the other hand, two subjects in the placebo food group dropped out, one to receive medication for diarrhea and one for personal reasons. As for the safety analysis, two subjects were excluded; one dropped out before the start of the study, and one rejected data used for this study. Regarding the efficacy analysis, we excluded six subjects who dropped out or discontinued or deviated from the protocol.

As for the allotment, the number of subjects is shown with the subject's age, anthropometric values (including height, weight, BMI, and body fat percentage), MMSE score, education periods, and intake rate (Table 2). The intake rate is expressed as a percentage of the actual number of days of test food intake out of the prescribed number of days (168 days).

Two independent *t* tests were performed, and there was no significant difference among the subjects. In the efficacy analysis, no significant difference was observed.

**Evaluation of cognitive function.** As for the change in MMSE score from the baseline to the other time points, significantly higher values were observed in the active test food group than the placebo food group for specific questions, the sum score at 24 weeks ($p = 0.024$), and temporal orientation at 12 weeks ($p = 0.046$) and 24 weeks ($p = 0.046$) (Table 3 and Fig. 3A). In addition, the score for copying the diagram was also improved in the active test food group ($p = 0.024$) compared to the placebo food group at 12 weeks.

Furthermore, to exclude the potential effects of the long-term use of medication on the overall results, we carried out a subclass analysis after excluding subjects on medication. Among the 62 subjects who completed the test, 38 subjects (19 each in the two groups) were included.

In the subclass analysis of MMSE, the change in the total score in the active test food group significantly improved compared to the placebo food group 24 weeks after intake ($p = 0.028$) (Table 4 and Fig. 3B), similar to the result for all subjects (Table 3 and Fig. 3A). For specific questions, however, the score for the stage command was statistically significantly higher in the active food group at 24 weeks after intake compared to the placebo food group ($p = 0.018$).

As for CADi2, we examined each individual test assessing cognitive function, including the total score and total response time, and emotion function, including mood [Self-Rating Depression Scale (SDS)] and motivation (apathy scale) (Table 5). No significant difference was observed in the total cognitive function score between the two groups at any time point. As for the total response time, the active test food group showed a significantly higher value at 12 weeks compared with the placebo food group ($p = 0.047$) (Table 5). As for the SDS, we found significant improvement in the active test food group ($p = 0.020$) (Table 5 and Fig. 4A). Similarly, regarding the change in score on the apathy scale, the active food test group showed a significantly low value 24 weeks after intake compared with the placebo food group ($p = 0.021$) (Table 5 and Fig. 4B). In the verbal recall test, regarding the changes of values, no significant difference was observed between the two groups at any time point.

In the subclass analysis of CADi2, the total score and total response time in cognition showed little difference between the two groups at any time point (Supplemental Table 2*). On the other hand, for the emotional function evaluation, the SDS was

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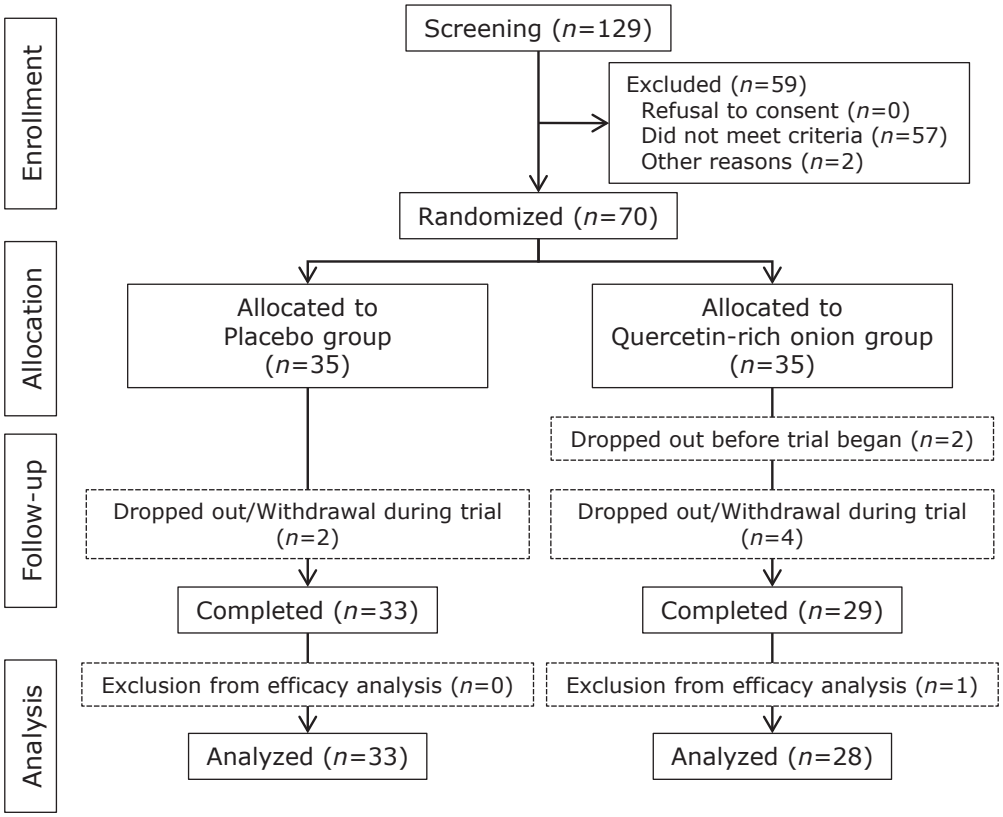


**Fig. 2.** Flowchart of subject selection.

**Table 2.** Characteristics of subjects and intake rates

| Characteristic | Placebo group | Quercetin-rich onion group | *p* |
|---|---|---|---|
| Subjects (*n*) | 33 | 28 | — |
| Male (*n*) | 15 | 12 | 1.00 |
| Age (year) | 71 ± 4 | 69 ± 4 | 0.13 |
| Body fat percentage (%) | 25.9 ± 6.0 | 27.0 ± 6.5 | 0.51 |
| Body mass index (kg/m$^2$) | 22.3 ± 3.0 | 22.6 ± 3.1 | 0.71 |
| MMSE (score) | 27.5 ± 1.4 | 27.1 ± 1.5 | 0.40 |
| Education periods (year) | 12 ± 2 | 13 ± 2 | 0.073 |
| Intake rate (%) | 98.3 ± 2.4 | 98.3 ± 3.2 | 0.62 |

Values are shown as mean ± SD. Fisher's exact probability test was used for sex, and the Mann–Whitney *U* test was used for Mini-Mental State Examination (MMSE) score, education periods, and intake rate. Student's *t* test was used for other characteristics.

significantly lower (improved) in the active test food group than in the placebo food group 24 weeks after intake ($p = 0.004$). In the emotional function evaluation apathy scale, some tendency, though not statistically significant, was observed between the two groups in the change in scores 24 weeks after intake ($p = 0.099$).

**NPI-NH.** The Mann–Whitney *U* test was performed to analyze the changes in values (Table 6). Regarding the changes in NPI-NH severity scores, no significant difference was observed between the two groups at any time point. However, the change in scores for burden on study partner was significantly lower in the active test food group than the placebo food group at 12 weeks ($p = 0.012$) and 24 weeks ($p = 0.022$) after intake. In subclass analysis, the NPI-NH scores for both severity of subject's symptoms and burden showed no difference under any conditions (Supplemental Table 3*).

