Long-Term Resveratrol Intake for Cognitive and Cerebral Blood Flow Impairment in Carotid Artery Stenosis/Occlusion

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Background and Purpose Carotid artery stenosis or occlusion (CASO) is a causative disease of vascular cognitive impairment (VCI) attributed to cerebral hypoperfusion, even without the development of symptomatic ischemic stroke. Preclinically, resveratrol has been demonstrated to play an important role in improving cognitive function in rodent CASO models. This study investigated the association between long-term resveratrol intake and improvements in cognitive and cerebral hemodynamic impairments in patients with CASO.

Methods A retrospective cohort study was conducted on patients with asymptomatic carotid artery stenosis of ≥50% or occlusion who underwent ^{15}O-gas positron emission tomography (^{15}O-gas PET) and neuropsychological tests such as Montreal Cognitive Assessment (MoCA) and Alzheimer's Disease Assessment Scale-Cognitive Subscale 13 (ADAS-Cog) twice between July 2020 and March 2022 allowing >125-day interval. Patients were administered 30 mg/day resveratrol after the first ^{15}O-gas PET and neuropsychological tests were compared with those who were not.

Results A total of 79 patients were enrolled in this study; 36 received resveratrol and 43 did not. Over a mean follow-up of 221.2 and 244.8 days, long-term resveratrol treatment significantly improved visuospatial/executive function (P=0.020) in MoCA, and memory domain (P=0.007) and total score (P=0.019) in ADAS-Cog. Cerebral blood flow demonstrated improvement in the right frontal lobe (P=0.027), left lenticular nucleus (P=0.009), right thalamus (P=0.035), and left thalamus (P=0.010) on ^{15}O-gas PET. No adverse events were reported.

Conclusion Long-term daily intake of oral resveratrol may prevent or treat VCI by improving the cerebral blood flow in asymptomatic patients with CASO.

Keywords Resveratrol; Carotid artery stenosis; Carotid artery occlusion; ^{15}O-gas positron emission tomography; Cerebral blood flow; Vascular cognitive impairment

Introduction

Carotid artery stenosis or occlusion (CASO) is a major etiological factor for cerebral infarction that can cause cognitive deterioration, even without the development of symptomatic ischemic stroke. Additionally, CASO causes vascular cognitive impairment

(VCI). Asymptomatic carotid artery stenosis of ≥50% can cause VCI even in the absence of focal symptoms and induce morphological change in the brain such as reducing cortical thickness.[1-4] Therefore, carotid artery stenosis of ≥50% increases the risk of VCI, indicating that asymptomatic CASO carries symptomatic implications. However, a large population exists with undetected cognitive morbidities. This finding has important public health implications. Moreover, the prevention or treatment of VCI, which may result from hypoperfusion in patients with CASO, is an urgent concern.

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a polyphenol that is naturally occurring in the skin of red grapes, wine, blueberries, and peanuts.[5] Resveratrol plays an important role in the inhibition of various human diseases, such as brain disorders, via pleiotropic effects.[6] In the preclinical stage, resveratrol improved cognitive performance in rat models subjected to bilateral common carotid artery occlusion, mimicking chronic cerebral hypoperfusion by suppressing oxidative stress and inflammation and promoting autophagy.[7-9] Furthermore, the longevity gene *SIRT1* which can be activated by resveratrol[10] revealed cognitive improvement and cerebral blood flow (CBF) preservation via deacetylation of endothelial nitric oxide synthesis (eNOS) in a mouse CASO model.[11,12] However, clinical evidence is limited, and only a few reports are available on the positive effects on cognition in healthy participants. Daily intake of oral resveratrol 200 mg/day for 26 weeks significantly improved memory and hippocampal functional connectivity in healthy participants aged 50–75 years.[5] Another study revealed that healthy postmenopausal women aged 45–85 years who received resveratrol 150 mg/day for 14 weeks displayed significant improvements in verbal memory and overall cognitive function.[13] However, the efficacy of resveratrol in asymptomatic patients with CASO remains unclear.

This study aimed to evaluate the association between long-term resveratrol intake and improvements in cognitive performance, with a focus on cerebral hemodynamic state preservation on ^{15}O-gas positron emission tomography (^{15}O-gas PET) in patients with asymptomatic CASO.

Methods

Study design

This retrospective cohort study was conducted at the National Cerebral and Cardiovascular Center (NCVC) of Japan, in accordance with the Declaration of Helsinki. The study was approved by the Research Ethics Committee of the NCVC (approval number: R20113). Patient participation was obtained through an opt-out methodology, which meant that participants were included in the study unless they expressed their decision to be excluded.

The inclusion criteria were as follows: (1) patients with asymptomatic CASO who presented with a peak systolic velocity of >130 cm/s at a stenotic lesion or occlusion on carotid Doppler ultrasonography; (2) patients who underwent ^{15}O-gas PET for the assessment of the CBF, cerebral metabolic rate of oxygen (CMRO$_2$), and oxygen extraction fraction (OEF), and neuropsychological examinations including Montreal Cognitive Assessment (MoCA) and Alzheimer's Disease Assessment Scale-Cognitive Subscale 13 (ADAS-Cog) twice between July 2020 and March 2022 with an interval of >125 days between visit 1 and visit 2 (Supplementary Figure 1). A peak systolic velocity of >130 cm/s at a stenotic lesion indicates a stenotic diameter of approximately ≥50%, which is comparable to moderate or severe stenosis.[14,15] Asymptomatic status was confirmed by the absence of stroke or transient ischemic attack attributed to the development of a carotid lesion within 180 days, with a modified Rankin Scale score ≤1.[16] Patients with ≥60% asymptomatic carotid stenosis are at a high risk of stroke, even when receiving the best medical treatment.[17] Therefore, patients with asymptomatic moderate or severe CASO generally need to undergo functional brain imaging such as PET to validate whether surgical revascularization is indicated. Patients who received management strategies for metabolic and vascular risk factors and orally received 30 mg/day resveratrol (resveratrol group) and those who received management of metabolic and vascular risk factors without resveratrol (non-resveratrol group) at our neurology clinic were compared. The resveratrol group was administered the polyphenol at a dose of 30 mg/day (DHC Corporation, Tokyo, Japan) after visit 1 (Supplementary Figure 1). Data on medical history, blood test results, and medications were collected. The primary outcome was cognitive preservation or improvement following the resveratrol treatment. The secondary outcome was the preservation or improvement of cerebral hemodynamic values on ^{15}O-gas PET.

^{15}O-gas PET measurements

All patients underwent a series of ^{15}O-gas PET examinations to assess CBF, CMRO$_2$, and OEF (Supplementary Figure 2A). Radioactive ^{15}O was produced by accelerating a deuteron (d) beam via a ^{14}N(d,n)^{15}O nuclear reaction using a cyclotron (CYPRIS HM-12, Sumitomo Heavy Industry, Tokyo, Japan). Furthermore, 0.3% oxygen (O$_2$) in the nitrogen (N$_2$) target gas was used to produce ^{15}O-O$_2$ and ^{15}O-carbon monoxide (CO) gases, while 1.0% carbon dioxide (CO$_2$) in the N$_2$ target gas was used to produce ^{15}O-CO$_2$ gas.

