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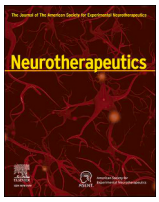

Original Article

Revisiting elevated HDL cholesterol, cerebral hemodynamic improvement in asymptomatic carotid artery disease: A longitudinal ^{15}O-Gas PET study

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ABSTRACT

Interventional trials with high-density lipoprotein cholesterol (HDL-C)-raising drugs have generally failed to demonstrate a beneficial effect on cardiovascular outcomes, although low HDL-C levels confer the risk of cerebrocardiovascular diseases. Previous experimental studies indicate that HDL-C promotes angiogenesis/arteriogenesis. Therefore, we aimed to clinically investigate whether high blood HDL-C levels could clinically predict cerebral hemodynamic improvements in patients with asymptomatic carotid artery stenosis/occlusion showing cerebral hypoperfusion. This longitudinal retrospective observational study included a total of 66 hemispheres governed by asymptomatic carotid artery stenosis/occlusion in patients who underwent 2-time multi-parametric ^{15}O-gas positron emission tomography (PET). The longitudinal changes of multiple parameters, including cerebral blood flow (CBF), cerebral blood volume (CBV), cerebral metabolic rate of oxygen, and oxygen extraction fraction (OEF) values were scrutinized between patients with high and low baseline blood HDL-C levels. The cerebral hemodynamic parameters were normalized to the bilateral cerebellum. The median interval between PET examinations was 212.0 and 219.0 days for patients with low and high HDL-C levels, respectively ($p = 0.91$). A high blood HDL-C level was an independent predictor of increasing CBF (β [mean difference]: 0.035, 95 % confidence interval [CI]: 0.010–0.060), and CBV (β: 0.26, 95 % CI: 0.023–0.50), and decreasing OEF (β: −0.041, 95 % CI: −0.077 to −0.006) in the anterior circulation territory. A high blood HDL-C level was clinically an independent predictor of cerebral hemodynamic improvement. HDL-C could be an important therapeutic target for ischemic stroke prevention by improving cerebral hemodynamic parameters presumably via angiogenesis and arteriogenesis especially in patients showing cerebral hypoperfusion.

Introduction

Blood high-density lipoprotein cholesterol (HDL-C) levels are inversely correlated with coronary heart disease [1] and ischemic stroke risk [1,2] as indicated by epidemiological studies for many decades. Despite of the results in various epidemiological studies, to date, clinical trials with HDL-C-elevating drugs have failed to demonstrate beneficial effects on cardiovascular events [3]. Thus, a full understanding of the effect of HDL-C level on stroke and stroke subtypes is warranted. In contrast, several *in vitro* and *in vivo* studies demonstrate that HDL-C indeed promotes arteriogenesis and angiogenesis [4–6]. Collectively, these findings suggest that HDL-C could clinically contribute to cerebral hemodynamic improvement, particularly in patients experiencing chronic cerebral hypoperfusion. Thus, a better understanding of the effect of HDL-C level on stroke and stroke subtypes is warranted.

Extracranial atherosclerotic carotid artery stenosis or occlusion (CASO) is a major cause of cerebral infarction and vascular cognitive impairment. Carotid artery stenosis of ≥50 % is responsible for 8 % of all stroke events and increases the risk of recurrence to 16 % over 5 years following the first episode [7]. Patients with asymptomatic carotid stenosis of ≥60 % receiving the best medical treatment are at high risk of ischemic stroke [8]. One of the reasons is that CASO can restrict the blood flow to the brain and cause cerebral hypoperfusion [9]. Therefore, we clinically examined the association between blood HDL-C levels and

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longitudinal changes in cerebral hemodynamic parameters in depth using multi-parametric ^{15}O-gas positron emission tomography (PET), which can fully quantify the cerebral blood flow (CBF), cerebral metabolic rate of oxygen ($CMRO_2$), oxygen extraction fraction (OEF), and cerebral blood volume (CBV) in patients with asymptomatic extracranial atherosclerotic CASO.

Methods

Study design

This longitudinal retrospective observational study was conducted at the National Cerebral and Cardiovascular Center (NCVC) in Japan between April 2013 and June 2021. The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the Research Ethics Committee of NCVC (approval number: R20113). Due to the anonymized state of the data, the Research Ethics Committee waived the requirement for individual consent for inclusion in the registry. Instead, the opt-out consent method was used. Patients provided written informed consent for the NCVC Biobank to measure blood APOA1, APOB, and lipoprotein A levels by enzyme-linked immunosorbent assay (ELISA). To investigate the associations between the blood HDL-C level and cerebral hemodynamics in extracranial atherosclerotic asymptomatic CASO, we included patients meeting the following criteria: First, asymptomatic extracranial atherosclerotic CASO patients showed a peak systolic velocity of ≥130 cm/s at the stenotic lesions or occlusions using carotid Doppler ultrasonography. A peak systolic velocity ≥130 cm/s and ≥200 cm/s corresponds to angiographic stenosis of ≥50 % (moderate) and ≥70 % (severe), respectively [10,11]. Bilateral extracranial atherosclerotic CASO means that patients have bilateral carotid arteries with ≥50 % stenosis or occlusion. Second, the patients underwent two ^{15}O-gas PET scans to assess CBF, $CMRO_2$, OEF, and CBV with an interval of 4–24 months between visits 1 and 2 (eFig. 1). Patients with asymptomatic carotid stenosis of ≥60 % are more predisposed to stroke events when receiving best medical treatment alone [8]. That is, all the patients in the study clinically underwent ^{15}O-gas PET to determine whether surgical revascularization was indicated. The exclusion criteria comprised patients undergoing carotid endarterectomy or artery stenting; those taking phosphodiesterase inhibitors, such as cilostazol due to their potential effects on cerebral circulation [12,13]; and patients with worsening carotid artery stenosis during PET scans. Worsening was defined as progression from moderate to severe stenosis or occlusion, or from severe stenosis to occlusion (Fig. 1). Patients' medical histories, blood tests, and medication were collected from their medical records. Smoking was defined as current or former smoking, and alcohol consumption was defined as current alcohol use at the time of