**Lipid and glucose metabolism.** An independent two-sample *t* test was performed to assess lipid and glucose metabolism, as shown in Table 7. Significant differences were observed between the two groups at only a few time points. In brief, the change in TC level was significantly greater in the active test food group than in the placebo food group at 12 weeks ($p = 0.043$) and 24 weeks ($p = 0.026$) after intake. There was no significant difference in HDL-C, but LDL-C was significantly higher in the active food group than the placebo food group at 12 weeks after intake ($p = 0.029$). No significant difference was observed in BG level or HbA1c between the two groups at any time point. In the subclass analysis, with regard to lipid and glucose metabolism, no significant difference was observed between the two groups in the change in scores at any time point (Supplemental Table 4*).

*See online. https://doi.org/10.3164/jcbn.21-17

**Table 3.** MMSE

| Variable | | *n* | Baseline | ΔWeek 12 | ΔWeek 24 |
|---|---|---|---|---|---|
| MMSE Sum score (score) | Placebo | 33 | 27.5 ± 1.4 | 1.2 ± 1.9 | 0.9 ± 1.7 |
| | Active | 28 | 27.1 ± 1.5 | 1.6 ± 1.9 | 1.7 ± 1.9 |
| | *p* | | 0.401 | 0.450 | 0.024* |
| MMSE Temporal orientation (score) | Placebo | 33 | 5.0 ± 0.0 | 0.0 ± 0.2 | 0.0 ± 0.2 |
| | Active | 28 | 4.9 ± 0.3 | 0.1 ± 0.3 | 0.1 ± 0.3 |
| | *p* | | 0.083 | 0.046* | 0.046* |
| MMSE Spatial orientation (score) | Placebo | 33 | 4.0 ± 0.8 | 0.6 ± 0.8 | 0.6 ± 0.9 |
| | Active | 28 | 4.4 ± 0.7 | 0.3 ± 1.0 | 0.4 ± 0.9 |
| | *p* | | 0.096 | 0.224 | 0.446 |
| MMSE Registration (score) | Placebo | 33 | 3.0 ± 0.0 | –0.1 ± 0.5 | 0.0 ± 0.0 |
| | Active | 28 | 2.9 ± 0.6 | 0.1 ± 0.6 | 0.1 ± 0.6 |
| | *p* | | 0.212 | 0.184 | 0.212 |
| MMSE Attention (score) | Placebo | 33 | 4.2 ± 1.0 | 0.6 ± 1.1 | 0.3 ± 1.0 |
| | Active | 28 | 4.0 ± 0.9 | 0.5 ± 0.9 | 0.6 ± 1.2 |
| | *p* | | 0.478 | 0.795 | 0.286 |
| MMSE Remote memory (score) | Placebo | 33 | 2.8 ± 0.5 | 0.0 ± 0.7 | 0.1 ± 0.6 |
| | Active | 28 | 2.7 ± 0.5 | 0.2 ± 0.5 | 0.3 ± 0.6 |
| | *p* | | 0.746 | 0.239 | 0.292 |
| MMSE Naming two objects (score) | Placebo | 33 | 2.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Active | 28 | 2.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | *p* | | 1.000 | 1.000 | 1.000 |
| MMSE Repeat (score) | Placebo | 33 | 1.0 ± 0.0 | 0.0 ± 0.2 | –0.1 ± 0.2 |
| | Active | 28 | 0.8 ± 0.4 | 0.1 ± 0.4 | 0.0 ± 0.4 |
| | *p* | | 0.022* | 0.112 | 0.275 |
| MMSE Stage command (score) | Placebo | 33 | 2.6 ± 0.6 | 0.2 ± 0.7 | 0.2 ± 0.7 |
| | Active | 28 | 2.8 ± 0.4 | 0.0 ± 0.7 | 0.1 ± 0.6 |
| | *p* | | 0.100 | 0.341 | 0.821 |
| MMSE Writing a complete sentence (score) | Placebo | 33 | 1.0 ± 0.0 | 0.0 ± 0.2 | 0.0 ± 0.2 |
| | Active | 28 | 1.0 ± 0.2 | 0.0 ± 0.2 | 0.0 ± 0.3 |
| | *p* | | 0.326 | 0.161 | 0.601 |
| MMSE Reading and obey (score) | Placebo | 33 | 0.9 ± 0.3 | –0.1 ± 0.2 | –0.1 ± 0.3 |
| | Active | 28 | 0.9 ± 0.4 | 0.0 ± 0.4 | –0.1 ± 0.4 |
| | *p* | | 0.534 | 0.458 | 0.859 |
| MMSE Copy the diagram (score) | Placebo | 33 | 1.0 ± 0.2 | 0.0 ± 0.2 | –0.1 ± 0.2 |
| | Active | 28 | 0.9 ± 0.4 | 0.1 ± 0.4 | 0.1 ± 0.4 |
| | *p* | | 0.136 | 0.024* | 0.067 |

Values are shown as mean ± SD. ΔWeek 12 and 24: change in value from baseline to week 12 and 24, respectively. Mann–Whitney *U* test was performed. *$p<0.05$ vs placebo group. MMSE, Mini-Mental State Examination.

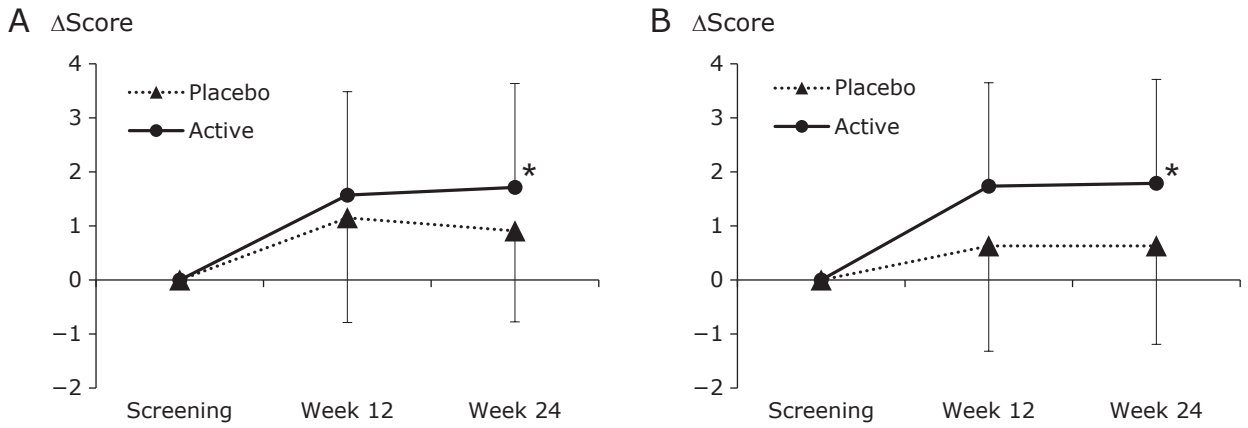


**Fig. 3.** Changes in Mini-Mental State Examination: (A) total subjects and (B) subclass analysis (placebo food group: $n = 19$; active test food group: $n = 19$). Values are shown as mean ± SD. Mann–Whitney *U* test was conducted for data analysis. *$p<0.05$ vs placebo group.



