

Decreased ^{13}N -labeled ammonia uptake in the ipsilateral and contralateral hemispheres following carotid endarterectomy

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Abstract. Carotid artery plaques are a leading cause of ischemic stroke, and carotid endarterectomy (CEA) is one of the major treatment approaches for this disease. Changes in cerebral metabolism following CEA remain unclear. The present study aimed to evaluate the effect of cerebral ammonia metabolism following CEA using ^{13}N -labeled ammonia positron emission tomography (PET) in humans. A total of 20 patients were enrolled in the present study, with a mean age of 59.5 years, comprising 16 males and four females. Of these patients, eight underwent right CEA and 12 underwent left CEA. The rate of carotid artery stenosis was between 50-69% in six of the patients, between 70-99% in 11 of the patients and was at 100% (thrombosis) in three of the patients, measured by computerised tomography digital subtraction angiography prior to CEA. ^{13}N -labeled ammonia (137 MBq) PET scanning was performed prior and subsequent to CEA surgery for each patient. The first ammonia PET scan was performed 1 day prior to CEA, while the second PET scan was performed 1-4 weeks following CEA. Following injection of ^{13}N -labeled ammonia, static PET was acquired for 10 min. The region of interest (ROI), covering the major cerebral hemisphere, was selected and ammonia uptake in the ROI was determined in the ipsilateral and contralateral hemispheres. No hyperperfusion syndrome was observed in the patients subsequent to CEA. No significant change in cerebral hemisphere ammonia uptake was observed between the ipsilateral and contralateral hemispheres prior to (ratio = 0.98; $P > 0.01$) or following (ratio = 1.09;

$P > 0.01$) CEA. Ammonia uptake in the ipsilateral and contralateral hemispheres was significantly reduced to 23.2 and 23.5%, respectively, following CEA. Using ^{13}N -labeled ammonia PET to evaluate cerebral ammonia metabolism following CEA in patients with severe carotid artery stenosis, the present study demonstrated that uptake of ammonia in the ipsilateral and contralateral hemispheres was significantly reduced.

Introduction

Stroke is a major cause of morbidity and mortality in developed and developing countries worldwide. At present, it is recognized that rupture of the carotid atherosclerotic plaque and subsequent formation of thrombi or emboli are the primary causes of ischemic stroke (1-3). Treatment for carotid artery stenosis includes carotid endarterectomy (CEA) and carotid artery stenting (CAS), which are two approaches that have been previously demonstrated to prevent future stroke (4-6).

Improvements in cerebral blood flow following CEA have been observed using various imaging modalities, including transcranial Doppler, single-photon emission computed tomography (SPECT), perfusion computerised tomography (CT) and magnetic resonance imaging (7-11). For the measurement of cerebral tissue perfusion, the use of ^{15}O -labeled water with PET is considered the gold standard, due to the fact that it possesses a high first-pass extraction (12). With ^{15}O -labeled water PET, Rijbroek *et al* (12) identified that, 1 day following CEA, absolute cerebral blood flow is increased in all arterial territories on the ipsilateral and contralateral hemispheres. In addition, the same study demonstrated no difference in cerebral blood flow between the ipsilateral and contralateral hemispheres prior to and following CEA.

Following carotid artery revascularization, several studies have observed changes in neuropsychological and cognitive functions in humans, including memory, attention, psychomotor speed and executive functions (8,13). In the mammalian cerebral cortex, $\geq 90\%$ of neurons are glutamatergic or GABAergic, and neuropsychological functions depend on neurotransmitter trafficking between neurons and astrocytes, with ammonia involved in the glutamate/glutamine cycle (14-16).

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At present, to the best of our knowledge, there have been no reports focussing on CEA regarding the cerebral uptake of ammonia using ^{13}N -labeled ammonia with PET. The present study aimed to examine the changes in neuropsychological functions following surgery in patients with carotid artery stenosis. It was hypothesized that cerebral ammonia metabolism is altered following CEA, and that this alteration may be associated with GABAergic neuron functions. Therefore, the purpose of the present PET study was to evaluate alterations in ammonia uptake in cerebral hemispheres prior and subsequent to CEA.

