European Review for Medical and Pharmacological Sciences 2022; 26: 8700-8712

Effect of quercetin glycosides on cognitive functions and cerebral blood flow: a randomized, double-blind, and placebo-controlled study

Y. NAKAMURA[1], H. WATANABE[1], A. TANAKA[2], J. NISHIHIRA[2], N. MURAYAMA[1]

[1]Suntory Global Innovation Center Ltd. Research Institute, Kyoto, Japan
[2]Department of Medical Management and Informatics, Hokkaido Information University, Hokkaido, Japan

Abstract. – OBJECTIVE: This study aimed to examine the effects of quercetin glycoside-containing beverages on cognitive function and cerebral blood flow (CBF) in adult men and women aged between 60 and 75 years.

PATIENTS AND METHODS: Eighty healthy men and women with no cognitive impairment and aware of ageing-related forgetfulness underwent a placebo-controlled, randomized, double-blind, and parallel-group trial. They regularly consumed 500 mL of beverage containing 110 mg of quercetin glycoside as isoquercitrin for 40 weeks. Cognitive function assessment by Cognitrax was the endpoint of the study. The participants were assessed for CBF, health-related quality of life, as well as physical, biological, and hematological parameters, and lateral index.

RESULTS: Cognitrax demonstrated that the reaction time significantly improved in the quercetin glycoside intake group. The CBF measurement suggested that quercetin glycoside intake could likely suppress the decrease in cerebral blood volume, CBF, and cerebral activity owing to stress alleviation and inhibition of the accumulation of amyloid β (Aβ), a waste product in the brain, although there were no significant differences between the groups.

CONCLUSIONS: Quercetin glycoside intake as a beverage could improve reaction time and may potentially inhibit the decrease in CBF and suppress Aβ accumulation.

Key Words:
Quercetin glycosides, Cognitive function, Reaction time, Cerebral blood flow, Amyloid β.

Abbreviations

Aβ, amyloid β; ALB, albumin; BMI, body mass index; CBF, cerebral blood flow; CBV, cerebral blood volume; COVID-19, coronavirus disease; Hb, hemoglobin; HDL, high-density lipoprotein; IGF-1, insulin-like growth factor; LDL, low-density lipoprotein; LI, lateral index; MCI, mild cognitive improvement; MMSE, Mini-Mental State Examination; NCI, Neurocognitive Index; NIRS, near-infrared spectroscopy; O_2Hb, oxygenated haemoglobin; QOL, quality of life; RP, role physical; SD, standard deviation; StO_2, tissue oxygen saturation; TP, total protein; VT, vitality.

Introduction

Age-related cognitive decline is a serious health problem worldwide. According to a report by the World Health Organization, dementia is a rapidly growing public health problem that affects approximately 50 million people worldwide[1]. Approximately 10 million new cases are diagnosed each year, and this number will expectedly triple by 2050. Dementia is a major cause of disability and dependency in the elderly and can devastate the lives of the affected individuals, their caregivers, and families. According to a report by the Ministry of Health, Labor and Welfare in Japan, the number of people with dementia is estimated to be approximately 7 million by 2025 based on an epidemiological study[2] conducted in Hisayama; the number will increase to one in five people aged over 65 years. Despite the availability of drugs that can inhibit dementia progression, there is no fundamental cure. Mild cognitive impairment (MCI) is considered a precursor to dementia; 5-15% of people with MCI develop dementia every year, whereas MCI persists in the remaining patients, or 16-41% return to normal[3,4]. Notwithstanding various factors that contribute to the development of dementia including genetic and environmental factors, controllable factors comprise education, hearing loss, hypertension, obesity, smoking, depression, diabetes, physical inactivity, and social isolation[5]. The prevention and improvement of cognitive decline before MCI in the elderly

Corresponding Author: Yumi Nakamura, Ph.D; e-mail: Yumi_Nakamura@suntory.co.jp

may avert the onset of dementia. Moreover, preventive and improvement measures are expected to be beneficial through controllable diet, exercise, sleep, and other lifestyle changes.

Quercetin is a flavonoid widely found in onions, broccoli, and tea. In a study[6] in Europe and the United States, people who consumed high levels of flavonoids displayed lower mortality from myocardial infarction. In Japan, people who consumed high amounts of quercetin had lower levels of low-density lipoprotein (LDL)-cholesterol in their blood[7]. Quercetin also prevents and improves obesity and metabolic syndrome[8]. Recently, researchers have developed onions with high quercetin content, and improved cognitive function is one of their benefits[9]. Quercetin glycosides may additionally reduce body fat[10] and improve vascular endothelial function[11,12]. In contrast, quercetin does not display good absorption efficacy upon ingestion. Nonetheless, quercetin glycosides are structured such that glucose is bound to quercetin and get hydrolyzed to quercetin in the small intestine upon oral consumption, thereby increasing their absorption efficacy[13]. In this study, we hypothesized that quercetin glycosides would enhance cognitive function by improving vascular function.

We aimed to determine the effects of the continuous intake of quercetin glycosides on cognitive function and cerebral blood flow (CBF) in healthy participants concerned about ageing-related forgetfulness. We intended to measure CBF using near-infrared spectroscopy (NIRS), which comprises a time-resolved spectroscopy method and can capture the hemoglobin concentration as an absolute quantity[14]. This novel study evaluated the effect of quercetin glycosides on CBF in humans.

Patients and Methods

Study Design

We conducted a placebo-controlled, randomized, double-blind, and parallel-group study. From the screening test to the end of the intervention period, the study was conducted at Hokkaido Information University (Ebetsu, Hokkaido, Japan) from December 2019 to October 2020. Table I summarizes the study schedule.

Participants

We recruited 162 participants based on the following inclusion criteria: individuals who (1) completely understood the significance, content, and purpose of the study and provided written consent to participate; (2) were Japanese men and women aged between 60 and 80 years; (3) were aware of ageing-related cognitive decline; (4) had Mini-Mental State Examination (MMSE) scores ≥24; and (5) had a relatively low standardized score on the Cognitrax Neurocognitive Index (NCI).

The exclusion criteria were as follows: individuals who (1) were receiving treatment or medications that affect the endpoints; (2) had a history

Table I. Clinical trial schedule.

Parameters	Guidance agreement screening (baseline)	Pre-observation period (1 week)	Test period		
			The beginning intervention		
			Week 0	Week 12	Week 40
Visit	●	–	●	●	●
Informed consent	●	–	–	–	–
Medical interview	●	–	●	●	●
Physical measurement	●	–	●	●	●
Vital signs	●	–	●	●	●
Blood sampling	●	–	●	●	●
MMSE	●	–	●	–	●
Cognitrax	●	–	●	–	●
NIRS measurement	●	–	●	–	●
QOL questionnaire	●	–	●	–	●
Diary record	–	●	●	●	●

● Refers to points that have been implemented. MMSE, mini-mental state examination; NIRS, near-infrared spectroscopy; QOL, quality of life.

of or are suspected of having a neurological or psychiatric disorder; (3) had severe anemia; (4) regularly consumed one or more onions per day; (5) regularly took medications, health foods, or supplements that may affect their cognitive function, (6) were excessive smokers and alcoholics; and (7) had irregular lifestyles.

