Understanding a role for hypoxia in lesion formation and location in the deep and periventricular white matter in small vessel disease and multiple sclerosis

Santiago Martinez Sosa & Kenneth J. Smith

Department of Neuroinflammation
And Queen Square MS Centre
UCL Institute of Neurology
Queen Square
London WC1N 3BG

Abstract
The deep and periventricular white matter is preferentially affected in several neurological disorders, including cerebral small vessel disease (SVD) and multiple sclerosis (MS), suggesting that common pathogenic mechanisms may be involved in this injury. Here we consider the potential pathogenic role of tissue hypoxia in lesion development, arising partly from the vascular anatomy of the affected white matter. Specifically, these regions are supplied by a sparse vasculature fed by long, narrow end-arteries/arterioles that are vulnerable to oxygen desaturation if perfusion is reduced (as in SVD, MS and diabetes), or if the surrounding tissue is hypoxic (as in MS, at least). The oxygen crisis is exacerbated by a local preponderance of veins, as these can become highly desaturated ‘sinks’ for oxygen that deplete it from surrounding tissues. Additional haemodynamic deficiencies, including sluggish flow and impaired vasomotor reactivity and vessel compliance, further exacerbate oxygen insufficiency. The cells most vulnerable to hypoxic damage, including oligodendrocytes, die first, resulting in demyelination. Indeed, in pre-clinical models, demyelination is prevented if adequate oxygenation is maintained by raising inspired oxygen concentrations. In agreement with this interpretation, there is a predilection of lesions for the anterior and occipital horns of the lateral ventricles, namely regions located at arterial watersheds, or border zones, known to be especially susceptible to hypoperfusion and hypoxia. Finally, mitochondrial dysfunction due to genetic causes, as occurs in leukodystrophies, or due to free radical damage, as occurs in MS, will compound any energy insufficiency resulting from hypoxia. Viewing lesion formation from the standpoint of tissue oxygenation not only reveals that lesion distribution is partly predictable, but may also inform new therapeutic strategies.

Summary statement (for broad audience)
The selective vulnerability of the parts of the brain to injury can give clues to pathological mechanisms. We explore the role of inadequate oxygenation and selective cell death arising from the vascular anatomy, and consider the consequent therapeutic opportunities.

Short Title
Importance of tissue hypoxia in causing demyelination

Key words
Introduction
The periventricular and deep white matter is preferentially affected in a range of neurological disorders, notably small vessel disease (SVD) and multiple sclerosis (MS), but also including some leukodystrophies, mitochondrial diseases, and diabetes. The fact that so many aetiologically distinct disorders cause lesions with similar topography suggests not only that these sites may be distinctly susceptible to insult, but also that lesion genesis may arise from shared mechanisms. Here we note the known vulnerability of the periventricular white matter to ischaemia, and focus attention more specifically on an inadequacy of the oxygen supply to this region as the mechanism responsible for the white matter lesions. We accept, of course, that ischaemia is a more profound insult than the hypoxia it causes, with an attendant reduction in the provision of glucose and other substrates, and a compromised removal of products such as carbon dioxide and lactate, and all these factors will contribute to the toxic milieu. Indeed, other factors such as inflammation-mediated nitric oxide and superoxide may also play a role in lesion formation in intensely inflammatory diseases such as MS. However, recent findings have revealed that even in intensely inflammatory lesions it is the paucity of adequate oxygen that is decisive in causing demyelination [1]. Indeed, the cells most vulnerable to the deleterious effects of tissue hypoxia are oligodendrocytes, which are selectively killed under hypoxaemic conditions, resulting in the observed pattern of demyelination. Notably, this demyelination can be prevented by breathing oxygen-supplemented ‘air’ (see below) [1]. This review focuses on the pivotal role of low oxygen (as distinct from ischaemia per se) in determining lesion formation and location, in the belief that this more precise focus may reveal the importance of particular types of therapy. We draw evidence mainly from observations in SVD and MS, as these disorders have been the focus of much attention, and indeed a contribution from SVD to MS has been discussed [2]. The mechanisms we describe do not, of course, provide a universal description of lesion formation, nor could they because cerebral SVD and MS involve a range of disease processes e.g. [3;4]. Moreover, we recognise that SVD and MS are separate pathological entities, and do not suggest that the origins of the diseases, or their clinical manifestations, are the same. Rather, we address the common involvement of the periventricular and deep white matter in both to highlight how a lack of adequate oxygenation may play a role in each disease. Focusing on hypoxia may illuminate a pathogenic mechanism that plays a more important role in neurological disease than is currently appreciated.

