Sex-specific differences in white matter microvascular integrity after ischaemic stroke

Mark R Etherton [ORCID],[1] Ona Wu,[1,2,3] Pedro Cougo,[1,4] Svetlana Lorenzano,[1,5] Hua Li,[3] Lisa Cloonan,[1] Mark J R J Bouts,[1,2,6] Arne Lauer,[1,7] Ken Arai,[3] Eng H Lo,[1,3] Steve K Feske,[8] Karen L Furie,[9] Natalia S Rost[1]

Correspondence to
Dr Mark R Etherton;
metherton@partners.org

ABSTRACT

Background and purpose Functional outcomes after ischaemic stroke are worse in women, despite adjusting for differences in comorbidities and treatment approaches. White matter microvascular integrity represents one risk factor for poor long-term functional outcomes after ischaemic stroke. The aim of the study is to characterise sex-specific differences in microvascular integrity in individuals with acute ischaemic stroke.

Methods A retrospective analysis of subjects with acute ischaemic stroke and brain MRI with diffusion-weighted (DWI) and dynamic-susceptibility contrast-enhanced (DSC) perfusion-weighted imaging obtained within 9 hours of last known well was performed. In the hemisphere contralateral to the acute infarct, normal-appearing white matter (NAWM) microvascular integrity was measured using the K_2 coefficient and apparent diffusion coefficient (ADC) values. Regression analyses for predictors of K_2 coefficient, DWI volume and good outcome (90-day modified Rankin scale (mRS) score <2) were performed.

Results 105 men and 79 women met inclusion criteria for analysis. Despite no difference in age, women had increased NAWM K_2 coefficient (1027.4 vs 692.7×10^{-6}/s; p=0.006). In women, atrial fibrillation (β=583.6; p=0.04) and increasing NAWM ADC (β=4.4; p=0.02) were associated with increased NAWM K_2 coefficient. In multivariable regression analysis, the K_2 coefficient was an independent predictor of DWI volume in women (β=0.007; p=0.01) but not men.

Conclusions In women with acute ischaemic stroke, increased NAWM K_2 coefficient is associated with increased infarct volume and chronic white matter structural integrity. Prospective studies investigating sex-specific differences in white matter microvascular integrity are needed.

INTRODUCTION

Sex disparities in acute ischaemic stroke (AIS) outcomes represent an important clinical issue. Compared with men, women have worse functional outcomes.[1 2] Women are known to be older at the time of stroke,[3] have higher rates of antecedent disability and atrial fibrillation,[2] and lower rates of treatment with thrombolytic therapy.[4] Even after adjusting for these differences, however, functional outcomes are worse in women.[5] Understanding the factors that contribute to this sex disparity in stroke

outcomes represents an opportunity to improve patient care and stroke outcomes.

The decreased structural integrity of cerebral white matter and increased blood–brain barrier (BBB) permeability are factors that negatively affect poststroke outcomes.[6–9] Evidence of cerebral microvascular dysfunction can be detected on T2-weighted MRI as white matter hyperintensities (WMH), enhanced BBB permeability on perfusion-weighted imaging (PWI), or measured in normal-appearing white matter (NAWM) as loss of microstructural integrity on diffusion-tensor imaging (DTI), and reflected as an increase in the apparent diffusion coefficient (ADC) values.[7 10] Both increased diffusivity and BBB permeability have been linked to poor outcomes after AIS,[6–8] with a potential sex-specific contribution.[11] We have also reported a novel association between increasing NAWM K_2 coefficient, as a potential surrogate of BBB permeability, and worse functional outcomes after AIS.[7] Two important findings were observed in that analysis: (1) increased NAWM K_2 coefficient in women compared with men and (2) increased matrix metalloproteinase-2 (MMP-2) levels, as a measure of acute BBB disruption,[12] were associated with worse long-term outcomes. The underlying aetiology of these sex-specific differences in BBB permeability and influences on ischaemic tissue and functional outcomes, however, was unclear.

In this study, we evaluated sex-specific clinical and radiographic variables associated with NAWM K_2 coefficient and their impact on infarct volume and 90-day functional outcomes after AIS.

METHODS

Standard protocol approvals, registrations and patient consents

This study and the use of human participants was approved by the local Institutional Review Board. Written informed consent was obtained from all participating subjects or their surrogates.

Subjects and inclusion/exclusion criteria

We performed a retrospective analysis of a prospective biomarker cohort study of consecutive adult AIS subjects admitted between February 2007 and 2011 (National Institutes of Health Specialized Program of Translational Research in Acute Stroke).[13 14]

All subjects presenting to two academic medical centres within 9 hours of the last known well (LKW) and with signs and symptoms of AIS were screened for eligibility. To be eligible for inclusion in this study, subjects were required to undergo brain MRI within 9 hours from LKW containing diffusion-weighted imaging (DWI), dynamic-susceptibility contrast (DSC) perfusion-weighted MRI, and fluid-attenuated inversion recovery (FLAIR) MRI sequences; have a confirmed acute infarct on DWI; and have plasma MMP-2 levels collected at the time of study enrollment. We excluded patients with artefactual MMP-2 recordings and those with acute infarcts in both hemispheres.