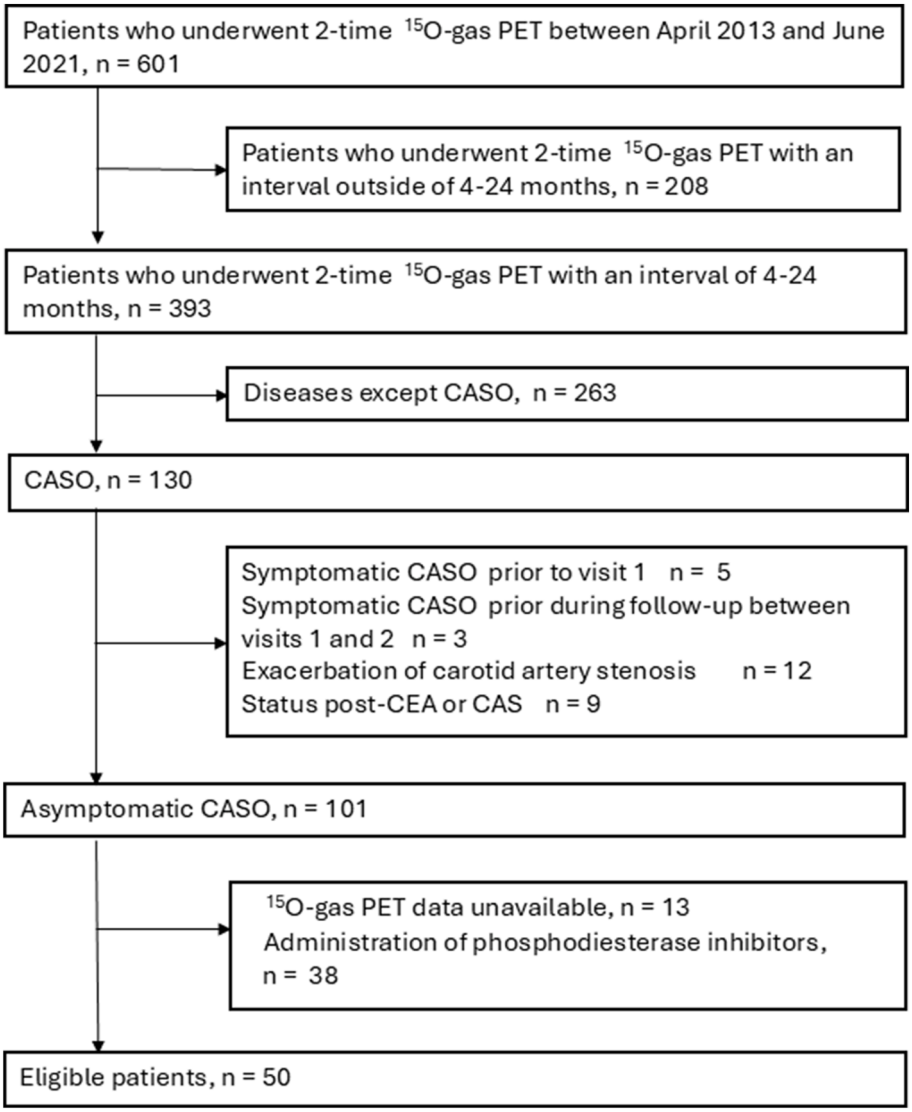

Fig. 1. Flowchart of the study.

assessment. We longitudinally evaluated improvements in CBF, $CMRO_2$, OEF, and CBV in hemispheres governed by asymptomatic carotid arteries with ≥50 % stenosis or occlusion. The primary study outcomes were associations between blood HDL-C levels at visit 1 and longitudinal changes of cerebral hemodynamic parameters. Blood HDL-C levels <40 mg/dL were defined as hypo-HDL cholesterolemia based on diagnostic guidelines of dyslipidemia [14,15]. This study compared patients with blood HDL-C levels <40 mg/dL and those with ≥40 mg/dL at visit 1.

^{15}O-gas PET measurements and regional value analysis

All patients underwent a series of ^{15}O-gas PET examinations for CBF (mL/100 g/min), $CMRO_2$ (mL/100 g/min), OEF (%), and CBV (mL/100 g) assessments (Fig. 2A). Radioactive ^{15}O was produced by accelerating a deuteron (d) beam via the ^{14}N (d,n)^{15}O nuclear reaction using a cyclotron (CYPRIS HM-12, Sumitomo Heavy Industry, Tokyo, Japan). We used 0.3 % O_2 in the N_2 target gas to produce the $^{15}O–O_2$ and $^{15}O–CO$ gases and 1.0 % CO_2 to produce the $^{15}O–CO_2$ gas.

We utilized the Biograph mCT (Siemens Healthineers, Erlangen, Germany) PET scanner for our investigations. Each patient inhaled $^{15}O–CO$ for 2 min. After 3 min, a PET scan was conducted for 4 min. An additional dynamic PET scan was performed for 8 min, with a 1 min sequential inhalation of $^{15}O–O_2$ and $^{15}O–CO_2$ gases at a 4.5 min interval. Two-channel rapid gas chromatography (Micro 990, Agilent Technologies Inc., Santa Clara, USA) revealed a radiochemical purity of >99 % before radio gas inhalation in each patient [16].