Patients and methods

Patients. The present study included 20 patients (four females; 16 males) with a mean age of 59.5 years (range, 33–75 years), who presented with unilateral chronic stenosis between 2011 and 2012 at the Affiliated Hospital of Inner Mongolia Medical University (Hohhot, China). The clinical characteristics of the patients are presented in Table I. No cortical infarctions were observed in the patients on conventional CT prior to CEA. Of the 20 patients, six exhibited carotid artery stenosis of 50–69%, 11 exhibited carotid artery stenosis of 70–99% and three patients exhibited 100% stenosis, termed thrombosis, measured by CT digital subtraction angiography prior to CEA. No patients exhibited occlusion or stenosis $\geq 50\%$ in the contralateral hemisphere. All study procedures were approved by the Ethics Committee in the Affiliated Hospital of Inner Mongolia Medical University, and written informed consent was obtained from all subjects prior to their enrolment in the study.

Surgical protocol. All patients received antiplatelet therapy (aspirin, 100–200 mg/day) until the morning of the day on which CEA was performed. All patients underwent surgery under general anaesthesia (propofol; initial bolus 1.5–2.5 mg/kg, infusion 100–140 $\mu\text{g/kg/min}$). Blood pressure was maintained at a stable level, in the range of their preoperative level $\pm 20\%$, throughout the procedure by adjusting the depth of anaesthesia or, if required, by intravenous administration of a vasodilator (nitroglycerin, ~ 100 mg/min) or a vasoconstrictor (adrenaline, ~ 2 mg/min). No intraluminal shunt was used in these procedures. The mean duration of intracranial artery clamping was 30 min, ranging between 20 and 40 min. An angiograph is presented in Fig. 1 as an example prior to and following surgery.

Ultrasound. High resolution B-mode and Doppler ultrasonography (Vivid 7; GE Healthcare, Milwaukee, WI, USA) of the carotid arteries were performed for each patient prior to surgery to evaluate the severity of vessel stenosis.

PET and CT protocol. ^{13}N -ammonia was prepared by the $^{16}\text{O}(\text{p},\alpha)^{13}\text{N}$ nuclear reaction in a water target with a cyclotron (MINItrace; GE Healthcare Life Sciences, Pittsburgh, PA, USA). The target, containing $^{16}\text{O}-\text{H}_2\text{O}$, was exposed to a 25 μA current for 10–15 min, following which the in-line-produced $^{13}\text{N}-\text{NH}_3$ was passed through a Sep-Pak CM column (Plus Accell CM; Waters, Brehamwood, UK), and was separated chromatally by positive ion exchange and use of a filter (EMD Millipore, Billerica, MA, USA), prior to its delivery to patients intravenously.

The PET imaging was performed using a PET/CT scanner (Discovery ST8; GE Healthcare). The CT imaging was performed 3 min prior to the injection of 137 MBq ^{13}N -labeled ammonia. Following injection, PET images were captured immediately for 10 min. The PET images were reconstructed using an ordered subset expectation maximization iterative algorithm, comprising eight subsets; a 128x128 matrix; 3 mm slice thickness and no overlap (12). The PET investigation and scanning protocols used in the present study are illustrated in Fig. 2A.