A PET scanner (Biograph mCT; Siemens Healthineers, Erlangen, Germany) was used in this study. A ^{15}O-gas PET scan was initiated 3 min after 2-min inhalation of ^{15}O-CO inhalation for 4 min. An additional dynamic ^{15}O-gas PET scan was performed

JoS

Hattori et al.   Efficacy of Resveratrol in Asymptomatic CASO

for 8 min during sequential inhalation of ^{15}O-O$_2$ and ^{15}O-CO$_2$ gases for 1 min each at 4.5-min intervals. The radiochemical purity was confirmed to be >99% before radio gas inhalation in each patient via two-channel rapid gas chromatography (Micro 990, Agilent Technologies, Inc., Santa Clara, CA, USA), as described in a previous study.[18]

^{15}O-gas PET images were reconstructed using vendor software with an adequately selected methodology that considered the presence of gaseous ^{15}O-radioactivity surrounding the face during the inhalation period.[19] Functional images of CBF, CMRO$_2$, and OEF were obtained according to a previously validated technique (dual-table autoradiography technique).[20] The arterial input function was obtained from the radioactivity concentration in the arterial blood, which was continuously withdrawn from the brachial artery.[20] The metabolized ^{15}O-water in the arterial blood generated from ^{15}O-O$_2$ was estimated by modeling the physiological oxygen metabolism.[21]

Neuropsychological assessment
All patients underwent neuropsychological examinations, including the MoCA and ADAS-Cog, performed by senior clinical neuropsychologists (CK and MY) blinded to the intervention groups.

Concentration of resveratrol and its metabolites
The concentrations of resveratrol and its metabolites were measured via liquid chromatography-mass spectrometry in patients who provided written informed consent to participate in the NCVC biobank. For a detailed description, see the Supplementary Methods.

Statistical analysis
All ^{15}O-gas PET images were anatomically standardized using linear and nonlinear transformations with SPM2[22] to ensure that the brain images of all patients had the same anatomical format. Three-dimensional (3D) Stereotaxic Region-of-interest (ROI) Template (3DSRT, PDRadiopharma Inc., Tokyo, Japan) was used (Supplementary Figure 2B).[23,24] Twelve ROI segments grouped according to arterial supply were examined in each hemisphere: callosomarginal, pericallosal, precentral, central, parietal, angular, temporal, posterior, hippocampus, lenticular nucleus, thalamus, and cerebellum[25] (Supplementary Figure 2B). The entire hemisphere comprised the area, except for the ipsilateral cerebellum. The frontal lobe comprises the callosomarginal, precentral, and central artery areas. The counts under each ROI were normalized to the average count within the bilateral cerebellum to quantify relative CBF, CMRO$_2$, and OEF.

Continuous variables with a normal distribution were expressed as means±standard deviations. Differential analysis was performed

by subtracting each cognitive subscale and the total MoCA and ADAS-Cog scores, as well as the relative CBF, CMRO$_2$, and OEF in each ROI between the time points to compare temporal changes in cognitive functions and cerebral hemodynamics between the two groups using the Student's t-test. To identify independent predictors of cognitive function and CBF preservation in patients with CASO, multivariate linear regression analyses were conducted after adjusting for age, sex, hypertension, diabetes mellitus, dyslipidemia, and coronary artery disease, as appropriate. No data regarding the primary and secondary outcomes was missing. All reported P-values were two-tailed, and P-values <0.05 were considered statistically significant. All analyses were performed using the SPSS software version 27 (IBM Corp., Armonk, NY, USA) and GraphPad PRISM (Ver 8.4.3; GraphPad Software, Boston, MA, USA).

Results

Baseline characteristics of the participants
This study enrolled 79 asymptomatic patients with moderate or severe CASO who underwent neuropsychological assessments and ^{15}O-gas PET. Among the 79 patients, 43 received the best medical treatment for metabolic and vascular risk factors and 36 received resveratrol 30 mg/day. The mean age (76.4±7.4 vs. 77.0±8.6 years) and the proportion of male patients (34 [79.1%] vs. 27 [75.0%]) were comparable between the non-resveratrol and resveratrol groups. The baseline mean total MoCA scores of the non-resveratrol and resveratrol groups were 23.6±3.6 and 23.7±3.6, respectively. Moreover, the baseline mean total ADAS-Cog scores were 14.6±5.4 and 15.8±7.2 in the non-resveratrol and resveratrol groups, respectively. Hence, these patients present with mild cognitive impairment (MCI) or mild dementia.[26] The mean observational periods between visit 1 and visit 2 in Supplementary Figure 1 were 244.8±86.9 and 221.2±61.9 days (P=0.18) (Table 1).

Resveratrol intake and blood concentration of resveratrol and its metabolites
In humans, oral resveratrol is rapidly metabolized into glucuronide and sulfate conjugates, with the parent molecules and metabolites initially peaking in the plasma 30–90 minutes after dosing.[27,28] The resveratrol group demonstrated a significant increase in blood concentrations of resveratrol and its metabolites, including resveratrol 3-O-glucuronide, resveratrol 4-O-glucuronide, and resveratrol 3-O-sulfate (Supplementary Table 1). The mean interval (±standard deviation) between the time of resveratrol intake and that of blood collection was 4 h 6 min (±3 h 49 min). Therefore, the blood concentration of resveratrol was

Table 1. Baseline characteristics of the patients

Characteristic	Non-resveratrol group (n=43)	Resveratrol group (n=36)	P
Age (yr)	76.4±7.4	77.0±8.6	0.75
Male sex	34 (79.1)	27 (75.0)	0.79
Smoking	18 (41.9)	8 (22.2)	0.09
Alcohol intake	22 (51.2)	16 (44.4)	0.65
PMH of coronary artery disease	12 (27.9)	6 (16.7)	0.29
Atrial fibrillation	9 (20.9)	3 (8.3)	0.21
Antiplatelet use	26 (60.5)	27 (75.0)	0.23
Anticoagulant use	9 (20.9)	2 (5.6)	0.06
Antihypertensive drug use	39 (90.7)	29 (80.6)	0.21
Statin use	39 (90.7)	30 (83.3)	0.50
Systolic blood pressure (mm Hg)	134.0±18.4	141.2±23.8	0.13
Diastolic blood pressure (mm Hg)	71.1±13.3	71.0±13.6	0.97
Routine blood glucose (mg/dL)	129.6±40.6	107.0±19.3	0.07
HbA1c (%)	6.2±0.6	6.1±0.8	0.34
TG (mg/dL)	131.7±70.7	153.2±97.4	0.26
LDL-C (mg/dL)	79.7±22.5	85.6±29.8	0.32
HDL-C (mg/dL)	52.0±12.6	52.8±12.7	0.76
CRP (mg/dL)	0.19±0.67	0.18±0.33	0.93
Side of carotid stenosis			0.27
Right	13 (30.2)	10 (27.8)	
Left	17 (39.5)	20 (55.6)	
Bilateral	13 (30.2)	6 (16.7)	
Initial total MoCA score	23.6±3.6	23.7±3.6	0.91
Initial total ADAS-Cog score	14.6±5.4	15.8±7.2	0.38
Observational period (days)	244.8±86.9	221.2±61.9	0.18

Categorical variables were expressed as actual numbers and proportions (%) inside parentheses. Continuous variables were expressed as means± standard deviations.
PMH, past medical history; HbA1c, hemoglobin A1c; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein; MoCA, Montreal Cognitive Assessment; ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale 13.

substantially lower than that of its metabolites. We assumed that the resveratrol group consumed resveratrol daily as displayed in Supplementary Table 1.