Eighty eligible participants were randomly assigned to active or placebo group by the stratified block randomization method. The assignment factors were age, sex, MMSE scores, and the left and right mean of tissue oxygen saturation (StO_2) in the anterior forehead by NIRS measurement. A third-party allocation agency managed allocation information and ensured that double-blindness was maintained until the data were fixed. The sample size was set at the maximum feasible number in this exploratory study.

Intervention

We used 500 mL of PET bottled barley tea beverage as the intervention. The test beverage consumed by the active group comprised a beverage containing 110 mg of quercetin glycosides as isoquercitrin, whereas the placebo group consumed a beverage that did not contain quercetin glycosides. Each beverage had similar ingredients, except for the presence of quercetin glycosides. The contents of the beverages are shown in Table II. Quercetin glycosides products contain enzymatically modified quercetin (EMIQ®, San-Ei Gen F.F.I., Incorporated, Osaka, Japan), which is standardized as isoquercitrin. The beverages were prepared to be identical in appearance and flavor to maintain the blindness of the participants. Test foods were provided by Suntory Beverage & Food Limited. We requested the participants to drink 500 mL of the beverage per day as much as possible in the morning, and to consume the entire amount every day for 40 weeks. The intake rate was calculated using the following formula:

$$\text{Intake rate (\%)} = \frac{\text{The actual number of days of intake}}{\text{The specified number of days of intake}} \times 100$$

The actual number of days was considered 1 day upon consumption of the entire daily amount, and the prescribed number of days consumed was 280 days from the test day before the start of intake to the day before the test day 40 weeks after intake.

Table II. Nutrient composition of the active test beverage and placebo beverage (500 mL).

	Active test beverage	Placebo food
Calories (kcal)	0	0
Water (g)	498	498
Protein (g)	0	0
Lipids (g)	0	0
Carbohydrates (g)	2.7	2.7
Ash (g)	0	0
Sodium (mg)	0.03-0.08	0.03-0.08
Quercetin glycoside as isoquercitrin	110	-

Study Outcomes

We evaluated the difference between each index at baseline and post 40 weeks of intake (Week 40). Cognitive function assessment by the MMSE and Cognitrax (Health Solution Co., Ltd.) were the endpoints. We conducted CBF assessment by tNIRS-1 (Hamamatsu Photonics K.K.), the health-related quality of life (QOL) scale assessment by the Short Form Health Survey version-2.0 (SF-36v2, Qualitest Co., Ltd.), physical examination, and blood tests.

Cognitive Function Assessment

The MMSE was assessed by the total scores following an interview with a physician. Cognitrax is a computerized cognitive function test based on an assessment developed by CNS Vital Signs, Inc. in the USA[15]. It was conducted online and consists of the following seven test items: verbal memory test, visual memory test, finger tapping test, symbol digit coding test, Stroop test, attention shift, and sustained processing test. We assessed the NCI, composite memory, verbal memory, visual memory, psychomotor speed, reaction time, complex attention, cognitive flexibility, processing speed, executive function, simple attention, and motor speed. The normalized scores were corrected by comparison with similar age groups.

Cerebral Blood Flow Measurement

CBF was measured using a non-invasive cerebral oxygen monitor tNIRS-1. We measured the time response waveform of the transmitted light using short-pulsed light as the irradiation, which is highly quantitative, and the reproducibility between measurements. A disposable pad was attached to each side of the forehead to avoid

the superior sagittal sinus, hair, eyebrows, and blood seeds. Furthermore, it was connected to the irradiation probe (outside) and detection probe (inside). The left and right sides were connected as Ch1 and Ch2, respectively. Considering the forehead area and the size of the pad, there was no room for choice in the position of application. Therefore, the pad was each time applied to almost a similar place. We measured a strongly curved banana-shaped area centered at a depth of 3 cm from the cranium and approximately 1 cm from the surface of the brain parenchyma. This product reportedly has excellent reproducibility, with a small variation of 1-2% in the position of application and from one measurement session to the next, compared with the conventional Modified Beer-Lambert method of NIRS[16].

We conducted the measurements in the resting state after requesting the participants to rest for 5 min in a semi-sitting position. These measurements were recorded every 5 s for 1 min, and we used the average value of the last 10 points of the 12 measurement points.

We evaluated the total hemoglobin (total Hb) concentration, oxygenated hemoglobin (O_2Hb) concentration, and StO_2. Total Hb is an index reflecting the cerebral blood volume (CBV), whereas O_2Hb reflects the CBF and brain activity.

Health-related Quality of Life Scale Assessment

The health-related QOL scale was evaluated using the SF-36v2 standard version (Qualitent Limited) and analyzed according to the SF-36 scoring program[17,18].

Physical, Hematological and Biological Assessments

The blood pressure and pulse at the time of visit were measured on the upper arm opposite the dominant arm using a digital automatic blood pressure monitor HEM-7080IC (Omron). After the participant rested for at least 10 min, we measured blood pressure thrice in a sitting position and used the median value of the three measurements as the blood pressure value for that day. Height was measured using a common manual measuring device. Body weight, body fat percentage, and body mass index (BMI) were measured with a DC-430A body composition meter (Tanita Corporation). Conventional blood tests included the following: general items (white blood cells, red blood cells, Hb, hematocrit, platelet count test), liver function indicators (aspartate aminotransferase, alanine transaminase, gamma-glutamyl transferase, alkaline phosphatase, and lactate dehydrogenase levels), renal function indicators (blood urea nitrogen and creatinine levels, urinalysis, and estimated glomerular filtration rate), blood lipid indicators [total cholesterol, LDL cholesterol (Cho), high-density lipoprotein (HDL) Cho, Non-HDL Cho, apolipoprotein B, apolipoprotein AI, triglycerides], glycaemic index (fasting glucose, glycated haemoglobin, glycoalbumin, and fasting insulin), and serum proteins [total protein (TP), albumin (ALB)]. We assessed insulin-like growth factor-1 (IGF-1) using an electrochemiluminescence immunoassay. Plasma amyloid-β 40, amyloid-β 42, Tau, neurofilament light chain, tumor necrosis factor-alpha, interleukin-6, and interferon-gamma were measured by sensitive enzyme-linked immunoassay using Single Molecule Array (Simoa).