Clinical assessments

Age, medical history and antecedent medications were obtained from the subject or their surrogates or otherwise from the review of the medical record at the time of admission. Admission systolic and diastolic blood pressures were acquired from the first documented vital signs. Treatment with intravenous alteplase was recorded. Tobacco use was dichotomised as current or past use versus never. Alcohol use was defined and dichotomised as current versus none. Anticoagulant use was defined as prehospital use of lovenox or coumadin.

Imaging analysis

MRI protocol

The majority of MRIs were performed on a 1.5T General Electric Signa HDx scanner with the following acquisition parameters. DTI: repetition time (TR)/echo time (TE)=5000/85.3 ms, 220 mm field-of-view, 5 mm slice thickness with 1 mm gap, in-plane resolution of $0.86 \times 0.86 \, mm^2$ (128×128 acquisition matrix up-sampled to 256×256), three $0 \, s/mm^2$ (b-zero) and $1000 \, s/mm^2$ in 25 directions. FLAIR: TR/TE/TI=10 002/145/2200 ms, 220 mm field-of-view, 5 mm slice thickness with 1 mm gap, and in-plane resolution of $0.86 \times 0.86 \, mm^2$. For 10 subjects, FLAIR scans were acquired at a separate time from the original DWI and DSC sequences due to an unavailable or unusable FLAIR scan on the original MRI. DSC: 80 acquisitions, TR/TE=1500/40 ms, 220 mm field-of-view, 5 mm slice thickness with 1 mm gap, $1.72 \times 1.72 \, mm^2$ in-plane resolution. DSC sequences were acquired using bolus tracking of a single dose of gadolinium-based contrast agent (0.1 mmol/kg) administered with a power injector. Motion and eddy current distortions were corrected in all DTI datasets and the ADC maps were then derived from the high-b-value and low-b-value DTI sequences.[15]

To estimate BBB permeability, the K_2 coefficient (/s) was mathematically derived from the concentration-time curves using the Boxerman-Weisskoff model.[16] In brief, the effective transverse relaxation rate due to the contrast agent, a parameter representing the per voxel concentration of contrast agent, is represented as:

$$\Delta \bar{R}_2^* (t) \approx K_1 \cdot \Delta \bar{R}_2^* (t) - K_2 \int_0^1 \Delta \bar{R}_2^* \left(t^{'} \right) dt^{'}$$

A least-squares fit to this equation can subsequently be used to solve for the K_2 coefficient on a voxelwise basis.

Mean transit time (MTT) maps were calculated from deconvolved DSC data converted to concentration changes over time using a tracer arrival timing-insensitive technique.[17 18]

Volumetric lesion mapping

The DWI and MTT lesions were outlined using an intensity threshold-based method. All DWI lesions were cross-referenced to ADC maps to exclude subacute and chronic infarcts as well as infarcts involving the bilateral cerebral hemispheres. The acute infarct volume and ipsilateral perfusion deficit, assessed on DWI sequences (DWIv) and MTT maps (MTTv), respectively, were calculated semiautomatically using MRIcro software (University of Nottingham School of Psychology, Nottingham, UK; www.mricro.com).

WMH lesions in the hemisphere contralateral to the acute infarct were outlined in a semi-automated manner using MRIcro software as described previously.[11 19] All images were analysed by trained research staff that were blinded to all outcome data.

NAWM segmentation and analysis

NAWM segmentation in the contralateral hemisphere was performed as described previously (figure 1).[7] The mean ADC and K_2 coefficients were measured in contralateral NAWM as defined by the probabilistic mask.

Stroke and outcomes assessment

All subjects were evaluated at the time of admission by a trained neurologist and stroke severity was determined using the National Institutes of Health Stroke Scale (NIHSS). Stroke subtype was categorised according to the Causative Classification of Stroke System (CCS) score.[20] Haemorrhagic transformation was defined as the presence of any petechial or parenchymal haematoma on follow-up CT or MRI (obtained within 48 hours of LKW). Functional outcome at 90 days was measured using the mRS score, which was obtained either through an in-person or validated telephone-based interview.[21] Good functional outcome was defined as a 90-day mRS score of <2.