SVD and the tendency to periventricular lesions
Cerebral SVD has been defined as “all pathological processes that affect the small vessels of the brain”, thereby encompassing a number of underlying mechanisms including arteriosclerosis, amyloid angiopathy, inflammatory vasculitis, and inherited mutations, among others [5]. How SVD results in tissue damage is not well understood, but both ischaemic and haemorrhagic causes have been recognized [5], together with breakdown of the blood-brain barrier [6], and activation of innate immune mechanisms [7]. SVD lesions occur preferentially in sub-cortical structures, such as in the cerebellar and brainstem white matter, the basal ganglia and thalamus, and, notably, the deep and periventricular white matter [8]. These white matter lesions, which appear hyperintense on MRI (termed leukoaraiosis), are most consistently found in the frontal and occipital horns of the periventricular white matter [9], and their likelihood decreases with distance from the ventricles [10]. Notably, the distribution of periventricular leukoaraiosis is similar even when caused by different pathological SVD mechanisms, suggesting that injury to the white matter arises from a regional vulnerability [9;11].

MS and the tendency to periventricular lesions
The most detailed examinations of white matter lesion location have been performed in the MS brain. MS lesions are often described as having a semi-random distribution within the white matter, but in fact they have a clear predilection for certain areas, including the junction of the cortex and white matter [12], the spinal white matter tracts [13-15], the optic nerves [13;16;17], and, as with SVD, especially the periventricular white matter [12;18-20](figure 1a). Here we focus on the periventricular white matter as an exemplar which can reveal local cellular and vascular factors critical to lesion formation that may also be instrumental at other sites, but remain less obviously expressed.

**Why is the periventricular white matter vulnerable?**

The particular vulnerability of the dorsal periventricular white matter to lesion formation appears to be imposed by the anatomy of the arterial blood vessels, namely that the tissue is supplied by an array of long, narrow, end arteries/arterioles [12;21-23]. Each of these features, coupled with the fact that this region of the brain is poorly vascularized (figure 2a &b) [24], renders the integrity of the local oxygen supply precarious. The arterioles are unusually long at approximately five centimetres in length, penetrating from the pial surface through the brain tissue to their periventricular destination (figure 2c). Moreover, they have a very narrow diameter of only 100-200μm, even at their origins, making it ‘difficult’ for viscous blood to pass along the vessels: indeed, Poiseuille’s law teaches that flow varies with the fourth power of the radius, and is thus substantially reduced by the diameter of narrow vessels. The motive power for flow is provided by the gradient in blood pressure from the proximal to distal parts of the long vessels, so hypotension affecting cerebral arteries will disproportionately reduce perfusion and oxygenation of periventricular tissue as compared with other brain regions. Furthermore, the periventricular arterioles are end-arterioles that independently supply particular fields, imposing a limited capacity for compensatory collateral replacement if oxygen supply is impaired. Moreover, with age, the penetrating arterioles can also become tortuous (reviewed in [25]). Not only will this impose a higher imperative on maintaining blood pressure to preserve adequate perfusion [26], but it will also increase the opportunity for oxygen desaturation by impeding and slowing flow, thereby providing more time and surface area over which oxygen can be lost.