Functional images of CBF, $CMRO_2$, OEF, and CBV were generated using the Dual-table Autoradiography technique [17], which has demonstrated OEF values in cynomolgus monkeys closely aligned with those obtained by A–V difference across a wide physiological range [17]. The theoretical framework of this method is consistent with the three-step autoradiography technique [18,19], which has been applied in multiple clinical studies. The CBV correction in the process of calculating the OEF and $CMRO_2$ was identical to that in the earlier work, with the exception that the Dual-table technique allows for a shorter interval between the administration of $^{15}O–O_2$ and $^{15}O–CO_2$. A novel automated system for radio-gas production and inhalation control was applied to ensure reproducible operations and qualification of radio-gas purity [16]. Radiochemical analysis with two-channel rapid gas chromatography (Micro 990, Agilent Technologies Inc., Santa Clara, USA) confirmed a purity greater than 99 % prior to each patient's radio-gas inhalation [16]. The arterial input function was determined from a constantly monitored radioactivity concentration in the arterial blood continuously withdrawn from the brachial artery [20]. In the arterial blood, the metabolized ^{15}O-water, produced as a result of whole-body metabolism from $^{15}O–O_2$, was estimated using a physiological oxygen metabolism model [21]. PET images were reconstructed using Vendor, via an adequately selected method that considers the presence of gaseous ^{15}O-radioactivity surrounding the face during inhalation [22].

All ^{15}O-gas PET images were analyzed using a three-dimensional stereotaxic region-of-interest template (3DSRT) (PDRadiopharma, Tokyo, Japan), standalone software that incorporates the spatial normalization algorithm developed by SPM2, to automatically set 12 segments of region of interest in each hemisphere [23,24]. (Fig. 2B). The following 12

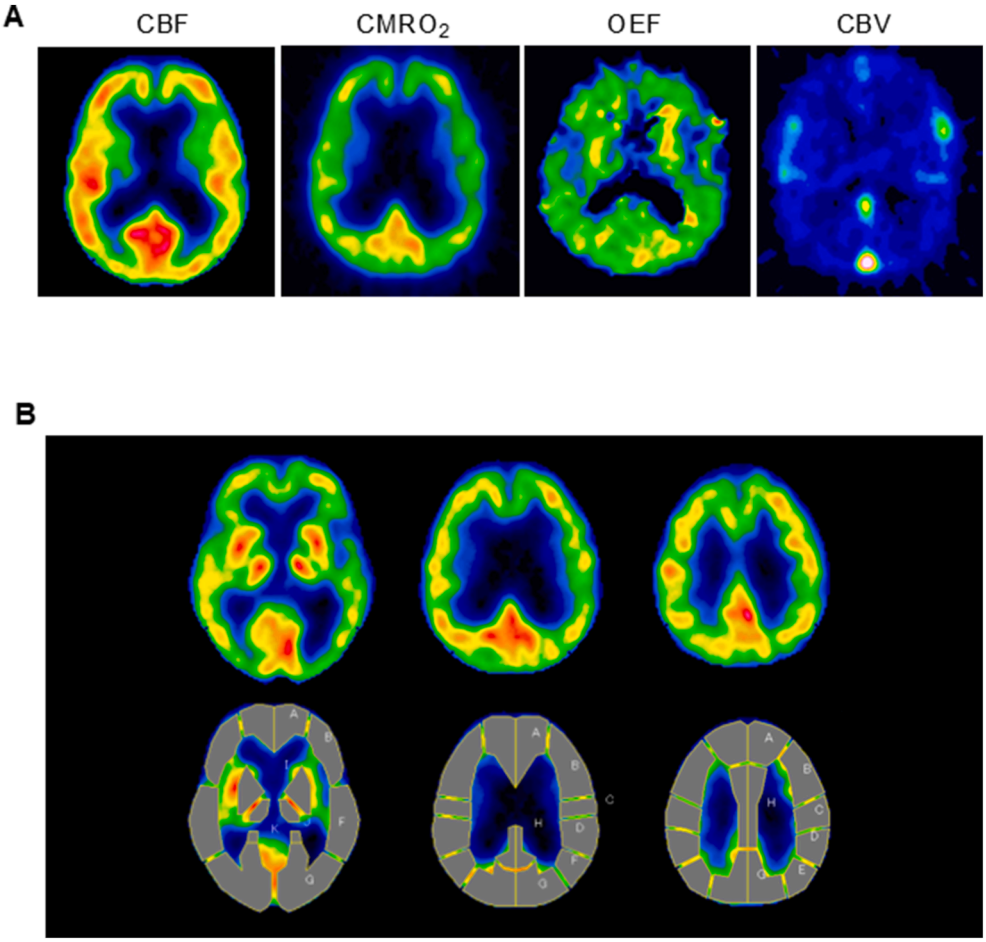

Fig. 2. Representative images of ^{15}O-gas positron emission tomography (PET) and a three-dimensional stereotaxic region-of-interest template software. (A) Representative images showing cerebral blood flow (CBF), cerebral metabolic rate of oxygen ($CMRO_2$), oxygen extraction fraction (OEF), and cerebral blood voleme (CBV) assessed with ^{15}O-gas PET. (B) The region-of-interest segments in the three-dimensional stereotaxic region-of-interest template software are as follows: A, the prefrontal area; B, the precentral artery area; C, the central artery area; D, the parietal artery area; E, the angular artery area; F, the temporal lobe; G, the occipital lobe; H, the pericallosal artery area; I, the lenticular nucleus; J, the thalamus; K, the hippocampus.

region-of-interest segments, grouped according to arterial supply, were examined in each hemisphere: territories of pericallosal, precentral, central, parietal, and angular arteries, prefrontal area, temporal lobe, occipital lobe, hippocampus, lenticular nucleus, thalamus, and cerebellum (Fig. 2B). The anterior circulation territory comprised the precentral, central, parietal, angular, and pericallosal artery territories, prefrontal area, temporal lobe, and lenticular nucleus, all of which fall within the territories of the anterior and middle cerebral arteries [23,24] (Fig. 2B).