Statistical analysis

WMH volume (WMHv) and DWI volume (DWIv) were normalised to the intracranial area.[22] Univariable analysis was performed using linear regression to determine factors associated with K_2 coefficient and DWIv and logistic regression to evaluate factors associated with good functional outcome. Variables that displayed an association, *a priori* set at p<0.05, in univariable analysis were inserted into a backward stepwise elimination
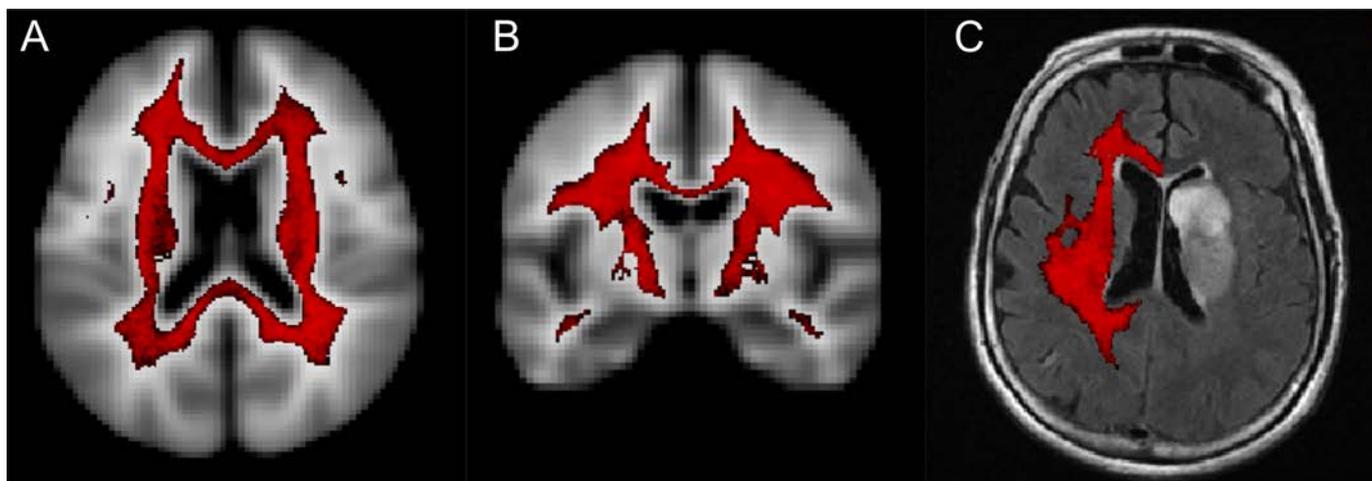
Figure 1 NAWM segmentation. (A) Axial and (B) coronal views of probabilistic white matter mask with threshold of >95% probability white matter (red) superimposed on the International Consortium for Brain Mapping 425 atlas. (C) Representative image of final contralesional normal-appearing white matter mask (red) overlaid on FLAIR image.

multivariable regression model. To minimise influences of collinearity, WMHv and NAWM ADC, as markers of global structural integrity, were input into separate multivariable regression models when significant in univariable analysis. Admission NIHSS was excluded from multivariable regression models for DWIv and good outcomes due to the known strong association between NIHSS and DWIv.[23] [24] Statistical significance was set at a p value of<0.05.

RESULTS

A total of 105 men and 79 women met inclusion criteria for analysis in this study. The baseline demographics are shown in table 1. Women were more likely to have atrial fibrillation and cardioembolic strokes. Men had a greater incidence of hyperlipidaemia and strokes attributed to large-artery atheroembolism. Treatment rates with intravenous alteplase were similar between men and women and only one patient in the cohort underwent intra-arterial therapy. There was no difference in WMHv, DWIv, MTT volume or NAWM ADC between men and women. Women, however, had significantly greater NAWM K_2 coefficient (table 1).

In women, WMHv, NAWM ADC and atrial fibrillation were associated with greater NAWM K_2 coefficient in univariable linear regression analysis (table 2). In multivariable analyses, increasing NAWM ADC was associated with increased NAWM K_2 coefficient in women, whereas WMHv was not (table 3). In contrast, in men, WMHv and age were associated with NAWM K_2 coefficient in univariable linear regression but only WMHv remained an independent predictor in the multivariable analysis (table 3). In the entire cohort, we observed no association between strokes attributed to large-artery atheroembolism (β=75.0; p=0.84) or cardioembolism (β=163.1; p=0.58) and the NAWM K_2 coefficient.

In both men and women, the admission NIHSS was a significant predictor of DWIv in univariable

analysis (table 4). In women, administration of intravenous alteplase, haemorrhagic transformation and increasing NAWM K_2 coefficient were independently associated with DWIv. In comparison, in men, only atrial fibrillation was associated with increased DWIv. Subsequent linear regression analysis including sex, NAWM K_2 coefficient and an interaction term (male sex • NAWM K_2 coefficient) showed that NAWM K_2 coefficient (β=0.006; p=0.037) and the interaction term (β=−0.007; p=0.027) were independent predictors of DWIv for the entire cohort.

In multivariable logistic regression analysis, increasing NAWM ADC was an independent predictor of the decreased likelihood of good outcome for both sexes (table 5).

DISCUSSION

We report that the NAWM K_2 coefficient, as a measure of microvascular integrity, is significantly increased in women compared with men with AIS. In addition, in women we observe sex-specific relationships: NAWM K_2 coefficient is independently associated with NAWM ADC values and a predictor of infarct volume. These findings suggest that increased NAWM K_2 coefficient in women, as a surrogate of BBB dysfunction, is associated with increased infarct size, which predisposes to worse long-term outcomes after stroke.

