Mapping human brain capillary water lifetime: high-resolution metabolic neuroimaging

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INTRODUCTION

Every eukaryotic cell has a plasma membrane P-type ATPase ion pump; for animals, this is Na⁺,K⁺-ATPase (NKA) (1,2). Since its activity maintains the transmural K⁺ and Na⁺ gradients and thus, respectively, the membrane potential and secondary active transport, NKA is vital for life. The normal “forward” reaction is the following, where the i and o subscripts indicate intra- and extra-cellular, respectively.

\[ \text{ATP}_i + 2 \text{K}^{+o} + 3 \text{Na}^{+i} \xrightarrow{\text{NKA}} \text{ADP}_i + \text{P}_i + 2 \text{K}^{+i} + 3 \text{Na}^{+o} \]

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**Abbreviations used:** ATP, adenosine triphosphate; CA, contrast agent; CBV, cerebral blood volume; CEST, chemical exchange spin lock/saturation transfer; CMRoxphos, cerebral metabolic rate of ATP synthesis from oxidative phosphorylation; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; F, cerebral blood flow (CBF); FDG, fluorodeoxyglucose; Fe-tol, ferumoxytol (Ferdaheme); FOV, field of view; FXL, fast-exchange limit; FXR, fast-exchange regime; GBM, glioblastoma multiforme; GdHPDO3A, gadoteridol (ProHance); GM, gray matter; HP, hyperpolarized; IR, inversion recovery; MRav, metabolic rate of glucose (consumption); MRS, magnetic resonance spectroscopic imaging; MS, multiple sclerosis; NAA, normal appearing brain tissue; MT, magnetization transfer; NAGM, normal-appearing gray matter; NAWM, normal-appearing white matter; NGM, normal-appearing GM; NKA, Na⁺,K⁺-ATPase; NMR, normal MRI; PCr, phosphocreatine; PET, positron emission tomography; P, inorganic phosphate (P/O⁺); ROI, region of interest; RRMS, relapsing–remitting MS; SPECT, single photon emission computed tomography; SSt, shutter-speed paradigm; SXR, slow-exchange regime; TI, inversion time; TP, tracer paradigm; TSC, tissue sodium concentration; WEI, water exchange index; WM, white matter; 2SX, two-site exchange.

Shutter-speed analysis of dynamic-contrast-agent (CA)-enhanced normal, multiple sclerosis (MS), and glioblastoma (GBM) human brain data gives the mean capillary water molecule lifetime (\(\tau_w\)) and blood volume fraction (\(\phi_v\); capillary density–volume product (\(\rho_C V\)) in a high-resolution \(^1\)H\(_2\)O MRI voxel (40 \(\mu\text{L}\)) or ROI. The equilibrium water extravasation rate constant, \(k_{po}(\text{in} \mu\text{s}^{-1})\), averages 3.2 and 2.9 s\(^{-1}\) in resting-state normal white matter (NWM) and gray matter (NGM), respectively (n = 6). The results (italicized) lead to three major conclusions. (A) \(k_{po}\) differences are dominated by capillary water permeability (\(P_w\)), not size, differences. NWM and NGM voxel \(k_{po}\) and \(v_o\) values are independent. Quantitative analyses of concomitant population-averaged \(k_{po}\) \(v_o\) variations in normal and normal-appearing MS brain ROIs confirm \(P_w\) dominance. (B) \(P_w\) is dominated (>95%) by a trans(endothelial)cellular pathway, not the \(P_{CA}\) paracellular route. In MS lesions and GBM tumors, \(P_{CA}\) increases but \(P_{W}\) decreases. (C) \(k_{po}\) tracks steady-state ATP production/consumption flux per capillary. In normal, MS, and GBM brain, regional \(k_{po}\) correlates with literature MRSI ATP (positively) and Na⁺ (negatively) tissue concentrations. This suggests that the \(P_w\) pathway is metabolically active. Excellent agreement of the relative NGM/NWM \(k_{po}\)\(v_o\) product ratio with the literature \(^{31}\)PMRSI-MT CMRoxphos ratio confirms the flux property. We have previously shown that the cellular water molecule efflux rate constant (\(k_{ro}\)) is proportional to plasma membrane P-type ATPase turnover, likely due to active transmembrane water cycling. With synaptic proximities and synergistic metabolic cooperativities, polar brain endothelial, neuroglial, and neuronal cells form “gliovascular units.” We hypothesize that a chain of water cycling processes transmits brain metabolic activity to \(k_{po}\) letting it report neurogliovascular unit Na⁺,K⁺-ATPase activity. Cerebral \(k_{po}\) maps represent metabolic (functional) neuroimages. The NGM 2.9 s\(^{-1}\) \(k_{po}\) means an equilibrium water efflux of \(\sim10^{15} \text{H}_2\text{O}\) molecules s\(^{-1}\) per capillary (in 1 \(\mu\text{L}\) tissue): consistent with the known ATP consumption rate and water co-transporting membrane symporter stoichiometries. © 2015 The Authors NMR in Biomedicine Published by John Wiley & Sons Ltd.

**Keywords:** high resolution; MRI; brain; metabolism; Na⁺,K⁺ pump; activity
Even in homeostasis, the NKA pump experiences continual turnover (there are return pathways). It has been estimated that it consumes over 50% of brain adenosine triphosphate (ATP) (3). Methods for measuring NKA activity have been adapted to the experimental sample. For solubilized, purified enzyme or tissue homogenate preparations, spectrophotometric (4) or radiola beled ($^{32}$P) (5) ATP hydrolysis rate assays suffice. For intact cells in culture or in tissue preparations, voltage clamp current, ion-selective ($^{86}$Rb$^+$) microelectrode response, radio-isotope ($^{22}$Na$^+$, $^{36}$Cl$^-$, $^{85}$Rb$^+$, $^{38}$K$^+$) uptake/release (6–9), or $^{23}$Na$^+$/$^{86}$Rb$^+$ MRSI (10,11) methods measure NKA-driven transmembrane ion transport kinetics. Phospholipid vesicles reconstituted with purified NKA allow measurement of both ATP hydrolysis and ion transport kinetics (5).

Each of these methods is best suited to macroscopically homogeneous samples. None are particularly appropriate for normally heterogeneous tissue, since there is no spatial encoding. Furthermore, many of these methods directly measure only net NKA activity, not homeostatic turnover. The radioisotope approach has been generally abandoned for about 20 years; deemed too problematic for even tissue preparations (12). As far as we are aware, NKA turnover has never been measured, let alone mapped, in a living animal or human subject. Doing so would provide a very fundamental view of ongoing metabolism, a new form of metabolic imaging. Since metabolic thermodynamics and kinetics need no necessary relationship (important example below), it is imperative to distinguish these aspects in imaging.

Mapping metabolic thermodynamics

Restricting ourselves essentially to human studies, so far metabolic imaging has been mostly accomplished by positron emission tomography (PET) (13) and magnetic resonance spectroscopic imaging (MRSI) (14,15), with some single photon emission computed tomography (SPECT) (16). Very often, this is mapping of metabolic thermodynamics (kinetics) and magnetic resonance spectroscopic imaging has been mostly accomplished by positron emission tomography (PET) (13) and magnetic resonance spectroscopic imaging (14,15,17,27,28). For extant metabolic imaging, it is understandably often thought of as providing only certain molecular nuclear magnetization from equilibrium. Monitoring the (relatively slow) metabolism recovery with spatial encoding allows mapping of faster metabolic fluxes, again with proper modeling (17,29,30). For example in the brain, the integrated cellular creatine kinase flux and ATP production/consumption rates have been determined (17).

Spatial resolution

Extant metabolic imaging has revealed a tremendous amount about normal and pathological biochemistry, as it actually exists in vivo. However, new approaches can be attractive. The PET and HP-$^{13}$CMRSI modalities are costly. Typical nominal spatial resolutions and voxel volumes for human modalities are the following: $^{31}$PMRSI, (1.3 cm)$^3 = 2.2$ mL (17); $^{1}$HMRISI, (1 cm)$^3 = 1$ mL (20); SPECT, (1 cm)$^3 = 1$ mL (16); HP-$^{13}$CMRSI, (7 mm)$^3 = 340$ μL (15); PET, (5 mm)$^3 = 125$ μL (16); $^{23}$NaMRSI, (4 mm)$^3 = 64$ μL (19). These are often insufficient for discriminating significant metabolic changes. For example, the cerebral gray matter (GM)/white matter (WM) boundary usually cannot be clearly distinguished. In comparison, MRI – generated from the relatively strong $^1$H$_2$O signal – commonly provides higher spatial resolution: (1 mm)$^3 = 1$ μL or better. Metabolic images are almost always accompanied by high-resolution MRI (sometimes computed tomography) views of the same tissue. Therefore, though MRI is relatively inexpensive (compared with PET and HP-$^{13}$CMRSI) and employs no ionizing radiation, it is understandably often thought of as providing only anatomical and/or vascular information. Of course, it has long mapped some tissue functions, as in cine cardiovascular MRI and functional MRI. The new metabo-CESL/CEST techniques approach anatomical $^1$H$_2$O resolution (22), since they employ this strong signal for indirect metabolite detection.

$^1$H$_2$O mapping of NKA flux

Here we introduce a method exploiting a newly discovered aspect of the biology of water itself – active trans-membrane cycling. The (dynamic-contrast-enhanced) DCE-MRI $^1$H$_2$O method is in wide clinical use. It employs any of a number of approved paramagnetic, monomeric Gd(III) chelates as contrast agents (CAs). For tissues manifesting extensive CA extravasation, a proper pharmacokinetic analysis of the CA bolus DCE-MRI time-course yields the mean lifetime ($\tau_i$) of water molecules inside the cells within a voxel (31). The reciprocal ($\tau_i^{-1}$) is the first-order rate constant ($k_{\text{u}}$) for the unidirectional, equilibrium cellular water molecule efflux. We have recently documented the
evidence, from enzymatic manipulations spanning a number of different cell types and models (from cells to animals to humans), that the magnitude of $k_{po}$ is proportional to P-type ATPase turnover (32). This is likely due to active transmembrane water cycling that accompanies the osmolyte cycling driven by the membrane ion pump (32,33).