ELISA for APOA1, APOB, and lipoprotein A

The protein concentration of human APOA1, APOB, and lipoprotein A in the blood were measured using a specific ELISA kit according to the manufacturer's recommendation (abcam, Cambridge, UK). The catalog numbers were as follows: human APOA1, ab189576; human APOB, ab190806; and human lipoprotein A, ab212165.

Evaluation of brain magnetic resonance imaging and angiography (MRA)

Vertebrobasilar artery stenosis was assessed using brain magnetic resonance angiography (MRA). Stenosis severity was categorized as normal (no signal reduction), mild (<50 % signal reduction), moderate (≥50 % signal reduction), or severe (focal signal loss with preservation of the distal arterial signal) [25]. Large cortical infarcts were defined as lesions with the largest diameter of >1 cm covering the gray matter on fluid-attenuated inversion recovery sequences, as described elsewhere [26]. Silent cerebral infarcts were defined as focal lesions with a diameter of ≥3 mm that exhibited central hypointensity with a surrounding rim of hyperintensity on fluid-attenuated inversion recovery sequences [27].

Statistical analysis

We presented continuous variables as the median (IQR: interquartile range) and categorical variables as frequencies with percentages in the baseline characteristics. Differences in CBF levels at visit 1 according to the severity of carotid stenosis or occlusion were evaluated using the one-way analysis of variance. We longitudinally evaluated changes in CBF, $CMRO_2$, OEF, and CBV in each hemisphere governed by an asymptomatic carotid artery with ≥50 % stenosis or occlusion. The CBF, $CMRO_2$, OEF, and CBV in the anterior circulation territory were normalized to an average count within the bilateral cerebellum [23]. To assess the association between HDL-C and cerebral hemodynamic

Table 1
Baseline characteristics of patients.

	Patients	Affected hemispheres		
	All (n = 50)	HDL-C <40 mg/dL[a] (n = 13)	HDL-C ≥40 mg/dL[a] (n = 53)	*p* value[b]
Age	76.0 (70.8–80.0)	74.0 (73.0–80.0)	76.0 (70.5–80.5)	0.72
Female	10 (20.0)	0 (0.0)	13 (24.5)	0.046
Unilateral CASO	34 (68.0)	–	–	
Bilateral CASO	16 (32.0)	–	–	
CASO grade				0.58
50 % ≤ carotid stenosis <70 %	–	3 (23.1)	17 (32.1)	
70 % ≤ carotid stenosis	–	5 (38.5)	23 (43.4)	
Carotid occlusion	–	5 (38.5)	13 (24.5)	
Smoking	–	10 (76.9)	23 (43.4)	0.030
Alcohol	–	3 (23.1)	26 (49.1)	0.091
Hypertension	–	13 (100.0)	47 (88.7)	0.20
Diabetes mellitus	–	5 (38.5)	14 (26.4)	0.39
Dyslipidemia	–	13 (100.0)	49 (92.5)	0.31
Coronary artery disease	–	8 (61.5)	14 (26.4)	0.016
Atrial fibrillation	–	2 (15.4)	11 (20.8)	0.66
Antiplatelets	–	8 (61.5)	33 (62.3)	0.96
Anticoagulants	–	2 (15.4)	11 (20.8)	0.66
Antihypertensive drugs	–	13 (100.0)	46 (86.8)	0.17
Statins	–	9 (69.2)	47 (88.7)	0.080
Eicosapentaenoic acids	–	3 (23.1)	7 (13.2)	0.37
Ezetimibe	–	3 (23.1)	13 (24.5)	0.91
Fibrates	–	0 (0.0)	1 (1.9)	0.62
Systolic blood pressure [mmHg]	–	139.0 (122.0–147.5)	135.5 (121.5–144.0)	0.64
Diastolic blood pressure [mmHg]	–	68.0 (53.5–94.0)	70.0 (62.0–78.8)	0.90
Hemoglobin A1c [%]	–	6.2 (6.0–7.2)	6.1 (5.6–6.6)	0.055
Triglyceride [mg/dL]	–	157 (118–202)	125 (81–168)	0.21
Low-density lipoprotein cholesterol [mg/dL]	–	89.0 (60.5–92.0)	85.0 (69.0–97.5)	0.97
High-density lipoprotein cholesterol [mg/dL]	–	36.0 (33.5–36.0)	52.0 (44.0–59.0)	–
APOA1 [ng/mL]	–	4.21 (3.54–6.00)	4.48 (4.01–5.22)	0.57
APOB [ng/mL]	–	491.59 (400.16–567.48)	393.80 (274.14–481.80)	0.033
APOB/APOA1 ratio	–	114.45 (108.23–140.43)	90.77 (61.23–124.94)	0.058
Lipoprotein A [ng/mL]	–	279.73 (206.41–715.47)	313.42 (81.19–840.77)	0.92
C-reactive protein [mg/dL]	–	0.100 (0.045–0.220)	0.05 (0.03–0.12)	0.30
Time interval of ^{15}O-gas PET between visits 1 and 2 [days]	–	212.0 (196.0–311.5)	219.0 (190.0–318.5)	0.91
Arterial partial pressure of CO_2 during ^{15}O-gas PET at visit 1 [mmHg]	–	38.10 (37.08–40.72)	39.40 (36.62–40.95)	0.60
Arterial partial pressure of CO_2 during ^{15}O-gas PET at visit 2 [mmHg]	–	35.33 (28.20–40.48)	38.35 (36.40–40.67)	0.43
Arterial hemoglobin during ^{15}O-gas PET at visit 1 [g/dL]	–	13.00 (11.77–13.55)	12.53 (11.33–13.65)	0.34
Arterial hematocrit during ^{15}O-gas PET at visit 1 [%]	–	39.87 (35.32–41.57)	38.00 (34.70–40.67)	0.29
Moderate or severe vertebrobasilar artery stenosis on brain MRA	–	0 (0)	0 (0)	>0.99
Large cortical infarcts on brain MRI	–	0 (0)	0 (0)	>0.99
Silent cerebral infarcts on brain MRI	–	0.0 (0.0–0.5)	0.0 (0.0–0.5)	0.78