Our finding of sex-specific differences in NAWM K_2 coefficient, as a putative measure of BBB dysfunction, is of clinical interest. We have previously reported an association between NAWM structural integrity (fractional anisotropy) and sex-specific functional outcomes.[11] Together, these observations offer some insight into the potential factors contributing to the disparity in stroke outcomes between sexes as they suggest that sex-specific differences in NAWM microvascular integrity predispose to worse outcomes in women. There are several potential explanations for the observed sex-specific differences in

Table 1 Comparison of admission and clinical variables in men and women with AIS

Variable	Women (N=79)	Men (N=105)	P values
Age, years, mean (SD)	68.5 (16.3)	68.1 (14.0)	0.55
Medical history, n (%)			
Anticoagulant use	10 (12.7)	19 (18.1)	0.43
Atrial fibrillation	29 (36.7)	21 (20.0)	**0.02**
Coronary artery disease	15 (19.0)	29 (27.6)	0.24
Diabetes	13 (16.5)	25 (23.8)	0.30
Hyperlipidaemia	27 (34.2)	55 (52.4)	**0.02**
Hypertension	52 (65.8)	77 (73.3)	0.35
Prior stroke/TIA	18 (22.8)	26 (24.8)	0.89
CCS subtype classification, n (%)			
Cardioembolic	48 (60.8)	46 (43.8)	**0.03**
Large artery	6 (7.6)	33 (31.4)	**<0.001**
Small vessel	4 (5.1)	5 (4.8)	1
Other/Undetermined	21 (26.6)	20 (19.0)	0.30
Clinical variables			
Admission NIHSS, median (IQR)	6 (3,12)	5 (2, 11)	0.05
Body mass index, kg/m^2, mean (SD)	27.8 (6.5)	28.3 (5.5)	0.52
Systolic blood pressure, mm Hg, median (IQR)	148 (136–173)	158 (140–174)	0.14
Diastolic blood pressure, mm Hg, median (IQR)	84 (72–92)	84 (76–89)	0.83
MMP-2 level, ng/mL, median (IQR)	304.6 (242.7–372.1)	301.7 (237.8–369.7)	0.80
IV alteplase treatment, n (%)	30 (38.0)	35 (33.3)	0.62
Radiographic variables			
Haemorrhagic transformation, n (%)	16 (27.1)	24 (22.9)	0.79
Parenchymal haematoma, n (%)	5 (5.9)	11 (11.1)	0.46
WMHv, cm^3, median (IQR)	2.0 (0.8–5.5)	1.7 (0.9–3.8)	0.55
DWIv, cm^3, median (IQR)	6.9 (1.2–30.2)	7.5 (1.7–21.9)	0.76
MTTv, cm^3, median (IQR)	32.1 (5.3–81.0)	23.5 (5.8–78.2)	0.92
ADC ($\times 10^{-6}$) NAWM, mm^2/s, mean (SD)	804.0 (69.2)	791.9 (48.7)	0.19
K_2 coefficient ($\times 10^{-6}$) NAWM, /s, mean (SD)	1027.4 (1192.9)	692.7 (2460.9)	**0.006**
NAWM mask volume, cm^3, median (IQR)	44.0 (35.4–51.5)	53.4 (43.5–63.1)	**<0.001**
Time from last seen well to MRI, min, median (IQR)	346 (256–438)	345.5 (250.8–437.8)	0.90
Functional outcomes			
90-day good outcome (mRS <2), n (%)	29 (46.8)	55 (58.5)	0.20

Bold values indicate P value < 0.05.
ADC, apparent diffusion coefficient; AIS, acute ischaemic stroke; CCS, causative classification system; DWIv, diffusion-weighted imaging volume; IQR, interquartile range; IV, intravenous; MMP, matrix metalloproteinaise; MTTv, mean transit time volume; NAWM, normal-appearing white matter; NIHSS, National Institutes of Health Stroke Scale; TIA, transient ischemic attack; WMHv, white matter hyperintensity volume.

NAWM K_2 coefficient. First, we observed no substantial difference in age, hypertension, diabetes, anticoagulant use, intravenous alteplase treatment, rate of haemorrhagic transformation, WMHv or stroke severity in women. A relationship between NAWM BBB permeability and white matter structural integrity has been reported in individuals with cerebral small-vessel disease.[25–27] In our acute cohort, however, we observed no difference in strokes attributed to small-vessel occlusive disease between sexes.