Because the normal blood–brain barrier is CA impermeable, cerebral DCE-MRI data do not directly yield $\tau_i$. However, the mean capillary water lifetime ($\tau_{w}$) is readily determined. Fortunately, the metabolic activity of cerebral neurons is exquisitely symbiotically connected with those of neuroglia (oligodendocytes, astrocytes) and thence capillary endothelial cells (34–37) – all within synaptic proximities. The terms “neurovascular unit” and “gliovascular unit” have been coined (36) to connote this. Here, we present results from normal and multiple sclerosis normal-appearing (MS-NA) brain, along with MS lesion and glioma tumor, indicating that $\tau_{w}^{-1}$ ($k_{po}$) is proportional to metabolic turnover within the neurogliovascular unit. We show the first human brain $k_{po}$ maps (40 µL voxels), and present evidence that these are metabolic flux maps at $^1$H$_2$O resolution.

TECHNICAL BACKGROUND

Since many different principles are involved here, we present some technical background material.

Mean brain intra-capillary blood water molecule lifetime ($\tau_{w}$)

The average erythrocyte speed through cerebral cortical capillaries is 2 mm s$^{-1}$ or less (38–40); this measures blood velocity. In a common (1 mm)$^3$ high-resolution human $^1$H$_2$O MRI voxel, a conservatively small estimate of the average, tortuous capillary path-length is 2 mm (40,41). Thus, the mean voxel transit time for a blood water molecule is at least 1 s. Many things happen to the molecule during this period. The mean lifetime inside an erythrocyte ($\tau_e$) is 10 ms (42–45). By equilibrium mass action, for a 40% hematocrit the mean plasma lifetime before entering a red cell ($\tau_{p}$) is 15 ms. Thus, any given water molecule enters and leaves erythrocytes about 40 times during its voxel passage (also, the entire red cell water content is exchanged ~100 times). The 3D Einstein diffusion equation, $\langle r^2 \rangle = 6D_{b}t$ (40), allows estimation of $^1$H$_2$O molecule capillary wall encounter frequency: $r$ is the capillary radius, $D$ the water diffusion coefficient, and $t_{f}$ the average time to diffuse a distance $r$. Inserting the mean feline $r$ value, 2.6 µm (40), and a conservatively small $D$ value (1.5 µm$^2$ ms$^{-1}$, half the pure water $D$), we obtain $t_{f} = 0.8$ ms. Even an $^1$H$_2$O molecule in the center of the capillary lumen would encounter the capillary wall more than 1000 times during its voxel passage. It is a very good approximation that capillary blood water is “well mixed.” Thus, we can estimate the probability of a water molecule escaping the capillary. The $\tau_{w}$ inverse, $\tau_{w}^{-1}$, is the unidirectional first-order rate constant, $k_{po}$, for water extravasation (47).

For a well-mixed lumen, this is $\tau_{w}^{-1} = P_{w}[A_{ca}/V_{ca}]$, where $P_{w}$ is the transendothelial water permeability coefficient, $A_{ca}$ the individual capillary surface area, and $V_{ca}$ the individual capillary lumen volume (48). For a cylindrical microvessel: $\tau_{w}^{-1} = 2[P_{w}/r]$. Some time ago (49), we noted that if $r = 2.6$ µm and a primate $P_{w}$ value of 2 µm s$^{-1}$ (50,51) predict 650 ms for $\tau_{w}$. The $k_{po}(\tau_{w}^{-1})$ value (1.5 s$^{-1}$) corresponds to about 78% probability (=100[1 – exp (–$k_{po}$)]): it is the capillary transit time (~1 s) that any given water molecule will exchange out of the blood space (to be replaced by an extravascular $^1$H$_2$O molecule) during its capillary passage. Because blood velocity causes no net change in the number of (indistinguishable) capillary $^1$H$_2$O molecules, the $\tau_{w}$ quantity is not influenced by the blood flow ($F$; CBF) magnitude. This contrasts with the situation for the extraction of labeled water (50), which is surely “perfusion limited.”

It is important to note that $\tau_{w}/r$ is also independent of the intensive capillary density ($\rho$) property. With tracer studies (e.g. intracarotid $^{15}$O$_2$ (50)) and sacrificial autoradiography (e.g. IV $^3$H$_2$O (52)), one obtains the intensive $P_{w}$’s product, where $S$ is the total region-of-interest (ROI) vascular surface area per unit tissue volume – dependent on the vasculature. The latter is measured by the blood volume fraction ($V_{b}$; CBV): the $\rho'V$ product ($V$ is the mean $V_{ca}$). In $P_{w}/r$, $r$ is the mean ROI vascular radius, and is related only to the $V$ factor of the $\rho'V$ product. Thus, theory demands that $\tau_{w}$ is independent of $\rho'$, and therefore a potentially powerful new type of imaging biomarker: we characterize it as supra-intensive. We show below that $k_{po}$ is also experimentally independent of $V_{b}$, a very meaningful finding, and that it can distinguish cerebral pathology undetectable with ordinary intensive biomarkers.

Inter-compartmental $^1$H$_2$O exchange effects in in vivo MR

It has been known for 40 years that a sufficiently concentrated paramagnetic solute localized in a cell suspension extracellular space can cause non-mono-exponential longitudinal and/or transverse $^1$H$_2$O relaxation. A two-site exchange (2SX) analysis of the recovery yields $\tau_{u}$, the mean intracellular water molecule lifetime (reviewed in (32,43–45)). This is equilibrium transcytosomeal $^1$H$_2$O water exchange. Longitudinal relaxation for yeast cell samples (33,49,53) exemplifies this. The extracellular CA increases the intrinsic outside water proton signal ($^1$H$_2$O$_o$) longitudinal relaxation rate constant, $R_{io}$ (≡ ($T_{1o}$)$^{-1}$). Though we generalized and systematized the exchange picture (and introduced the term) only in 1999 (54), this approach increases the longitudinal transcytosomeal “shutter-speed,” $\tau_{ic}^{-1}$ (≡ [$R_{io} - R_{il}$]), sufficiently that this water exchange NMR system is moved out of its fast-exchange-limit (FXL) condition ($\tau_{ic}^{-1}$ < ($\tau_{w}^{-1} + \tau_{o}^{-1}$); $R_{il}$ is the intrinsic inside ($^1$H$_2$O$_i$) relaxation rate constant). A sufficient outside CA concentration, [$CA_o$], allows the NMR system to reach the slow-exchange-regime (SXR) condition. This is characterized by non-mono-exponential magnetization recovery, but is distinct from the slow-exchange- and no-exchange-limit conditions (31,33,43,47,55). It is quite customary to achieve the SXR condition with cell suspensions (33,43–45,49), but there is no convincing evidence that the SXR can be reached in vivo with approved CAs. However, it has been shown that when the [$CA_o$] value is only modest, and the system can attain only the fast-exchange-regime (FXR) condition, it is still possible to measure $\tau_{i}$ by varying $\tau_{ic}^{-1}$ (by varying [$CA_o$]) (49). The FXR condition features mono-exponential longitudinal recovery: i.e. $R_i$ is single valued, but with a non-linear [$CA_o$] dependence. In the FXL condition, this dependence is linear (31,33,43,49,54). The in vivo implementation of these principles generally employs some variant of DCE-MRI, the serial acquisition of $\tau_{i}$ by varying $\tau_{ic}^{-1}$ (by varying [$CA_o$]) (49)
biomarkers. Overviews of these concepts have been published (31,33,43,55).

It was gratifying when, in 1997, multiple infusions of an intra-vascular CA were used to vary the murine brain transendothelial shutter-speed, \( \tau_{1e}^{-1} = |R_{te} - R_{1e}\text{ox}}\) (\(^1\text{H}_2\text{O}_{b}\) and \(^1\text{H}_2\text{O}_{\text{ox}}\) are the intra- and extravascular signals, respectively) and reach the FXR condition \( (\tau_{1e}^{-1} \to (\tau_{b}^{-1} + \tau_{\text{ox}}^{-1})^{-1}) ; \tau_{\text{ox}}^{-1}\) is the mean extravascular water molecule lifetime) for this water exchange system. Variation of the plasma CA concentration, \([\text{CA}]_{p}\), allowed the exchange kinetics to be measured (56). The cortical \( \tau_{b} \) value we calculate from these results is 295 ms – of the magnitude we had earlier predicted from literature parameters (see above). In 2002, an approach using a single intravascular CA injection was demonstrated in the rat (57). The \([\text{CA}]_{p}\) value was not varied, but the acquisition was combined with an arterial spin labeling (ASL) variant. This approach yields only \( P_{W}^{-1}S \) and, because ASL is used, the \( F \) value must be included in order to obtain \( P_{W}^{-1}S \) correctly (57). In 2003, we introduced an SSP DCE-MRI method whereby a single CA bolus injection can be used to determine human brain \( \tau_{b} \) (58). This approach is used here. Seven years ago, the water exchange index (WEI), an approximate, dimensionless, non-linear \( \tau_{b}^{-1} \), estimate, was demonstrated in the mouse, an approach also requiring a single intravascular CA injection (59). Recently however, the authors of Reference 59 themselves showed that, unfortunately, the WEI approximation formally depends on the \( v_{o} \) value (60). As noted above, an important feature of the actual \( \tau_{b}^{-1} (k_{p}) \) biomarker is its \( v_{o} \) independence if \( r \) does not vary.

Indirect detection

There are crucial differences between the tracer and shutter-speed paradigms. Classic tracers (radiolabeled molecules, electron-dense compounds, etc.) are detected directly: the tracer molecule is also the signal molecule. Though the CA of DCE-MRI electron-dense compounds, etc.) are detected directly: the tracer molecule is also the signal molecule. These species are never detecting only the labeled water. In DCE-MRI, there is no compartmental selection in the initial water proton magnetization perturbation: all \(^1\text{H}_2\text{O}^\dagger \) signals are (usually) inverted, and the return to magnetic equilibrium of each of them is monitored simultaneously. Only magnetization equilibrium is perturbed. These methods can lead to common parameters (e.g. \( P_{W}^{-1} \) here), and thus can support each other. However, the experimental results must be analyzed with different paradigms as appropriate. For example, though the SSP assumes that brain capillary water is “well mixed” – justified above – it does not require the same assumption of extravascular water, which is in fact not well mixed. Although \( \tau_{b} \) and \( \tau_{o} \) values are typically hundreds of milliseconds, that of \( \tau_{\text{ox}}^{-1} \) is typically tens of seconds (see later) – because of the relatively sparse microvessel density. Yet the extravascular (“parenchymal”) MR system is in the fast-exchange-limit condition even though transcytomedial exchange is not particularly fast. With no extravascular CA, the \( \tau_{1c}^{-1} \) values are much smaller than \( (\tau_{1c}^{-1} + \tau_{b}^{-1}) \). Thus, the non-well-mixed nature of the parenchyma is of no consequence to the DCE-MRI experiment, but “unstirred layer” effects can be significant for \(^1\text{H}_2\text{O}^\dagger \) re-intravasation kinetics, or for any tracer study (12).