However, perhaps the greatest problem associated with long, narrow arteries/arterioles is one that is less appreciated, namely that these are prone to substantial losses of oxygen through the arterial wall [27-32], a phenomenon that has been clearly illustrated in experiments exploring the consequences of inspiratory hypoxia on cerebral mitochondrial function [33;34] (figure 3). Figure 3 shows how oxygen diffuses from arteries to sustain mitochondrial oxidative phosphorylation in the surrounding tissues (figure 3A &B), and the precise superimposition of mitochondrial FAD fluorescence with mitochondrial polarisation (figure 3B). These observations have been made in rodent cerebral arteries, and while rodent vessel walls are arguably thinner and presumably more permeable to oxygen than human arteries, this consideration must be balanced against the fact that human vessels are very much longer, permitting greater opportunity for oxygen depletion. This opportunity is potentiated if surrounding brain tissue is hypoxic, as this steepens the gradient for oxygen diffusion from vasculature to tissue. In health, diffusion gradients are low in the well-oxygenated brain, and oxygen loss is consequently small, but the MS brain is characterized by widespread tissue hypoxia (see below) and so the risk of significant blood de-oxygenation is much enhanced. In fact, this risk is greater still because the MS brain is also marked by a global slowing of cerebral blood flow (see below), which allows more time for de-oxygenation to occur.
For the considerations above, the risk of detrimental blood deoxygenation is substantial, and this problem will typically be at its most severe within veins, which are plentiful in the periventricular white matter. Different venules and veins have a heterogeneous oxygen concentration so that Vovenko, for example, found “A distinct difference in colour between separate streams of blood, the result of confluence of flows from small proximal venules, could be seen with the microscope.”, but despite this heterogeneity, “…all studied venules released oxygen to the tissue under normoxic conditions.” [30]. Thus, notably, veins normally act to supply oxygen to surrounding brain tissue [29;30;35], and this tissue is presumably dependent on the venous provision. An indication that veins provide oxygen to tissue can be seen in figure 3b (arrows) in which the vein to the left is surrounded by a narrow halo. Of course the halo is narrower than that around arteries, because the venous concentration of oxygen is less than that in arteries, but, nonetheless, venular oxygen is sustaining function in the mitochondria of the surrounding cells. However, rather than functioning in such a supply capacity, veins containing highly de-oxygenated blood become sinks for oxygen [29], draining it from surrounding oligodendrocytes and axons and rendering these even more hypoxic. These considerations can easily explain the well-known predilection for MS lesions to form around veins [13;36-43], now demonstrated in living patients [44-48]. Whereas early neuropathologists have understandably supposed that ‘something bad leaks from veins’ to induce perivenular demyelination (see [49]), a more complete explanation includes the fact that something good, oxygen, can fail to leak from veins.

Thus, a combination of deleterious conditions renders the periventricular white matter at severe risk of suffering hypoxia, and this risk will be exacerbated if other co-incident factors exist that promote it, such as infection, anaemia, sleep apnoea, low atmospheric oxygen pressures or increased altitude.

The influence of vascular watersheds
In addition to the vulnerability of the periventricular white matter in general, there is a specific vulnerability of the white matter at the anterior and occipital horns of the ventricles [18]. Indeed, 90% or more of MS patients have a lesion pinpointed at these specific locations (i.e. a lesion here is almost a certainty) establishing that lesion distribution is partly predictable. In fact, the tendency of lesions to form precisely at the horns is as remarkable as the zero percent chance of a lesion in the immediately adjacent tissue: heat maps of lesion location reveal that, out of 1249 lesions, none occur at that location (figure 1b) [18]. The particular vulnerability of the anterior and posterior horns appears to arise due to the superimposition of another inherent attribute of their vascular anatomy, namely that these locations are at the very ends of three arterial territories, at the watershed (border zone) formed by the confluence of the anterior and middle cerebral arteries (ventriculopetal branches) and the lenticulostrate arteries (figure 1) [50;51]. This correlation between lesion location and vascular watersheds has been noted previously [12;18;19;22;23], and the concept may explain the predilection of MS lesions to form at other watersheds, including the optic nerves [17];[52;53], spinal cord [15;54-56], and between the various cerebral arteries [12;57]. Unsurprisingly, watersheds are distinguished by their susceptibility to hypoxia, and blood flow in cerebral border zones in healthy individuals is indeed significantly (p<0.001) lower than in non-border zone regions, with longer arterial transit times [57;58] (allowing more time for oxygen loss). Accordingly, infarcts form selectively at watershed sites if global perfusion is impaired [59].
When considering heat map distributions of lesions (figure 1) it is important to note that these maps may omit less severe lesions, or lesions effectively rendered invisible due to repair by remyelination. Thus, Holland and colleagues [18] note that lesions are distributed more widely in early relapsing–remitting MS, but that they may persist in poorly perfused regions. In this way, the periventricular lesions shown on the maps may represent lesions that, due to their location, are either less well repaired, or rendered more severe with persistent damage. Accordingly, although remyelination can be extensive in MS, it occurs less commonly in the periventricular white matter [60]. Failure of remyelination may reflect the fact that the oligodendrocyte precursor cells required for remyelination can be especially vulnerable to hypoxia [61], or that repair mechanisms are ‘worn out’ by repeated challenges. Experimental evidence against the latter possibility has been provided [62], but here the repeated demyelinating challenge was not inflammatory (it was effected by ethidium bromide) and so the number of precursor cells would probably not be depleted.