We also observed no difference in baseline MMP-2 levels between sexes, a molecule thought to play a role in BBB remodelling.[7] Another study showed that diastolic blood pressure and blood glucose were independently associated with BBB permeability abnormalities,[28] however, we observed no differences in admission blood pressure or rates of hypertension between sexes. Another hypothesis is the potential hormonal dysregulation of the BBB in women. Different murine models of oestrogen depletion

Table 2 Univariable analysis of sex-specific associations with NAWM K_2 coefficient

Variable	Women		Men	
	Estimate	P values	Estimate	P values
Age	11.3	0.17	36.0	**0.04**
BMI	−41.8	0.06	−64.0	0.20
Anticoagulant use	523.8	0.20	2.9	0.99
Atrial fibrillation	583.6	**0.04**	−935.3	0.12
Diabetes	−118.6	0.75	603.2	0.29
Hyperlipidaemia	82.6	0.77	613.2	0.20
Hypertension	−123.4	0.67	−362.2	0.51
Prior stroke/TIA	336.7	0.30	650.6	0.24
Cardioembolic stroke	194.6	0.48	66.7	0.89
Admission NIHSS	40.4	0.07	31.4	0.40
Systolic blood pressure	0.9	0.86	8.0	0.36
MMP-2 level	0.4	0.69	1.4	0.45
IV alteplase treatment	195.5	0.48	−732.6	0.15
HT	413.4	0.17	357.1	0.52
WMHv	31.8	**0.04**	131.1	**0.005**
NAWM ADC	4.4	**0.02**	2.2	0.66
NAWM mask volume	−17.8	0.11	−24.0	0.13

Bold values indicate P value < 0.05.
ADC, apparent diffusion coefficient; BMI, body mass index; HT, haemorrhagic transformation; IV, intravenous; MMP, matrix metalloproteinase; NAWM, normal-appearing white matter; NIHSS, National Institutes of Health Stroke Scale; TIA, transient ischemic attack; WMHv, white matter hyperintensity volume.

have shown an association with increased BBB permeability.[29 30] These observations could suggest that in our female patient population, which is likely predominantly postmenopausal based on age, relative oestrogen deficiency contributes to increased NAWM K_2 coefficient. Future studies investigating the association between menopausal status and BBB permeability could therefore be informative.

We also demonstrate an association between increasing white matter structural injury and alterations in NAWM BBB permeability. In both men and women, increased WMH burden is associated with NAWM K_2 coefficient.

Table 3 Multivariable backward stepwise elimination linear regression analysis for sex-specific predictors of NAWM K_2 coefficient

	Model	Variables	Estimate	P values
Women	1	Atrial fibrillation	–	–
		NAWM ADC	4.4	**0.02**
	2	Atrial fibrillation	468.5	0.10
		WMHv	25.1	0.11
Men	1	Age	27.3	0.11
		WMHv	115.7	**0.01**

Bold values indicate P value < 0.05.
ADC, apparent diffusion coefficient; NAWM, normal-appearing white matter; WMHv, white matter hyperintensity volume.

These findings support the hypothesis that early microvascular dysfunction in NAWM, causing BBB leakage, precedes white matter damage and WMH development. Corroborating this hypothesis, in patients with the small-vessel disease, increased BBB permeability is associated with WMH burden and hypertension.[31] We go on to show in women that NAWM ADC, a marker of altered water mobility, is associated with increased BBB permeability. These findings suggest that there may be sex-specific differences in the early relationship between NAWM BBB permeability and abnormal water mobility or structural integrity, which represents a risk factor for incident stroke and poor outcomes.[8 27 32]

In women, NAWM K_2 coefficient is associated with larger acute infarct volume. This occurred despite no difference between groups in age, intravenous alteplase administration, small-vessel occlusive stroke subtype, WMH burden and rates of haemorrhagic transformation. One interpretation of these results is that increased NAWM K_2 coefficient predisposes, or is a surrogate, for larger acute infarct volume. In support of this hypothesis, prior work in patients with AIS has shown that the K_2 coefficient of the DWI lesion is inversely associated with rates of reperfusion post alteplase,[33] and positively associated with the DWI volume and severity of intracranial haemorrhage postendovascular thrombectomy.[34] Based on what has been shown previously on the relationship of increasing BBB permeability and poor functional

Table 4 Sex-specific associations with acute infarct volume

| Variable | Women | | | | Men | |
| | Univariable | | Multivariable | | Univariable | |
	Estimate	P values	Estimate	P values	Estimate	P values
Age	−0.1	0.60			0.3	0.20
BMI	−0.55	0.25			−0.84	0.21
Anticoagulant use	−4.5	0.61			−6.5	0.45
Atrial fibrillation	−1.2	0.84			20.8	**0.01**
Diabetes	−11.8	0.14			−3.6	0.64
Hyperlipidaemia	0.6	0.92			−6.4	0.34
Hypertension	−5.7	0.36			12.8	0.09
Prior stroke/TIA	6.1	0.38			13.7	0.07
Cardioembolic stroke	1.8	0.76			−1.1	0.87
Admission NIHSS	2.7	**<0.001**			3.0	**<0.001**
Systolic blood pressure	−0.1	0.48			0.02	0.85
IV alteplase treatment	13.3	**0.03**	12.6	**0.03**	7.8	0.27
HT	20.6	**0.005**	16.9	**0.01**	13.0	0.11
WMHv	0.3	0.43			0.13	0.84
NAWM ADC	0.02	0.66			0.08	0.24
NAWM K_2	0.006	**0.01**	0.007	**0.01**	−0.001	0.50
NAWM mask volume	0.15	0.54			−0.1	0.78

Multivariable Regression Equation.