Categorical variables are presented as frequencies (%) and continuous variables are presented as medians (interquartile ranges).
Abbreviation: CASO, carotid artery stenosis/occlusion; CO_2, carbon dioxide; HDL-C, high-density lipoprotein cholesterol; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; PET, positron emission tomography.
[a] The blood HDL-C levels were examined at visit 1.
[b] *p* value indicates difference between HDL-C < and ≥40 mg/dL.

changes, we conducted multivariable linear regression analyses in which we adjusted for potential confounding factors, including age, sex, ipsilateral and contralateral CASO grades, smoking, alcohol consumption, hypertension, diabetes mellitus, medical history of coronary artery disease, low-density lipoprotein cholesterol, APOB, interval days of PET between visits 1 and 2, and statin use. Additionally, to assess whether the effect of HDL-C on changes in CBF varied by medication use, we included an interaction term.

Receiver operating characteristic (ROC) analyses were also conducted to determine the most appropriate cutoff blood HDL-C levels at visit 1 for indicating preserved or improved CBF on the basis of the Youden index. Multivariable logistic regression analyses estimating the cutoff blood HDL-C levels at visit 1 associated with preserved or improved CBF were performed while adjusting for age, sex, ipsilateral CASO grade, hypertension, diabetes mellitus, and low-density lipoprotein cholesterol.

A sensitivity analysis was conducted. We compared changes of CBF in the anterior circulation territory between patients with HDL-C ≥40 mg/dL at both visits 1 and 2 (sustained high HDL-C group), and others (low or nonsustained high HDL-C group).

All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant. We used SPSS (IBM Corporation, Armonk, NY, USA), GraphPad PRISM (GraphPad Software, Boston, MA, USA), and R statistical software version 4.5.0 (R foundation for Statistical Computing, Vienna, Austria) for all statistical analyses.

Results

Baseline clinical characteristics of patients

As shown in Table 1, we assessed cerebral hemodynamic parameters in 66 cerebral hemispheres among 50 patients. Among 66 cerebral hemispheres governed by asymptomatic CASO, 20 (30.3 %) carotid arteries had 50 % ≤ stenosis <70 %, 28 (42.4 %) had 70 % ≤ stenosis and 18 (27.3 %) had occlusion.

Among the 66 affected cerebral hemispheres, 13 and 53 had HDL-C levels of <40 and ≥ 40 mg/dL, respectively, at visit 1. The group with HDL-C ≥40 mg/dL had significantly higher number of women, but lower frequency of smoking, and medical history of coronary artery disease. There were no statistically significant differences between the two groups in CASO grades, vascular risk factors, such as hypertension, diabetes mellitus, and dyslipidemia, anti-dyslipidemia drugs, including statins, eicosapentanoic acids, ezetimibe, and fibrates, blood triglyceride and low-density lipoprotein cholesterol levels, and time interval of ^{15}O-gas PET between visits 1 and 2. Blood APOB levels were significantly higher in the HDL-C <40 mg/dL group than the HDL-C ≥40 mg/dL group. No patients took selective peroxisome proliferator-activated receptor modulators, while one took fibrates (Table 1).

The severity of carotid stenosis or occlusion did not determine the severity of cerebral hemodynamics

First, we compared CBF at visit 1 according to the severity of CASO. The CBF did not differ among patients with carotid stenosis of ≥50 % and ≥70 % and those with occlusion within each group of patients (eFig. 2).

Baseline high blood HDL-C levels were significantly associated with longitudinal improvement in cerebral hemodynamic parameters

The group with HDL-C ≥40 mg/dL exhibited significantly greater increases in CBF (−0.013 vs. 0.0036) and $CMRO_2$ (−0.013 vs. 0.010) compared with the HDL-C <40 mg/dL group, along with a trend toward a greater increase in CBV (−0.098 vs. 0.075) (Fig. 3A–C). The reduction in OEF was also greater in the group with HDL-C ≥40 mg/dL (0.020 vs. −0.0040) (Fig. 3D).