Lateral Index

We calculated the lateral index (LI) from O_2Hb measurements by NIRS using the following formula:

$$LI = \frac{(\text{Right } O_2\text{Hb-left } O_2\text{Hb})}{(\text{Right } O_2\text{Hb+left } O_2\text{Hb})}$$ [19]

The LI refers to an index of the right dominant state of the prefrontal cortex, which reflects the stress state[19].

Statistical Analysis

Numerical data are expressed as mean ± standard deviation (SD). We performed an independent two-sample *t*-test and the corresponding *t*-test for between- and within-group comparisons, respectively, for the amount of change from baseline to Week 40. Moreover, we performed Fisher's exact probability test for the sex ratio. Correlation analysis was performed by linear single regression analysis, and the correlation coefficient and analysis of variance were used to assess significant differences. The significance level was set at 5%. Data aggregation and statistical analysis were performed using Microsoft Excel 2013, JMP v. 14.0.0 (SAS Institute Inc., Cary, NC, USA).

Ethics Approval

This study was conducted in accordance with the Ethical Guidelines for Medical Research Involving Human Subjects (partially revised in 2017 by the Ministry of Education, Culture, Sports, Science and Technology and the Min-

istry of Health, Labor and Welfare) and was in compliance with the tenets of the Declaration of Helsinki (revised in October 2013 by the World Medical Association Fortaleza General Assembly). Prior to implementation, it was reviewed and approved by the Bioethics Committee of Hokkaido Information University (Approval number: 2019-26; Approval date: October 28, 2019). The summary of this study was registered in the University Hospital Medical Information Network Clinical Trial Registration System (UMIN-CTR) (UMIN000038593) on December 02, 2019.

In response to the spread of coronavirus disease (COVID-19), the contents of this study were changed for safety reasons. However, each change was made following approval from the Bioethics Committee of Hokkaido Information University. Primarily, the intake period was changed from 48 weeks to 40 weeks to ensure the safety of all participants. They provided re-consent for this change and continued participation in the study.

Results

Participant Flow and Baseline Characteristics

Figure 1 depicts the flowchart of the study participants. We selected 80 eligible participants from 162, and the study was conducted with 40 participants each in the active and placebo groups. During the study, there were two dropouts due to personal reasons, one from each group. To ensure safety from COVID-19, six participants aged ≥75 years and one with borderline diabetes considered at high risk of aggravation were excluded from undergoing the examination at Week 40. Thus, a total of seven participants (four in the active group and three in the placebo group) dropped out. Therefore, 71 participants completed the study (35 and 36 participants in the active and placebo groups, respectively). Subsequently, we excluded three participants from the analysis due to the long-term use of medications that may affect cognitive function and violate the exclusion criteria (active group), the beginning of dyslipidemia medication (active group), and multiple medication use (placebo group). Therefore, the safety and efficacy analysis groups comprised 80 and 68 participants (33 in the active group and 35 in the placebo group) (Figure 1).

There were no safety issues in conducting the study or the intake of the test beverage. However, we could not deny a causal relationship between frequent urination and the study. Owing to the likely association between increased water intake and the study beverage, the aforementioned causal relationship was considered 'possible'. No other adverse events were causally related to the study.

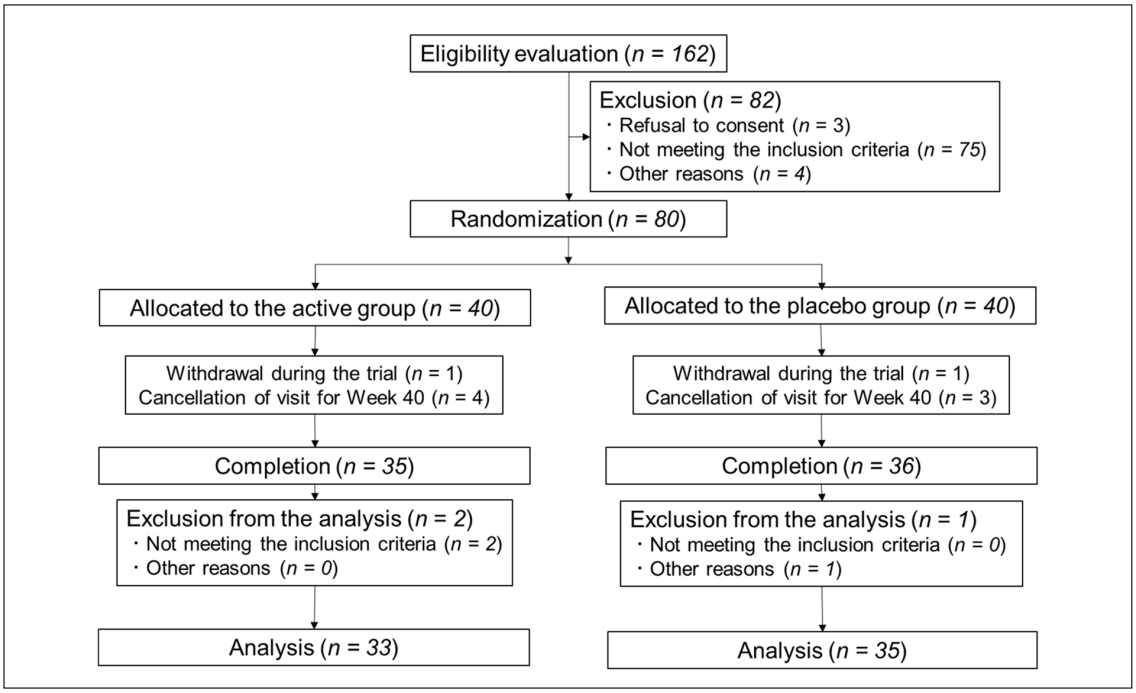

Figure 1. Flow diagram of participant recruitment during the trial.

Table III summarizes the subject background. The mean ages of the participants were 67.1 ± 3.8 years and 65.3 ± 3.6 years for the active and placebo groups, respectively. The active group was characterized by a significantly higher age than the placebo group. No other allocation factors, such as gender, MMSE scores, and StO_2 were significantly different between the groups. There was no significant difference in the height, weight, BMI, and body fat percentage between the groups. The intake of the test beverage was >99% in both groups, indicating high compliance (Table III).

Effects on Cognitive Function and Quality of Life

MMSE total scores, the primary endpoint, significantly increased within the groups from baseline to Week 40 in both groups, without a significant difference between the groups (Table IV). The Cognitrax test revealed that the NCI, which indicates overall cognitive function, cognitive flexibility, and executive function, improved within the active group from baseline to Week 40, whereas it did not significantly change in the placebo group; however, there were no significant differences between the groups (Table IV). Changes in reaction time, which is one of the cognitive functions, from baseline to Week 40 showed a significant improvement in the active group *vs.* the placebo group (Table IV). The SF-36v2 Standard Version revealed that the active group displayed significantly improved within-group mental component summary scores (2MCS_U), role-physical (RP), and vitality (VT) from baseline to Week 40, whereas these parameters did not vary significantly in the placebo group, despite there being no significant differences between the groups (Table V).