Women : DWIv = $\beta_0 + \beta_1 \cdot$ (*IV alteplase*) + $\beta_2 \cdot$ (*HT*) + $\beta_3 \cdot$ (*NAWMK₂*)
Men: NA.

Bold values indicate P value < 0.05.

ADC, apparent diffusion coefficient; BMI, body mass index; DWIv, diffusion-weighted imaging lesion volume; HT, haemorrhagic transformation; IV, intravenous; NA, not applicable; NAWM, normal-appearing white matter; NIHSS, National Institutes of Health Stroke Scale; TIA, transient ischemic attack; WMHv, white matter hyperintensity volume.

outcomes after AIS,[7 8 35] our findings are promising and hypothesis generating for a role of microvascular integrity in the sex-disparity of stroke outcomes.

There are several limitations of this study that warrant consideration. First, this was a retrospective analysis of a hospital-based biomarker study of AIS subjects who underwent a clinical MRI with PWI within 9 hours from LKW. Non-isotropic voxels were used for analysis of diffusivity anisotropy, which could raise the possibility of partial volume effects as confounders of the MRI measurements.[36] This was also an analysis of clinical MRIs performed on patients with acute stroke where differences in patient head orientation, for example, could introduce registration errors of the automated NAWM mask. By automatically applying the same probabilistic white matter mask to each subject, however, this approach reduces human bias from manually placed region-of-interests. An automated approach for selecting NAWM regions-of-interest is therefore a clinically feasible method for measuring global NAWM microstructural and microvascular integrity in patients with AIS.

The interpretation of changes in the K_2 coefficient also warrants discussion as this approach is relatively new for measuring BBB permeability in AIS subjects. At the individual voxel level the K_2 coefficient is a representation of the contribution of the T1 leakage effects due to contrast agent kinetics and the T2* leakage effects due to the tissue microstructure.[37 38] For these reasons, the K_2 coefficient may reflect differences in other microvascular and tissue microstructural properties. The measurement of K_2 in the acute setting is also likely a dynamic process with possible global influence from the acute ischaemic event.[39] As compared with dynamic contrast-enhanced (DCE) MRI and K_{trans}, the measurement of NAWM K_2 coefficient might have reduced sensitivity in the setting of delayed bolus arrival. However, by restricting our analyses to the hemisphere contralateral to the acute infarct we attempted to account for this limitation. We also cannot exclude the possibility that AIS can influence blood regulation and BBB permeability on remote sites or that differences in contralateral NAWM K_2 reflect differences in reactivity in the acute setting. A DSC-based approach has, however, previously been shown to be feasible for characterising BBB disruption in AIS[33 34] and there are distinct advantages of the DSC technique for this population. DCE-MRI has a long acquisition time, which limits its feasibility for studying AIS subjects. In contrast, DSC image acquisition takes 1–2 min. Several prior reports

Table 5 Sex-specific associations with good outcomes

	Women				Men			
	Univariable		Multivariable		Univariable		Multivariable	
Variable	Estimate	P values	Estimate	P values	Estimate	P values	Estimate	P values
Age	−0.01	0.40			−0.05	**0.006**		
BMI	0.07	0.18			0.04	0.31		
Anticoagulant use	0.5	0.56			0.3	0.57		
Atrial fibrillation	−0.1	0.88			−1.0	0.06		
Diabetes	−1.1	0.20			0.03	0.95		
Hyperlipidaemia	0.1	0.92			−0.4	0.34		
Hypertension	0.7	0.17			−1.0	0.06		
Prior stroke/TIA	−1.2	0.10			−0.7	0.16		
Cardioembolic stroke	0.1	0.88			0.1	0.80		
Admission NIHSS	−0.3	**<0.001**			−0.2	**<0.001**		
Systolic blood pressure	−0.01	0.93			−0.01	0.44		
IV alteplase treatment	−0.5	0.36			−1.4	**0.002**	−1.3	**0.01**
HT	0.02	0.97			−0.6	0.20		
WMHv	−0.1	0.07			−0.2	**0.02**		
DWIv	−0.03	**0.04**	−0.03	0.05	−0.04	**0.006**	−0.04	**0.01**
NAWM ADC	−0.01	**0.03**	−0.01	**0.04**	−0.02	**0.0007**	−0.02	**0.003**
NAWM K_2	−0.0004	0.06			−0.0001	0.42		

Multivariable Regression Equation.
Women : *Good outcome* = $\beta_0 + \beta_1 \cdot DWIv + \beta_2 \cdot (NAW\ ADC)$
Men : *Good outcome* = $\beta_0 + \beta_1 \cdot DWIv + \beta_2 \cdot (NAW\ ADC) + \beta_3 \cdot (IV\ alteplase) + \beta_4 \cdot (Age)$
Bold values indicate P value < 0.05.
ADC, apparent diffusion coefficient; BMI, body mass index; DWIv, diffusion-weighted imaging lesion volume; HT, haemorrhagic transformation; IV, intravenous; NAWM, normal-appearing white matter; TIA, transient ischemic attack; WMHv, white matter hyperintensity volume.

have also demonstrated a strong correlation between K_2 and K_{trans}.[38 40] Lastly, although we show sex-specific differences in NAWM K_2 coefficient and the relationship with infarct volume, the aetiology of this observation is less clear. Future stroke studies applying DSC-MRI to characterise sex-specific differences in BBB permeability, stratified for comorbidities, are therefore necessary.