Active trans-membrane water cycling

There is obviously considerable interest in the brain capillary \( \tau_{b} \) \( (k_{p}) \) quantity. From the above, we see that \( \tau_{b} \) variation can reflect a change in capillary \( r \), in \( P_{W}^{-1} \), or in both. Vasodilation or vasoconstriction \( (r \) alteration) would respectively decrease or increase \( k_{p} \) \( (\tau_{b}) \). However, the rate constant for \( r \) changes is orders of magnitude smaller than \( k_{p} \) itself (33,62). Changes in \( k_{p} \) not attributable to capillary size alteration are ascribed to \( P_{W}^{-1} \) variation and, until recently, this has been conceived as resulting from alterations in passive molecular processes \( (P_{W}^{-1} \) passive). These include (a) paracellular water passage through endothelial tight junctions, (b) simple, transcellular water diffusion across cell membrane lipid bilayers, and (c) transcellular transport through membrane aquaporin protein water channels (63,64) and/or transcellular leakage through membrane transporters (65). However, NMR studies have recently revealed the cell membrane water permeability coefficient \( (P_{W} \) active) to have an active component \( (P_{W} \) active) that dominates over the passive component (32,33). This is due to active transmembrane water cycling accompanying active transmembrane osmolyte cycling, which is paced by the driving cell membrane P-type ATPase ion pump (32,33). For animal cells, this is NKA (32,33,66–68). The molecular mechanism likely involves water cotransporting membrane symporters (69,70), and supports a cycling flux of \( 10^{12} \) water molecules \( s^{-1} \) per cell.

**EXPERIMENTAL**

**Subjects**

Healthy (2M/4F, 30 (±10) years), relapsing–remitting-MS (RRMS) (2M/4F, 46 (±7) years, 18–55 years), and glioblastoma multiforme (GBM) (3M/2F, 19–57 years) subjects gave informed consent to OHSU Institutional Review Board approved protocols. The MS group was early in disease, but with positive MRI findings. An additional 52 year old female late-stage RRMS subject was also studied. The GBM subjects had prior surgical biopsies or resections and chemo-radiation therapy.
Healthy and MS subject DCE-MRI

A 7 T whole-body MRI instrument (Siemens, Erlangen, Germany), with quadrature transmission and 24-channel phased-array receive head RF coils, was used. Dynamic measurements employed a single-slice inversion recovery (IR) turboFLASH technique (71), sampling magnetization at eight post-inversion times (TI values): the inversion pulse was non-selective. The 128 × 96 image matrix covered a (256 × 192) mm² field of view (FOV) (nominal pixel, (2 mm)²), and a 6° flip angle RF pulse selected a 10 mm transverse slice superior to the lateral ventricles (nominal voxels, (2 × 2 × 10) mm³ = 40 μL). Gadoteridol (ProHance; Bracco Diagnostics, Cranberry, NJ) was injected into an antecubital vein catheter at 1.0 mL s⁻¹ using a power injector (Medrad, Warrenville, PA) to deliver a dose of 28 μmol kg⁻¹ (typically ~5 mL), followed by a 20 mL saline flush at the same rate. For each CA injection, 50 IR image sets were collected with 2.3 s temporal resolution. Total acquisition time was 1.9 min. Parametric R₁ maps were calculated on a voxel basis by fitting the signal magnitude at each TI with a full Bloch simulation incorporating all RF pulses and delays. The IR was modeled with a two-parameter single exponential, using a gradient expansion algorithm.

GBM subject DCE-MRI

Data were acquired using a 3 T Tim Trio (Siemens) instrument, body transmit and 12-channel phased-array head receive RF coils, and a full volume 2D gradient-recalled-echo-planar imaging sequence. Non-selective IR pulses were sampled at 24–36 TI values. The 128² image matrix covered a 256² FOV (nominal (2 mm)² isotropic resolution; 8 μL nominal voxels) (72). ProHance DCE-MRI was similar to the controls and MS subjects. The next day, the intravascular FeO nanoparticle CA ferumoxytol (Fe-tol; Feraheme; AMAG Pharmaceuticals, Waltham, MA) was used. Points were obtained in the CA steady states before and after three IV Fe-tol injections (73), fractionated into doses (1:2:4) totaling 4 mg(Fe) kg⁻¹ (72 μmol(Fe) kg⁻¹, ~12 nmol(Fe-tol) kg⁻¹), each at 3 mL s⁻¹. The 5 min acquisitions were initiated 120 s after each Fe-tol injection, during the steady-state period when [CAₚ] was uniform and constant. (The 14 hour Fe-tol plasma t₁/₂ ensures [CAₚ] is larger after each successive injection.) All four acquisitions were completed in 40 min. Pre- and post-CA session R₁ maps were co-registered to pre-CA T₁-w MPRAGE maps using rigid body transformations. This protocol yielded four pharmacokinetic time points, sufficient to characterize high quality R₁ values versus R₁b measurements such as those in NA brain (see Fig. 5 later). This sparse temporal sampling approach was originally designed for optimization of brain coverage and spatial resolution. Unlike gadoteridol (GdHPDO₃A) DCE-MRI, in which [CAₚ] reaches large values only transiently – during the first pass (Fig. 1), Fe-tol steady-state [CAₚ] can attain quite high and sustained levels.

DCE-MRI pharmacokinetic modeling

Non-linear modeling (IDL; Exelis, Boulder, CO) was used to extract accurate estimates of R₁exv, νb, and τb for selected ROIs (and also on a voxel-wise basis) using the equation

\[ R_{1}(t) = \frac{1}{2} \left[ \left( R_{1}\text{exv} + R_{1b}(t) + \frac{1 - \nu_b}{\nu_b} \right) \frac{\tau_b}{\nu_b} \right] \]

\[ + \left( \frac{R_{1}\text{exv} - R_{1b}(t) - \frac{1 - \nu_b}{\nu_b} \frac{\tau_b}{\nu_b}}{\nu_b} \right)^2 \]

\[ + 4(1 - \nu_b)^2 \left( \frac{\tau_b}{\nu_b} \right)^2 \]

\[ \frac{1}{2} \frac{\tau_b}{\nu_b} \]

where \( \nu_b \) is the mole fraction of tissue water in blood, \( R_{1b} \) is the response function, and \( f_{vw} \) is the tissue volume fraction accessible to mobile aqueous solutes (55)). The independent modeling measure is

Figure 1. Normal ¹H₂O DCE-MRI data. These arise from a 22 year old female control subject. Panel D shows axial R₁ maps pre- and at one point post-CA administration. The CA injection is indicated by blue arrows in the R₁ time-courses in panels A and B (A, blood ROI R₁b response function; B, WM ROI R₁; response function). The WM ROI is indicated by the yellow circles in panel D. Panel C plots the R₁b versus R₁. The points exhibit a clear deviation from the linearity demanded by the TP, which embodies the FXL constraint (dashed line). The points are well fitted with the SSP (solid curve), Equation [1], with parameters given in the text. Population-averaged parameter values are presented in Table 1.
$R_{1b}(t)$, which is determined by $[C_{Ab}](t)$ (defined by $R_{1b}(t) = R_{1b0} + r_{1b}[C_{Ab}](t)$, with $R_{1b0}$ the pre-CA $R_{1b}$ and $r_{1b}$ the CA relaxivity). $R_{1b}$ (t) was measured directly from an $R_1$ map ROI placed fully within the sagittal sinus. Equation [1] describes a 2SX NMR system spanning the FXL and FXR conditions, depending on $[C_{Ab}]$. The biomarkers are (a) assumes it zero. 

**RESULTS**

$k_{po}$ and $v_b$ values in normal and normal-appearing MS brain

Figure 1 displays 7 T 1H2O IR turboFLASH DCE-MRI data, and SSP analysis (58), for a 22 year old female control subject. Panels A and B show $R_1$ time-courses: GdHPD30A was injected at about 35 s (arrows). The Figure 1(A) data are from a blood (sagittal sinus) ROI, those in Figure 1(B) from the normal white matter (NWM) ROI indicated by circles in panel D; axial $R_1$ maps before and after CA administration. The CA passes through the brain without appreciable extravasation. Panel C shows the $R_{1b}$ dependence of $R_1$, from Figure 1(A), (B). If the TP obtained, the $R_1$ vs. $R_{1b}$ plot would be linear. This is indicated by the dashed line in Figure 1(C). The non-linear data are well fitted with the 2SX SSP expression of Equation [1] ($v_w = 0.8$), spanning the FXL and FXR conditions (31,42,55,58) (solid curve] with $\tau_b = 560$ ms, $v_b = 0.018$, and $R_{1exv} = 0.85 \text{ s}^{-1}$ (the intrinsic extravascular $1^1H_2O$ $R_1$). If the TP is forced to the data, the dashed FXL line must pivot about their origin ($R_{1b}(0), R_1(0)$) and its slope, $v_b$, is significantly decreased. In this case, the TP gives $v_b = 0.015$, a 17% underestimation. Of course, TP also denies access to $\tau_b$, since it assumes it zero.

Figure 2 displays axial voxel-by-voxel parametric maps for the subject of Figure 1. The biomarkers are (a) $R_{1exv}$, (b) $v_b$, and (c) $k_{po}$ ($\tau_b$). As expected, $R_{1exv}$ is greater in NWM than in normal gray matter (NGM), and the $v_b$ map exhibits greater NGM (0.03) than NWM (0.01) values. The latter approximate rather well absolute CBV fractions, not relative values. Though such maps are quite important, they exhibit the natures of the $R_{1exv}$ and $v_b$ properties. The larger NWM $R_{1exv}$ values reflect the greater macromolecular volume fractions of NWM (74) and the larger NGM $v_b$ values reflect the well-known greater NGM vasculature. As far as we are aware, Figure 2(c) is the first $k_{po}$ map. As discussed above, $\tau_b$ is a super-intensive parameter. It is very interesting that the $k_{po}$ map (Fig. 2(c)) exhibits greater intensity in NWM, averaging 3.0 $\text{s}^{-1}$, than in NGM, 2.5 $\text{s}^{-1}$.