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**Table 4.** MMSE (subclass analysis)

| Variable | | *n* | Baseline | ΔWeek 12 | ΔWeek 24 |
|---|---|---|---|---|---|
| MMSE Sum score (score) | Placebo | 19 | 27.9 ± 1.1 | 0.6 ± 1.9 | 0.6 ± 1.5 |
| | Active | 19 | 27.1 ± 1.6 | 1.7 ± 1.8 | 1.8 ± 1.9 |
| | *p* | | 0.100 | 0.113 | 0.028* |
| MMSE Temporal orientation (score) | Placebo | 19 | 5.0 ± 0.0 | –0.1 ± 0.2 | 0.0 ± 0.0 |
| | Active | 19 | 4.9 ± 0.3 | 0.1 ± 0.3 | 0.1 ± 0.3 |
| | *p* | | 0.152 | 0.086 | 0.152 |
| MMSE Spatial orientation (score) | Placebo | 19 | 4.3 ± 0.7 | 0.3 ± 0.7 | 0.4 ± 0.8 |
| | Active | 19 | 4.3 ± 0.7 | 0.5 ± 0.8 | 0.5 ± 0.8 |
| | *p* | | 0.860 | 0.386 | 0.550 |
| MMSE Registration (score) | Placebo | 19 | 3.0 ± 0.0 | –0.2 ± 0.7 | 0.0 ± 0.0 |
| | Active | 19 | 2.9 ± 0.2 | 0.0 ± 0.3 | 0.1 ± 0.2 |
| | *p* | | 0.317 | 0.553 | 0.317 |
| MMSE Attention (score) | Placebo | 19 | 4.4 ± 0.7 | 0.5 ± 0.8 | 0.4 ± 0.6 |
| | Active | 19 | 4.0 ± 1.0 | 0.6 ± 0.9 | 0.5 ± 1.4 |
| | *p* | | 0.285 | 0.975 | 0.345 |
| MMSE Remote memory (score) | Placebo | 19 | 2.7 ± 0.6 | 0.1 ± 0.7 | 0.2 ± 0.5 |
| | Active | 19 | 2.7 ± 0.6 | 0.2 ± 0.4 | 0.3 ± 0.6 |
| | *p* | | 0.723 | 0.453 | 0.708 |
| MMSE Naming two objects (score) | Placebo | 19 | 2.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Active | 19 | 2.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | *p* | | 1.000 | 1.000 | 1.000 |
| MMSE Repeat (score) | Placebo | 19 | 1.0 ± 0.0 | –0.1 ± 0.2 | –0.1 ± 0.3 |
| | Active | 19 | 0.8 ± 0.4 | 0.1 ± 0.5 | 0.1 ± 0.4 |
| | *p* | | 0.037* | 0.179 | 0.188 |
| MMSE Stage command (score) | Placebo | 19 | 2.7 ± 0.5 | 0.1 ± 0.7 | 0.0 ± 0.6 |
| | Active | 19 | 2.7 ± 0.5 | 0.0 ± 0.8 | 0.3 ± 0.5 |
| | *p* | | 1.000 | 0.889 | 0.142 |
| MMSE Writing a complete sentence (score) | Placebo | 19 | 1.0 ± 0.0 | –0.1 ± 0.2 | 0.0 ± 0.0 |
| | Active | 19 | 0.9 ± 0.2 | 0.1 ± 0.2 | 0.1 ± 0.2 |
| | *p* | | 0.317 | 0.163 | 0.317 |
| MMSE Reading and obey (score) | Placebo | 19 | 0.9 ± 0.3 | –0.1 ± 0.2 | –0.1 ± 0.3 |
| | Active | 19 | 0.8 ± 0.4 | 0.0 ± 0.3 | –0.1 ± 0.3 |
| | *p* | | 0.636 | 0.574 | 1.000 |
| MMSE Copy the diagram (score) | Placebo | 19 | 0.9 ± 0.2 | 0.0 ± 0.0 | –0.1 ± 0.2 |
| | Active | 19 | 0.8 ± 0.4 | 0.2 ± 0.4 | 0.1 ± 0.5 |
| | *p* | | 0.297 | 0.075 | 0.179 |

Values are shown as mean ± SD. ΔWeek 12 and 24: change in value from baseline to week 12 and 24, respectively. Mann–Whitney *U* test was performed. *$p<0.05$ vs placebo group. MMSE, Mini-Mental State Examination.

**Subject background and food safety.** With regard to body weight, body fat percentage, and BMI, no significant difference was observed between the two groups at any time point (Table 8). Minimal changes were observed in CBC parameters (WBC, RBC, Hb, Ht, and Plt), liver function (AST, ALT, γ-GTP, ALP, and LDH), renal function (BUN, CRE, and UA), Alb, TP, and BP. These results indicate that consuming quercetin-rich onion ('Sarasara Gold') had no or minimal unfavorable effects, even in the amount present in the active test food in this study.

## Discussion

In this study, we demonstrated that 24-week continuous intake of quercetin-rich onion improved cognitive function as measured by MMSE scores in a randomized, double-blind, placebo-controlled, parallel-group comparative clinical trial (number of participants = 70). Although the mechanism of quercetin with regard to cognitive function is not fully understood, it is suggested that quercetin-rich onion could exert favorable action on cognition by improving emotional functions of depression and motivation, as examined by CADi2.

It is generally considered that improving cognitive function takes time, possibly one year or more. We here demonstrated an improvement in cognitive function within a relatively short duration (24 weeks) associated with consumption of quercetin-rich onion. Recently, two double-blind clinical trials revealed that supplementation with functional foods containing anserine/carnosine (54 participants)[29] or polyunsaturated fatty acids (46 participants)[30] could improve cognitive function within 3 and 6 months, respectively. These data, including our current data, indicate that preclinical and mild cognitive decline could be improved within a relative shorter period time, e.g., 3–6 months, and be well evaluated by a relatively small number of participants, e.g., 50 subjects (25 subjects for active foods, 25 for placebo).