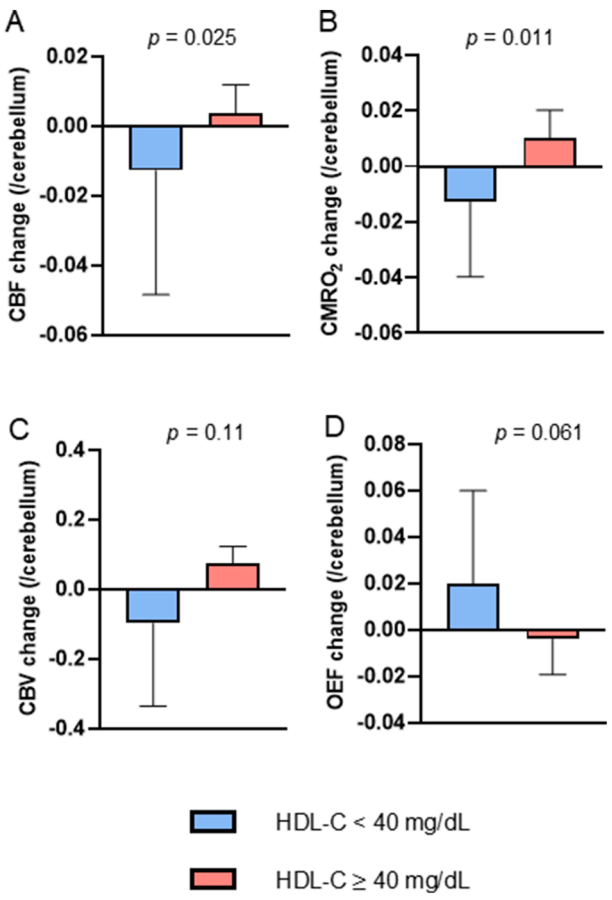

Fig. 3. Longitudinal changes of cerebral blood flow (CBF), cerebral metabolic rate of oxygen ($CMRO_2$), cerebral blood volume (CBV), and oxygen extraction fraction (OEF) assessed with ^{15}O-gas positron emission tomography in the groups with blood high-density lipoprotein cholesterol (HDL-C) <40 mg/dL and ≥40 mg/dL. Bar graphs show the median longitudinal changes in CBF (A), $CMRO_2$ (B), CBV (C), and OEF (D) in the anterior circulation territory.

To evaluate whether blood HDL-C ≥40 mg/dL predicts cerebral hemodynamic improvement, we conducted multivariable linear regression analyses. HDL-C ≥40 mg/dL emerged as an independent predictor of increased CBF and CBV, as well as decreased OEF in the anterior circulation territory (Fig. 4A–C). Inside the anterior circulation territory, most of the territories consisting of the precentral, central, parietal, and angular arteries, prefrontal area, and temporal lobe showed that blood HDL-C ≥40 mg/dL was an independent predictor of CBF increase (eTable 1). However, the blood HDL-C ≥40 mg/dL was not associated with $CMRO_2$ increase in the anterior circulation territory (Fig. 4D). No significant interaction with medication use was detected in the association between HDL-C and changes in CBF (eTable 2). No patients developed new silent cerebral infarcts on brain magnetic resonance imaging during the follow-up period. Thus, high HDL-C levels were significantly associated with improvements in CBF, CBV, and OEF, suggesting that CBF improvement primarily occurs presumably via angiogenesis and arteriogenesis.

Next, we performed ROC analyses to determine the cutoff blood HDL-C level that could improve CBF. The cutoff blood HDL-C level for preserving or improving CBF was 44.5 mg/dL with an area under the curve of 0.722 (Fig. 5). The blood HDL-C level of 44.5 mg/dL was an independent predictor of CBF preservation or improvement in a multivariable logistic regression analysis (Table 2).

Regarding longitudinal changes in blood HDL-C levels, blood HDL-C levels did not differ between visits 1 and 2 in either group (eTable 3). Further comparison revealed that the median change in blood HDL-C levels