Resveratrol intake and vascular risk factors
Figure 1 illustrates the longitudinal changes in blood pressure and blood test findings. The systolic blood pressure of the resveratrol group significantly decreased (-10.1±21.3 mm Hg), meanwhile, the systolic blood pressure of the non-resveratrol group increased slightly (5.8±19.8 mm Hg) (P=0.003). The routine blood glucose level of the resveratrol group significantly decreased by -3.0±16.9 mg/dL. However, the routine blood glucose level of the non-resveratrol group increased by 15.0±44.9 mg/dL (P=0.029).

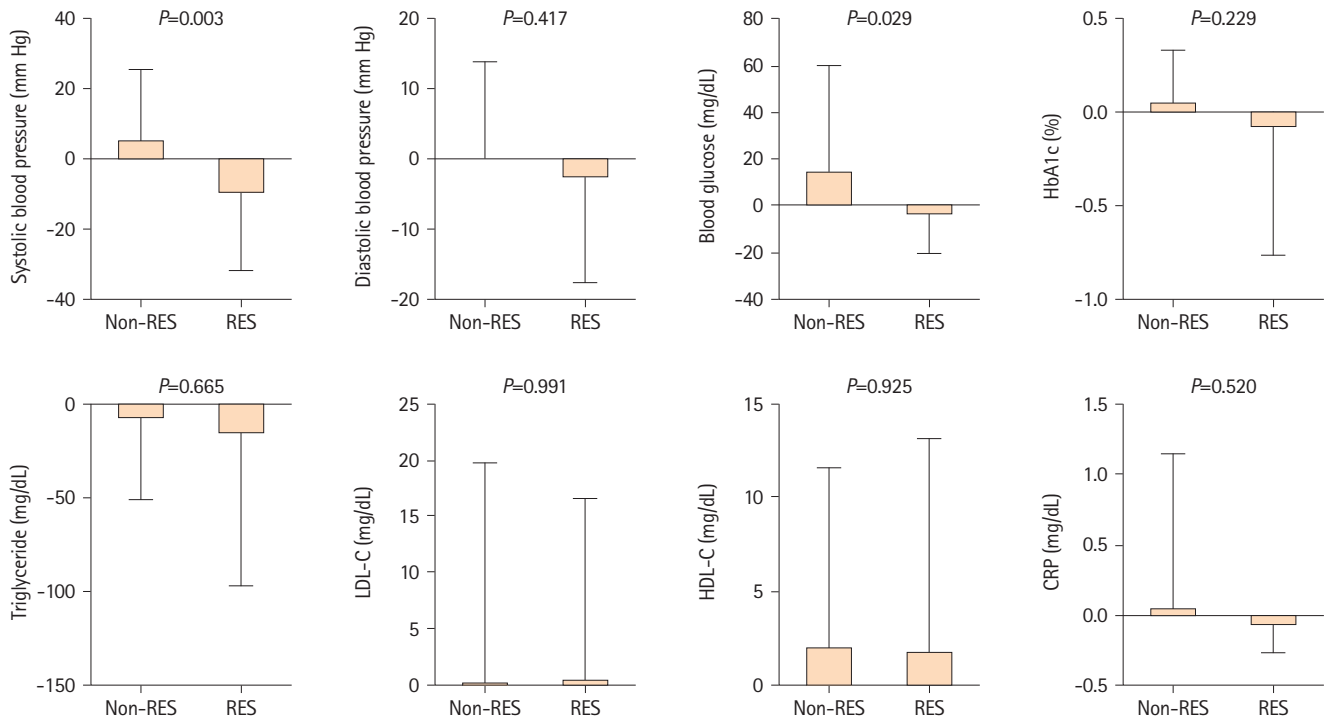Diastolic blood pressure (P=0.417) and glycated hemoglobin (HbA1c) levels (P=0.229) in the resveratrol group were more likely to improve (Figure 1). No significant changes were observed in lipid metabolism, such as in triglyceride, low-density lipoprotein cholesterol, or high-density lipoprotein cholesterol levels (Figure 1).

Association between resveratrol intake and cognitive improvement in patients with asymptomatic CASO
We examined the MoCA and ADAS-Cog scores to investigate cognitive changes related to resveratrol treatment. CASO mainly induces visuospatial, executive, and memory impairment.[4,16] Resveratrol intake was significantly associated with visuospatial/ executive improvement (non-resveratrol group: -0.2±1.3 vs. resveratrol group: 0.4±0.9; P=0.020) based on MoCA. However, the MoCA total score did not demonstrate a significant difference (-0.4±3.2 vs. 0.4±2.6) (Figure 2). Furthermore, according to ADAS-Cog, a significantly high interval change was observed in the delayed word recall (0.5±1.6 vs. -0.9±1.8; P=0.001), memory domain (0.4±3.3 vs. -1.6±3.1; P=0.007), and total score (0.5±3.7 vs. -1.5±3.4; P=0.019) between the non-resveratrol and resveratrol groups (Figure 3). Therefore, multivariate linear regression model analysis was performed after adjusting for age, sex, hypertension, diabetes, dyslipidemia, and coronary artery disease. Resveratrol intake was an independent predictor of improvement in visuospatial/executive function (β=0.26, 95% confidence interval [CI]: 0.06–1.15, P=0.030) and memory (memory domain: β=-0.26, 95% CI: -3.23 to -0.15, P=0.032; delayed word recall: β=-0.37, 95% CI: -2.16 to -0.54, P=0.001) (Table 2). Thus, resveratrol may contribute to the amelioration of visuospatial, executive, and memory impairments in patients with CASO.

Association between resveratrol intake and CBF improvement in patients with asymptomatic CASO
To identify the reason for the significantly high interval change in visuospatial and executive functions and memory in the resveratrol group, cerebral hemodynamics, including relative CBF, $CMRO_2$, and OEF, were assessed using ^{15}O-gas PET. Initially, the CBF changes in each hemisphere were analyzed. Compared with the non-resveratrol group, the resveratrol group was more likely to present with CBF improvement in the entire right (0.0011± 0.034 vs. 0.016±0.032; P=0.051) and left (-0.0011±0.035 vs. 0.014±0.031; P=0.054) hemispheres. To examine the aforemention changes in detail, we analyzed CBF in the frontal lobe, basal ganglia, and thalamus, which govern visuospatial and executive function[29] and memory. Longitudinally, long-term resveratrol in-