PET data analysis. Human cerebral hemisphere templates were created covering the majority of the cerebral tissue, including the white matter, grey matter and basal ganglia, as shown in Fig. 2B, in Carimas (a PET data analysis package developed in Turku PET Centre of Finland; <http://www.turku-petcentre.fi/carimas>). For individual cases, these templates were loaded and modified accordingly to fit the cerebral hemispheres. Additional volume of interest (VOI), covering the lateral skin, was created for each case. The preoperative and postoperative PET image data were loaded into Carimas. Automatic registration using an algorithm developed by Maes *et al* (17) implemented in Carimas was performed, and all cases exhibited geometric alignment of the PET image with the CT image, as shown in Fig. 2. Ammonia uptake was calculated by taking the average radioactivity in all pixels inside the hemisphere or skin VOIs from the preoperative images and postoperative images. Furthermore, ammonia uptake in the cerebral hemisphere was normalized by the corresponding contralateral skin VOI. The reason for replacing standardized uptake value (SUV) with contralateral skin VOI normalization was due to the fact that the remains of radioactivity in the syringe following the injection were not measured for all cases. Without the measurement of remaining radioactivity, the introduction of bias requires inclusion in the calculation of SUV. All the ammonia uptake numerical data generated in Carimas were saved in ASCII format files for further statistical analysis. An overview of the PET data analysis protocol is presented in Fig. 3.

Statistical analysis. All statistical analyses were performed using Origin 7.0 (OriginLab Corporation, Northampton, MA, USA). Student's t-test was used for the analysis of differences in paired data. $P < 0.01$ was considered to indicate a statistically significant difference.

Results

A total of 20 patients were involved in the present study. The patients' characteristics, including age, gender, side of surgery, postoperative PET scanning time (in days) and stenosis, estimated by ultrasound, are listed in Table I. In total, $>50\%$ of the patients (13 patients) suffered from stenosis $>80\%$.

The ratio of ammonia uptake prior to and following CEA for the ipsilateral and contralateral hemispheres is shown in Fig. 4 for each individual patient. The changes in the uptake of ammonia were not significantly different between the ipsilateral and contralateral hemispheres. Ammonia uptake was observed to be higher following CEA in four patients, whereas the uptake in two patients remained almost the same

Table I. Characteristics of patients.

Patient	Age (years)	Gender	Side of surgery	PET (days ^a)	Stenosis (%)
1	56	Male	Left	23	70-99
2	75	Female	Left	10	50-69
3	73	Male	Left	30	50-69
4	66	Female	Left	14	50-69
5	72	Male	Right	13	50-69
6	36	Male	Right	13	100
7	46	Male	Left	10	70-99
8	72	Male	Left	10	70-99
9	57	Male	Right	14	50-69
10	54	Male	Left	11	50-69
11	58	Male	Right	9	70-99
12	52	Male	Right	10	70-99
13	67	Male	Left	12	100
14	62	Female	Right	10	70-99
15	71	Female	Right	10	70-99
16	57	Male	Left	7	70-99
17	51	Male	Left	9	70-99
18	58	Male	Left	8	70-99
19	33	Male	Left	12	70-99
20	75	Male	Right	7	100

^aDays of the second PET scanning following CEA. Stenosis estimation was based on ultrasound investigation. PET, positron emission tomography; CEA, carotid endarterectomy.