Figure 3 shows the voxel-by-voxel $k_{po}$ versus $v_b$ scatter plot of many of the data of Figure 2(b), (c). The voxels were chosen from $50^2$ in a square slab ROI centered on and covering about 75% of the brain image slice of Figures 1 and 2. The $R_{1exv}$ relaxation rate constant spectrum (histogram) was used to assign the voxels (74). The 649 voxels with $R_{1exv}$ values between 0.80 and 0.92 $\text{s}^{-1}$ were identified as NWM, and yield the pink points in Figure 3. The 670 voxels between 0.62 and 0.72 $\text{s}^{-1}$ are labeled NGM, and give the olive points in Figure 3. As expected, the NWM points cluster below $v_b = 0.02$. The NGM points cluster about a $v_b$ value (0.06) somewhat greater than expected, because a number represent voxels with some partial-volume averaging of vessels larger than capillaries – especially near the cortical surface (Fig. 2(c)). Interestingly, the NGM $k_{po}$ (($\tau_b$, $v_b$)) values are essentially independent of $v_b$, and the NWM $v_b$ values are essentially independent of $k_{po}$. The basically horizontal and vertical orthogonal NGM and NWM clusters are consistent with parameters not numerically correlated by data fittings. The interesting trends seen in Figure 3 are physiological. The parameter $v_b = \rho^* V$. By definition, $k_{po}$ is $\rho^*$ independent, and is dependent on only $V^{-1/2}$. Since $k_{po}$ is experimentally independent of $v_b$, $k_{po}$ variations must be due to $P^{*}$ variations (Background). In NWM voxels these are large but $v_b$ is small and apparently regulated (likely $\rho^*$ regulation), while in NGM voxels $k_{po}$ seems regulated. The very slight downward slope of the green point cluster at larger $v_b$ is due to the partial-volume averaging mentioned above. Larger $v_b$ values reflect larger mean $r$ values, and there is a slight $k_{po}$ decrease due to this, but mostly $k_{po}$ is constant in NWM.
Now, we turn to population averages. Table 1 presents (about 3.6 mL) ROI biomarker values averaged for six healthy controls and six RRMS subjects with "non-enhancing" WM lesions. Precision is generally quite good: the SEMs listed are due mostly to inter-subject variation. The fact that the $k_{po}$ values in NWM and NGM are more similar than the $v_b$ values is due to the aforementioned supra-intensive nature of $\tau_b$. The $R_{1exv}$ values are reduced in NGM, normal-appearing gray matter (NAGM), and MS lesions because of decreased macromolecular volume fractions (74). The $v_b$ values are increased in normal-appearing white matter (NAWM) and NAGM, and decreased in MS lesions. More interesting is the fact that $k_{po}$ is decreased in MS-NAWM and MS-NAGM, and even more-so in lesions.

For a cylindrical capillary, $k_{po} = 2P_w r^{-1}$; the quantity $r$ is a 1D measure of capillary size. With a conservatively large $r$ value (3 μm (39,40)), $k_{po} = 0.7P_w r^{-1}$ (in s $^{-1}$, $P_w$ in μm s $^{-1}$). With a typical $P_w$ value (2 μm s $^{-1}$ (50,51)), $k_{po} = 4r^{-1}$ (r in μm). Thus, $k_{po}$ is linearly related to both $P_w$ and $r^{-1}$, with different coefficients. In the Discussion section, we compare concomitant relative (%) changes in the population-averaged $v_b$ and $k_{po}$ parameters for the NWM → NAGM (Table 1) transition. A deductive quantitative analysis shows that the $k_{po}$ decrease is dominated by the $P_w$ decrease. The analogous exercise indicates an even greater $P_w$ decrease in MS-NAWM. $k_{po}$ ($\tau_b$ $^{-1}$) is dominated by the $P_w$ factor, not the $r^{-1}$ factor.

Decreased $k_{po}$ in the MS lesion

In non-enhancing MS lesions, the $k_{po}$ value is decreased even further: the average for the six Table 1 RRMS subjects is 1.8 s $^{-1}$. However, these represent relatively early-stage disease. Figure 4 shows results for a 52 year old female late-stage RRMS subject. Quite large chronic demyelinated WM lesions appear hypointense in the $R_{1exv}$ map (Fig. 4(a)) – indicating extensive macrovascular loss, consistent with demyelination and gliosis. These lesions are many months past their last high CA-enhancement stage. The $v_b$ map (Fig. 4(b)) is rather similar to that of the control (Fig. 2(b)), but reduced (<0.01) in lesion areas and NAWM. However, the $k_{po}$ map (Fig. 4(c)) is dramatically altered. Unlike NWM (Fig. 2(c)), the WM region is extremely hypointense. The $k_{po}$ values in the lesions themselves (−1.5 s $^{-1}$) are decreased below the MS-NAWM mean (2.2 s $^{-1}$) and even the RRMS lesion mean (1.8 s $^{-1}$) (Table 1). Furthermore, compared with the RRMS NAGM mean (2.0 s $^{-1}$) (Table 1), the $k_{po}$ values (−2.9 s $^{-1}$) are considerably increased in this advanced subject NAGM. (It is hard to discern the NAWM situation because the lesions are so large.)

Decreased $k_{po}$ in the GBM tumor

For GBM capillaries, clinical monomeric Gd(III) chelate CAs extravasate too rapidly to allow $k_{po}$ determination. Thus, we used the intravascular, coated superparamagnetic iron oxide nanoparticle Fe-tol as CA (73). This agent has a molecular mass of 750 000 Da (10$^4$ times that of GdHPDO3A; 588 Da). Its $k^{trans}$ in normal and NA brain tissue is $\sim 10^8$ min $^{-1}$ (75). (The biomarker $k^{trans} \approx P_{CA}$, where $P_{CA}$ is the endothelial CA permeability coefficient (76).) During the first pass, it remains intravascular even in very advanced GBM tumors with extremely permeable capillaries. Figure 5 shows results from a 52 year old male GBM subject. In the center is an $R_1$ map obtained 30 min after GdHPDO3A injection. The large CA-enhancing tumor is clearly visible at the bottom left. Twenty-four hours after GdHPDO3A, the subject received IV Fe-tol. Inset are data (points) obtained from four representative ROIs (frontal WM, thalamus, putamen (white ellipses), and tumor (red circle)) during the Fe-tol injections. Each plot shows the $R_{1exv}$ dependence of $R_1$ (as in Fig. 1(C)). If the $R_{1exv}$ $\rightarrow$ 0) held, the $R_1$ vs. $R_{1exv}$ plots would be linear. None are, and all are well fitted by Equation 1: 2SX SSP expression curves with $R_{1exv}$ $\rightarrow$ 0, $\tau_b$ varied. The $R_{1exv}$ $v_b$ and $\tau_b$ parameter values returned are 1.24 s $^{-1}$, 0.008, 0.29 s (frontal WM); 0.99 s $^{-1}$, 0.013, 0.32 s (thalamus); 0.94 s $^{-1}$, 0.014, 0.43 s (putamen), and 0.77 s $^{-1}$, 0.028, 1.52 s (tumor). For five subjects, the population- and ROI-averaged parameter values are given in Table 1. For this 3 T study the $R_{1exv}$ values are greater than the normal and MS brain 7 T entries. Tissue macromolecular relaxivity is greater at smaller field (74). In the GBM normal appearing (GBM-NA) brain, the $v_b$ values are generally smaller than normal. They are large in the tumor.

Table 1. Population-averaged resting-state human brain ROI parameter values

|                      | SSP DCE-MRI (′$H_2$O) | $R_{1exv}$ (s$^{-1}$) | $v_b$ (μm s$^{-1}$) | $\tau_b$ (s) | $k_{po}$ ($\tau_b$ $^{-1}$) (s$^{-1}$)$^c$ |
|----------------------|-----------------------|----------------------|---------------------|--------------|------------------------------------------|
| **Healthy controls (n = 6)$^a$** |                       |                      |                     |              |                                          |
| NWM                  | 0.831 (±0.021)         | 0.014 (±0.002)       | 0.35 (±0.04)        | 3.2 (±0.56)  |                                          |
| NGM                  | 0.679 (±0.015)         | 0.031 (±0.004)       | 0.41 (±0.06)        | 2.9 (±0.59)  |                                          |
| **Relapsing–remitting MS (n = 6)$^b$** |                       |                      |                     |              |                                          |
| NAWM                 | 0.810 (±0.022)         | 0.019 (±0.002)       | 0.48 (±0.05)        | 2.2 (±0.20)  |                                          |
| NAGM                 | 0.672 (±0.009)         | 0.045 (±0.004)       | 0.50 (±0.03)        | 2.0 (±0.13)  |                                          |
| Lesion               | 0.624 (±0.009)         | 0.012 (±0.003)       | 0.59 (±0.14)        | 1.8 (±0.45)  |                                          |
| **Glioblastoma (n = 5)$^b$** |                       |                      |                     |              |                                          |
| NA-frontal WM        | 1.10 (±0.027)          | 0.008 (±0.001)       | 0.44 (±0.04)        | 2.6 (±0.31)  |                                          |
| NA-thalamus          | 0.90 (±0.009)          | 0.017 (±0.001)       | 0.38 (±0.05)        | 2.9 (±0.37)  |                                          |
| NA-putamen           | 0.78 (±0.013)          | 0.012 (±0.005)       | 0.43 (±0.03)        | 2.5 (±0.22)  |                                          |
| Tumor                | 0.67 (±0.013)          | 0.046 (±0.013)       | ≥5.6                | ≤0.18        |                                          |

$^a$ProHance, 7 T.

$^b$Ferumoxytol, 3 T.

$^c$(Kpo) ($\tau_b$ $^{-1}$). Uncertainties are ±SEM.
The $k_{po}$ values in the GBM-NA brain are fairly normal. This supports the general accuracy of the three Fe-tol injection steady-state protocol. (The latter favored spatial resolution over pharmacokinetic temporal resolution, just the opposite of the GdHPDO3A protocol used to obtain the Table 1 normal and MS-NA values.) Importantly, the Figure 5 tumor tissue $k_{po}$ is decreased by more than a factor of five. The example tumor ROI shows why: the $R_1t$ vs. $R_1b$ plot has a much sharper hyperbolic shape. Though the Fe-tol protocol yielded only four pharmacokinetic points, this behavior is confirmed by the population ($n = 5$) averaging (Table 1), where the tumor $k_{po}$ decrease is over an order of magnitude. Since there is likely a family of fittings of the four data points for which the $k_{po}$ values are even smaller, it is best to consider the Table 1 GBM tumor $k_{po}$ entry as an upper limit. The very large GBM tumor tissue $r_2$ values were unexpected. Future Fe-tol protocols will acquire more than four points while still achieving full brain coverage and good spatial resolution.