The vulnerability of oligodendrocytes in watershed tissue is well illustrated by the demyelinating Pattern III lesion induced by the direct intraspinal injection of lipopolysaccharide in rodents [63-65]. Interestingly, this lesion selectively forms not at the site of lipopolysaccharide injection, but rather at a neighbouring vascular watershed in the spinal cord [1]. In contrast, demyelinating agents that are directly myelinolytic or oligodendrocyte-toxic form lesions centred on the site of injection [66-68], as expected. The shift in location from the site of injection to the watershed, although subtle in terms of distance, reveals the vulnerability of the oligodendrocytes to hypoxia: it is not the lipopolysaccharide that kills the oligodendrocytes, but rather the tissue hypoxia which forms in response to the inflammation, and this is most severe within the watershed tissue. In the rodent spinal cord, this watershed lies at the termination of three arterial supplies (two dorsal and one ventral artery) [69;70], and the human anatomy is closely similar. It is therefore unsurprising that, in humans, systemic hypoperfusion also produces lesions due to spinovascular insufficiency in the same “endangered frontier zone” or watershed tissue [71]. This region of the dorsal columns also shows a relative paucity of blood vessels [1].

**Patchy distribution of oxygen in normal brain due to vessel density & configuration**

It is notable that the oxygen concentration is far from homogeneous across even the normal healthy brain. Instead, there exist steep gradients between vessels [72-74], such that tissues most proximal to arteries are marked by high oxygen levels [75], while niches between adjacent capillaries can fall to near anoxia [35;73;75;76]. The likelihood of such niches increases with reduced vascular density, and the periventricular white matter is inherently poorly vascularised [24]. Indeed, oligodendrocytes located only 35–40µm from a capillary become much more hypoxic than more proximal cells, and this vulnerability is easily exposed in animals breathing 10% oxygen [1]. Moreover, this vulnerability will increase in circumstances where the arterial/arteriolar blood is partly desaturated on its journey, as discussed above. The consequences of such desaturation will be exacerbated in some vessels by the fact that at bifurcations the red cells preferentially enter the branch with the higher flow (phase separation and Fahraeus effects), so that after multiple bifurcations the vessel haematocrit can be quite heterogeneously distributed across the tissue, and some vessels are poorly provided with red cells [77;78]. The vessel haematocrit is also dynamic, because it will change as vessels change their diameter to satisfy local electrical (and other) demand. Capillary dysfunction and the associated disruption of capillary flow patterns also limits the extraction efficacy of oxygen [79], further contributing to tissue oxygen insufficiency. The resulting mosaic distribution of oxygen concentrations even in the normal brain renders particular cells and micro-niches inherently susceptible to hypoxia, i.e. they habitually live
near a ‘cliff edge’, and the superimposition of additional deleterious factors can topple cells over the edge into fatally low oxygen levels. The most vulnerable cells will die first, principally the oligodendrocytes (see below). In accord with these considerations, larger MS lesions tend to be further from blood vessels large enough to be detectable by MRI, and the vessels nearest to the lesions tend to be of smaller diameter, consistent with the lesions having a hypoxic aetiology [80].

An interesting consideration is that veins are fed by blood from different capillary territories and if some of these are less affected by hypoxia the veins to which they drain can have a higher oxygen concentration than the tissue through which they pass. In this case they will instead supply oxygen to the hypoxic tissue (as normal), providing an explanation for why Pattern III demyelinating lesions in MS often contain a thin rim of preserved myelination around veins despite demyelination beyond that border [4;81].

Vessels in both SVD and MS can exhibit a widened perivascular space, which can contain inflammatory cells. Both these features will exacerbate the hypoxia experienced by the adjacent white matter. First, the widened space may partly be at the expense of the diameter of the lumen, which will clearly impair flow. Additionally, the presence of the perivascular fluid imposes an impediment to oxygen diffusion from the blood to the tissue. The additional presence of perivascular cells (as occurs in SVD and the ‘perivascular cuff’ of MS) will further impede oxygen diffusion. Moreover, these cells may also consume oxygen themselves, reducing supply to adjacent tissues.