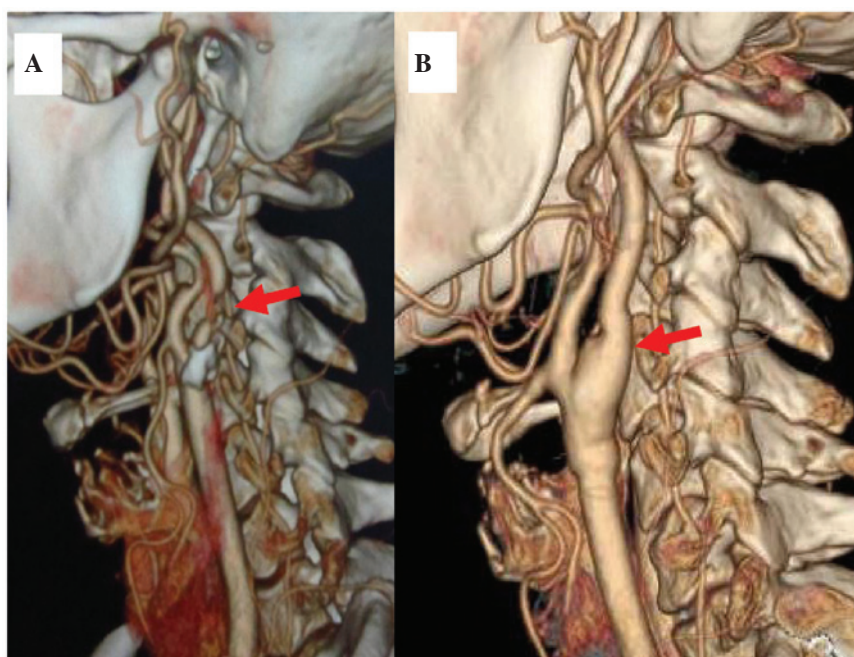


Figure 1. Computed tomography angiography images (A) prior to and (B) following carotid endarterectomy operation. The red arrow indicates stenosis of the carotid artery in (A) and revascularization following surgery in (B).

and the remaining patients exhibited significantly reduced uptake.

The mean values of ammonia uptake prior and subsequent to CEA for the ipsilateral and contralateral hemispheres were

examined (Fig. 5). Following surgery, the uptake of ammonia was observed to decrease between 3.04 ± 0.42 and 2.54 ± 0.35 (23.2%) in the ipsilateral hemisphere, and between 3.10 ± 0.47 and 2.48 ± 0.36 (23.5%) in the contralateral hemisphere. Changes