Flavonoids such as quercetin can have multiple beneficial

**Table 5.** CADi2 iPad version, verbal recall test

| Variable | | *n* | Baseline | ΔWeek 12 | ΔWeek 24 |
|---|---|---|---|---|---|
| CADi2 total score (score) | Placebo | 33 | 8.8 ± 1.0 | 0.3 ± 1.2 | 0.2 ± 1.0 |
| | Active | 28 | 9.3 ± 1.0 | 0.0 ± 1.0 | 0.0 ± 0.9 |
| | *p* | | 0.014* | 0.501 | 0.262 |
| CADi2 total response time (s) | Placebo | 33 | 94.5 ± 22.1 | –9.7 ± 26.6 | –7.3 ± 21.5 |
| | Active | 28 | 98.9 ± 34.8 | 2.2 ± 48.1 | –11.9 ± 33.4 |
| | *p* | | 0.761 | 0.047* | 0.728 |
| Self-rating Depression Scale (score) | Placebo | 33 | 33.1 ± 5.9 | 0.3 ± 8.0 | –0.1 ± 4.9 |
| | Active | 28 | 32.5 ± 7.7 | –0.3 ± 3.9 | –2.4 ± 5.0 |
| | *p* | | 0.473 | 0.936 | 0.020* |
| Apathy Scale (score) | Placebo | 33 | 4.8 ± 4.1 | –0.6 ± 3.6 | –0.1 ± 3.0 |
| | Active | 28 | 6.4 ± 4.8 | –0.3 ± 3.6 | –2.6 ± 3.8 |
| | *p* | | 0.217 | 0.832 | 0.021* |
| Verbal recall test (score) | Placebo | 33 | 10.2 ± 1.8 | 0.6 ± 1.7 | 1.2 ± 2.2 |
| | Active | 28 | 9.6 ± 2.5 | 1.0 ± 1.7 | 1.8 ± 3.1 |
| | *p* | | 0.468 | 0.622 | 0.640 |

Values are shown as mean ± SD. ΔWeek 12 and 24: change in value from baseline to week 12 and 24, respectively. Mann–Whitney *U* test was performed. *$p<0.05$ vs placebo group. CADi2, Cognitive Assessment for Dementia iPad version.

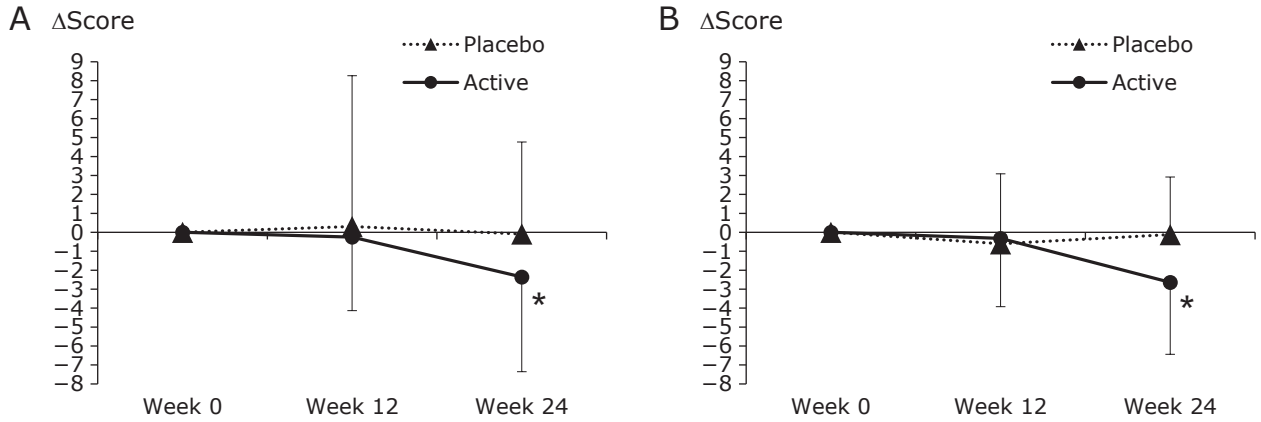


**Fig. 4.** Changes in (A) Self-Rating Depression Scale and (B) Apathy Scale. Value are shown as mean ± SD. Mann–Whitney *U* test was conducted for data analysis. *$p<0.05$ vs placebo group.

**Table 6.** NPI-NH

| Variable | | *n* | Baseline | ΔWeek 12 | ΔWeek 24 |
|---|---|---|---|---|---|
| NPI-NH Severity of subject's symptom (score) | Placebo | 33 | 0.6 ± 2.1 | –0.5 ± 2.0 | –0.3 ± 1.0 |
| | Active | 28 | 0.6 ± 0.9 | –0.5 ± 0.9 | –0.5 ± 0.8 |
| | *p* | | 0.103 | 0.128 | 0.225 |
| NPI-NH Burden on study partner (score) | Placebo | 33 | 0.4 ± 1.3 | –0.3 ± 1.3 | –0.1 ± 0.5 |
| | Active | 28 | 0.6 ± 1.1 | –0.6 ± 1.1 | –0.6 ± 1.1 |
| | *p* | | 0.065 | 0.012* | 0.022* |

Values are shown as mean ± SD. ΔWeek 12 and 24: change in value from baseline to week 12 and 24, respectively. Mann–Whitney *U* test was performed. *$p<0.05$ vs placebo group. NPI-NH, Neuropsychiatric Inventory Nursing Home version.

effects on the vascular system, which leads to changes in cerebrovascular blood flow, which in turn can alter the neural morphology that causes neurogenesis and angiogenesis. Additionally, the antioxidative biological system of flavonoids protects neurons from neurotoxin-induced injury. Consumption of flavonoids suppresses neurodegeneration and prevents cognitive decline with age.[31] Flavonoid-rich plants (e.g., spinach, strawberry, blueberry, and tea) and food supplements (e.g., γ-aminobutyric acid, carotenoids, vitamins C and E) improve the ability to protect vulnerable neurons by enhancing existing neural functions or stimulating neuronal generation.[32] In this report, we demonstrated that consumption of quercetin-rich onion for 24 weeks improved cognitive function, with the MMSE score as a primary endpoint.


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**Table 7.** Lipid and blood glucose profiles

| Variable | | *n* | Baseline | ΔWeek 12 | ΔWeek 24 |
|---|---|---|---|---|---|
| TC (mg/dl) | Placebo | 33 | 240 ± 38 | −6 ± 18 | −12 ± 30 |
| | Active | 28 | 233 ± 37 | 5 ± 22 | 2 ± 17 |
| | *p* | | 0.460 | 0.043* | 0.026* |
| HDL-C (mg/dl) | Placebo | 33 | 72 ± 16 | −4 ± 6 | −4 ± 6 |
| | Active | 28 | 76 ± 21 | −3 ± 12 | −1 ± 6 |
| | *p* | | 0.423 | 0.666 | 0.074 |
| LDL-C (mg/dl) | Placebo | 33 | 147 ± 32 | −4 ± 17 | −8 ± 26 |
| | Active | 28 | 139 ± 33 | 5 ± 14 | 1 ± 14 |
| | *p* | | 0.343 | 0.029* | 0.093 |
| TG (mg/dl) | Placebo | 33 | 116 ± 55 | 8 ± 58 | −2 ± 48 |
| | Active | 28 | 106 ± 70 | 10 ± 39 | 6 ± 50 |
| | *p* | | 0.538 | 0.883 | 0.535 |
| BG (mg/dl) | Placebo | 33 | 95 ± 10 | 0 ± 8 | 1 ± 6 |
| | Active | 28 | 96 ± 10 | −1 ± 7 | 0 ± 5 |
| | *p* | | 0.667 | 0.639 | 0.765 |
| HbA1c (%) | Placebo | 33 | 5.6 ± 0.4 | 0.0 ± 0.1 | −0.1 ± 0.1 |
| | Active | 28 | 5.4 ± 0.3 | −0.1 ± 0.2 | −0.1 ± 0.1 |
| | *p* | | 0.100 | 0.413 | 0.967 |

Values are shown as mean ± SD. ΔWeek 12 and 24: change in value from baseline to week 12 and 24, respectively. Student's *t* test was performed. *$p<0.05$ vs placebo group. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; BG, blood glucose; HbA1c, hemoglobin A1c.