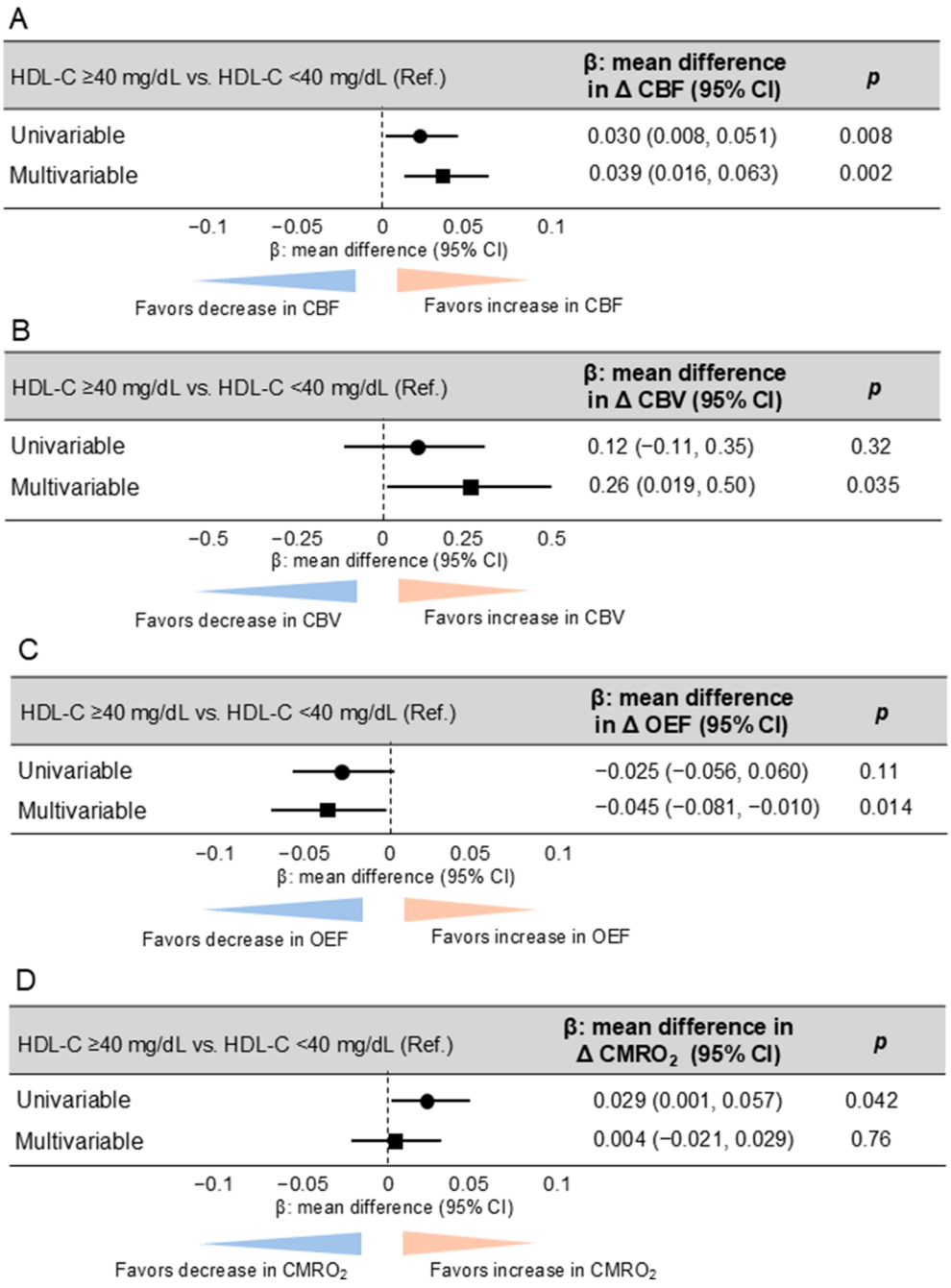

Fig. 4. Associations of cerebral hemodynamic parameters with blood high-density lipoprotein cholesterol (HDL-C) level ≥40 mg/dL. Linear regression analysis estimating changes of cerebral blood flow (CBF) (A), cerebral blood volume (CBV) (B), oxygen extraction fraction (OEF) (C), and cerebral metabolic rates of oxygen ($CMRO_2$) (D), associated with blood HDL-C level ≥40 mg/dL. The multivariable models were adjusted for age, sex, ipsilateral and contralateral carotid artery stenosis/occlusion grades, smoking, alcohol consumption, hypertension, diabetes mellitus, medical history of coronary artery disease, low-density lipoprotein cholesterol, APOB, interval days of PET between visits 1 and 2, and statin use.

between visits 1 and 2 was also comparable between the patients with an HDL-C of <40 mg/dL and those with an HDL-C of ≥40 mg/dL (−1.00 mg/dL [IQR, −1.50 to 3.00] and 0.00 mg/dL [IQR, −4.00 to 5.00]; $p = 0.75$).

Arterial partial pressure of CO_2, hemoglobin, and hematocrit levels showed no significant differences between the two ^{15}O-gas PET examinations in the HDL-C <40 mg/dL and ≥40 mg/dL groups, respectively (eTable 4). Likewise, changes in arterial partial pressure of CO_2 (−0.28 [IQR: −4.26 to 0.21] mmHg vs. −0.76 [−2.10 to 0.57] mmHg; $p = 0.33$), hemoglobin (−0.67 [−0.80 to 0.28] g/dL vs. −0.10 [−0.67 to 0.28] g/dL; $p = 0.34$), and hematocrit (−2.00 [−2.45 to 0.97]% vs. −0.40 [−1.97 to 0.85]%; $p = 0.35$) during ^{15}O-gas PET were not significantly different between the two groups.

Sustained high blood HDL-C levels remained significantly associated with longitudinal improvements in cerebral hemodynamic parameters in the sensitivity analysis

Among the 66 affected cerebral hemispheres, the number of affected cerebral hemispheres with high blood HDL-C levels ≥40 mg/dL at visits 1 and 2 (sustained high HDL-C group) was 49, and the others (low or nonsustained high HDL-C group) were 17. The sustained high HDL-C group had a significantly lower number of patients with a medical history of coronary artery disease. CASO grades, the female sex, and vascular risk factors, such as hypertension, diabetes mellitus, and dyslipidemia did not differ significantly between the two groups. Time intervals of ^{15}O-gas PET between visits 1 and 2 also did not differ (eTable 5).

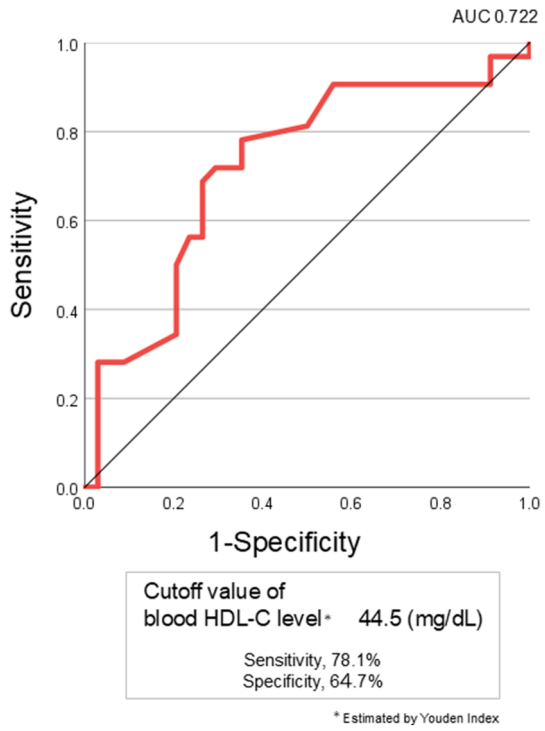

Fig. 5. Receiver operating characteristic curve to determine the most appropriate cutoff blood high-density lipoprotein cholesterol (HDL-C) level to preserve or improve cerebral blood flow.

Compared to the low- or nonsustained high HDL-C group, a sustained blood HDL-C ≥40 mg/dL was an independent predictor of CBF increase (eFig. 3).

Discussion

This study demonstrated that a high blood HDL-C level was significantly associated with improvement in CBF, CBV, and OEF, but not linked to changes in $CMRO_2$ in patients with asymptomatic CASO. These findings suggested that CBF improvement occurred through angiogenesis and arteriogenesis, rather than increased metabolic demand.