The fact that $k_{po}$ decreases in MS lesions and in GBM tumors is very significant. We show below that this is due to $P_{W}^{\dagger}$ decreases. However, these are both pathological tissues well known to have leaky capillaries: $K_{trans}$ is clearly increased in each. Furthermore, the increase of the $P_{CA}^{\dagger}$ factor dominates $K_{trans}$. This is what is meant by the colloquial phrase “increased capillary permeability.” The facts that $P_{W}^{\dagger}$ decreases while $P_{CA}^{\dagger}$ increases mean that water and CA molecules do not exchange across the capillary wall by the same dominant mechanism. This important finding is elaborated in the Discussion section.
\[ \tau_{\text{exv}} \]

Finally, equilibrium mass action demands \( \tau_{\text{exv}} = \tau_0 (1 - P_0/P_\infty) \), where \( P_0 \) is the mole fraction of water that is vascular (= \( V_0/V_\infty \)). Combining the Table 1 \( V_0 \) and \( \tau_0 \) values, we obtain \( \tau_{\text{exv}} \) values of 19 s and 10 s for NWM and NGM, respectively. These are comparable to the first-order lifetime, 45 s, calculated (49) from the observed 31 s \( \tau_1/2 \) for brain parenchyma \( ^{15} \text{O}_2 \) intravasation (50). As detailed above, this 2SX expression does not require the assumption that parenchymal water is “well mixed,” only that its MR systems are in their FXL conditions.

**DISCUSSION**

**\( k_{\text{po}} \) variations are due to \( P_W \cdot \)**

As noted above, the essential independence of the experimental \( k_{\text{po}} \) and \( V_0 \) parameters in the Figure 3 voxel scatter plot signifies that \( k_{\text{po}} \) is dominated by capillary wall water permeability. Further, quantitative deductive analyses of concomitant population-averaged \( \tau_0 \) and \( V_0 \) ratios in normal brain, MS-NA brain, GBM-NA brain, MS lesions, and GBM tumors show that variations in the capillary equilibrium water efflux rate constant (\( k_{\text{po}} \)) are dominated by differences in microvessels wall water permeability (\( P_W \cdot \)), not capillary radius. Example analyses are detailed in Appendix A.

Briefly, we use the relationships \( k_{\text{po}}(A)/k_{\text{po}}(B) = [P_W(A)/P_W(B)] (r/\rho_f) \), and \( [V_0(B)/V_0(A)]^{1/2} = (r/\rho_f)^{1/2} (r/\rho_f) \), for ROIs A and B. For example, for \( A = \text{NAGM} \) and \( B = \text{NGM} \) we plot in 3D capillary property space the trace of all points that simultaneously satisfy the experimental population-averaged \( k_{\text{po}}(A)/k_{\text{po}}(B) \) and \( [V_0(B)/V_0(A)]^{1/2} \) ratios (Fig. A1). The experimental data are incompatible with the mean capillary radius and density simultaneously remaining invariant from NGM to NAGM. The brain literature generally indicates it more likely that chronic \( V_0 \) differences are due to capillary density (\( \rho_f \)) differences than to capillary dilation or constriction (\( r \) changes) (38-41). (Even in an acute hypercapnic perturbation, the microvessel radii for the dominant capillary volume fraction remain unchanged (41). The very smallest capillaries, normally effectively occluded, are opened during the hypercapnia – there is some “recruitment” – but in most capillaries there is a blood velocity increase (41:). Therefore, the Figure 1A results clearly indicate that the mean capillary water permeability in MS NAGM is reduced from its value in NGM (the NGM \( \rightarrow \) NAGM transition). For equal mean capillary radii, \( P_W^\ast(\text{NAGM}) = 0.8P_W(\text{NGM}) \), \( P_W^\ast \) is reduced by 20%. Recall that \( r \) is the average for a large number of capillaries. Only 100 capillaries \( \mu L^{-1} \) means 4000 per 40 \( \mu L \) voxel. The Table 1 ROIs represent 80–100 voxels. 100 voxel ROIs in six subjects yield averages over 2 400 000 capillaries.

**Capillary water exchange is dominated by transcellular pathways**

There are many possible pathways water molecules can use for capillary egress and ingress. Figure 6 summarizes these. It depicts (a) paracacellular water passage through endothelial tight junctions (endothelial cells are colored gray), (b) simple, transcellular water diffusion across cell membrane lipid bilayers, and (c) transcellular transport through membrane aquaporin protein water channels (63,64,77) and/or leakage through membrane transporters (65). (Inspirations for this diagram are found in References 35, 36, and 63. It emphasizes water effluxibra, and is otherwise greatly simplified.) The Figure 6(d) transcellular process will be elaborated below.

The facts that, in MS lesions and GBM tumors, \( P_W^\ast \) decreases while \( P_W^\ast \) increases mean that water and CA molecules exchange via different pathways. CA molecules are universally thought to employ the para(endothelial)cellular pathway (Fig. 6(a)), and we previously thought that this would be a major mechanism for water as well. In Appendix B, however, we compare \( k_{\text{po}} \) with \( k_{\text{pe}} \) (for CA extravasation) to show that, for the normal brain, by far the vast majority (>95%) of capillary water efflux (and influx) occurs via one or more transcellular processes (Fig. 6(b)–(d)). This is a fundamental finding, and we are not aware that it has been previously known.

**Equilibrium transendothelial water exchange is a metabolically active process**

Which, if any, of the transcellular pathways (Fig. 6(b)–(d)) dominates \( k_{\text{po}} \)? Mechanisms 6(b) and 6(c) (bilayer diffusion, and passage through trans-membrane aquaporin and/or protein channels, respectively) are passive, i.e. require no energy expenditure. However, comparison of our results with literature metabolic imaging studies of the same tissues indicates that the process measured by \( k_{\text{po}} \) is metabolically active.

This is shown in Table 2. The second and third columns repeat the Table 1 \( V_0 \) and \( k_{\text{po}} \) entries and place them adjacent to results of pertinent quantitative \( ^{31} \text{PMRSI} \) (78) and \( ^{23} \text{NaMRSI} \) (19,79) studies. Because of \( ^{31} \)PMRSI spatial resolution limitations, it is important that WM/GM image segmentation be accomplished with co-registered \( ^1 \text{H}_2 \text{O} \) maps, and then applied to apportion the MRSI measurements (78). Such results are listed in Table 2. It is clear that \( k_{\text{po}} \) exhibits a positive correlation with tissue ATP concentration, \([\text{ATP}]_n \), comparing NWM with NGM, NWM with NAWM, NGM with NAGM, or NAWM with NAGM. For example, \([\text{ATP}]_n \) decreases from normal brain in both NAWM (13%) and NAGM (20%). The phosphocreatine concentration, \([\text{PCr}]_n \), also decreases (not shown) in rough proportion to \([\text{ATP}]_n \) (78), consistent with the maintenance of overall ATP/PCr equilibrium. In contrast, \( k_{\text{po}} \) correlates negatively with TSC, \([\text{Na}]_n \), which increases in NAWM (39%), NAGM (17%) (19), and GBM tumor (51%) (79). Since there is insignificant extracellular ATP, \([\text{ATP}]_n \) reflects \([\text{ATP}]_n \). An \([\text{Na}]_n \), TSC, increase could reflect an \([\text{Na}]_n \) increase. An \([\text{ATP}]_n \) decrease and a possible \([\text{Na}]_n \) increase signify compromised metabolism: decreased ATP hydrolysis chemical potential and trans-mural Na⁺ gradient electrochemical potential, respectively. The brain \( k_{\text{po}} \) values are correlated with metabolic thermodynamic properties.

However, \( k_{\text{po}} \) is a kinetic parameter (of dimension reciprocal time). In order to validate a flux measurement, one must compare it with the gold standard flux measurement. Normal homoeostatic neuronal cell metabolic rates have been measured with \( ^{31} \text{PMRSI-MT} \), using \( ^1 \text{H}_2 \text{O} \) segmentation (17). For NGM, the ATP synthesis (mostly by oxidative phosphorylation, \( \text{CMR}_{\text{oxphos}} \) Fig. 6) flux is 0.16 nmol s⁻¹ \( \mu L^{-1} \) (17), and of course the consumption rate is the same. The fluxes between ATP and phosphocreatine (PCr) are seven times larger, 1.15 nmol s⁻¹ \( \mu L^{-1} \) in each direction (17); integrated over the neuron, ATP and PCr are in effective equilibrium. The NGM and NWM \( \text{CMR}_{\text{oxphos}} \) values, 160 and 50 pmol(\( \text{ATP} \)) s⁻¹ \( \mu L^{-1} \), respectively (17), are entered in Table 2, as is the \( \text{CMR}_{\text{oxphos}}(\text{NGM})/\text{CMR}_{\text{oxphos}}(\text{NWM}) \) ratio, 3.2. By definition, \( k_{\text{po}} \) is proportional to the \( ^1 \text{H}_2 \text{O} \) flux per capillary and \( V_0 \) to the number of capillaries per unit tissue.
Figure 6. A neurogliovascular unit chain mechanism. Water exchange processes determine mean water molecule lifetimes in blood (τ_b, beige), interstitial (τ_o, aqua), endothelial (τ_i′, gray), neuroglial (τ_i, pink), and neuronal (τ_i″, blue) cell spaces. The equilibrium paracellular (a), simple diffusion (b), facilitated transcellular (c), and active water cycling (d, stars) pathways are indicated, as are “Magistretti steps” (e–g). We suggest the d steps couple unit metabolic activity to τ_b.