Elderly people, particularly those with delirium or dementia, can only cooperate well for short periods on cognitive function tests. MMSE is a cognitive function test that is used internationally and can be performed in about 10 min, so the burden on the subject is small.[24] It is also used during outpatient medical checkups for cognitive decline to assess the effect on cognitive function of functional foods such as soybean-derived phosphatidylserine, which is given to elderly patients with memory complaints;[33] diosgenin-rich yam extract, given to improve cognitive function in healthy adults;[34] and auraptene, a citrus coumarin.[35]

The current study enrolled 70 Japanese men and women between 60 and 80 years of age to evaluate the effects of continuous intake of quercetin-rich onion for 24 weeks on cognitive function. As a result, the sum score of MMSE, the primary endpoint in the clinical trial, was significantly improved in the active test food group compared with the placebo food group. Regarding the MMSE score, temporal orientation significantly improved after 24 weeks of intake.

Our previous clinical study followed the same study design, but with a group of 50 Japanese men and women between 65 and 84 years of age.[4] The overall analysis showed no significant difference in the MMSE between the groups, and improvement was limited to those younger than 72 years. On the other hand, the current study, which considered the sample size and amount of quercetin in the test food, shows the possibility that continuous intake of quercetin-rich onion could delay cognitive decline even in elderly people.

Elderly people are more likely than younger people to develop cognitive decline as a result of taking medications.[36] We examined 38 subjects who were not taking medications, such as antihypertensive and antihyperlipidemic drugs, in order to exclude the potential effects of long-term or consistent medication use. As a result, the total MMSE score was significantly improved in the active test food group compared to the placebo food group at 24 weeks after intake. Concerning individual test items, for the stage command (oral instruction), cognitive function improved significantly in the active test food group at 24 weeks compared with the placebo food group. From the above results, we concluded that quercetin-rich onion could help improve cognitive function and that the chronic medication taken by some subjects did not affect this conclusion. Interestingly, for the change in MMSE sum score from baseline to 24 weeks, the difference between the two groups was bigger in subclass analysis of subjects with no medication compared to full efficacy analysis.

Concerning cognitive improvement by quercetin, *in vivo* effects of this flavonoid compound have been reported. Plasma exosomes loaded with quercetin ameliorated the declining cognitive function in mice with okadaic acid-induced Alzheimer's disease, assisted by increased bioavailability of quercetin by the mechanism of inhibition of cyclin-dependent kinase 5.[22] Moreover, intake of dietary advanced glycation products (dAGEs) induced cognitive dysfunction in aged ICR mice, which was ameliorated by quercetin intake.[23] These animal experiments demonstrating the anti-cognitive decline function of quercetin support our current results of the clinical trial by quercetin-rich onion. However, we have to further investigate the bioavailability of onion-derived quercetin *in vivo*, and the precise mechanism of quercetin should be elucidated.

CADi2, which is known as a dementia mass screening test, has been widely used with elderly people.[25–27] CADi2 has high sensitivity and specificity, and has shown significant correlations with existing neuropsychological tests, such as the MMSE and Frontal Assessment Battery. CADi2 shows good correlation with the MMSE, especially regarding the scores in word fluency tasks. In fact, a significant negative correlation was observed between MMSE score and total response time on the intake start date in this study. However, little correlation was observed between the MMSE scores at baseline and 24 weeks and the total score and total response time at 24 weeks. In the subclass analysis after excluding those who regularly took medication, neither the total score nor the total response time was improved by consuming the active test food.

Regarding the emotional function evaluation, in both the mood survey (SDS) and the motivation survey (apathy scale), the active test food group was significantly improved 24 weeks after intake