HDL-C promotes angiogenesis and arteriogenesis via sphingosine-1-phosphate 3-dependent vascular endothelial growth factor receptor 2 activation [5], miR-24-3p suppression to increase vinculin expression and stimulate nitric-oxide production [4], and the prevention of oxidized low-density lipoprotein-induced inhibition of endothelial nitric-oxide synthase localization and activation in caveolae [6], as demonstrated in *in vitro* and *in vivo* studies. However, clinical trials of drugs designed to elevate blood HDL-C level have not shown a corresponding reduction in cerebrocardiovascular events [3]. The experimental studies [4–6] and our clinical longitudinal observational study may offer insights into appropriate patient selection to explore the therapeutic potential of HDL-C. Patients with conditions causing chronic cerebral hypoperfusion, such as CASO, may be ideal candidates for clinical trials investigating whether HDL-C has beneficial effects in preventing ischemic events and cognitive impairment. However, extremely high HDL-C levels warrant caution. Recent studies have reported a U-shaped relationship between HDL-C and cardiovascular risk, indicating that very high HDL-C levels (i.e., ≥80–90 mg/dL) are paradoxically linked to increased mortality from cardiovascular events [28–30]. In our study, only 2 patients had HDL-C ≥80 mg/dL. Consequently, HDL-C levels ≥40 mg/dL were significantly associated with improvements in cerebral hemodynamics in this cohort. Future research with larger sample sizes is needed to determine whether extremely high HDL-C levels influence cerebral hemodynamic changes.

In this study, CBF, $CMRO_2$, CBV, and OEF values were normalized to the bilateral cerebellum to minimize inter-examination variability in repeated measurements. This normalization helps account for potential differences in brain counts that may result from variations in breathing depth across separate ^{15}O-gas PET examinations [31]. Previous studies have shown that cerebral hemodynamic parameters normalized to the cerebellum was employed in patients with CASO [23] as well as Alzheimer's disease and moyamoya disease [32–34], which are not supposed to affect the cerebellar perfusion. Furthermore, three-dimensional stereotactic surface projection (3D-SSP) is widely used to improve PET's diagnostic performance [35]. Thus, cerebral hemodynamic parameters after cerebellar normalization were justified in this study.

Patients with asymptomatic carotid stenosis of ≥60 % who receive best medical therapy remain at high risk for ischemic stroke [8]. One contributing factor is that even asymptomatic CASO can limit cerebral blood flow, leading to hypoperfusion [9]. Therefore, longitudinal monitoring of cerebral hemodynamic status is essential to assess potential decline and determine the need for surgical revascularization [36] using nuclear medicine techniques such as PET imaging. This study included patients with asymptomatic carotid artery stenosis exhibiting a peak systolic velocity of ≥130 cm/s at the stenotic lesions, corresponding to ≥50 % angiographic stenosis by carotid Doppler ultrasonography. These patients require ongoing follow-up to evaluate cerebral hemodynamics over time.

Nevertheless, this study has several limitations. First, its retrospective design comes with a risk of selection bias. This bias could originate at the time of enrolling the CASO patients of the study because all the patients clinically underwent ^{15}O-gas PET to consider the indication of surgical revascularization, although this study investigated the relationship between cerebral hemodynamic parameters and blood HDL-C levels, because patients with asymptomatic carotid stenosis of ≥60 % are more predisposed to stroke events when receiving best medical treatment alone [8]. Second, the sample size appeared to be relatively small in general. However, it is not always small in clinical studies utilizing ^{15}O-gas PET. To the best of our knowledge, longitudinal studies involving 50−100 participants are considered larger cohorts [23,37,38].

Table 2
Multivariable logistic regression analysis estimating a cutoff blood high-density lipoprotein cholesterol level associated with preserved or improved cerebral blood flow (CBF).

	Univariable			Multivariable		
	Odds ratio for preserved or improved CBF	95 % CI	*p* value	Odds ratio for preserved or improved CBF	95 % CI	*p* value
HDL-C 44.5 mg/dL	7.99	2.58–24.77	<0.001	8.68	2.44–30.86	0.001
Age				0.99	0.92–1.073	0.83
Sex (women)				0.82	0.20–3.42	0.82
Ipsilateral CASO grade				1.37	0.60–3.14	0.46
Hypertension				0.69	0.083–5.80	0.74
Diabetes				2.29	0.62–8.53	0.22
LDL-C level				0.99	0.96–1.013	0.33

Abbreviation: CI, confidence interval; CASO, carotid artery stenosis or occlusion; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Accordingly, this study, which enrolled 66 hemispheres affected by CASO, may be considered one of the larger studies in this research field. Third, HDL-C is a marker for increased exercise. Thus, exercise duration, intensity, and regularity should be controlled in future prospective studies. However, this study suggested that elevated blood HDL-C levels resulted in contributing to the longitudinal improvement of cerebral hemodynamics whatever various interventions, including medication and exercise were.

In conclusion, a high blood HDL-C level clinically identified as an independent predictor of cerebral hemodynamic improvement in the anterior circulation territory. Thus, medication targeting blood HDL-C levels could potentially reduce the risk of ischemic stroke and vascular cognitive impairment in patients suffering from chronic cerebral hypoperfusion, such as CASO. Randomized clinical trials may be considered to determine whether increasing blood HDL-C levels can prevent cerebral hemodynamic exacerbation in patients with asymptomatic CASO.

Author contribution

Conceptualization: YH. Study design: YH. Enrollment and Data collection: YH, SA, and YK. Statistical analysis: YH, SA, YK, YN, SO, KN. Writing—original draft: YH. Writing—review & editing: all authors. Funding acquisition: YH. Approval of final manuscript: all authors.

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Declaration of competing interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurot.2026.e00849.

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