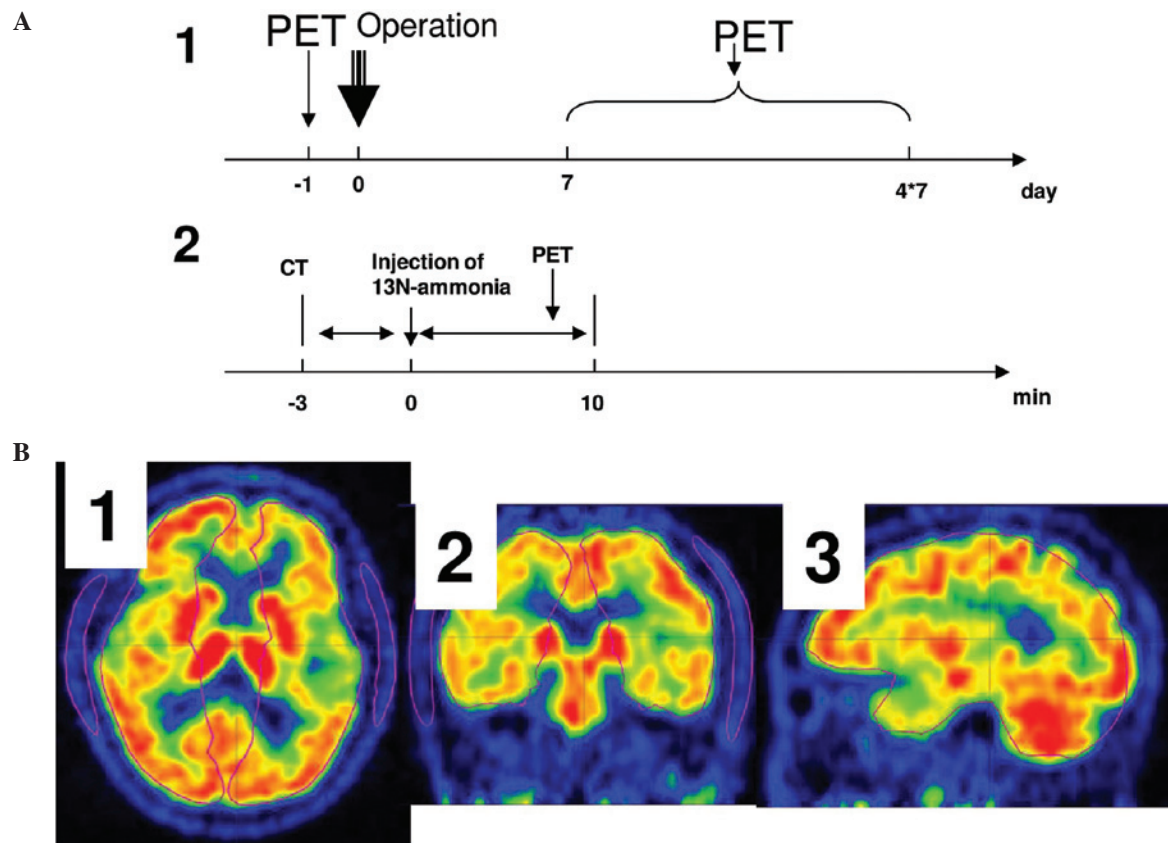


Figure 2. (A) Study protocols. (1) PET scanning and CEA. All patients underwent PET imaging 1 day prior to CEA, and were imaged for a second time with PET 1-4 weeks post-CEA; (2) static PET data were collected between 15 and 20 min following injection of ^{13}N -ammonia. (B) Template of the brain region of interest, including the left and right hemispheres in (1) transverse, (2) coronal and (3) sagittal sections. PET, positron emission tomography; CEA, carotid endarterectomy; CT, computed tomography. Red and yellow indicate regions of increased ^{13}N -ammonia uptake; green and blue indicate decreased uptake.