Figure 1. Temporal changes in conventional vascular risk factors. Bar graphs demonstrate temporal changes in systolic and diastolic blood pressure and routine blood glucose, glycated hemoglobin (HbA1c), triglyceride, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and C-reactive protein (CRP) levels between the non-resveratrol (non-RES) and resveratrol (RES) groups. Error bars indicate standard deviation.
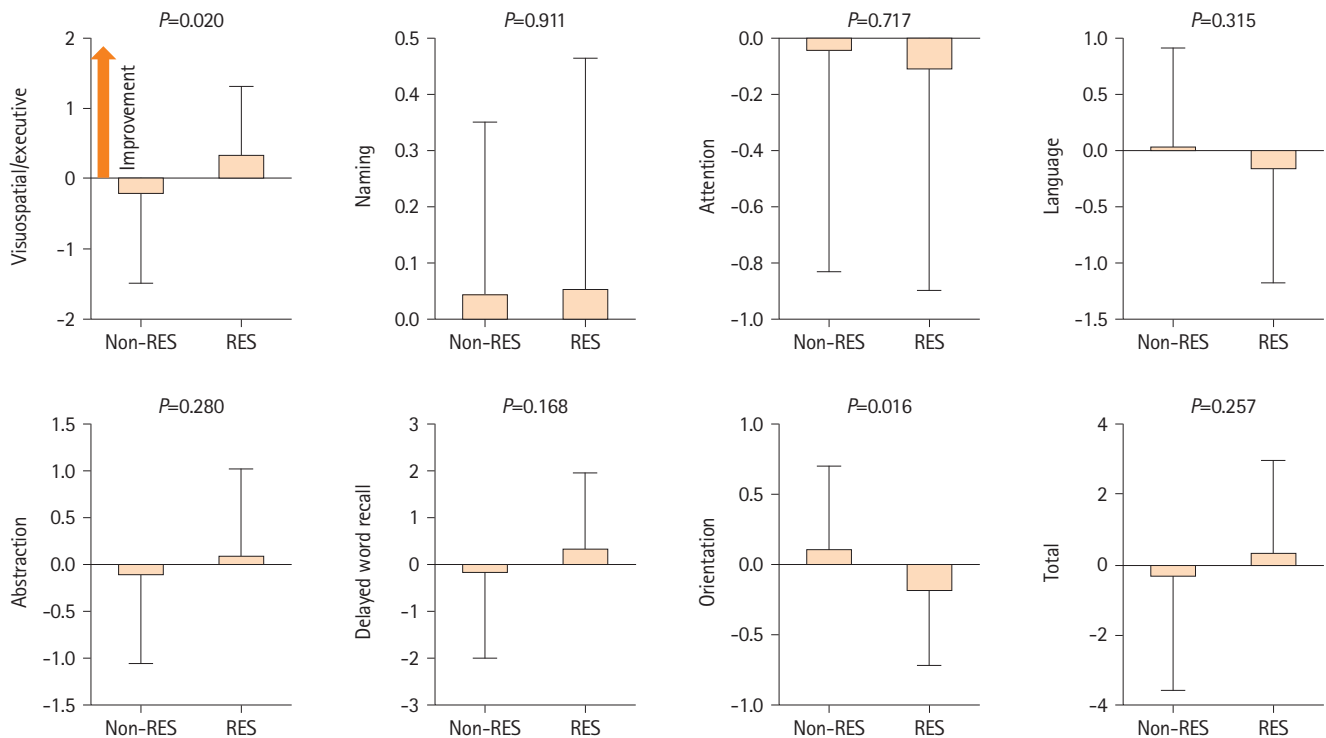
Figure 2. Temporal cognitive changes assessed using the Montreal Cognitive Assessment (MoCA). Bar graphs display temporal cognitive changes assessed with the MoCA between the non-resveratrol (non-RES) and resveratrol (RES) groups. Error bars indicate standard deviation.

take was associated with a significant increase in relative CBF in the right frontal lobe (non-resveratrol group: 0.001±0.037 and resveratrol group: 0.019±0.033; P=0.027) (Figure 4). In the frontal lobe, the right (−0.004±0.041 vs. 0.018±0.034; P=0.013) and left (−0.003±0.040 vs. 0.017±0.042; P=0.031) callosomarginal artery areas, and the right precentral artery area (0.002±0.040 vs. 0.021±0.035; P=0.028) demonstrated associations between significant increased CBF and long-term resveratrol intake (Fig-
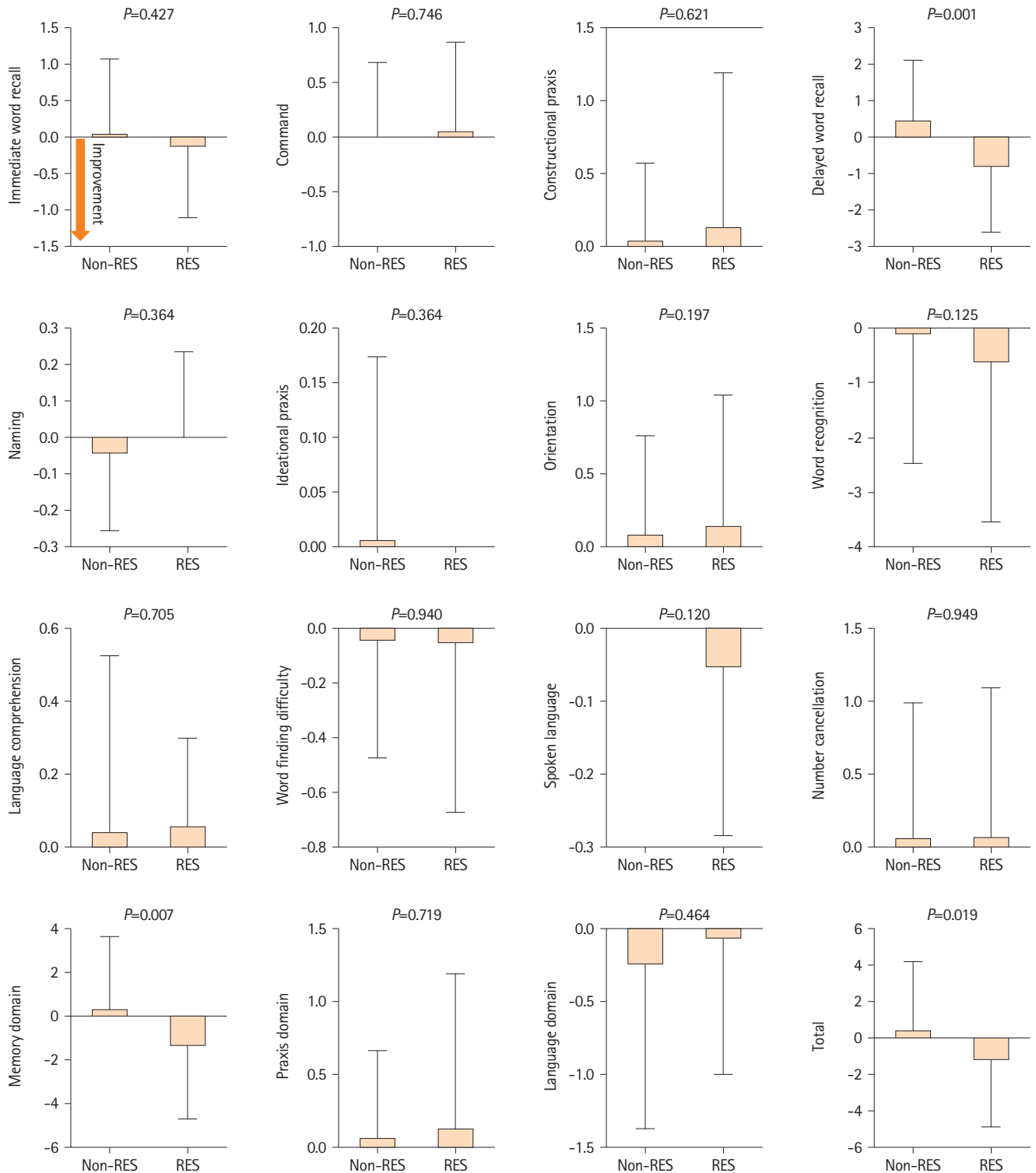

Figure 3. Temporal cognitive changes assessed using the Alzheimer's Disease Assessment Scale-Cognitive Subscale 13 (ADAS-Cog). Bar graphs display temporal cognitive changes assessed with ADAS-Cog between the non-resveratrol (non-RES) and resveratrol (RES) groups. Error bars indicate standard deviation.