Changes in Cerebral Blood Flow

We evaluated CBV and CBF and cerebral activity at rest by total Hb and O_2Hb concentrations, respectively. The total Hb and O_2Hb concentrations significantly decreased in the placebo group on the left side, compared with no significant change in the active group. In other words, the active group displayed suppression of the decrease in concentrations (Table VI). The total Hb concentration on the right side displayed an upward movement in both groups, despite there being no significant difference between the groups (Table VI). The right side O_2Hb concentration in the placebo group significantly increased within the group from baseline to Week 40. Moreover, the active group revealed an upward movement, without a significant difference (Table VI).

Correlation between Cognitive Function, Cerebral Blood Flow, and Each Index

IGF-1 was significantly elevated in the active group from baseline to Week 40 within the group, whereas the placebo group did not reveal a significant change (Figure 2a). The ratio of plasma Aβ (Aβ42/Aβ40) revealed a downward movement from baseline to Week 40 in both groups; nonetheless, only the placebo group displayed a significant difference (Figure 2b). The O_2Hb concentration (left) and Cognitrax reaction time scores at Week 40 revealed a weak positive correlation trend in the active group, whereas there was no correlation in the placebo group (Figure 2c). Changes in the total Hb concentration and blood IGF-1 were positively correlated in the active group (Figure 2d). **Supplementary Table I** summarizes the results of other physical, hematological, and biological assessments.

Table III. Baseline characteristics of the study participants.

Parameters	Active (n = 33)	Placebo (n = 35)	*p*-value
Age (years)	67.1 ± 3.8	65.3 ± 3.6	0.048
Sex (male, %)	30.3	37.1	0.614
Height (cm)	158.5 ± 8.0	160.9 ± 8.3	0.243
Weight (kg)	57.7 ± 7.8	58.4 ± 10.2	0.752
BMI (kg/m^{-2})	23.0 ± 2.7	22.5 ± 3.0	0.492
Body fat (%)	29.7 ± 7.3	28.5 ± 6.4	0.480
MMSE	27.5 ± 1.3	27.5 ± 1.4	0.857
NCI	100.5 ± 7.3	101.8 ± 7.9	0.503
StO_2_bilateral mean	67.1 ± 3.8	65.3 ± 3.6	0.672
Intake rate (%)	99.5 ± 0.5	99.3 ± 0.9	0.347

MMSE, mini-mental state examination; BMI, body mass index; NCI, Neurocognitive Index using Cognitrax; and StO_2, tissue oxygen saturation.

Table IV. Changes in each parameter of cognitive function owing to the intervention

Cognitive function domain	Group	N	Baseline	Week 40	Δ values	*p*-value
MMSE	Active	33	27.5 (1.3)	28.2 (1.6)*	0.7 (1.7)	0.766
	Placebo	35	27.5 (1.4)	28.3 (1.9)*	0.8 (1.9)	
NCI	Active	30	100.5 (7.3)	103.3 (7.7)**	2.8 (5.2)	0.188
	Placebo	33	101.8 (7.9)	102.8 (8.0)	1.1 (4.3)	
Composite memory	Active	33	103.1 (13.6)	104.6 (13.0)	1.5 (12.5)	0.736
	Placebo	35	105.3 (13.2)	106.6 (11.4)	1.3 (11.3)	
Verbal memory	Active	33	100.1 (16.4)	103.8 (14.8)	3.7 (14.5)	0.369
	Placebo	35	105.8 (12.2)	106.5 (13.1)	0.7 (13.6)	
Visual memory	Active	33	105.6 (11.3)	104.2 (11.8)	-1.4 (13.1)	0.480
	Placebo	35	103.7 (14.1)	105.2 (12.4)	1.5 (14.8)	
Psychomotor speed	Active	33	105.8 (9.9)	109.5 (10.9)**	3.7 (7.5)	0.777
	Placebo	35	10.5.9 (10.0)	108.3 (10.2)*	2.4 (5.6)	
Reaction time	Active	32	89.6 (13.3)	93.6 (14.2)*	4.0 (10.7)	0.037
	Placebo	35	93.8 (11.6)	94.3 (13.0)	0.5 (8.6)	
Complex attention	Active	30	107.2 (8.7)	108.3 (9.0)	1.1 (6.4)	0.880
	Placebo	33	106.7 (9.6)	106.6 (10.0)	-0.1 (8.5)	
Cognitive flexibility	Active	31	97.8 (10.2)	100.9 (10.1)*	3.1 (7.5)	0.906
	Placebo	34	97.4 (11.7)	98.7 (12.1)	1.4 (8.9)	
Processing speed	Active	33	111.7 (8.4)	113.4 (7.9)	1.7 (7.1)	0.946
	Placebo	35	111.8 (11.2)	112.3 (11.3)	0.6 (8.3)	
Executive function	Active	33	96.8 (11.9)	100.8 (10.0)*	4.0 (9.7)	0.956
	Placebo	35	96.5 (11.8)	98.3 (12.2)	1.8 (8.5)	
Simple attention	Active	32	106.8 (4.4)	105.7 (8.6)	-1.1 (8.7)	0.807
	Placebo	34	105.8 (7.0)	106.3 (4.7)	0.5 (5.7)	
Motor speed	Active	33	100.1 (11.3)	103.5 (11.8)*	3.4 (7.5)	0.956
	Placebo	35	100.3 (9.7)	103.1 (9.7)**	2.9 (5.5)	

Data are presented as mean (SD). *Paired *t*-test was performed for intragroup comparisons from baseline. *$p < 0.05$, **$p < 0.01$. *p*-values indicate differences between groups for changes from baseline to Week 40. Two independent sample t-tests were performed. NCI, Neurocognitive Index; MMSE, mini-mental state examination.

Table V. Changes in each quality-of-life parameter owing to the intervention.