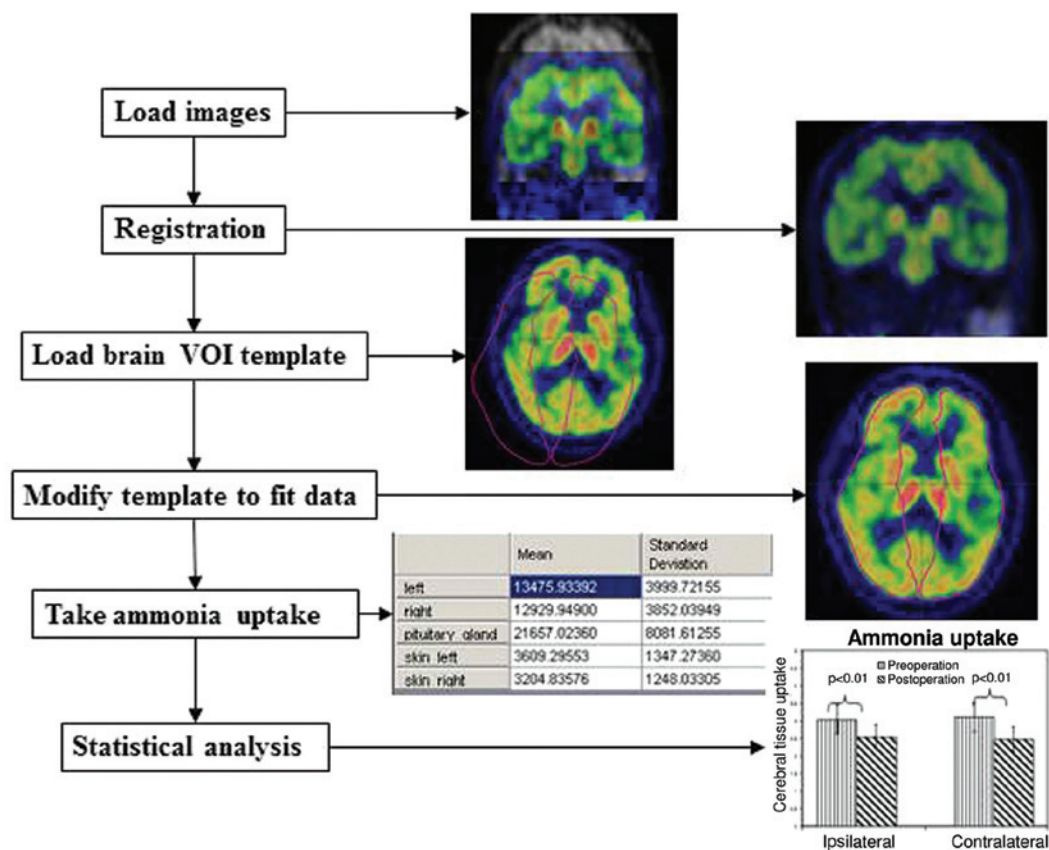


Figure 3. Flow chart illustrating the positron emission tomography data analysis. VOI, volume of interest.

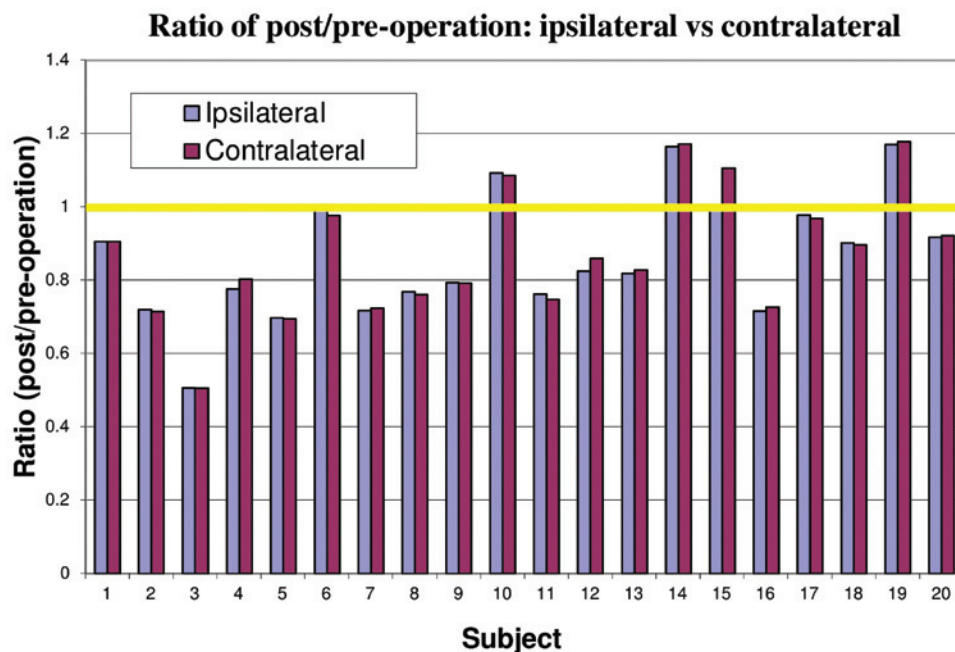


Figure 4. Ratio of post/pre-operative ^{13}N -ammonia uptake. Blue indicates ipsilateral and red indicates contralateral. The value of the yellow line is one.