In summary, several factors render particular brain regions severely and selectively vulnerable to hypoxia. The periventricular white matter exemplifies this: it is inherently poorly vascularized, and its limited vascular supply consists of long, fine, end-arterioles that are vulnerable to loss of oxygen by diffusion through vessel walls, particularly if the brain is hypoxic and vascular flow is slow. The paucity of anastomoses between local arterioles exacerbates this by rendering their distal irrigation fields at increased risk of inadequate perfusion. Moreover, portions of the periventricular white matter are located at poorly-perfused, ‘at risk’ watershed regions at the boundaries of converging arterial territories, which increases the probability of inadequate oxygenation. The veins will contain particularly de-oxygenated blood, and cells and tissue dependent on venular oxygen will therefore become severely hypoxic, resulting in selective oligodendrocyte death (see below) and demyelination. More severe hypoxia will cause axonal degeneration.

**Haemodynamic deficiencies imposed by SVD & MS**

The vulnerabilities to inadequate tissue oxygenation imposed by the brain’s inherent vascular architecture may lie dormant for the lifetime of an individual, but they are likely to be exposed from time to time by adverse events, such as infections and other disease, or simply by age-related pathology, like SVD, that have deleterious haemodynamic consequences. Here we explore the extent of haemodynamic alterations present in SVD and MS, like hypoperfusion and vasomotor reactivity, in order to draw attention to their possible contributions to lesion genesis.

**Haemodynamic consequences of SVD**

Arteriosclerosis of cerebral vessels (Type 1 SVD) [5] causes partial occlusion of the vessel lumen, and the resulting hypoperfusion corresponds with the severity of ischaemic damage in deep and “irregular” periventricular lesions [82], suggesting that reduced tissue perfusion (and by extension reduced oxygenation), may be pathogenic in the white matter lesions of
SVD. This point has been examined by Shi and colleagues, who systematically reviewed 34 cross-sectional and 4 longitudinal studies regarding cerebral blood flow in SVD to clarify any cause-and-effect relationship. Meta-analysis of 24 cross-sectional studies revealed that higher burdens of white matter hyperintensities (WMH) correlate with lower cerebral blood flow, globally and in most grey and white matter regions [83]. Evidently, there is a close association between global hypoperfusion and white matter damage, but our understanding of the cause and effect relationship of these phenomena remains incomplete. This review focuses on the mechanisms by which falling blood flow can precipitate white matter damage by impairing its oxygen delivery. These mechanisms necessitate declining blood flow to precede white matter damage, as indeed occurs in the periventricular white matter [84]. Here, reductions in global cerebral blood flow over time correlate with the progression of periventricular WMH loads [84]. Intriguingly, the same is not true of the deep white matter, where declining global blood flow does not impact the risk of lesion development [84]. This discrepancy suggests that these regions are differentially predisposed to lesion formation by insufficient blood flow. This interpretation is supported by analysis of regional (rather than global) blood flow. In patients with ischaemic leukoaraiosis, reductions to cerebral blood flow occur preferentially in the periventricular normal appearing white matter, remaining normal in the grey matter and deep normal appearing white matter [85]. This regional vulnerability is not surprising given the unique vascular architecture of the periventricular white matter (outlined above). Moreover, just as falling blood flow can precipitate white matter damage by impairing its oxygen supply, so too may vascular deficits arise as a result of WM damage, given that damaged tissue is likely to be non-functional and therefore have reduced oxygen demands. Once white matter becomes damaged by an initial perfusion deficit, further vascular compromise might ensue, exacerbating the original insult.

An early precursor to arteriosclerosis, endothelial dysfunction also predisposes to haemodynamic alterations in the form of aberrant vasomotor reactivity, an important autoregulatory haemodynamic control mechanism. Indeed, the degree of endothelial dysfunction in patients with SVD is associated with the severity of WMH loads and cerebral microbleeds [86]. Specifically, endothelial-mediated vasodilation correlates inversely with WMH volume, suggesting that endothelial dysfunction, and therefore altered vascular autoregulation, may be pathogenic in cerebral SVD [87]. In agreement, cerebral vasomotor reactivity is inversely associated with total white matter lesion loads, and, unsurprisingly, periventricular lesions exhibit the strongest correlations [88;89]. In fact, in some SVD patients impaired vasomotor reactivity (i.e. impaired dynamic control of blood flow) may be a better predictor of disease severity than resting cerebral blood flow [89].