JoS

Hattori et al.   Efficacy of Resveratrol in Asymptomatic CASO

Table 2. Univariate and multivariate linear mixed model analyses of cognitive improvement associated with resveratrol intake

Cognitive components	Univariate analysis			Multivariate analysis		
	β	95% CI	P	β	95% CI	P
MoCA						
Visuospatial/executive	0.26	0.09–1.10	0.022	0.26	0.06–1.15	0.030
ADAS-Cog						
Memory domain	–0.30	–3.41 to –0.55	0.007	–0.26	–3.23 to –0.15	0.032
Delayed word recall	–0.37	–2.12 to –0.59	0.001	–0.37	–2.16 to –0.54	0.001
Total	–0.26	–3.57 to –0.32	0.019	–0.18	–2.94 to –0.32	0.113

Multivariate regression analyses were performed after adjusting for age, sex, hypertension, diabetes mellitus, dyslipidemia, and coronary artery disease.
MoCA, Montreal Cognitive Assessment; ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale 13; CI, confidence interval.
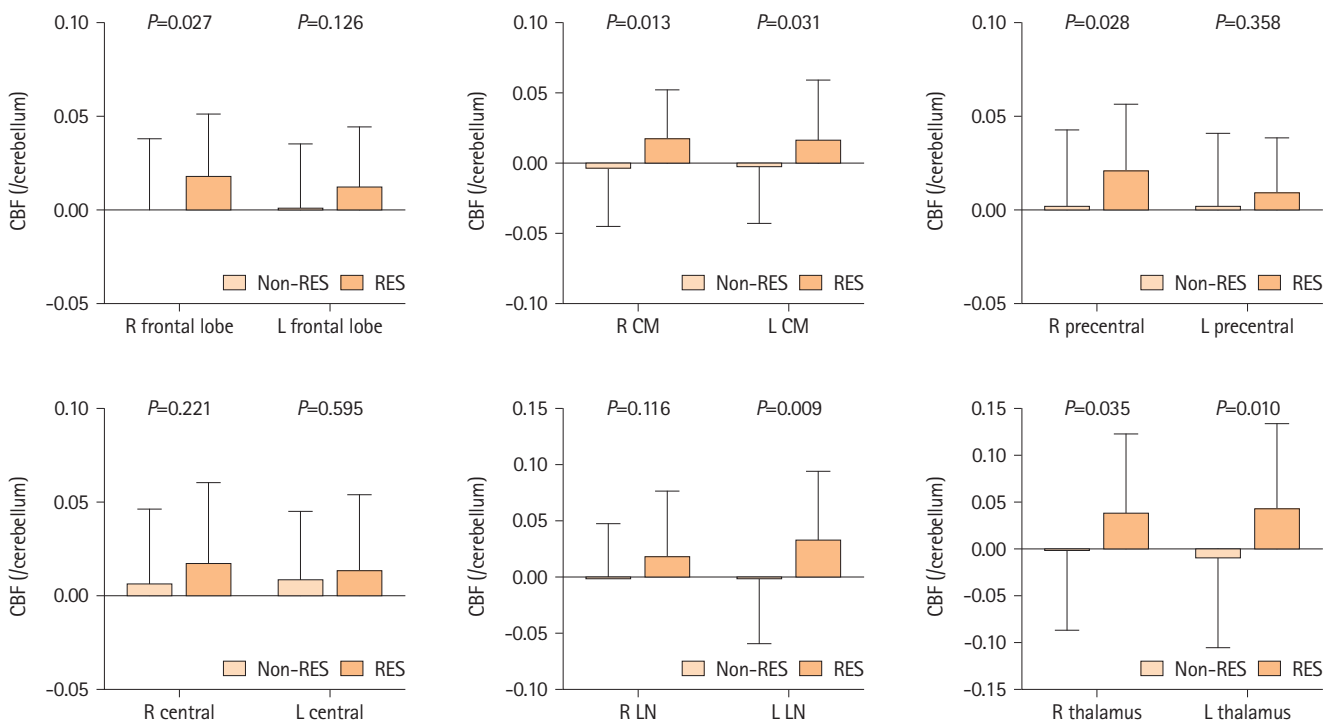
Figure 4. Temporal changes in cerebral blood flow (CBF). Bar graphs indicate the temporal changes in cerebral blood flow in the right (R) and left (L) frontal lobes, callosomarginal artery area (CM), precentral artery area, central artery area, lenticular nucleus (LN), and thalamus in the non-resveratrol (non-RES) and resveratrol (RES) groups. Error bars indicate standard deviation.

ure 4). Furthermore, the left lenticular nucleus (–0.002±0.06 vs. 0.033±0.06; $P=0.009$), right thalamus (–0.002±0.085 vs. 0.039± 0.85; $P=0.035$), and left thalamus (–0.011±0.095 vs. 0.045±0.090; $P=0.010$) also demonstrated significant associations (Figure 4). After adjusting for age, sex, hypertension, diabetes, dyslipidemia, and coronary artery disease, the multivariate linear regression models displayed that long-term resveratrol intake was an independent predictive factor of increased CBF in the right frontal lobe (β=0.25, 95% CI: 0.001–0.035; $P=0.039$), the right callosomarginal artery area (β=0.29, 95% CI: 0.005–0.041; $P=0.013$), the right precentral artery area (β=0.25, 95% CI: 0.001–0.037; $P=0.042$), the left lenticular nucleus (β=0.31, 95% CI: 0.009–0.067; $P=0.011$), and the left thalamus (β=0.29, 95% CI: 0.011–

0.102; $P=0.016$) in patients with asymptomatic CASO (Table 3). The relative $CMRO_2$ changes were comparable between the two groups in the entire right (0.0044±0.035 vs. 0.0095±0.037; $P=0.53$) and left (0.0033±0.035 vs. 0.0069±0.033; $P=0.65$) hemispheres as well as in the bilateral frontal lobe, lenticular nucleus, and thalamus (Supplementary Figure 3). Similarly, the relative OEF changes were also comparable between the two groups in the entire right (–0.0028±0.39 vs. –0.0040±0.48; $P=0.91$) and left (–0.0028±0.42 vs. –0.0001±0.48; $P=0.79$) hemispheres as well as in the bilateral frontal lobe, lenticular nucleus, and thalamus (Supplementary Figure 4). Thus, long-term resveratrol intake may contribute not only to cognitive improvement but also to increased CBF. No adverse events were reported.

Table 3. Univariate and multivariate linear mixed model analyses of cerebral blood flow changes associated with resveratrol intake

Regions of interest	Univariate analysis			Multivariate analysis		
	β	95% CI	P	β	95% CI	P
Right frontal lobe	0.25	0.002–0.034	0.027	0.25	0.001–0.035	0.039
Right callosomarginal artery	0.28	0.005–0.039	0.013	0.29	0.005–0.041	0.013
Left callosomarginal artery	0.24	0.002–0.039	0.031	0.27	0.003–0.042	0.027
Right precentral artery	0.25	0.002–0.036	0.028	0.25	0.001–0.037	0.042
Left lenticular nucleus	0.29	0.009–0.062	0.009	0.31	0.009–0.067	0.011
Thalamus						
Right	0.24	0.003–0.079	0.035	0.23	0.00–0.082	0.052
Left	0.29	0.014–0.097	0.010	0.29	0.011–0.102	0.016

Multivariate regression analyses were performed after adjusting for age, sex, hypertension, diabetes mellitus, dyslipidemia, and coronary artery disease.
CI, confidential interval.

Discussion

This study demonstrated that long-term intake of oral resveratrol might improve cognitive functions, including visuospatial and executive function, and memory. Furthermore, CBF was associated with cognitive function in asymptomatic patients with moderate or severe CASO.