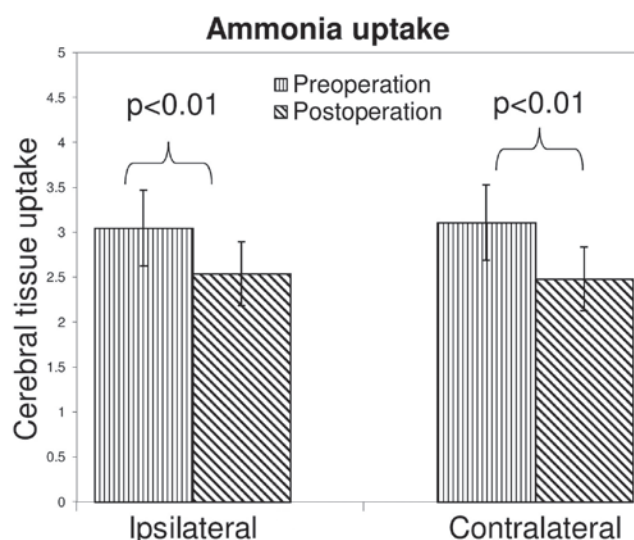


Figure 5. Statistical analysis of ^{13}N -ammonia uptake pre- and post-operatively. Statistically significant decreases in ^{13}N -ammonia uptake were observed following carotid endarterectomy in the ipsilateral and contralateral hemispheres. Error bars indicate the standard deviation.

in ammonia uptake were statistically significant for the right and left hemispheres between the preoperative and postoperative states. However, no significant difference was identified between the two hemispheres preoperatively and postoperatively.

Discussion

In the present study, ^{13}N -labeled ammonia PET was used to examine cerebral ammonia metabolism in patients with severe carotid artery stenosis, by comparing the cerebral ammonia uptake prior to and following CEA. The two key observations were as follows: i) Uptake of ammonia in the ipsilateral and contralateral cerebral hemispheres reduced significantly following treatment; ii) no significant difference in the

uptake of ammonia was identified between the ipsilateral and contralateral hemispheres prior to and following CEA.

As an effective treatment approach for symptomatic and asymptomatic carotid artery stenosis, CEA has been in use since the 1950s (18), and CAS has been used more widely and frequently since the 1990s, as an alternative treatment option for this disease (18). A large clinical randomized trial, termed the Carotid Revascularization Endarterectomy vs. Stenting Trial (CREST), sponsored by the National Institutes of Health, demonstrated that carotid stenting and endarterectomy were associated with similar rates of mortality and disabling stroke (19). However, at least among symptomatic patients in the CREST trial, CEA exhibited notably low stroke and mortality rates (20).

Several studies have demonstrated the increase of cerebral blood flow in ipsilateral cerebral territories or hemispheres following CEA or CAS in the short- and long-term, including a study by Yang *et al* (10) using perfusion CT and a study by Araki *et al* (21) using transcranial Doppler, SPECT and PET. Of note, using ^{15}O -labeled water, which is known to be the gold standard for measurement of cerebral blood/perfusion, Rijbroek *et al* (12) observed that blood flow and oxygen consumption are increased in the ipsilateral and contralateral hemispheres following CEA, with no significant difference of these parameters between the ipsilateral and contralateral hemispheres, either preoperatively or postoperatively. The present study demonstrated that the effects of carotid artery stenosis and the improvements of CEA on cerebral blood flow and metabolism were equal to the two hemispheres. This observation can be explained by the effective collateral circulation, or circle of Willis, in the human brain (12). Furthermore, since blood supply is the basis of all metabolism, the observation that ammonia uptake did not change in the present study, was reasonable, based on uniform hemodynamic changes pre- and postoperatively. Combining the findings of the present study with those of Rijbroek *et al* (12) suggests that unilateral surgery for carotid artery stenosis improves cerebral blood supply and metabolism, not only for the ipsilateral hemisphere, but also for the contralateral hemisphere.

Several studies have investigated the neurological and neuropsychological effects of carotid artery revascularisation, including carotid artery stenting and endarterectomy, in patients with severe carotid artery stenosis or occlusion (22,23). For example, in patients with chronic internal carotid artery occlusion, Lin *et al* (22) identified that successful carotid artery stenting significantly improves global cognitive function, and attention and psychomotor processing speed. Another study (23) demonstrated that unilateral carotid artery stenting is beneficial to patients with severe asymptomatic stenosis by improving executive function. In humans, neurotransmitters are important in the modulation of neurological and neuropsychological functions, therefore, neurotransmitters are likely to be involved in the neurological and neuropsychological effects following CEA.