**Table 8.** Body composition, vital signs, complete blood counts, liver function, renal function, and protein

| Variable | | *n* | Baseline | ΔWeek 12 | ΔWeek 24 |
|---|---|---|---|---|---|
| Body weight (kg) | Placebo | 35 | 55.5 ± 11.5 | −0.1 ± 0.7 | 0.2 ± 0.8 |
| | Active | 33 | 58.3 ± 10.0 | 0.1 ± 1.1 | 0.5 ± 1.4 |
| | *p* | | 0.286 | 0.406 | 0.322 |
| Body fat percentage (%) | Placebo | 35 | 25.0 ± 6.3 | −0.4 ± 1.5 | 0.5 ± 1.3 |
| | Active | 33 | 26.8 ± 7.4 | 0.1 ± 1.1 | 1.0 ± 1.2 |
| | *p* | | 0.291 | 0.178 | 0.154 |
| BMI (kg/m$^2$) | Placebo | 35 | 22.0 ± 2.9 | −0.1 ± 0.3 | 0.1 ± 0.3 |
| | Active | 33 | 22.9 ± 3.2 | 0.0 ± 0.5 | 0.2 ± 0.5 |
| | *p* | | 0.214 | 0.407 | 0.325 |
| SBP (mmHg) | Placebo | 35 | 130 ± 16 | −2 ± 12 | −2 ± 11 |
| | Active | 33 | 129 ± 17 | −3 ± 14 | 1 ± 13 |
| | *p* | | 0.803 | 0.644 | 0.405 |
| DBP (mmHg) | Placebo | 35 | 77 ± 10 | −1 ± 5 | −2 ± 6 |
| | Active | 33 | 77 ± 10 | −3 ± 8 | 1 ± 8 |
| | *p* | | 0.935 | 0.220 | 0.208 |
| Pulse rate (bpm) | Placebo | 35 | 77 ± 12 | −2 ± 9 | 0 ± 8 |
| | Active | 33 | 73 ± 13 | 0 ± 9 | 3 ± 11 |
| | *p* | | 0.197 | 0.542 | 0.201 |
| WBC (×10$^3$/μl) | Placebo | 35 | 5.4 ± 1.4 | 0.1 ± 1.0 | −0.4 ± 0.7 |
| | Active | 33 | 5.6 ± 1.2 | 0.6 ± 0.9 | 0.0 ± 1.0 |
| | *p* | | 0.541 | 0.036* | 0.110 |
| RBC (×10$^4$/μl) | Placebo | 35 | 456 ± 48 | −6 ± 17 | −12 ± 18 |
| | Active | 33 | 450 ± 32 | −1 ± 16 | −5 ± 15 |
| | *p* | | 0.497 | 0.256 | 0.082 |
| Hb (g/dl) | Placebo | 35 | 14.0 ± 1.3 | −0.1 ± 0.5 | −0.3 ± 0.5 |
| | Active | 33 | 14.0 ± 1.1 | 0.0 ± 0.5 | −0.1 ± 0.5 |
| | *p* | | 0.896 | 0.483 | 0.069 |
| Ht (%) | Placebo | 35 | 42.8 ± 4.0 | −0.8 ± 1.5 | −1.3 ± 1.5 |
| | Active | 33 | 42.7 ± 3.1 | −0.6 ± 1.4 | −0.7 ± 1.7 |
| | *p* | | 0.891 | 0.611 | 0.162 |
| Plt (×10$^4$/μl) | Placebo | 35 | 22.2 ± 6.0 | 0.1 ± 2.6 | 0.1 ± 1.9 |
| | Active | 33 | 21.6 ± 4.4 | 0.3 ± 1.9 | 0.5 ± 2.3 |
| | *p* | | 0.611 | 0.705 | 0.418 |
| AST (U/L) | Placebo | 35 | 25 ± 4 | −1 ± 3 | 1 ± 4 |
| | Active | 33 | 25 ± 12 | −1 ± 11 | 0 ± 7 |
| | *p* | | 0.968 | 0.830 | 0.735 |
| ALT (U/L) | Placebo | 35 | 19 ± 9 | 1 ± 5 | 2 ± 5 |
| | Active | 33 | 21 ± 12 | 0 ± 8 | 2 ± 8 |
| | *p* | | 0.334 | 0.459 | 0.763 |
| γ-GTP (U/L) | Placebo | 35 | 27 ± 21 | 2 ± 5 | 0 ± 4 |
| | Active | 33 | 29 ± 17 | 5 ± 16 | 5 ± 12 |
| | *p* | | 0.643 | 0.372 | 0.062 |
| ALP (U/L) | Placebo | 35 | 222 ± 63 | −1 ± 23 | 0 ± 24 |
| | Active | 33 | 197 ± 43 | −1 ± 19 | 5 ± 29 |
| | *p* | | 0.060 | 0.928 | 0.398 |
| LDH (U/L) | Placebo | 35 | 208 ± 33 | −6 ± 17 | −5 ± 19 |
| | Active | 33 | 202 ± 32 | 2 ± 18 | −1 ± 14 |
| | *p* | | 0.480 | 0.073 | 0.329 |
| BUN (mg/dl) | Placebo | 35 | 16.9 ± 4.2 | −0.7 ± 3.4 | −1.2 ± 3.3 |
| | Active | 33 | 17.0 ± 4.0 | 0.3 ± 3.2 | −0.5 ± 2.7 |
| | *p* | | 0.882 | 0.222 | 0.367 |
| CRE (mg/dl) | Placebo | 35 | 0.79 ± 0.14 | 0.00 ± 0.08 | −0.02 ± 0.07 |
| | Active | 33 | 0.83 ± 0.18 | 0.00 ± 0.05 | 0.00 ± 0.05 |
| | *p* | | 0.259 | 0.912 | 0.409 |
| UA (mg/dl) | Placebo | 35 | 5.1 ± 1.1 | 0.2 ± 0.5 | −0.1 ± 0.5 |
| | Active | 33 | 5.1 ± 1.4 | 0.0 ± 0.5 | 0.0 ± 0.5 |
| | *p* | | 0.926 | 0.260 | 0.781 |
| TP (g/dl) | Placebo | 35 | 7.3 ± 0.3 | −0.1 ± 0.3 | −0.1 ± 0.2 |
| | Active | 33 | 7.3 ± 0.4 | 0.0 ± 0.2 | −0.1 ± 0.2 |
| | *p* | | 0.630 | 0.288 | 0.376 |
| Alb (g/dl) | Placebo | 35 | 4.6 ± 0.2 | 0.0 ± 0.2 | 0.0 ± 0.2 |
| | Active | 33 | 4.5 ± 0.2 | 0.0 ± 0.2 | 0.0 ± 0.1 |
| | *p* | | 0.514 | 0.958 | 0.673 |
| A/G ratio | Placebo | 35 | 1.65 ± 0.21 | 0.06 ± 0.10 | 0.03 ± 0.10 |
| | Active | 33 | 1.64 ± 0.19 | 0.01 ± 0.10 | 0.01 ± 0.09 |
| | *p* | | 0.793 | 0.098 | 0.405 |

Values are shown as mean ± SD. ΔWeek 12 and 24: change in value from baseline to week 12 and 24, respectively. Student's *t* test was performed. *$p$<0.05 vs placebo group. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; Plt, platelet; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltranspeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; TP, total protein; Alb, albumin; A/G ratio, albumin/globulin ratio.


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began compared with the placebo food group. It has been suggested that depressive symptoms and decreased motivation are the BPSDs that appear most frequently.[37,38] Depression, as well as the development of depressive symptoms, can lead to withdrawal for the elderly, which causes not only worsening dementia but also physical decline.[39] It is conceivable that consuming this food could improve depressive symptoms and motivation in elderly people and contribute to the suppression of cognitive decline and activation of total brain health. This result provides an important reminder that we need to understand the potential mechanism of action concerning the improvement of cognitive function by quercetin-rich onion.

The 2017 Dementia Disease Treatment Guideline (Japan Neurological Society) states that the diagnosis rate of mild dementia can be improved by adding a neuropsychological test such as a trail-making or word recall test to the MMSE.[40] Although a word recall test was performed in this study, an analysis of all subjects did not reveal any improvement as a result of consuming quercetin-rich food.

We reported that scores on the NPI-Q-J test improved 12 weeks after intake began in a previous study.[4] For the NPI-NH conducted in this study, the total score was not significantly different between the two groups, but the burden level was significantly improved after 12 and 24 weeks of intake. In the subclass analysis excluding those who regularly took medication, there was some improving tendency of the effect in the total score between the two groups, but no significant difference. This result may be due to an insufficient number of subjects in the subclass analysis. Although the NPI-NH is a standard questionnaire for assessing the symptoms of BPSD, it may not be suitable for subjects with mild cognitive decline, such as those enrolled in this study. We need further investigation.

As for the mechanism, Hayakawa *et al.*[20] demonstrated that GADD34 was induced in the brains of mice in an Alzheimer's disease model by intake of quercetin-containing food, leading to a delay in the deterioration in memory. The findings suggest that consuming food rich in quercetin may improve the memory.

A more detailed mechanism of action of quercetin in cognitive function has been proposed.[41] In brief, GADD34 suppressed the endoplasmic reticulum stress response in the brain by dephosphorylating eIF-2α. Overall, quercetin may reduce eIF-2α phosphorylation and activate transcription factor 4 expression through GADD34 induction in the brain. This particular pathway might improve the memory potential and delay the deterioration of memory in the early stage of Alzheimer's disease in aged mice. Thus, quercetin is expected to reduce the risk factors for cognitive decline caused by dysregulation of the lipid and glucose metabolisms, and to improve brain function through the suppression of endoplasmic reticulum stress. Following this report, the same research group focused on the effect of quercetin on cognitive function in humans with early stage Alzheimer's disease to investigate the function of quercetin. They demonstrated the effect of quercetin-rich onion on delaying cognitive decline in elderly people. In a future study, the mechanism of action by which quercetin improves cognitive function is expected to be demonstrated in a better-designed clinical trial that includes those other factors, e.g., quantity of quercetin and study duration.

Quercetin, a flavonoid, has beneficial effects on the vascular system, which lead to changes in cerebrovascular blood flow, which in turn can alter the neural morphology that causes neurogenesis and angiogenesis. Cognitive decline was reported in high-cholesterol-diet-loaded animals.[19] In spite of the available data regarding dyslipidemia, no improvement was observed in the active food group in this study, but TC and LDL-C were significantly improved in the placebo food group. This result may be due to the fact that there were two subjects in the placebo group who started receiving medication for dyslipidemia after the start of the study. When those two subjects were excluded from the statistics, no significant difference between the two groups was observed.