CONCLUSIONS
Despite no differences in age and stroke severity, the NAWM $K2$ coefficient is increased in women with AIS and associated with infarct volume. These observations suggest that sex-specific differences in microvascular integrity may contribute to the sex disparity in poststroke outcomes.

Author affiliations
[1]Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA
[2]Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA, United States
[3]Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States
[4]Medical School of Ribeirao Preto, University of Sao Paulo, Sao Paulo, Brazil
[5]Department of Human Neurosciences, Sapienza University of Rome, Rome, Italy
[6]Institute of Psychology, Leiden University, Leiden, Netherlands
[7]Department of Neuroradiology, Goethe-Universitat Frankfurt am Main, Frankfurt, Germany
[8]Department of Neurology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States
[9]Department of Neurology, Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

ORCID iD
Mark R Etherton http://orcid.org/0000-0002-4739-2449

REFERENCES

1. Appelros P, Stegmayr B, Terent A. Sex differences in stroke epidemiology: a systematic review. *Stroke* 2009;40:1082–90.
2. Phan HT, Blizzard CL, Reeves MJ, *et al*. Sex differences in long-term mortality after stroke in the INSTRUCT (International stroke outcomes sTudy): a meta-analysis of individual participant data. *Circ Cardiovasc Qual Outcomes* 2017;10.
3. Gall SL, Donnan G, Dewey HM, *et al*. Sex differences in presentation, severity, and management of stroke in a population-based study. *Neurology* 2010;74:975–81.
4. Lisabeth LD, Brown DL, Morgenstern LB. Barriers to intravenous tissue plasminogen activator for acute stroke therapy in women. *Gend Med* 2006;3:270–8.
5. Reeves MJ, Bushnell CD, Howard G, *et al*. Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. *Lancet Neurol* 2008;7:915–26.
6. Etherton MR, Wu O, Cougo P, *et al*. Integrity of normal-appearing white matter and functional outcomes after acute ischemic stroke. *Neurology* 2017;88:1701–8.
7. Rost NS, Cougo P, Lorenzano S, *et al*. Diffuse microvascular dysfunction and loss of white matter integrity predict poor outcomes in patients with acute ischemic stroke. *J Cereb Blood Flow Metab* 2018;38:75–86.
8. Wardlaw JM, Doubal FN, Valdes-Hernandez M, *et al*. Blood-Brain barrier permeability and long-term clinical and imaging outcomes in cerebral small vessel disease. *Stroke* 2013;44:525–7.
9. Arsava EM, Rahman R, Rosand J, *et al*. Severity of leukoaraiosis correlates with clinical outcome after ischemic stroke. *Neurology* 2009;72:1403–10.
10. Wardlaw JM, Smith EE, Biessels GJ, *et al*. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* 2013;12:822–38.
11. Etherton MR, Wu O, Cougo P, *et al*. Structural integrity of normal appearing white matter and sex-specific outcomes after acute ischemic stroke. *Stroke* 2017;48:3387–9.
12. Rosenberg GA, Estrada EY, Dencoff JE. Matrix metalloproteinases and TIMPs are associated with blood-brain barrier opening after reperfusion in rat brain. *Stroke* 1998;29:2189–95.
13. Corbin ZA, Rost NS, Lorenzano S, *et al*. White matter hyperintensity volume correlates with matrix metalloproteinase-2 in acute ischemic stroke. *J Stroke Cerebrovasc Dis* 2014;23:1300–6.
14. Lorenzano S, Rost NS, Khan M, *et al*. Oxidative stress biomarkers of brain damage: hyperacute plasma F2-isoprostane predicts infarct growth in stroke. *Stroke* 2018;49:630–7.
15. Sorensen AG, Wu O, Copen WA, *et al*. Human acute cerebral ischemia: detection of changes in water diffusion anisotropy by using MR imaging. *Radiology* 1999;212:785–92.
16. Boxerman JL, Schmainda KM, Weisskoff RM. Relative cerebral blood volume maps corrected for contrast agent extravasation significantly correlate with glioma tumor grade, whereas uncorrected maps do not. *AJNR Am J Neuroradiol* 2006;27:859–67.
17. Wu O, Ostergaard L, Weisskoff RM, *et al*. Tracer arrival timing-insensitive technique for estimating flow in Mr perfusion-weighted imaging using singular value decomposition with a block-circulant deconvolution matrix. *Magn Reson Med* 2003;50:164–74.
18. Copen WA, Deipolyi AR, Schaefer PW, *et al*. Exposing hidden truncation-related errors in acute stroke perfusion imaging. *AJNR Am J Neuroradiol* 2015;36:638–45.