Currently, no promising preventive or treatment strategies are available for CASO-related VCI. Carotid revascularization, such as carotid endarterectomy (CEA) and artery stenting (CAS), does not affect cognition, although restores cerebral perfusion. However, operative ischemia, including subclinical cerebral microembolic patterns that occur during revascularization, may worsen neuropsychological function.[30] One meta-analysis[31] revealed that CEA and CAS did not improve cognitive dysfunction. To prevent cognitive deterioration in patients with CASO, cerebrovascular integrity should be maintained without perioperative cerebral ischemia or silent microembolic cerebral injury. Furthermore, no evidence supports the efficacy of antithrombotic drugs in preventing VCI.[32]

In this setting, where no novel candidates had been established, the first report demonstrating that resveratrol treatment preclinically improved cerebrovascular dysfunction was published in 2014. Resveratrol preserves neurovascular function by reducing oxidative stress and improving vascular endothelial function in 24-month-old mice.[33] Thereafter, several *in vivo* studies have displayed that resveratrol improves cognitive performance via pleiotropic effects, including suppression of oxidative stress and inflammation, promotion of autophagy, and anti-apoptotic effects in rat models subjected to bilateral common carotid artery occlusion mimicking chronic cerebral hypoperfusion. For example, resveratrol activates autophagy and inhibits the expression of protein kinase B (AKT)/mechanistic target of rapamycin signaling pathway-related proteins, thereby reducing oxidative

stress-induced neuronal damage in the frontal cortex and hippocampus and neuronal apoptosis.[7] Resveratrol can improve cognitive function by activating protein kinase A and cyclic adenosine monophosphate (cAMP) responsive element-binding protein pathways, which prevent the inhibition of long-term potentiation and restore dendritic spinal impairment in rats.[8] Resveratrol reduces the inflammatory response during chronic cerebral hypoperfusion by downregulating the stimulator of interferon genes/TRAF family member-associated NF-κB activator (TANK)-binding kinase 1/interferon regulatory factor 3 signaling.[9]

Alternatively, resveratrol may activate the nicotinamide adenine dinucleotide (NAD$^+$)/Sirtuin 1 (SIRT1) pathway.[10] Resveratrol competitively inhibits cAMP-degrading phosphodiesterases, leading to elevated cAMP levels. Therefore, resveratrol increases NAD$^+$, resulting in increased SIRT1 activity.[10] Resveratrol also activates eNOS via SIRT1 activation.[34] Our previous study demonstrated that working memory, CBF, and white matter integrity were preserved in *Sirt1*-transgenic mice with bilateral common carotid artery stenosis. Brain eNOS was deacetylated in *Sirt1*-transgenic mice, leading to CBF and cognitive restoration.[11] In addition, we provided evidence for the promising protective role of *SIRT1* against bilateral common carotid artery occlusion by preserving CBF and restoring the cerebrovascular reserve in mice.[12] Thus, resveratrol may contribute to the aforementioned beneficial effects in the cerebrovascular system by activating the NAD$^+$/SIRT1-eNOS axis, which is the main pathway.

Notably, the resveratrol group demonstrated a significant reduction in systolic blood pressure. Blood pressure can decrease via endothelial restoration associated with the activation of the NAD$^+$/SIRT1-eNOS axis. Oral resveratrol supplementation improved endothelium-dependent vasodilation, as demonstrated by a significant increase (approximately 1.5 times) in flow-mediated dilation at a dose of 30 mg/day relative to the placebo.[35] Therefore, resveratrol was administered at a dose of 30 mg/day

JoS

Hattori et al.　Efficacy of Resveratrol in Asymptomatic CASO

in this study. Therefore, resveratrol may be effective in treating patients with hypertension. Furthermore, resveratrol intake was associated with decreased blood glucose and HbA1c levels in this study. Resveratrol potentially improves glucose delivery, uptake, and substrate utilization for energy production and reduces circulating blood glucose and insulin levels via eNOS activation and glucose transporter-4 translocation.[36]

This study demonstrated that cognitive improvement in the resveratrol group accompanied an increase of approximately 1.5%–7% in relative CBF (Supplementary Table 2). Methylthionine (138 mg/day), a tau aggregation inhibitor, induced an increase of approximately 1.5%–2.5% in CBF normalized to the cerebellum throughout the entire brain and a significantly larger ADAS-Cog effect size than the placebo in patients with mild Alzheimer's disease.[37] Therefore, such increase in the CBF may be clinically beneficial for cognitive improvement.

In this study, CBF, $CMRO_2$, and OEF values were normalized to the cerebellum to minimize the influence of inter-examination variability on repeated testing. This adjustment accounts for potential differences in whole-brain counts that may arise due to variations in the depth of breathing during different ^{15}O-gas PET examinations.[38] Studies have demonstrated that relative CBF normalized to the cerebellum is employed in patients with Alzheimer's disease and Moyamoya disease,[37,39,40] which are not expected to affect cerebellar perfusion. Moreover, 3D stereotactic surface projection, which enables quantitative data extraction and reliable localization of abnormalities through stereotaxic coordinates using the cerebellum as a reference region, has been widely used to improve the diagnostic performance of PET.[41] Thus, cerebellar normalization is a beneficial method for minimizing the influence of inter-examination variability during repeated testing using brain ^{15}O-gas PET.

This study has several limitations. First, patients receiving the placebo control were not compared with those receiving regular health advice and medications for controlling vascular risk factors. Based on these results, randomized, double-blind, placebo-controlled trials should be performed in the near future. Second, the sample size was small; thus, randomized controlled trials with large sample sizes must be conducted. Despite these limitations, our results revealed that cognitive function and CBF may be maintained or improved with long-term oral resveratrol intake in asymptomatic patients with moderate or severe CASO who present with cognitive functions ranging from MCI to mild dementia.

Conclusions

In conclusion, cognitive function and CBF could be maintained or improved with long-term intake of oral resveratrol, which has pleiotropic neuroprotective effects such as eNOS activation, in patients with asymptomatic moderate or severe CASO who have cognitive function ranging from MCI to mild dementia. As no disease-modifying therapies for dementia are available, including VCI, preventive therapies must be developed. Our results emphasize the need for a randomized, double-blind, placebo-controlled trial to analyze the effects of resveratrol in preserving cognitive function in patients with VCI caused by asymptomatic moderate or severe CASO.

Supplementary materials

Supplementary materials related to this article can be found online at https://doi.org/10.5853/jos.2023.02733.

Conflicts of interest

Yorito Hattori received a grant from Nature Holdings Co., Ltd. All remaining authors declare no competing interests.

Author contribution

Conceptualization: YH (Yorito Hattori), MI (Masafumi Ihara). Study design: YH (Yorito Hattori), MI (Masafumi Ihara). Methodology: YH (Yorito Hattori), YH (Yuji Hattori), HU, HI. Data collection: YH (Yorito Hattori), YK, YH (Yuji Hattori), MI (Mari Iwashita), HU, KN, TY. Investigation: YH (Yorito Hattori), YK, YH (Yuji Hattori), MI (Mari Iwashita), HU, KN. Statistical analysis: YH (Yorito Hattori), YK. Writing—original draft: YH (Yorito Hattori). Writing—review & editing: YH (Yorito Hattori), HI, MI (Masafumi Ihara). Funding acquisition: YH (Yorito Hattori). Approval of final manuscript: all authors.