Table 2. The biomarker k_po measures metabolic activity

|                      | SSP DCE-MRI (1H2O) | 31PMRSI | 23NaMRSI | SSP DCE-MRI (1H2O) | 31PMRSI-MT |
|----------------------|---------------------|---------|----------|---------------------|------------|
|                      | v_b, k_po (τ_b^-1) (s^-1) | [ATP] (mM) | [Na] (mM) | k_po v_b (s^-1) | CMR oxphos (pmol(ATP) s^-1 μL^-1) |
| **Healthy controls** |                     |         |          |                    |            |
| NWM                  | 0.014               | 3.2     | 2.43     | 19^a               | 0.045      | 50         |
| NGM                  | 0.031               | 2.9     | 1.62     | 31^a               | 0.090      | 160        |
| NGM/NWM              |                     |         |          |                    | 2.0        | 3.2        |
| **Relapsing–remitting MS** |             |         |          |                    |            |
| NAWM                 | 0.019               | 2.2     | 2.11     | 27^a               | 0.042      |            |
| NAGM                 | 0.045               | 2.0     | 1.29     | 36^a               | 0.090      |            |
| Lesion               | 0.012               | 1.8     | 35^a     |                    | 0.022      |            |
| **Glioblastoma**     |                     |         |          |                    |            |
| NA-frontal WM        | 0.008               | 2.6     |          | 13%b               | 0.021      |            |
| NA-thalamus          | 0.017               | 2.9     |          | ↓12%b              | 0.049      |            |
| NA-putamen           | 0.012               | 2.5     |          |                    | 0.030      |            |
| Tumor                | 0.046               | ≤0.18   |          | ↑51%b              | ≤0.008     |            |
| References           | this work           | 78      | 19, 79   | this work          | 17         |

^aReference 19.
^bRelative to NWM, Reference 79.
volume. Since CMR_{\text{oxphos}} is an ordinary intensive property (all that is accessible by directly detected $^{31}$PMRSI), we must multiply the supra-intensive $k_{\text{pxo}}$ by the intensive $V_b$ in order to compare. Thus, the $k_{\text{pxo}}V_b$ products and the $k_{\text{pxo}}V_b(\text{NGM})/k_{\text{pxo}}V_b(\text{NWM})$ ratio, 2.0, are displayed in Table 2. The agreement of the flux ratios for these two very different and independent techniques, $^{31}$PMRSI-MT and DCE-MRI ($^1$H$_2$O), is rather remarkable and strongly suggests $k_{\text{pxo}}$ is proportional to CMR_{\text{oxphos}} per capillary. The Table 2 NGM and NWM [ATP]/ and CMR_{\text{oxphos}} values exemplify the thermodynamics/kinetics distinction. While the steady-state ATP concentration (and thus free energy) per unit tissue volume is 1.5 times greater in NWM than NGM, the oxidative phosphorylation ATP flux in the same unit tissue volume is 3.2 times greater in NGM than NWM.

A neurogliovascular unit chain mechanism

Table 2 shows that $k_{\text{pxo}}$ is proportional to the ATP consumption flux per capillary (the core of the neurogliovascular unit). What could be a mechanism for this? The clue is in a recent report on the heterogeneity, and response to therapy, of cellular $r_i$, values within human breast tumors in vivo (32). That paper also assembles the evidence from model studies that $r_i^{−1}$ is increased by the gene dosage of, and substrates for, the driving cell membrane P-type ATP-ase ion pump, and decreased by specific inhibitors (33,66). Cellular $k_{\text{pxo}}(r_i^{−1})$ reflects P-type ATP-ase turnover, per cell (32). Figure 6(d) visualizes a cascade (or chain) of $r_i$, changes for cells within the neurogliovascular unit. These are $r_i$ (neuroglia, pink), $r_i^{−1}$ (endothelial cells), and $r_i^{∗}$ (neurons, blue). In the Figure 6 diagram, these active processes are indicated by trans-membrane water cycles (stars, 6(d)). The pink cells can be astrocytes, oligodendrocytes, pericytes, etc. (34–37). Combinations of neurons, glia, and microvessels have been termed “gliovascular units” (36), because of their crucial, exquisite symbiotic metabolic and energetic interactions (34,35,37). The Figure 6(d) pathways represent processes driven by NKA turnover, perhaps the most crucial ongoing cellular metabolic activity in the brain. These would affect each other by changes in transporter substrate concentrations (“paracrine communication”). This is plausible because these cells are within synaptic proximities (<50 nm), and have asymmetric transporter distributions. Microjets of water and substrates are continually injected into these confined spaces as transporters turn over. Obligate active trans-membrane water cycling (32) means that water is effectively a substrate for the reactions driven by NKA turnover – the steady-state system for which the forward reaction is given in the Introduction. Thus, it is possible that $r_{\text{pxo}}$ in turn reflects ongoing neurogliovascular unit metabolic turnover, particularly Na$^+$,K$^+$-ATP-ase activity: the smaller $r_{\text{pxo}}$, the greater NKA turnover, and vice versa – a cascade of altered active trans-membrane water cycling – a $k_{\text{pxo}}$ decrease reflecting $k_{\text{oxphos}}$ decreases within the unit. For example, the “Magistretti mechanism” (Fig. 6(e)-(g)) has neuroglia essentially conducting most glycolysis and transferring lactate to neurons for mostly oxidative phosphorylation: the capillary is intimately involved in this intercellular metabolic cooperativity (34,35). An increase in $k_{\text{pxo}}$ would reflect a speed-up of Magistretti-type processes (Fig. 6(e)-(g)), an exciting hypothesis. The increased $k_{\text{pxo}}$ in NWM (Figs. 2(c) and 3) may reflect increased metabolic activity in common tracts shared by fluctuating resting-state neural circuits. For example, the Figure 2c asymmetry (left centrum semiovale WM hyperintensity (image right)) could reflect a “rich club” node WM connection active at the time of this acquisition (80). Consistent with this, in other subjects (not shown) WM $k_{\text{pxo}}$, hot spot loci vary. The greater spatial resolution planned (see below) could prove informative in this regard. (We note that the BOLD effect reflects the coupling of metabolic activity with vascular properties.) The extravascular water lifetime $T_{\text{exv}}$ is a complicated function of water populations and lifetimes $T_{\text{exv}}$, $T_{\text{ox}}$, $T_{\text{pxo}}$, etc. (Fig. 6) (55).

Absolute quantification

Our results allow calculation of the equilibrium brain capillary water efflux. In 1 µL tissue, we estimated the average capillary length and radius as 2 mm and 2.6 µm, respectively, above. For a cylinder, this gives a mean capillary volume ($V$) of 42.5 µL. A 50 M $[^1$H$_2$O] yields $1.3 \times 10^{15}$ H$_2$O molecules per capillary. For NGM, $k_{\text{pxo}}$ is 2.9 s$^{-1}$ (Table 1). This gives the equilibrium water efflux $= 1.3 \times 10^{15} \times 2.9 = 3.8 \times 10^{15}$ H$_2$O molecules s$^{-1}$ per capillary (and, of course, an equal influx). Now, consider the homeostatic NGM CMR_{\text{oxphos}}, 160 pmol(ATP) s$^{-1}$µL$^{-1}$ (17). If 75% is used for NKA turnover (3), we have 120 pmol(ATP) s$^{-1}$µL$^{-1}$ consumption. For 100 capillaries µL$^{-1}$ (81), this is 1.2 pmol(ATP) s$^{-1}$ per capillary. The NGM water flux estimated above corresponds to 6 pmol(H$_2$O) s$^{-1}$ per capillary, and yields $5 \times 10^{15}$ H$_2$O molecules cycled per NKA turnover (one ATP molecule consumed). Some individual water co-transporting membrane sympotomers have H$_2$O stoichiometries approaching this order of magnitude (69,70,82), and there are likely a number of different sympotomers involved in the neurogliovascular unit chain (Fig. 6(d)). Thus, in addition to the remarkable agreement with relative CMR_{oxphos} values in Table 2, it is possible that brain $k_{\text{pxo}}$ values can be interpreted quantitatively. For a 44 µL rat brain ROI in vivo, $k_{\text{pxo}}$ was measured as 1.8 s$^{-1}$ using a very invasive intracerebroventricular CA infusion (83). The chain mechanism (Fig. 6(d)) suggests that $k_{\text{pxo}}$ should be similar to $k_{\text{ox}}$.

Clinical implications

This paper does not focus on clinical aspects. However, the approach introduced here has much to offer in this regard.

MS

The longstanding MS imaging hallmark is the “enhancing” WM lesion. In Appendix B, we mention that enhancement (with CA) is transient, and not always “caught” in an MRI study. The Figure 4 late-stage subject is an example. Though her WM lesions are chronic, and large, they were not especially CA-enhancing at the time of acquisition, and their conspicuity in the $R_{\text{exv}}$ map (essentially a $T_{1-W}$ image inverse) is poor. However, if we inspect the $T_2$ map (the Fig. 4(c) inverse) in Figure 7, the conspicuity is very high. Thus, the prospect for detecting lesions is much greater with a $T_2$ map.

Meanwhile, MS understanding is evolving. While long considered a WM disease, emerging data suggest that GM may be an early, or even the initial, disease target (84,85). A recent concept is that MS disease activity originates in brain regions other than WM, perhaps GM; the “outside-in” hypothesis (85). In early disease, pro-inflammatory cytokines are chronically upregulated and can reduce oxygen utilization despite sufficient delivery (“metabolic hypoxia” (86), mediate mitochondrial function, and decrease neurogenesis, and may increase overall neurodegeneration risk. Metabolic deficits of MS-NAGM are more extensive than those in MS-NAWM and include decreased oxygen
utilization (85), altered perfusion, and high-energy phosphate depletion (78).

If MS-NAGM and MS-NAWM NKA turnover is diminished by metabolic hypoxia then, according to our mechanism, the supra-intensive \( k_{po} \) will decrease. This is what we see (Table 2). The fact that, in RRMS, \( k_{po} \) decreases by the same amount (31%) from both NWM and NGM is strong evidence for whole brain involvement. Furthermore, our results predict this should not be detectable by \(^3\)P-MRSI-MT, which can access only the intensive CMRoxphos. Table 2 indicates that \( k_{po}V_b \) the CMRoxphos analog – does not decrease in RRMS NAGM or NAWM: the recruited \( p_i \) increase exactly compensates the \( p_{i0} \) decrease.