^{13}N -ammonia has been validated as a perfusion tracer for use in cardiac PET (24). In brain tissue, the uptake of ammonia is not as closely associated with blood flow as in the myocardium. For example, Phelps *et al* (24) demonstrated that doubling or halving basal cerebral blood flow resulted in alterations of ammonia uptake of 30-50% in the human brain, and further increases of blood flow were associated with progressively smaller alterations in ammonia uptake. Other studies have demonstrated that the uptake of ammonia is more dependent on the process of glutamine synthesis in the brain (14,16).

In the human cerebral cortex, >90% of neurons are glutamergic or GABAergic, and the high *in vivo* flux of the glutamate/glutamine cycle in brain cortex puts a significant demand on the return of ammonia, mediated by phosphate-activated glutaminase, between the neurons and astrocytes in order to maintain the nitrogen balance (16). Therefore, changes in the uptake of ^{13}N -labeled ammonia in the human brain are likely to be more sensitive than glutamine metabolism.

The reduced uptake of ammonia in the human brain following carotid CEA observed in the present study suggested two potential hypotheses: Washout by increased blood flow or a negative association between blood flow and uptake of ammonia in human brain tissue.

Washout effect. In human cortex tissue, neurons are surrounded by astrocytes. This anatomical structure in cerebral histology suggests that the exchange of contents between the blood and neurons is modulated by astrocytes. In the glutamate/glutamine and GABA/glutamine cycles, glutamate and ammonia are released in neurons and taken up by astrocytes, where glutamine is synthesized by glutamine synthetase using glutamate and ammonia (14). Previous studies have demonstrated that there is a high flux of ammonia trafficking between neurons and astrocytes. Therefore, the present study hypothesized that increased levels of cerebral blood flow following CEA wash out more ammonia from astrocytes, resulting in a reduction in ^{13}N -labeled ammonia uptake in the patients.

Negative association of cerebral blood flow with ammonia uptake. Hepatic encephalopathy is a worsening of brain function resulting from liver failure, due to the fact that the liver is no longer able to remove ammonia from the blood (25). In patients with hepatic encephalopathy and healthy individuals, blood-borne ammonia readily enters brain tissue and the metabolic trapping in it is correlated with the blood concentration of ammonia (26). However, how ammonia leaves the brain had been debated for a long time. Using ^{13}N -labeled ammonia PET, Sørensen *et al* revealed that backflux of ammonia from the brain to the blood does indeed occur in healthy individuals and patients with and without hepatic encephalopathy (27). Therefore, combining this finding with the present results, demonstrating a reduction of ammonia uptake in cerebral tissue, it is reasonable to assume that, after CEA, the improved cerebral blood flow accelerates the backflux of ammonia from the brain to the blood, or a negative association between cerebral blood flow and the ammonia uptake in brain tissue.

Certain limitations were identified in the present study. Firstly, no ^{15}O -labeled water was used to measure the cerebral blood flow simultaneously with ^{13}N -labeled ammonia. In addition, dynamic PET scanning was not performed, which has been reported to provide more accurate results for ammonia uptake in brain tissue. These two limitations are due to the lack of a ^{15}O -labeled water device and blood radioactivity measurement devices at the Department of Nuclear Medicine of Inner Mongolia University Hospital of China. Finally, the patients were only scanned 1 month following CEA and no long-term follow up, for example PET scanning 6 months or 1 year-post CEA, was performed. Therefore, whether the reduction of cerebral ammonia uptake is temporary or permanent following CEA remains to be elucidated. These limitations require attention in further investigations.

In conclusion, using ^{13}N -labeled ammonia PET to evaluate cerebral ammonia metabolism following CEA in patients with severe carotid artery stenosis, the present study demonstrated that the uptake of ammonia in the ipsilateral and contralateral hemispheres is significantly reduced. However, no significant differences in ammonia uptake were identified between the two hemispheres, or between ipsilateral and contralateral hemispheres, pre- or postoperatively.

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