Venous collagenosis, the type 5 SVD characterised by veins and venules with pathologically thickened walls [5], will also impair local haemodynamics. These stenotic venules, like their sclerosed arteriolar counterparts, will impair flow. Thus, arteriolar and venular pathology may synergise in promoting the conditions for tissue hypoxia. Indeed, the extent of venous collagenosis correlates with the severity of leukoaraiosis, suggesting venous disease (stenosis/occlusion) participates in white matter pathology [90]. In confluent periventricular lesions (caps and halos), “large tortuous venules, but not arteriolar sclerotic” changes are observed, and myelin loss is most severe around such venous structures [82].

In SVD the vascular and white matter pathology progress hand in hand over decades and it is therefore difficult to discern which pathology is primary, and which secondary. The sequence of events may therefore be easier to determine in MS, where the genesis of individual lesions occurs over a shorter time scale, and in younger patients.
**Haemodynamic consequences of MS**

The MS brain is affected by a number of haemodynamic disturbances that will tend to expose any inherent vulnerabilities to tissue hypoxia. Most importantly, a broad consensus has arisen that there is a widespread reduction in cerebral blood flow in MS (reviewed in [91;92]), although this view has been challenged [93] and some reports describe increases in white matter flow alongside reductions in the grey matter [94;95]. Nonetheless, the current balance of evidence favours a significant reduction in flow in both the grey and white matter in relapsing-remitting [20:96-104] and primary-progressive MS [102;103;105], as well as a disability-associated reduction in the thalamus [106] see also [107]. A significant reduction in cortical blood flow in the absence of structural differences has also been reported in cognitively impaired people with MS [108;109].

Of particular interest are observations on blood flow before disease is advanced in MS, such as in patients with clinically isolated syndrome and in normal appearing white matter, where clues may be found concerning factors contributing to the initiation or advance of the disease. For example, decreased perfusion has been reported in both the corpus callosum [110], normal appearing white matter [96;100], and deep grey matter [96] in clinically isolated syndrome, suggesting that reduced perfusion is a primary event in disease development, rather than a secondary response to reduced tissue demand [91;110] (see also [111]). The reduced flow in the normal appearing white matter and deep grey matter occurs in conjunction with increased blood volume and mean transit time, when compared with normal, or patients with relapsing remitting MS [96]. Increased blood volume is also observed in patients with clinically isolated syndrome and impaired executive function [112].

Within the lesions themselves the haemodynamic picture is more complex. Wuerfel and colleagues studied changes in perfusion at different stages of lesion development, including before the breakdown of the blood-brain barrier [113]. They found a significant increase in cerebral blood flow and blood volume both three weeks before, and during, the period of gadolinium leakage, and these measurements remained above baseline for the next several weeks. Other studies have also noted enhanced perfusion in active lesions in which the blood-brain barrier is disturbed [99;114], as well as in ring enhancing lesions, where the increase is restricted to the lesion circumference [113]. The inclusion of active lesion data may explain the increased perfusion in studies of MS white matter mentioned above [94;95], and must be considered when interpreting global or regional measures of blood flow. Most non-enhancing or chronic lesions show significantly reduced perfusion and blood volume [99;114]. Indeed, two of the lesions followed by Wuerfel et al. also developed reduced perfusion after several additional weeks [113], probably in response to tissue destruction during the inflamed period.

Hypoperfusion occurs in conjunction with a slowing of flow, resulting in substantially prolonged cerebral circulation times [99] (but see also [103;113]). In one study, the mean time in MS patients was 4.9 seconds (s.d. = 1.27 secs) versus 2.8 seconds (s.d. = 0.51 secs) in controls [115], and in another, the median longest time in MS was 6.47 seconds (range, 3.29-29.24 seconds) versus 5.54 seconds in controls (range, 2.57-7.63 seconds; p <0.001) [116]. In particular, it is the arterial flow which is slow, with prolonged arterial bolus arrival times [94;117] even in the presence of normal venous flow [117]. Slow arterial flow may be the result of raised circulating concentrations of the potent vasoconstrictive agent endothelin-1 [118-122], which is raised in the cerebrospinal fluid of relapsing-remitting patients suffering acute clinical attacks, in comparison with those in a stable phase [123]. Lower than normal
endothelin-1 concentrations have been reported in MS patients with non-active disease [124]. Additionally, arteries in young people with MS have been found to have significantly less compliance (the ability to expand and recoil with pulsations) than normal [125], a deleterious phenomenon that also occurs in small vessel disease [126], diabetes, and hypertension, and which may help to explain the significant reductions in cerebrovascular reactivity observed in MS [127;128]. In MS, reduced flow may also be due to deficits in astrocytic β2-adrenoceptors, which may impair astrocytic regulation of vessel tone and result in a chronically vasoconstricted state [119].