The TSC is the volume fraction-weighted average of the (extra- and intra-cellular) compartmental \( Na^+ \) concentrations within the voxel or ROI: \[ [Na^+]_T = \frac{v_v[Na^+]_v + v_i[Na^+]_i}{v_v + v_i} \] where \( v_v + v_i = 1 \). Globally in the brain, \( v_v \) is usually thought to be near 0.2 (thus \( v_i = 0.8 \)); \( [Na^+]_v \) is considered (highly regulated) near 140 mM; and \( [Na^+]_i \) near 12 mM in normal homeostasis (79). Thus, there are (at least) two general mechanisms for increasing \( [Na^+]_v \). The first (anatomic) is a \( v_v \) increase (with concomitant \( v_i \) decrease; cell shrinking; cell density decrease) without changing \( [Na^+]_v \) or \( [Na^+]_i \). This is often called the Hilal mechanism. The second (metabolic) is an \( [Na^+]_v \) increase due to a slow-down of membrane NKA turnover \( v_v \) or \( [Na^+]_i \). NKA activity can be regulated in several different ways (3). Of course, there are various possible combinations of the first and second mechanisms simultaneously. The opposite changes could decrease \( [Na^+]_v \). The good (negative) correlation of \( k_{po} \) with \( [Na^+]_v \) in both normal and NA-MS brain (Table 2) is suggestive of the second mechanism. However, we do not have an independent measure of \( v_v \) (and thus \( v_i \)), its interpretation remains ambiguous.

Also exciting is the significantly increased MS-NAGM \( k_{po} \) in the late-stage disease (Fig. 4(c)). If this is borne out in more subjects, it means that neurogliovascular unit NKA activity is increased in advanced MS-NAGM – a strong indication of global metabolic GM involvement, possibly indicating an RRMS to secondary-progressing MS conversion. Access to a metabolic imaging biomarker for this stage change would be of tremendous benefit. Inspection of the same map (Fig. 4(c)) suggests that the demyelinated lesions in WM have greatly diminished resting-state metabolic activity. Perhaps the NAGM activity is increased because of the necessity to employ "detour" circuitry because of blocked rich club node connections. Alternatively, perhaps neurogliovascular unit cells enter apoptosis in advanced MS disease. There is an interesting report that cells intentionally put into a defined apoptotic state exhibit a substantially increased \( k_{io} \) (87).

**GBM tumor**

In GBM tumors, \( k_{po} \) values less than 1 s\(^{-1}\) (Table 2) suggest that NKA turnover is exceptionally slow. This is consistent with tumor \( [Na^+]_v \) being increased by 51% over its value in NWM (79) (Table 2). Our finding that \( k_{po} \) values in GBM-NA tissue are similar to those in control brain is also consistent with the fact that tumor-NA tissue \( [Na^+]_v \) values differ little from controls (79) (Table 2). A recent qualitative \(^3\)P-MRSI investigation of human GBM in vivo suggests that \([ATP]_v\) and \([PCr]_v\) values are essentially the same in tumor tissue as in control brain (88). Also it has been found that, while MRglc is only slightly elevated over adjacent WM in GBM tumor (27), there is extensive hypoxia (89). In this condition, ATP production shifts from oxidative phosphorylation toward glycolysis. Within the neurogliovascular unit, this means that the locus of ATP synthesis shifts from neurons toward neuroglial cells (34,35). The consequence of this could be that a decrease in neuronal glucose consumption is slightly overcompensated by an increase in neuroglia, which also have proliferated in the tumor. However, net NKA turnover in the neurogliovascular unit, dominated by neurons, would decrease and consequently neuronal \([Na^+]_v\) would increase. The substantial GBM tumor \( k_{po} \) decrease (Table 2) may be a hypoxia signature. Unlike \(^{18}\)FDG PET or guco-CEST/CELS, the activity we ostensibly measure is catabolically downstream of an oxidative phosphorylation \( \rightarrow \) glycolysis shift. The turnover of NKA is a major end-point of central (intermediary) metabolism.

The population-averaged value of 0.18 s\(^{-1}\) for \( k_{po} \) in GBM tissue (Table 1) is quite surprisingly small. We find the tumor \( k_{io} \) value is substantial in the epithelial cancers (>2.0 s\(^{-1}\), breast carcinoma (32); >2.5 s\(^{-1}\), prostate adenocarcinoma (90)) we have examined (hot spots even larger). Perhaps \( k_{io} \) within the GBM tumor is also significant and the small \( k_{po} \) really reflects a breakdown of neurogliovascular unit \( k_{io}/k_{po} \) coupling. Preliminary analyses of Gd(III) CA DCE-MRI time-courses (not shown) suggest that the GBM tumor \( k_{io} \) values are not as small as \( k_{po} \). Of course, the GBM tumor could also have just a decreased metabolic activity.

**Stroke**

The acute and significant cerebral water apparent diffusion coefficient (ADC) drop after an ischemic event is of considerable clinical importance, though the mechanism has remained elusive. We have suggested (33) that an active trans-membrane water cycling decrease may contribute. Consistent with this, the ADC drops by 40% within 15 min of direct application of ouabain (a specific NKA inhibitor) to the striatum – before there is significant decrease of the NKA substrate ATP, (91).
Concluding remarks

We have designed a new acquisition pulse sequence that incorporates the “multiband” approach (92,93) and yields full brain coverage with nominal voxel volume less than half that in Figures 2, 4, and 7.

The existence of, or dominance of, an active cell membrane \( k_{\text{in}} \) has implications for many different types of in vivo MR experiment. In the metabo-CEST experiment, The RF-induced \( ^1\text{H}_2\text{O} \) intensity change depends on two factors: (1) the metabolite concentration, and (2) the probability per unit time of a water molecule encountering the metabolite molecule. Some water must cross a cell membrane to gain this access, and we have suggested (61) that this transport can contribute to the metabo-CEST signal. In gluco-CEST, for example, the contribution of intracellular glucose is “very small to negligible” (94). However, about 80% of water is intracellular. An aim of gluco-CEST is to determine relative glucose concentrations (23). Since \( k_{\text{in}} \) likely changes during a glucose challenge, the probability of intra- and extracellular \( ^1\text{H}_2\text{O} \) molecules crossing the membrane changes. The fact that this would also alter the temporal probability of water encountering glucose could affect the interpretation of gluco-CEST changes. This phenomenon may have already been manifest in the gluco-CEL experiment (24).

It is possible that water movement in living tissue is dominated by active trans-membrane water cycling. Significant shutter-speed effects are very common in cancer MRI (32). (Since the angiogenic microvessels of malignant tumors have larger intrinsic \( k_{\text{trans}} \) values than benign tumors, the use of SSP DCE-MRI makes it possible to contemplate eliminating most, if not all, unnecessary biopsies (i.e. those that find no malignancy) in breast (95–97) and prostate (31,90) cancer. These comprise about 70% of all breast and prostate biopsies. The \( k_{\text{trans}} \) values of malignant tumors are systematically suppressed by the TP.) These effects are now also being found in animal and human myocardium in vivo (66,68). The NMR shutter-speed concept has broad application.

Acknowledgements

We are grateful for NIH (RO1 NS40801; RO1 EB007258; UO1 CA154602; R44 CA180425; S10 RR027694, and U1R RR024140) and NMSS (RG 3168A1) support. We appreciate stimulating discussions with Drs Jeffrey Iliff, Craig Jahr, Damien Fair, Kenneth Krohn, Christopher Kroenke, Martin Pike, Wei Huang, Thomas Barbara, Michael Garwood, and Kamil Ugurbil, as well as the indispensable technical assistance of Mr Brendan Moloney.

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APPENDIX A

kpo VARIATIONS AND ACCOMPANYING Vb VARIATIONS

Consider (conservatively) the MS-induced GM kpo change—the smallest in Table 1. We have \( (\tau_1(NAGM))^{1-1/\tau_1(NGM)} \) = 2.0 s\(^{-1}\). This is reduced by 20% from that in NGM (\( k^{\text{t}} \)) = 0.8: the NAGM (\( k^{\text{t}} \)) is reduced by 31%. From the fundamental theoretical relationship, \( (\tau_1(NAGM))^{1-1/\tau_1(NGM)} \) = \( P_w(NAGM)/P_w(NGM)/\tau_1(NAGM)/\tau_1(NGM) \), where \( \tau_1(NAGM) \) and \( \tau_1(NGM) \) are the mean capillary radii in NGM and NAGM tissues, respectively. (Capillaries dominate vascular volume in most voxels (39,41).) Thus, the experimental relationship \( P_w(NAGM)/P_w(NGM)/\tau_1(NAGM)/\tau_1(NGM) \) = 0.80 must be satisfied. There is an infinite number of possibilities: if \( \tau_1(NAGM) = 1.25\times\tau_1(NGM) \), \( P_w(NAGM)/P_w(NGM) \) = \( P_w(NGM) \); if \( \tau_1(NAGM) = \tau_1(NGM) \), \( P_w(NAGM) = 0.8\times\tau_1(NGM) \).

It is extremely difficult, and invasive, to determine individual capillary radii in vivo (38,40,41). However, we can estimate capillary radius changes, if any, from our data. The blood volume fraction \( v_b = (n/v_r) V = \rho V \), where \( n/v_r \) is the number of
capillaries in the voxel or ROI total volume, the capillary number density, \( \rho^\tau \) (hundreds \( \mu L^{-1} \)) (81), and \( V \) is the mean individual capillary volume. Assuming cylindrical capillaries, \( r \sim V^{1/2} \) and the ratio \( v_b(NGM)/v_b(NAGM) = r_b^{-1/2} = \left( P_W(NGM)/P_W(NAGM) \right)^{1/2} \). Taking \( v_b(NGM) \) as 0.031 and \( v_b(NAGM) \) as 0.045 (Table 1) gives \( [v_b(NGM)/v_b(NAGM)]^{1/2} = 0.83 \).

The solid red curve in Figure A1 is the trace of all points that simultaneously satisfy the experimental relationships \( P_W(NAGM)/P_W(NGM) = 0.80 \) and \( [v_b(NGM)/v_b(NAGM)]^{1/2} \). Taking \( v_b(NGM) \) as 0.031 and \( v_b(NAGM) \) as 0.045 (Table 1) gives \( [v_b(NGM)/v_b(NAGM)]^{1/2} = 0.83 \). The dot-dashed red projection does not pass through the \( f_NAGM = f_{NGM} \), \( \rho_{NAGM} = \rho_{NGM} \) point. The experimental data are incompatible with the mean capillary radius and density simultaneously remaining invariant from NGM to NAGM. The brain literature generally indicates it is more likely that chronic \( v_b \) differences are due to capillary density (\( \rho^\tau \)) differences than to capillary dilation or constriction (\( r \) changes) (38–41). (Even in an acute hypercapnic perturbation, the microvascular radii for the dominant capillary volume fraction remain unchanged (41). The very smallest capillaries, normally effectively occluded, are opened during the hypercapnia – there is some "recruitment" – but in most capillaries there is a blood velocity increase (41).) When the (solid) red curve passes through \( f_{NAGM} = f_{NGM} \) (black point), \( \rho_{NAGM} = 1.44 \rho_{NGM} \), and \( P_W(NAGM) = 1.25 P_W(NGM) \). \( P_W \) is reduced in resting-state NAGM from its value in resting-state NGM.