As for glucose metabolism, studies on quercetin and cognitive function have reported improved cognitive impairment in diabetic model rats.[18] In this study, we failed to show any improvement in glucose level as a result of the intake of the active test food. Overall, we could not find a significant relationship between blood lipid profiles or glucose level and cognitive function in this study. We are currently re-examining the effect of the quercetin-rich food on these parameters.

Serious adverse or relevant clinical findings and abnormal changes were not observed in the laboratory test values. Therefore, it was concluded that the continuous intake of this food for 24 weeks had no adverse effect on health in terms of food safety.

Lastly, aging is the predominant factor in cognitive decline caused by neurodegeneration, but no single active drug is available for the prevention and treatment of neurodegenerative diseases. We here demonstrated the potential of quercetin-rich onion to protect against cognitive decline. To confirm the preventive effects of foods and nutrients against early cognitive decline, we require more precise diagnostic tools, such as MRI and single photon emission computed tomography (SPECT) for examination of brain function, image analysis of cerebral blood flow,[42,43] or a high-performance method of analyzing plasma-level amyloid β based on immunoprecipitation coupled with mass spectrometry.[44] More importantly, it is critical to understand the value of plasma concentration of quercetin in order to better understand the precise biological function of this flavonoid molecule. We measured plasma concentration of quercetin at the fourth visit (24 weeks) before daily intake of onion, but no measurable amount of quercetin was detected, possibly due to rapid excretion of quercetin ingested. To solve this issue, we need to explore more precise methods of measuring quercetin, which is under investigation. Nevertheless, the appropriate intake of functional foods and nutrients as well as a balanced diet are strongly recommended to reduce age-related cognitive dysfunction, as demonstrated by the intake of quercetin-rich onion in this study. Moreover, as shown in this study, it could be generalized that lifestyle diseases, including even mild cognitive decline, could be successfully managed, at least in part, by the consumption of appropriate functional foods.

In this study, we demonstrated that quercetin-rich onion, not quercetin-poor onion, possesses the valuable function of improving cognitive function in a randomized, double-blind, placebo-controlled, parallel-group comparative clinical trial. It is often described that clinical trials need years to evaluate the effects of agents (mostly pharmaceuticals) on cognitive function, and require large numbers of participants. From multiple studies about the effects of foods on cognition, including the current study, it is considered that evaluating food functionality focusing on participants with mild cognitive decline may require a relatively short period of time and a small number of participants. The mechanism of cognitive improvement by quercetin-rich onion is still unclear, but it may be due to improvement of emotional factors, as demonstrated by CADi2. On the other hand, it is sometimes difficult to evaluate the level of cognitive function due to various confounding factors. In particular, lifestyle includes a number of confounding factors, such as smoking, alcohol, medications, exercise, and so forth. We excluded these possible factors to obtain robust results, supporting the previous data.[4] Accordingly, the results of previous and current studies shed light on the management of cognitive decline by intervention with onions rich in quercetin. Cognitive decline is the major social issue to be solved, as average life expectancy has been unexpectedly prolonged. In the future, specifically designed foods such as 'Sarasara Gold' onions should be widely available to maintain brain health in an aging society.

## Author Contributions

Conceptualization, JN and MKo; methodology, JN, MN, and MKo; software, HK and HH; validation, JN, MN, MKu, HK, and MKo; formal analysis, MN, MKu, and HK; investigation, JN and MN; resources, TM; data curation, JN, MN, HK, and HH; writing—original draft preparation, JN and MKu; writing—review and editing, JN, MN, MKu, TN, and MKo; visualization, MN, MKu, and HK; supervision, JN and MKo; project administration, JN and MKo; funding acquisition, JN and MKo. All authors have read and agreed to the published version of the manuscript.


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## Abbreviations

| | |
|---|---|
| A/G ratio | albumin/globulin ratio |
| Alb | albumin |
| ALP | alkaline phosphatase |
| ALT | alanine aminotransferase |
| AST | aspartate aminotransferase |
| BG | blood glucose |
| BMI | body mass index |
| BUN | blood urea nitrogen |
| CADi2 | Cognitive Assessment for Dementia iPad version |
| CRE | creatinine |
| DBP | diastolic blood pressure |
| γ-GTP | γ-glutamyltranspeptidase |
| 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 |
| MMSE | Mini-Mental State Examination |
| NPI-NH | Neuropsychiatric Inventory Nursing Home version |
| Plt | platelet |
| RBC | red blood cell |
| SBP | systolic blood pressure |
| TC | total cholesterol |
| TG | triglyceride |
| TP | total protein |
| UA | uric acid |
| WBC | white blood cell |

## Conflicts of Interest

No potential conflicts of interest were disclosed.

## References


1 World Health Organization. *Risk Reduction of Cognitive Decline and Dementia: WHO Guidelines*. Geneva: World Health Organization, 2019; 1.

2 Bo Y, Zhang X, Wang Y, *et al*. The *n*-3 polyunsaturated fatty acids supplementation improved the cognitive function in the Chinese elderly with mild cognitive impairment: a double-blind randomized controlled trial. *Nutrients* 2017; **9**: 54.

3 Külzow N, Witte AV, Kerti L, *et al*. Impact of omega-3 fatty acid supplementation on memory functions in healthy older adults. *J Alzheimers Dis* 2016; **51**: 713–725.

4 Nishimura M, Ohkawara T, Nakagawa T, *et al*. A randomized, double-blind, placebo-controlled study evaluating the effects of quercetin-rich onion on cognitive function in elderly subjects. *Funct Food Health Dis* 2017; **7**: 353–374.

5 Najima M, Munekata M, Nakano T, Nadachi Y, Kimura H, Nagata H. Improvement in cognitive function by supplement contained plasmalogen for healthy Japanese—a randomized, double-blind, placebo-controlled study—. *Med Cons New-Remed* 2016; **53**: 1125–1135.

6 Sekikawa T, Kizawa Y, Li Y, Takara T. Cognitive function improvement with astaxanthin and tocotrienol intake: a randomized, double-blind, placebo-controlled study. *J Clin Biochem Nutr* 2020; **67**: 307–316.

7 Del Bo' C, Bernardi S, Marino M, *et al*. Systematic review on polyphenol intake and health outcomes: Is there sufficient evidence to define a health-promoting polyphenol-rich dietary pattern? *Nutrients* 2019; **11**: 1355.

8 Rajaram S, Jones J, Lee GJ. Plant-based dietary patterns, plant foods, and age-related cognitive decline. *Adv Nutr* 2019; **10 (Suppl 4)**: S422–S436.

9 Tsushida T, Suzuki M. Content of flavonol glucosides and some properties of enzymes metabolizing the glucosides in onion. Flavonoid in fruits and vegetables, Part II. *Nippon Shokuhin Kagaku Kogaku Kaishi* 1996; **43**: 642–649.

10 Stewart AJ, Mullen W, Crozier A. On-line high-performance liquid chromatography analysis of the antioxidant activity of phenolic compounds in green and black tea. *Mol Nutr Food Res* 2005; **49**: 52–60.