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JoS

Hattori et al.   Efficacy of Resveratrol in Asymptomatic CASO

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Supplementary Methods

Concentration of resveratrol and its metabolites

The concentrations of resveratrol and its metabolites including resveratrol 3-O-glucuronide, resveratrol 4-O-glucuronide, and resveratrol 3-O-sulfate in patients who provided a written informed consent for the National Cerebral and Cardiovascular Center (NCVC) biobank were measured. The liquid chromatography-mass spectrometry (LC-MS) quantification was performed using a mass spectrometer (Xevo TQ-XS; Waters, Milford, MA, USA) coupled to a liquid chromatography (UPLC I-Class, Waters) using resveratrol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan); trans-resveratrol 3-sulfate sodium salt (Toronto Research Chemicals, Toronto, ON, Canada); and trans-resveratrol 3-O-β-D-glucuronide, trans-resveratrol 4'-O-β-D-glucuronide, and trans-resveratrol-d4 (Cayman Chemical, Ann Arbor, MI, USA).

The protocol for the determination of resveratrol and its metabolites in human plasma was as follows. Initially, the solid phase extraction (SPE) cartridge (Oasis HLB 96-well µElution plate 2 mg; Waters) was conditioned with 200 µL of 2% formic acid followed by 200 µL of methanol. Subsequently, 200 µL of samples two-fold diluted internal standard solution (100 ng/mL of trans-resveratrol-d4 in 2% formic acid) loaded on the SPE cartridge, followed by washing the SPE cartridge with 200 µL of 2% formic acid. Finally, analytes were eluted twice with 100 µL of methanol/ammonia solution 28% (19:1). After centrifugation (500×g, 30 sec, 4°C), supernatants were dried for 20 min at 40°C under a nitrogen stream. The residues were reconstituted in 150 µL of 10 mM ammonium formate in water/methanol (9:1) and subjected to analysis.

We used a YMC Triart C18 column (3 µm, 3.0×50 mm, YMC, Kyoto, Japan) kept at 40°C for LC separation and the injected sample volume was 2 µL. The mobile phases A and B were 10 mM ammonium formate in water and acetonitrile, respectively. The flow rate was 2 mL/min, following the multistep gradient methods. The concentration of mobile phase B was increased from 10% to 90% at 7 min, and then decreased to 10% at 7.5 min, and equilibrated for 4.5 min. MS/MS with electrospray ionization was operated in negative ionization multiple-reaction monitoring mode and the transitions monitored were m/z (mass-to-charge ratio) 227 to 185 (resveratrol), m/z 403 to 113 (resveratrol 3-O-glucuronide and resveratrol 4-O-glucuronide), m/z 307 to 227 (resveratrol 3-O-sulfate) and m/z 231 to 189 (trans-resveratrol-d4).

JoS

Hattori et al.   Efficacy of Resveratrol in Asymptomatic CASO

Supplementary Table 1. Longitudinal change in the blood concentrations of resveratrol and its metabolites

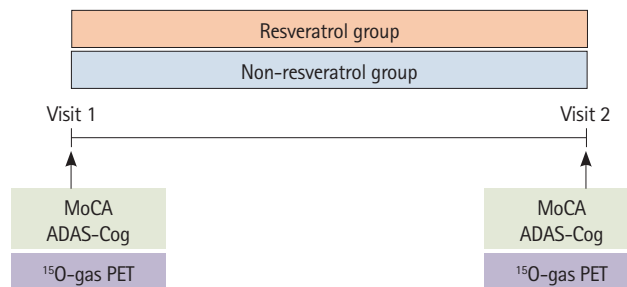|	Non-resveratrol group (n=42)	Resveratrol group (n=36)	P
Visit 1			
Resveratrol	0.00±0.00	0.00±0.00	>0.999
Resveratrol 3-O-glucuronide	0.63±4.11	0.00±0.00	0.358
Resveratrol 4-O-glucuronide	0.00±0.00	0.00±0.00	>0.999
Resveratrol 3-O-sulfate	0.12±0.77	0.00±0.00	0.358
Visit 2			
Resveratrol	0.00±0.00	0.21±0.49	0.018
Resveratrol 3-O-glucuronide	0.00±0.00	75.07±73.23	<0.001
Resveratrol 4-O-glucuronide	0.00±0.00	46.79±47.93	<0.001
Resveratrol 3-O-sulfate	0.00±0.00	206.52±223.57	<0.001

The units for all items are ng/mL. The variables were expressed as means± standard deviations.

Supplementary Figure 1. Schedule of the study. Resveratrol group started to orally take resveratrol 30 mg/day with control of metabolic and vascular risk factors after the neuropsychological assessments and ^{15}O-gas positron emission tomography (PET) at Visit 1. Non-resveratrol group received only control of metabolic and vascular risk factors. Second neuropsychological tests and ^{15}O-gas PET were performed at Visit 2. MoCA, Montreal Cognitive Assessment; ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale 13.
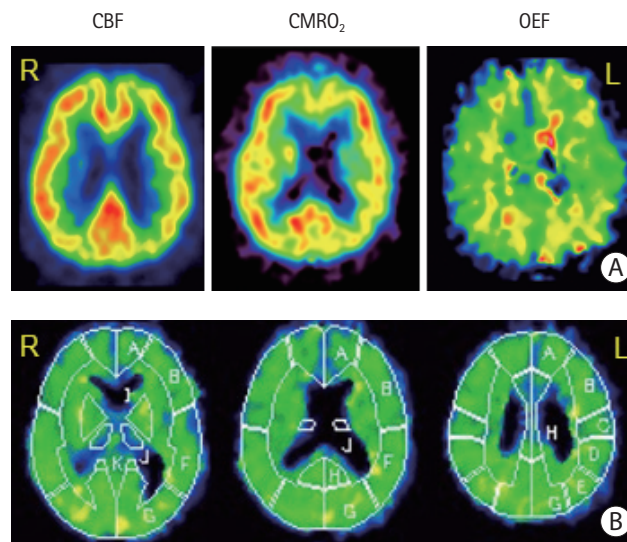
Supplementary Table 2. Mean increase rate of relative cerebral blood flow in the resveratrol group

Region of interest	Increase rate (%)
Frontal lobe	
Right	2.67±4.64
Left	1.93±4.54
Callosomarginal artery	
Right	2.57±4.87
Left	2.51±6.05
Precentral artery	
Right	2.89±4.66
Left	1.39±3.99
Central artery	
Right	2.61±6.31
Left	2.00±6.31
Lenticular nucleus	
Right	2.35±6.50
Left	3.91±6.76
Thalamus	
Right	6.18±12.39
Left	7.23±14.24

Values are presented as mean±standard deviation.