	Group	N	Baseline	Week 40	Δ values	*p*-value
Physical functioning	Active	33	90.9 (10.3)	89.4 (10.8)	-1.5 (8.0)	0.709
	Placebo	35	93.1 (6.4)	92.3 (7.9)	-0.9 (6.5)	
Role of physical	Active	33	84.9 (19.4)	93.0 (12.0)*	8.1 (21.0)	0.124
	Placebo	35	91.4 (14.4)	92.0 (14.3)	0.5 (19.3)	
Bodily pain	Active	33	78.9 (17.5)	79.7 (20.6)	0.7 (21.4)	0.558
	Placebo	35	75.2 (20.6)	73.0 (18.1)	-2.2 (19.2)	
Social functioning	Active	33	85.6 (14.4)	88.3 (16.5)	2.7 (21.4)	0.361
	Placebo	35	90.7 (15.3)	87.9 (22.2)	-2.9 (27.5)	
General health perceptions	Active	33	69.4 (12.7)	70.0 (13.9)	0.6 (13.1)	0.778
	Placebo	35	70.7 (14.4)	70.3 (16.0)	-0.4 (15.1)	
Vitality	Active	33	67.1 (13.7)	72.9 (15.4)*	5.9 (13.4)	0.309
	Placebo	35	70.9 (18.5)	72.9 (16.0)	2.0 (17.86	
Role emotional	Active	33	86.6 (16.7)	91.4 (14.2)	4.8 (16.7)	0.680
	Placebo	35	90.0 (18.4)	93.1 (13.3)	3.1 (17.3)	
Mental health	Active	33	76.4 (13.8)	80.5 (12.5)	4.1 (12.8)	0.316
	Placebo	35	79.4 (13.7)	80.4 (11.8)	1.0 (12.4)	
2PCS_U	Active	33	51.5 (6.0)	51.9 (6.6)	0.4 (6.7)	0.429
	Placebo	35	52.2 (4.9)	51.3 (5.9)	-0.8 (5.3)	
2MCS_U	Active	33	53.7 (7.1)	56.3 (5.5)*	2.6 (6.4)	0.302
	Placebo	35	55.7 (7.5)	56.6 (6.0)	0.9 (7.1)	

Data are presented as mean (SD). *Paired *t*-test was performed for intragroup comparisons from baseline. *$p < 0.05$, **$p < 0.01$. *p*-values indicate the difference between groups for changes from baseline to Week 40. Two independent sample *t*-tests were performed.

Table VI. Changes in cerebral blood flow parameter owing to intervention.

	Group	N	Baseline	Week 40	Δ values	*p*-value
Total Hb (Left)	Active	33	56.5 (6.4)	55.7 (6.7)	-0.8 (4.0)	0.102
	Placebo	35	57.3 (8.0)	54.6 (8.7)**	-2.8 (5.4)	
Total Hb (Right)	Active	33	55.7 (10.0)	56.8 (6.7)	1.1 (6.3)	0.974
	Placebo	35	56.5 (7.5)	57.6 (7.5)	1.2 (4.4)	
O2Hb_Left	Active	33	34.0 (3.7)	32.8 (3.5)	-1.2 (3.3)	0.117
	Placebo	35	34.5 (5.6)	32.0 (5.4)**	-2.5 (3.7)	
O2Hb_Right	Active	33	33.8 (6.0)	34.6 (4.1)	0.8 (4.6)	0.581
	Placebo	35	34.0 (5.3)	35.4 (5.3)*	1.4 (3.4)	

Data are presented as mean (SD). *Paired *t*-test was performed for intragroup comparisons from baseline. *$p < 0.05$, **$p < 0.01$. p-values indicate the difference between groups for changes from baseline to Week 40. Two independent sample *t*-tests were performed. Total Hb, total hemoglobin; O_2Hb, oxygen hemoglobin.

Lateral Index Status for Stress Assessment

The LI significantly increased from baseline to Week 40 in both groups, with a trend towards higher LI in the placebo group at Week 40 ($p = 0.05$ between the placebo and active groups) (Figure 3a). We identified a strong negative correlation between the change in LI and that in total Hb or O_2Hb concentrations in the placebo group; however, there was no correlation in the active group (Figure 3b, 3c).

Discussion

This placebo-controlled, randomized, double-blind, and parallel-group study compared the effects of quercetin glycoside-containing beverages for 40 weeks on cognitive function in Japanese men and women aged between 60 and 75 years, who were aware of age-related memory loss. MMSE total scores, the primary endpoint, significantly improved in both groups, without a difference between the groups (Table IV). MMSE

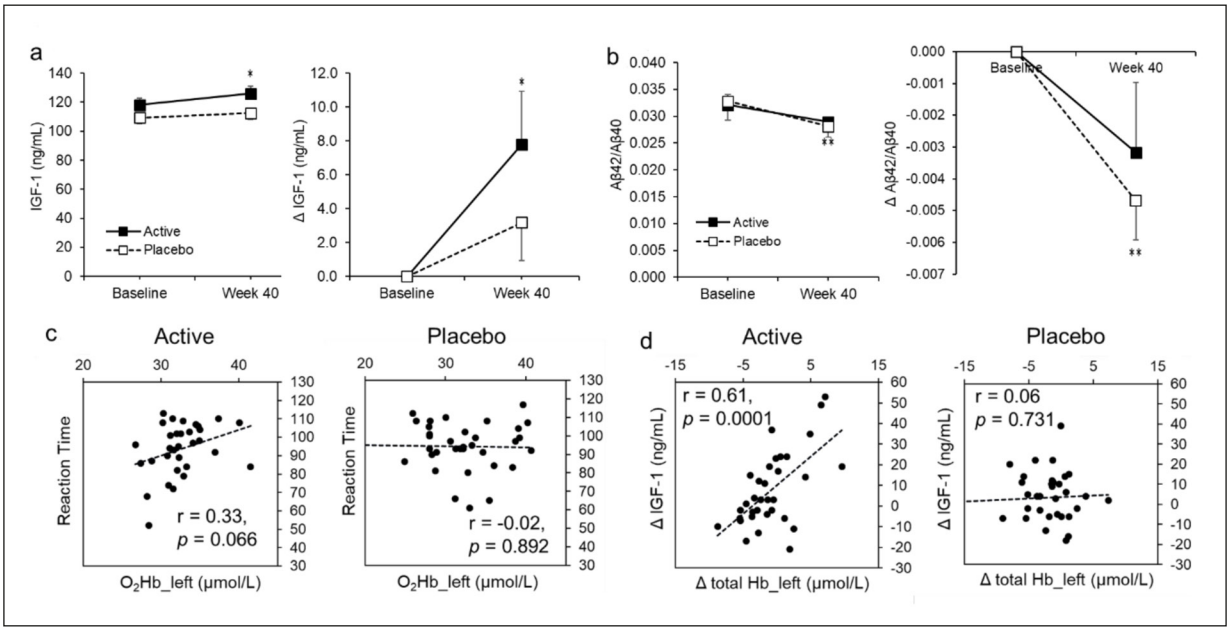

Figure 2. Lateral index. Lateral index (LI) (**a**), The correlation between changes in LI and total hemoglobin (Hb) concentration (*left side*) (**b**) and between changes in LI and oxygenated hemoglobin concentration (*left side*) (**c**). In **a**, * indicates within-group comparisons by the corresponding *t*-test. *$p < 0.05$, **$p < 0.01$. *p*-values indicate between-group comparisons by independent two-sample *t*-test. In **b-c**, r indicates the correlation coefficient by linear single correlation analysis, and the *p*-value indicates that by analysis of variance.