11 Meyers KJ, Rudolf JL, Mitchell AE. Influence of dietary quercetin on glutathione redox status in mice. *J Agric Food Chem* 2008; **56**: 830–836.

12 Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr* 2001; **74**: 418–425.

13 Kobori M, Masumoto S, Akimoto Y, Oike H. Chronic dietary intake of quercetin alleviates hepatic fat accumulation associated with consumption of a Western-style diet in C57/BL6J mice. *Mol Nutr Food Res* 2011; **55**: 530–540.

14 Kobori M, Takahashi Y, Sakurai M, *et al*. Quercetin suppresses immune cell accumulation and improves mitochondrial gene expression in adipose tissue of diet-induced obese mice. *Mol Nutr Food Res* 2016; **60**: 300–312.

15 Kawai Y, Nishikawa T, Shiba Y, *et al*. Macrophage as a target of quercetin glucuronides in human atherosclerotic arteries: implication in the anti-atherosclerotic mechanism of dietary flavonoids. *J Biol Chem* 2008; **283**: 9424–9434.

16 Takami M, Aoi W, Terajima H, Tanimura Y, Wada S, Higashi A. Effect of dietary antioxidant-rich foods combined with aerobic training on energy metabolism in healthy young men. *J Clin Biochem Nutr* 2019; **64**: 79–85.

17 Dok-Go H, Lee KH, Kim HJ, *et al*. Neuroprotective effects of antioxidative flavonoids, quercetin, (+)-dihydroquercetin and quercetin 3-methyl ether, isolated from Opuntia ficus-indica var. saboten. *Brain Res* 2003; **965**: 130–136.

18 Bhutada P, Mundhada Y, Bansod K, *et al*. Ameliorative effect of quercetin on memory dysfunction in streptozotocin-induced diabetic rats. *Neurobiol Learn Mem* 2010; **94**: 293–302.

19 Lu J, Wu DM, Zheng YL, *et al*. Quercetin activates AMP-activated protein kinase by reducing PP2C expression protecting old mouse brain against high cholesterol-induced neurotoxicity. *J Pathol* 2010; **222**: 199–212.

20 Hayakawa M, Itoh M, Ohta K, *et al*. Quercetin reduces eIF2α phosphorylation by GADD34 induction. *Neurobiol Aging* 2015; **36**: 2509–2518.

21 Yu X, Li Y, Mu X. Effect of quercetin on PC12 Alzheimer's disease cell model induced by A$\beta_{25\text{-}35}$ and its mechanism based on sirtuin1/Nrf2/HO-1 pathway. *Biomed Res Int* 2020; **2020**: 8210578.

22 Qi Y, Guo L, Jiang Y, Shi Y, Sui H, Zhao L. Brain delivery of quercetin-




doi: 10.3164/jcbn.21-17

loaded exosomes improved cognitive function in AD mice by inhibiting phosphorylated tau-mediated neurofibrillary tangles. *Drug Deliv* 2020; **27**: 745–755.
23 Yang S, Zhou H, Wang G, *et al*. Quercetin is protective against short-term dietary advanced glycation end products intake induced cognitive dysfunction in aged ICR mice. *J Food Biochem* 2020; **44**: e13164.
24 Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189–198.
25 Onoda K, Hamano T, Nabika Y, *et al*. Validation of a new mass screening tool for cognitive impairment: Cognitive Assessment for Dementia, iPad version. *Clin Interv Aging* 2013; **8**: 353–360.
26 Onoda K, Yamaguchi S. An iPad application on mass-screening for dementia: the development and validation of CADi. *High Brain Funct Res* 2014; **34**: 331–334.
27 Onoda K, Yamaguchi S. Revision of the Cognitive Assessment for Dementia, iPad version (CADi2). *PLoS One* 2014; **9**: e109931.
28 Wood S, Cummings JL, Hsu MA, *et al*. The use of the neuropsychiatric inventory in nursing home residents. Characterization and measurement. *Am J Geriatr Psychiatry* 2000; **8**: 75–83.
29 Masuoka N, Yoshimine C, Hori M, *et al*. Effects of anserine/carnosine supplementation on mild cognitive impairment with APOE4. *Nutrients* 2019; **11**: 1626.
30 Stavrinou PS, Andreou E, Aphamis G, *et al*. The effects of a 6-month high dose omega-3 and omega-6 polyunsaturated fatty acids and antioxidant vitamins supplementation on cognitive function and functional capacity in older adults with mild cognitive impairment. *Nutrients* 2020; **12**: 325.
31 Vauzour D, Vafeiadou K, Rodriguez-Mateos A, Rendeiro C, Spencer JP. The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes Nutr* 2008; **3**: 115–126.
32 Youdim KA, Joseph JA. A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. *Free Radic Biol Med* 2001; **30**: 583–594.
33 Kato-Kataoka A, Sakai M, Ebina R, Nonaka C, Asano T, Miyamori T. Soybean-derived phosphatidylserine improves memory function of the elderly Japanese subjects with memory complaints. *J Clin Biochem Nutr* 2010; **47**: 246–255.
34 Tohda C, Yang X, Matsui M, *et al*. Diosgenin-rich yam extract enhances cognitive function: a placebo-controlled, randomized, double-blind, crossover study of healthy adults. *Nutrients* 2017; **9**: 1160.
35 Igase M, Okada Y, Ochi M, *et al*. Auraptene in the peels of *Citrus kawachiensis* (Kawachibankan) contributes to the preservation of cognitive function: a randomized, placebo-controlled, double-blind study in healthy volunteers. *J Prev Alzheimers Dis* 2018; **5**: 197–201.
36 Moore AR, O'Keeffe ST. Drug-induced cognitive impairment in the elderly. *Drugs Aging* 1999; **15**: 15–28.
37 van der Linde RM, Dening T, Stephan BC, Prina AM, Evans E, Brayne C. Longitudinal course of behavioural and psychological symptoms of dementia: systematic review. *Br J Psychiatry* 2016; **209**: 366–377.
38 Kitching D. Depression in dementia. *Aust Prescr* 2015; **38**: 209–211.
39 Qiu WQ, Dean M, Liu T, *et al*. Physical and mental health of homebound older adults: an overlooked population. *J Am Geriatr Soc* 2010; **58**: 2423–2428.
40 Wouters H, Appels B, van der Flier WM, *et al*. Improving the accuracy and precision of cognitive testing in mild dementia. *J Int Neuropsychol Soc* 2012; **18**: 314–322.
41 Nakagawa T, Itoh M, Ohta K, *et al*. Improvement of memory recall by quercetin in rodent contextual fear conditioning and human early-stage Alzheimer's disease patients. *Neuroreport* 2016; **27**: 671–676.
42 Santos RF, Galduróz JC, Barbieri A, Castiglioni ML, Ytaya LY, Bueno OF. Cognitive performance, SPECT, and blood viscosity in elderly non-demented people using Ginkgo biloba. *Pharmacopsychiatry* 2003; **36**: 127–133.
43 Landin-Romero R, Tan R, Hodges JR, Kumfor F. An update on semantic dementia: genetics, imaging, and pathology. *Alzheimers Res Ther* 2016; **8**: 52.
44 Nakamura A, Kaneko N, Villemagne VL, *et al*. High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nature* 2018; **554**: 249–254.