Supplementary Figure 2. Representative images of ^{15}O-gas positron emission tomography (PET) and a three-dimensional stereotaxic region-of-interest template software. (A) Representative images displaying cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO$_2$), and oxygen extraction fraction (OEF) assessed using ^{15}O-gas PET in a patient with asymptomatic left carotid artery stenosis. (B) Region-of-interest segments in the three-dimensional stereotaxic region-of-interest template software are displayed as follows: A, callosomarginal artery area; B, precentral artery area; C, central artery area; D, parietal artery area; E, angular artery area; F, temporal lobe; G, occipital lobe; H, pericallosal artery area; I, lenticular nucleus; J, thalamus; K, hippocampus. R, right; L, left.
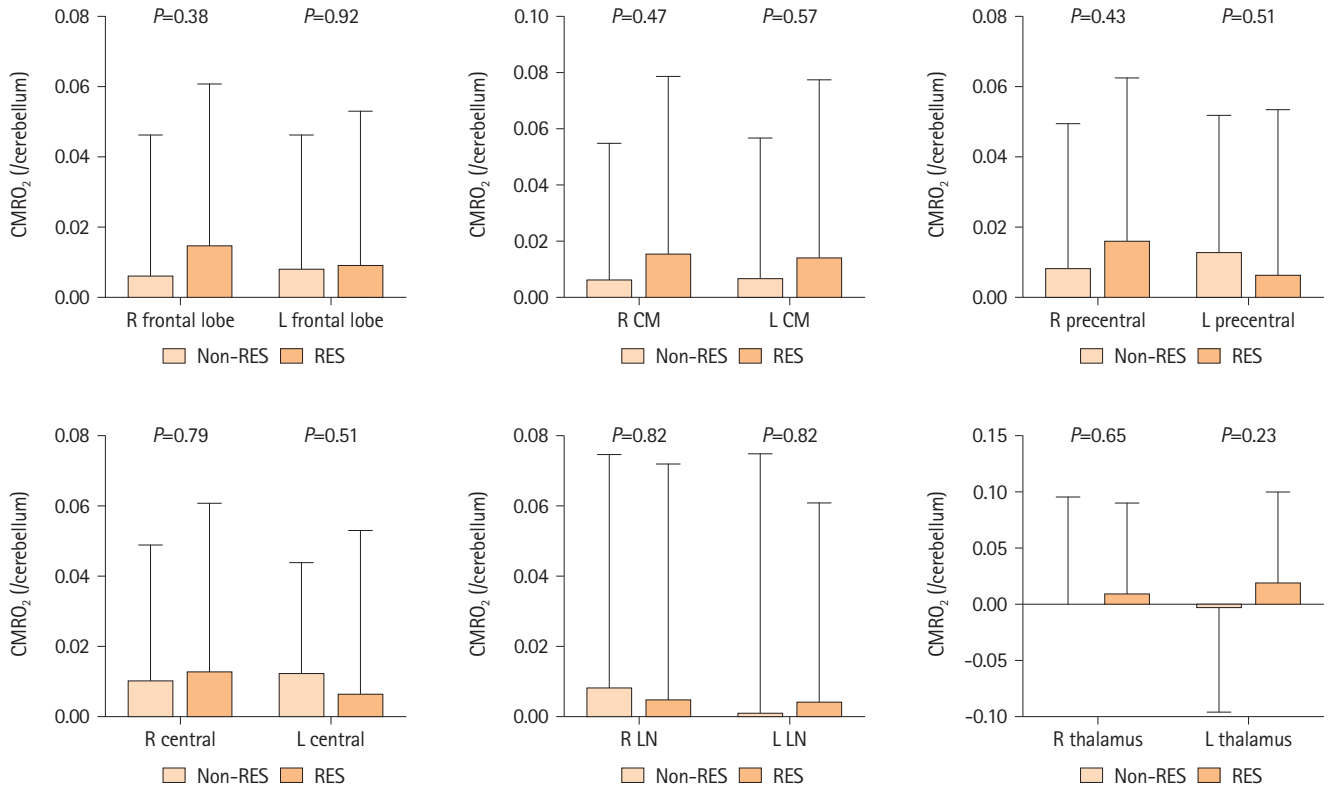
Supplementary Figure 3. Temporal changes in the cerebral metabolic rate of oxygen ($CMRO_2$) between the non-resveratrol (non-RES) and resveratrol (RES) groups. Bar graphs showing temporal changes in $CMRO_2$ in the right (R) and left (L) frontal lobe, callosomarginal artery area (CM), precentral artery area, central artery area, lenticular nucleus (LN), and thalamus. The error bars indicate standard deviation.

JoS

Hattori et al.   Efficacy of Resveratrol in Asymptomatic CASO
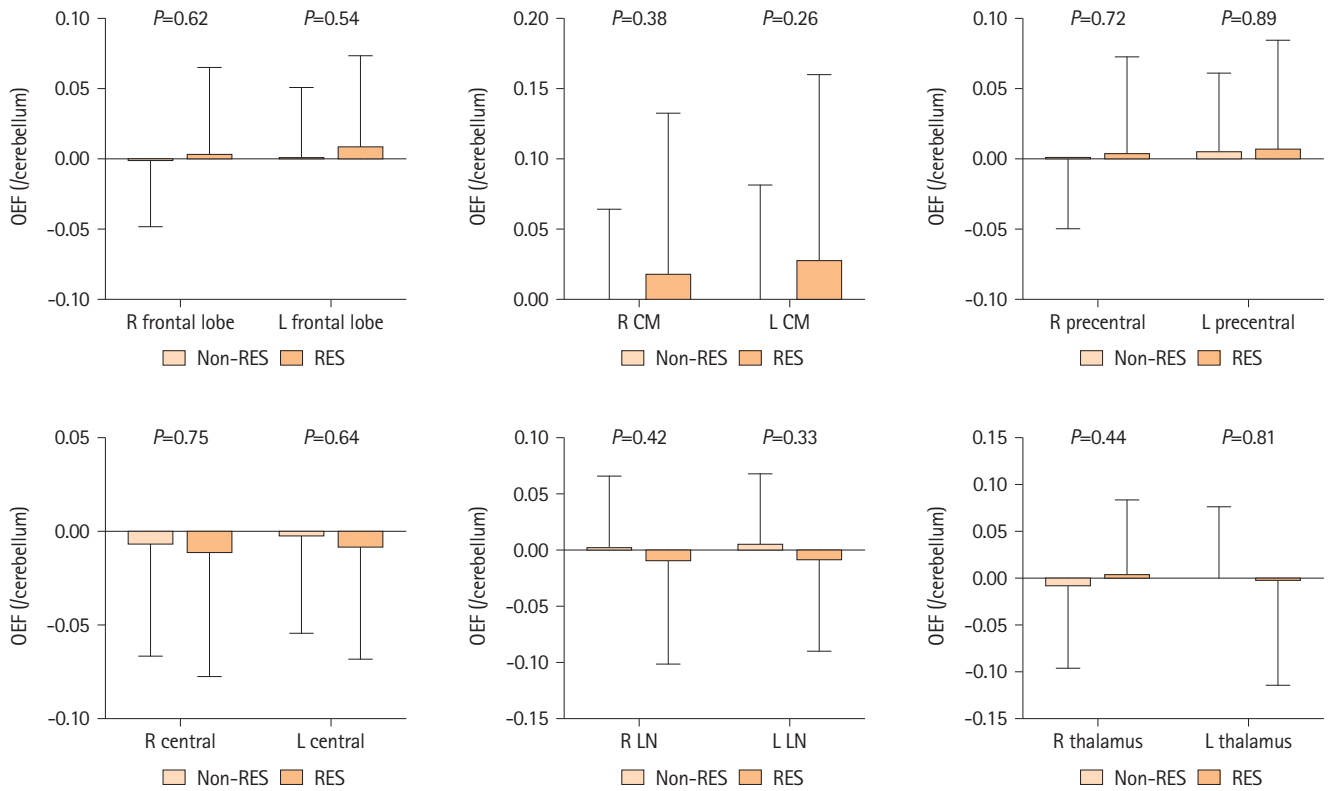
Supplementary Figure 4. Temporal changes in oxygen extraction fraction (OEF) between the non-resveratrol (Non-RES) and resveratrol (RES) groups. Bar graphs showing temporal changes in OEF in the right (R) and left (L) frontal lobe, callosomarginal artery area (CM), precentral artery area, central artery area, lenticular nucleus (LN), and thalamus. The error bars indicate standard deviation.