19. Rost NS, Sadaghiani S, Biffi A, *et al*. Setting a gold standard for quantification of leukoaraiosis burden in patients with ischemic stroke: the Atherosclerosis risk in Communities study. *J Neurosci Methods* 2014;221:196–201.
20. Ay H, Benner T, Arsava EM, *et al*. A computerized algorithm for etiologic classification of ischemic stroke: the causative classification of stroke system. *Stroke* 2007;38:2979–84.
21. Bruno A, Shah N, Lin C, *et al*. Improving modified Rankin scale assessment with a simplified questionnaire. *Stroke* 2010;41:1048–50.
22. Ferguson KJ, Wardlaw JM, Edmond CL, *et al*. Intracranial area: a validated method for estimating intracranial volume. *J Neuroimaging* 2005;15:76–8.
23. Tong DC, Yenari MA, Albers GW, *et al*. Correlation of perfusion- and diffusion-weighted MRI with NIHSS score in acute (. *Neurology* 1998;50:864–70.
24. Lovblad KO, Baird AE, Schlaug G, *et al*. Ischemic lesion volumes in acute stroke by diffusion-weighted magnetic resonance imaging correlate with clinical outcome. *Ann Neurol* 1997;42:164–70.
25. Topakian R, Barrick TR, Howe FA, *et al*. Blood-Brain barrier permeability is increased in normal-appearing white matter in patients with lacunar stroke and leucoaraiosis. *J Neurol Neurosurg Psychiatry* 2010;81:192–7.
26. Arba F, Leigh R, Inzitari D, *et al*. Blood-Brain barrier leakage increases with small vessel disease in acute ischemic stroke. *Neurology* 2017;89:2143–50.
27. Wardlaw JM, Doubal F, Armitage P, *et al*. Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. *Ann Neurol* 2009;65:194–202.
28. Bang OY, Saver JL, Alger JR, *et al*. Patterns and predictors of blood-brain barrier permeability derangements in acute ischemic stroke. *Stroke* 2009;40:454–61.
29. Wilson AC, Clemente L, Liu T, *et al*. Reproductive hormones regulate the selective permeability of the blood-brain barrier. *Biochim Biophys Acta* 1782;2008:401–7.
30. Maggioli E, McArthur S, Mauro C, *et al*. Estrogen protects the blood-brain barrier from inflammation-induced disruption and increased lymphocyte trafficking. *Brain Behav Immun* 2016;51:212–22.
31. Munoz Maniega S, Chappell FM, Valdes Hernandez MC, *et al*. Integrity of normal-appearing white matter: influence of age, visible lesion burden and hypertension in patients with small-vessel disease. *J Cereb Blood Flow Metab* 2017;37:644–56.
32. Farrall AJ, Wardlaw JM. Blood-Brain barrier: ageing and microvascular disease-systematic review and meta-analysis. *Neurobiol Aging* 2009;30:337–52.
33. Simpkins AN, Dias C, Leigh R. National Institutes of health natural history of stroke I. Identification of reversible disruption of the human blood-brain barrier following acute ischemia. *Stroke* 2016;47:2405–9.
34. Leigh R, Christensen S, Campbell BC, *et al*. Pretreatment blood-brain barrier disruption and post-endovascular intracranial hemorrhage. *Neurology* 2016;87:263–9.
35. Liu C, Yan S, Zhang R, *et al*. Increased blood-brain barrier permeability in contralateral hemisphere predicts worse outcome in acute ischemic stroke after reperfusion therapy. *J Neurointerv Surg* 2018.
36. Vos SB, Jones DK, Viergever MA, *et al*. Partial volume effect as a hidden covariate in DTI analyses. *NeuroImage* 2011;55:1566–76.
37. Liu HL, YY W, Yang WS, *et al*. Is Weisskoff model valid for the correction of contrast agent extravasation with combined T1 and T2* effects in dynamic susceptibility contrast MRI? *Med Phys* 2011;38:802–9.
38. Skinner JT, Moots PL, Ayers GD, *et al*. On the use of DSC-MRI for measuring vascular permeability. *AJNR Am J Neuroradiol* 2016;37:80–7.
39. Villringer K, Sanz Cuesta BE, Ostwaldt AC, *et al*. Dce-Mri blood-brain barrier assessment in acute ischemic stroke. *Neurology* 2017;88:433–40.
40. Bonekamp D, Deike K, Wiestler B, *et al*. Association of overall survival in patients with newly diagnosed glioblastoma with contrast-enhanced perfusion MRI: Comparison of intraindividually matched T1 - and T2 (*) -based bolus techniques. *J Magn Reson Imaging* 2015;42:87–96.