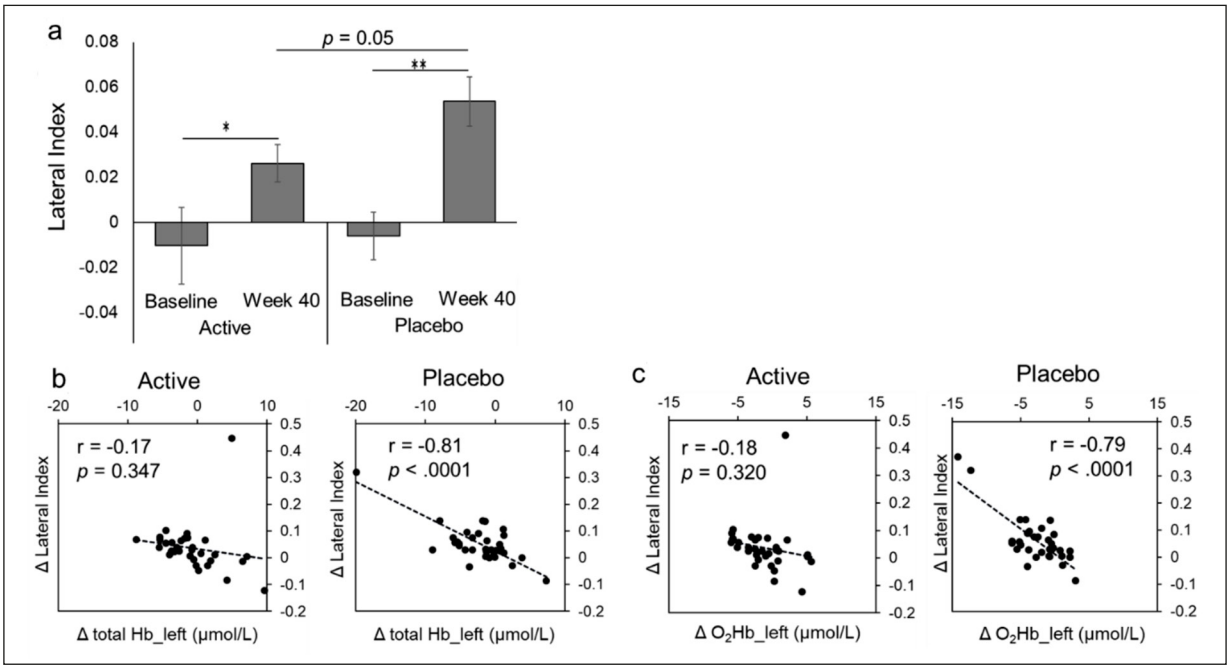

Figure 3. Lateral index. Lateral index (LI) (**a**), The correlation between changes in LI and total hemoglobin (Hb) concentration (*left side*) (**b**) and between changes in LI and oxygenated hemoglobin concentration (*left side*) (**c**). In **a**, *indicates within-group comparisons by the corresponding *t*-test. *$p < 0.05$, **$p < 0.01$. *p*-values indicate between-group comparisons by independent two-sample *t*-test. In **b-c**, r indicates the correlation coefficient by linear single correlation analysis, and the *p*-value indicates that by analysis of variance.

is a widely used screening test for dementia; however, it has insufficient power to detect MCI[20] and a ceiling effect[21]. A previous study[20] of quercetin-rich onions reported a significant improvement in MMSE scores in the active group compared with the placebo group; however, the current study comprised participants of a relatively younger age, which might have introduced the ceiling effect.

As the secondary endpoints, NCI, cognitive flexibility, and executive function measured using Cognitrax revealed significant within-group improvements only in the active group without a difference between the groups (Table IV). The change in reaction time from baseline to Week 40 revealed a significant improvement in the active group, compared with the placebo group (Table VI). The correlations between the initial value and the change in reaction time were r = -0.32 (p = 0.074) and r = -0.21 (p = 0.235) in the active and placebo groups, respectively, thus suggesting that the initial value did not exert a significant effect on the reaction time score. This score was calculated by the Stroop test, which evaluated the speed of information processing in terms of the speed of noticing and correctly responding to simple and increasingly complex instructions[15]. Therefore, the intake of quercetin glycosides improved the function of judging and processing information and attention. Cognitrax can measure a wide range of cognitive function domains and is highly sensitive to MCI and normal function, which allows it to detect mild cognitive abnormalities[15]. In addition, Cognitrax was able to detect improvements in cognitive function even in subjects in the healthy-to-MCI range owing to low learning and ceiling effects, which was difficult to assess with the MMSE. Age exerted a negligible effect on the assessment because of the use of age-normalized scores that were averaged for the same age group to be 100.

CBF measurement by NIRS revealed left-right differences (Table VI). Blood flow in the right frontal lobe increased under stress[19,22,23], which reflects the effects of stress; therefore, the effects on cognitive function were assessed by the results of left-sided measurements. The total Hb and O_2Hb concentrations on the left side revealed a significant within-group decrease in the placebo group from baseline to Week 40, whereas the active group displayed a suppressed decrease without any significant difference between the groups

(Table VI). The reasons for this decrease in the placebo group may include stressful conditions, increased water intake owing to the consumption of the test beverage, and the effects of ageing. In contrast, quercetin glycoside intake improves vascular endothelial function[12,13]; thus, the active group also demonstrated an increase in CBV and CBF owing to improved blood flow by quercetin glycoside intake. Eventually, the total Hb (left) and O_2Hb (left) concentrations suppressed the decrease. In other words, quercetin glycoside intake might be suppressed by decreased CBV, stress induced CBF, and other factors.

Regarding the relationship between CBF and cognitive function, the O_2Hb concentration (left) and reaction time scores at Week 40 displayed a weak positive correlation trend in the active group, compared with no correlation in the placebo group (Figure 2c). Therefore, CBF improvement enhanced reaction time, which is a part of cognitive function. In a previous study[24], the oral administration of quercetin improved CBF reduction and memory impairment in a mouse model of streptozotocin-induced Alzheimer's disease, thereby suggesting that quercetin-based CBF improvement is one of the mechanisms underlying improved cognitive function. Furthermore, IGF-1 significantly improved in the active group (Figure 2a) to support the aforementioned improvement. Changes in total Hb concentration (left) and IGF-1 revealed a positive correlation in the active group (Figure 2d), thus suggesting that CBF improvement may have led to neural activation. According to an epidemiological study[25,26] in the U.S. and Japan, middle-aged and elderly people, respectively, with high IGF-1 blood levels had a lower risk of developing dementia and MCI than those with low levels; people with high IGF-1 concentrations were found to have a larger cerebral volume than those with low concentrations[25]. In other words, increased IGF-1 concentration by quercetin glycoside intake may be involved in the mechanisms underlying improved cognitive function.