\( \rho_{NAGM}/\rho_{NGM} = 1.25 \rho_{NGM} \) (black point), \( P_W(NAGM) = 1.25 P_W(NGM) \) (dashed black lines). A conservatively large area for \( f_{NAGM} \) from 0.75 \( f_{NGM} \) to 1.25 \( f_{NGM} \) is shaded gray in the Figure A1 bottom plane. Over this area, the red curve \( f_{NAGM} \) coordinates range from 2.56 \( \rho_{NAGM} \) to 0.92 \( \rho_{NGM} \) and the \( P_W(NAGM) \) coordinates range from 1.67 \( P_W(NGM) \) to 1.0 \( P_W(NGM) \). These results clearly indicate that the mean capillary water permeability in MS NAGM is reduced from its value in NGM. For equal mean capillary radii, \( P_W(NAGM) = 0.8 P_W(NGM) \), \( P_W \) is reduced by 20%. Recall that \( r \) is the average for a large number of capillaries. Only 100 capillaries \( \mu L^{-1} \) means 4000 per 40 \( \mu L \) voxel. The Table 1 ROIs represent 80–100 voxels.

Figure A1. Capillary property space. A plot of biomarker inter-relationships for population-averaged NGM and NAGM ROI values in Table 1. Thus, \( (\tau_b(NAGM))^{-1}/(\tau_b(NGM))^{-1} = 0.80 \), and \( v_b(NAGM)/v_b(NGM) = 1.5 \), where \( \tau_b^{-1} = k_{\mu L} / t_w \) (\( t_w \) is the mean capillary water lifetime) and \( v_b \) the capillary volume fraction. The red curve is the trace of points that satisfy these experimental relationships simultaneously, in a 3D space of brain tissue microvascular properties. The vertical axis is \( P_W(NGM)/P_W(NAGM) \), the capillary water permeability coefficient ratio. The oblique axes are the inverse capillary radius ratio, \( r_b(NGM)/r_b(NAGM) \), and the capillary density ratio, \( \rho_b(NGM)/\rho_b(NAGM) \). The coordinates of the curve in regions of reasonable \( f_b(NAGM)/f_b(NGM) \) (gray shading) are consistent only with \( \tau_b \) being dominated by the \( P_W \) factor. When \( f_{NAGM} = f_{NGM} \) (black point), \( \rho_{NAGM} = 1.44 \rho_{NGM} \), and \( P_W(NAGM) = 1.25 P_W(NGM) \).
100 voxel ROIs in six subjects yield averages over 2 400 000 capillaries. The analogous exercise indicates an even greater $P_W^t$ decrease in MS-NAWM. $k_{po}$ ($r_{po}$) is dominated by the $P_W^t$ factor, not the $r^{-1}$ factor.

For the GBM tumor, we make the same analysis, using tissue ROI- and population-averaged parameter values from Table 1. There is a 93% decrease in tumor (T) $k_{po}$ relative to putamen (P). If the permeability coefficients were equal ($P_W^t(T) = P_W^t(P)$), then $r_T = 14r_P$ (with $r_T$ and $r_P$ the mean capillary radii in tumor and putamen tissue, respectively). It is even more unlikely than in the MS-NAGM tissue that capillaries would dilate by more than an order of magnitude in the tumor tissue. As above, the ratio $(v_b(P)/v_b(T))^{1/2} = (r_T/r_P)^{1/2}(r_P/r_T)$. Unlike the MS lesions (Table 1), however, the tumor $v_b(T)$, 0.046, is increased over normal-appearing tissue, $v_b(P) = 0.012$. (This reinforces that $k_{po}$ is indeed independent of $v_b$. It is decreased in MS lesions and in GBM tumors, though $v_b$ is decreased in the former and increased in the latter.) This gives $(v_b(P)/v_b(T))^{1/2} = 0.51$. Though extremely unlikely, if $r_T$ were actually 14$r_P$, then $r_T^2 = 0.020r_P^{-1}$: the tumor capillary density would be less than 3% of that of normal brain – even more unreasonable. An unchanged $P_W^t$ value is incompatible with both the $k_{po}$ and $v_b$ changes. If $r_T = r_P$, then $r_T^2 = 3.87r_P^{-1}$, and capillary density is increased almost fourfold in the tumor. This is plausible, and explains the $v_b$ ratio. (However, capillary density does not affect the supra-intensive $P_W^t$ value.) If $r_T = r_P$, $P_W^t(T) = 0.072P_W^t(P)$. Whatever the actual capillary radius change, if any, it seems certain that overall $P_W^t$ is much decreased in the tumor.

APPENDIX B

CALCULATION OF CAPILLARY TRANS-CELLULAR WATER FLUX

We pursue the significance of decreased MS lesion $k_{po}$ – decreased trans-endothelial water exchange. Consider lesion $K_{trans}^{\mu}$ values. The biomarker $K_{trans}^{\mu} = P_{CAn}^{\mu}S$, where $P_{CAn}^{\mu}$ is the endothelial CA permeability coefficient (76). The DCE-MRI enhancement of MS lesions is transient during disease progression, increasing and decreasing with time-constants of months (98,99), making them hard to “catch.” When measurable, however, the $K_{trans}^{\mu}$ values exhibit intra-lesion heterogeneity, and can reach magnitudes over $10^{-2}$ min$^{-1}$ (96,98,99). Thus, active MS lesions exhibit significantly increased $K_{trans}^{\mu}$ values, and these remain elevated above NWM values ($10^{-5}$ min$^{-1}$) for at least six months after maximum enhancement (98). Though the chronic Figure 4 lesions are no longer “active” in the clinical sense, their $K_{trans}^{\mu}$ values are still ten times normal (98). Since $v_b$ is decreased in the Figure 4 MS lesions, the $S$ quantity must be as well. Thus, $P_{CAn}^{\mu}$ must be significantly increased. There is little doubt that CA employs the para(endothelial)cellular pathway (Fig. 6(a)) for capillary extravasation: the endothelial cell junctions must open somewhat (36) in an MS lesion. The paracellular pathway must also constitute a component of the passive water permeability, $P_W^t$ (passive), contribution. Thus, it is highly likely that, in an MS lesion, $P_W^t$ (passive) is increased. However, we see that in the lesion overall $P_W^t$ is decreased ($k_{po}$ is decreased). The conclusion is that $P_W^t$ (active) is significantly decreased in an MS lesion.

Also, for monomeric Gd(III) chelate CAs, $P_{CAn}^{\mu}K_{trans}^{\mu}$ is greatly increased (four orders of magnitude) in GBM tumors (see Fig. 5, center) (75), mostly due to the $P_{CAn}^{\mu}$ factor (since the $v_b$ increase is less than one order of magnitude). There is little doubt that this is due to widened para(endothelial) cellular pores. Thus, paracellular water extravasation (a $P_W^t$ (passive) pathway) must also increase. However, this would make $k_{po}$ increase. Once again, we are left with the essentially inescapable conclusion that $P_W^t$ (active) is very much decreased in the brain pathology.

Previously, we calculated the equilibrium brain capillary water efflux in 1 μL tissue for an average capillary length and radius of 2 mm and 2.6 μm, respectively. For a cylinder, this gives a mean capillary volume (V) of 42.5 pL. A 50 M [H2O] yields $1.3 \times 10^{15}$ H2O molecules per capillary. For NMG, $k_{po}$ is 2.9 s$^{-1}$ (Table 1). This gives the equilibrium water efflux = $1.3 \times 10^{15} \times 2.9 = 3.8 \times 10^{15}$ H2O molecules s$^{-1}$ per capillary (and, of course, an equal influx). Extravasating CA molecules surely use the paracellular pathway (Fig. 6(a); for H2O). A typical maximum plasma CA concentration is 3 mM (100). The blood $[CA_{p,\text{max}}]$ = $(1 - h)[CA_{p,\text{max}}]$. A 0.4 hematocrit (h) yields $[CA_{p,\text{max}}] = 0.6 \times 3 = 1.8$ mM. Thus, at maximum, there are $1.8 \times 10^{-3} \times 42.5 \times 10^{-12} \times 6.0 \times 10^{23} = 4.6 \times 10^{10}$ CA molecules per capillary. The CA extravasation first-order rate constant ($k_{po}$) is $K_{trans}^{\mu}/v_b$ (31,32,43,47), also supra-intensive. A large $K_{trans}^{\mu}$ value, 0.1 min$^{-1}$, say for a GBM tumor capillary, and $v_b = (1 - h)v_b = 0.6 \times 0.03 = 0.02$, yields $k_{po}$ $= 8.3 \times 10^{-2}$ s$^{-1}$. This gives a maximum efflux = $4.6 \times 10^{10} \times 8.3 \times 10^{-2} = 3.8 \times 10^{8}$ CA molecules s$^{-1}$ per capillary. Thus the minimal H2O efflux/CA efflux ratio is 1 000 000. Even if 28 000 H2O molecules accompanied each CA molecule ([H2O]/[CAp,\text{max}]) through the paracellular tight junction pore (Fig. 6(a)), there would be 972 000 H2O molecules simultaneously exiting by transcellular pathways (Fig. 6(b)–(d)). Only 3% of water employs the paracellular pathway (Fig. 6(a)); 97% of equilibrium water flux is transcellular (Fig. 6(b)–(d)). This is for quite leaky capillaries: less permeable vessels would give an even greater transcellular percentage. (Though the $K_{trans}^{\mu}$ for head muscle tissue is greater, 0.15 min$^{-1}$ (96), $k_{po}$ may be more than 10 s$^{-1}$ (56). Consequently, even in that case less than 1% of the steady-state water flux is paracellular (Fig. 6(a))). Thus, for the normal brain ($K_{trans}^{\mu} \sim 10^{-2}$ min$^{-1}$) by far the vast majority of capillary water efflux occurs via one or more transcellular processes.