Next, we considered the inhibition of Aβ aggregation[27] and Tau phosphorylation[28] from *in vivo* data as a mechanism for improving cognitive function following quercetin action. Plasma Aβ42/Aβ40 levels were decreased in patients with amyloid-positive scans on positron emission tomography; the lower the value, the higher the Aβ accumulation and aggregation in the brain was found to be[29]. The average reference value provided by the Simoa measurement is approximately 0.02 to date according to the accumulated data of the measurement contractor. Compared with the aforementioned value, the participants of the present study initially accumulated less Aβ. However, both groups moved in the direction of accumulation from baseline to Week 40. Only the placebo group revealed a significant within-group difference (Figure 2b). Quercetin glycoside intake likely suppressed Aβ accumulation, thus warranting confirmation in studies with participants with initial Aβ accumulation and undergoing in the long-term intervention.

In the health-related QOL scale, the 2MCS_U, RP, and VT demonstrated a significant within-group improvement in the active group without any significant difference between the groups (Table V). Particularly, it has been found that patients with dementia experience a marked decline in their QOL. A study[30] that assessed QOL using the SF-36 in healthy elderly people, patients with MCI, and those with mild and Alzheimer's disease reported that mood disorder was a strong predictor of QOL decline in elderly people at a stage of cognitive decline. In addition, the onset of depressive symptoms leads to withdrawal in the elderly, thereby worsening dementia and a decline in physical function owing to withdrawal[31], which is a major factor contributing to QOL decline in this population. An intervention study[9] on the ingestion of onions containing high quercetin content for 24 weeks reported significant improvements in the depressive self-rating scale and motivation score. In this study, quercetin glycosides might have contributed to QOL improvement, such as vitality and mental health in old age, similar to previous reports[9].

Based on the results of physical examination and blood tests (**Supplementary Table I**), the physician responsible for this study judged that these parameters were within the range of physiological variation from a medical standpoint and were not clinically problematic. Regarding the nutritional status, such as TP and ALB, there was no significant between- and within-group difference (**Supplementary Table I**). Thus, variation in nutritional status did not affect our results.

As a secondary consideration, we analyzed the LI from NIRS measurements, which refers to an index of the right dominant state of the prefrontal cortex and is correlated with the State-Trait Anxiety Index for stress assessment, heart rate for stress response, and parasympathetic and exchange nerves[19,23]. The LI significantly increased in both groups from baseline to Week

40, suggesting increased stress during the study. Additionally, the LI at Week 40 tended to be higher in the placebo group than in the active group ($p = 0.05$) (Figure 3a). Therefore, the intake of quercetin glycosides may have alleviated the stress state. We did not directly examine stress; nonetheless, the improvement in the active group on the SF-36v2 health-related QOL scale (Table V) supported the possibility of improved mental status following quercetin glycoside intake. The study was conducted under stressful conditions, such as anxiety and behavioral restrictions, owing to the impact of the spread of COVID-19. O_2Hb concentration (left), which indicates brain activity, significantly decreased in the placebo group, whereas the active group had a suppression of this decrease (Table VI). Continued stress decreases brain activity[32,33], which is also termed as the brain fatigue state. Quercetin glycoside intake alleviated stress and suppressed decreased brain activity, which might have alleviated the brain fatigue state. Changes in LI and total Hb concentration (left) and those in the LI, total Hb, and O_2Hb concentration (left) revealed a strong negative correlation in the placebo group (Figure 3b, 3c). Therefore, stress might have induced a decrease in CBV and CBF in the participants of this study.

An advantage of this study is the approach adopted: a randomized controlled trial conducted for 40 weeks, using a variety of measures of the effects of quercetin glycosides on cognitive function and other health parameters. On the other hand, COVID-19-induced stress conditions were considered a limitation of the study. Since we did not use a subjective stress assessment tool, it is unclear how much stress was actually experienced by the study participants.

Conclusions

Quercetin glycoside intake as a beverage could improve reaction time and may potentially inhibit decreases in CBF and suppress Aβ accumulation in the brain.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Acknowledgements
We thank Dr. Kaoru Sakatani for his experimental support and advice.

Funding
This research received no external funding.

Authors' Contribution
Conceptualization: Yumi Nakamura, Hiroshi Watanabe and Norihito Murayama; Data curation: Yumi Nakamura and Aiko Tanaka; Investigation: Yumi Nakamura, Aiko Tanaka and Jun Nishihira; Project administration: Hiroshi Watanabe, Jun Nishihira and Norihito Murayama; Writing - original draft: Yumi Nakamura; Writing - review and editing: Hiroshi Watanabe, Aiko Tanaka, Jun Nishihira and Norihito Murayama.

ORCID ID
Yumi Nakamura: 0000-0002-5915-0498.

Ethics Approval
This study was conducted in accordance with the Ethical Guidelines for Medical Research Involving Human Subjects (partially revised in 2017 by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare) and was in compliance with the tenets of the Declaration of Helsinki (revised in October 2013 by the World Medical Association Fortaleza General Assembly). Prior to implementation, it was reviewed and approved by the Bioethics Committee of Hokkaido Information University (Approval number: 2019-26; Approval date: October 28, 2019). The summary of this study was registered in the University Hospital Medical Information Network Clinical Trial Registration System (UMIN-CTR) (UMIN000038593) on December 02, 2019.

Informed Consent
Participants provided consent for the participation in the study.

Trial Registration
December 2, 2019, Registration number: UMIN000038593.

References

1) World Health Organization. Risk Reduction of Cognitive Decline and Dementia [WHO guidelines]; World Health Organization: Geneva, 2019; Pp. 1.
2) Ninomiya T. Study on the Future Estimation of the Elderly Population With Dementia in Japan (Japanese Grant 2014 Special Research Project Funded by the Health Labor Sciences Research). Available at: https://mhlw-grants.niph.go.jp/system/files/2014/141031/201405037A_upload/201405037A0003.pdf.
3) Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia—me-

ta-analysis of 41 robust inception cohort studies. Acta Psychiatr Scand 2009; 119: 252-265.

4) Roberts R, Knopman DS. Classification and epidemiology of MCI. Clin Geriatr Med 2013; 29: 753-772.

5) Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, Brayne C, Burns A, Cohen-Mansfield J, Cooper C, Costafreda SG, Dias A, Fox N, Gitlin LN, Howard R, Kales HC, Kivimäki M, Larson EB, Ogunniyi A, Orgeta V, Ritchie K, Rockwood K, Sampson EL, Samus Q, Schneider LS, Selbæk G, Teri L, Mukadam N. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet 2020; 396: 413-446.

6) Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, Simic BS, Toshima H, Feskens EJM, Hollman PCH, Katan MB. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 1995; 155: 381-386.

7) Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, Kinae N. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J Nutr 2000; 130: 2243-2250.

8) Yoshimura M, Maeda A, Nakamura J, Kitagawa Y, Shibata H, Fukuhara. Effect of continuous intake of a beverage containing quercetin glycoside (enzyme-treated isoquercitrin) on body fat reduction in obese subjects. Jpn Pharmacol Ther (Pharmacol Ther) 2012; 40: 901-914.

9) Nishihira J, Nishimura M, Kurimoto M, Kagami-Katsuyama H, Hattori H, Nakagawa T, Muro T, Kobori M. 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. J Clin Biochem Nutr 2021; 69: 203-215.

10) Tateishi N, Egawa E, Kanzaki N, Kitagawa Y. Effect of quercetin glycosides on mouse dietary obesity model: lipolysis-promoting effect of quercetin. Jpn Pharmacol Ther (Pharmacol Ther) 2009; 37: 123-131.

11) Bondonno NP, Bondonno CP, Ward NC, Woodman RJ, Hodgson JM, Croft KD. Enzymatically modified isoquercitrin improves endothelial function in volunteers at risk of cardiovascular disease. Br J Nutr 2020; 123: 182-189.

12) Nakayama H, Tsuge N, Sawada H, Higashi Y. Chronic intake of onion extract containing quercetin improved postprandial endothelial dysfunction in healthy men. J Am Coll Nutr 2013; 32: 160-164.

13) Terao J. Factors modulating bioavailability of quercetin-related flavonoids and the consequences of their vascular function. Biochem Pharmacol 2017; 139: 15-23.

14) Ohmae E, Ouchi Y, Oda M, Suzuki T, Nobesawa S, Kanno T, Yoshikawa E, Futatsubashi M, Ueda Y, Okada H, Yamashita Y. Cerebral hemodynamics evaluation by near-infrared time-resolved spectroscopy: correlation with simultaneous positron emission tomography measurements. Neuroimage 2006; 29: 697-705.

15) Gualtieri CT, Johnson LG. Reliability and validity of a computerized neurocognitive test battery, CNS vital signs. Arch Clin Neuropsychol 2006; 21: 623-643.

16) Nakano M, Murayama Y, Hu L, Ikemoto K, Uetake T, Sakatani K. Effects of Antioxidant Supplements (BioPQQ™) on cerebral blood flow and oxygen metabolism in the prefrontal cortex. Adv Exp Med Biol 2016; 923: 215-222.

17) Fukuhara S, Bito S, Green J, Hsiao A, Kurokawa, K. Translation, adaptation, and validation of the SF-36 Health Survey for use in Japan. J Clin Epidemiol 1998; 51: 1037-1044.

18) Fukuhara S, Ware JE Jr, Kosinski M, Wada S, Gandek B. Psychometric and clinical tests of validity of the Japanese SF-36 health survey. J Clin Epidemiol 1998; 51: 1045-1053.

19) Tanida M, Katsuyama M, Sakatani K. Relation between mental stress-induced prefrontal cortex activity and skin conditions: a near-infrared spectroscopy study. Brain Res 2007; 1184: 210-216.

20) Tierney MC, Szalai JP, Dunn E, Geslani D, McDowell I. Prediction of probable Alzheimer disease in patients with symptoms suggestive of memory impairment. value of the Mini-Mental State Examination. Arch Fam Med 2000; 9: 527-532.

21) Oiwake C, Obigata C, Tabata C. An examination of the floor effect of the Japanese version of the Montreal cognitive assessment (MoCA-J) on delayed play: a comparison with the Mini-Mental State Examination (MMSE). Geriatr J Psychiatry 2015; 26: 531-538.

22) Ueda K, Okamoto Y, Okada G, Yamashita H, Hori T, Yamawaki S. Brain activity during expectancy of emotional stimuli: an fMRI study. Neuroreport 2003; 14: 51-55.

23) Ishikawa W, Sato M, Fukuda Y, Matsumoto T, Takemura N, Tsujii T, Sakatani K. New method of analyzing NIRS data from prefrontal cortex at rest. Adv Exp Med Biol 2013; 789: 391-397.

24) Tota S, Awasthi H, Kamat PK, Nath C, Hanif K. Protective effect of quercetin against intracerebral streptozotocin induced reduction in cerebral blood flow and impairment of memory in mice. Behav Brain Res 2010; 209: 73-79.

25) Westwood AJ, Beiser A, DeCarli C, Harris TB, Chen TC, He XM, Roubenoff R, Pikula A, Au R, Braverman LE, Wolf PA, Vasan RS, Seshadri S. Insulin-like growth factor-1 and risk of Alzheimer dementia and brain atrophy. Neurology 2014; 82: 1613-1619.

26) Doi T, Shimada H, Makizako H, Tsutsumimoto K, Hotta R, Nakakubo S, Suzuki T. Association of

insulin-like growth factor-1 with mild cognitive impairment and slow gait speed. Neurobiol Aging 2015; 36: 942-947.

27) Jiménez-Aliaga K, Bermejo-Bescós P, Benedí J, Martín-Aragón S. Quercetin and rutin exhibit antiamyloidogenic and fibril-disaggregating effects in vitro and potent antioxidant activity in APPswe cells. Life Sci 2011; 89: 939-945.

28) Sabogal-Guáqueta AM, Muñoz-Manco JI, Ramírez-Pineda JR, Lamprea-Rodriguez M, Osorio E, Cardona-Gómez GP. The flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in aged triple transgenic Alzheimer's disease model mice. Neuropharmacology 2015; 93: 134-145.

29) Schindler SE, Bollinger JG, Ovod V, Mawuenyega KG, Li Y, Gordon BA, Holtzman DM, Morris JC, Benzinger TL, Xiong C, Fagan AM, Bateman RJ. High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. Neurology 2019; 93: e1647-e1659.

30) Weiss EM, Papousek I, Fink A, Matt T, Marksteiner J, Deisenhammer EA. Quality of life in mild cognitive impairment, patients with different stages of Alzheimer disease and healthy control subjects. Neuropsychiatr 2012; 26: 72-77.

31) Qiu WQ, Dean M, Liu T, George L, Gann M, Cohen J, Bruce ML. Physical and mental health of homebound older adults: an overlooked population. J Am Geriatr Soc 2010; 58: 2423-2428.

32) Lim J, Wu WC, Wang J, Detre JA, Dinges DF, Rao H. Imaging brain fatigue from sustained mental workload: an ASL perfusion study of the time-on-task effect. Neuroimage 2010; 49: 3426-3435.

33) Pang CY, Zhao CH, Xu FJ. Study on brain fatigue based on EEG signals. Adv Mater Res 2011; 396-398: 1523-1526.

34) Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A, Collaborators Developing the Japanese Equation for Estimated GFR. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009; 53: 982-992.