Several of the abnormalities reported in the brains of people with MS are also present in the retina, where it is possible to view blood flow directly, together with the dimensions of the vessels. Here, reductions in blood flow volume and velocity have been observed in both retinal arteries and veins [129;130]. Most interestingly, even patients with no history of optic neuritis exhibit retinal arteries that are significantly thinner than normal, as well as significantly thicker venules [131] (see also [44]). Such arterial thinning, perhaps due to constriction, coupled with venular widening, is consistent with many of the vascular observations of MS. Perivenous sheathing has also been observed in optic neuritis [132], in a cohort where more than half the patients subsequently developed MS. Given that the retina is devoid of myelin, the authors revived the possibility that venous pathology may be primary in MS (developing observations first made over 150 years ago [37]).

In summary, a pattern emerges in MS of an overall reduction in cerebral blood flow, which nonetheless shows a local and temporary increase during lesion formation in response to local inflammation. One interpretation is that the overall reduction in flow predisposes particular niches to hypoxia, and that the subsequent superimposition of additional impairments results in the focal degeneration of oligodendrocytes, consequent demyelination and generation of myelin breakdown products. An inflammatory response, perhaps augmented by autoreactive lymphocytes, results in a temporary hyperaemia, which later resolves to a reduction in blood flow in response to tissue destruction.

Other factors contributing to hypoxic damage
In addition to aberrant haemodynamics, tissue hypoxia is also promoted by several other factors. Inflammation, for example, can augment oxygen demand, reduce its supply, or raise the oxygen concentration threshold required for continued oxidative phosphorylation. Oxygen demand is increased by the recruitment of lymphocytes, which, unlike myeloid cells, primarily use oxidative phosphorylation for energy production (reviewed in [133]). Furthermore, substantial amounts of oxygen are consumed by the respiratory burst of activated neutrophils and microglia [134;135]. Inflammation can also promote dysfunctional vasoconstriction and leukocyte-capillary plugging, reducing capillary perfusion and tissue oxygenation [136]. Cells in inflammatory lesions additionally produce reactive oxygen and nitrogen species that are deleterious to normal mitochondrial function [137-141]. In particular, the prominent expression of NADPH oxidase and the inducible form of nitric oxide synthase (iNOS) can produce large quantities of the damaging free radicals superoxide and nitric oxide, and oxidative damage is prominent in MS lesions [139-141]. Additionally, nitric oxide competes with oxygen for the same binding site on mitochondrial cytochrome-c oxidase [142-147] thereby raising the Km for oxygen such that even normal oxygen concentrations become inadequate to maintain mitochondrial function. Thus, nitric oxide can create a condition of ‘virtual hypoxia’ [148] that exacerbates the harmful effects of actual hypoxia on cell (e.g. oligodendrocyte) survival, even under conditions where either problem can be tolerated alone [149]. Widespread occurrences of virtual hypoxia are therefore one...
explanation for reduced oxygen utilisation of the MS brain [150]. In fact, the enhanced production of nitric oxide has been implicated in the pathogenesis of several demyelinating and neurodegenerative diseases [1;64;65;151-156].

**Evidence that the MS brain is indeed hypoxic**

There is substantial evidence that the MS brain is not only vulnerable to hypoxia, as described above, but that it is actually hypoxic. Histological evidence of hypoxia includes the expression of hypoxia-related antigens [157-161], and the presence of lesions characterized by a Pattern III (hypoxia-like) type of demyelination. This pattern of demyelination is similar to that found surrounding acute ischaemic lesions, and characterized by the preferential loss of myelin associated glycoprotein [81;162]. Furthermore, microarray analysis of normal appearing white matter from MS brains reveals the upregulation of genes induced by ischaemic preconditioning, including the upregulation of hypoxia inducible factor-1α and genes involved in vascular remodelling such as vascular endothelium derived growth factor (VEGF) receptor 1 [163;164]. More indirect evidence for hypoxia in MS includes a raised concentration of lactate within acute lesions, as assessed by magnetic resonance spectroscopy e.g. [165], and in cerebrospinal fluid [166;167]. Raised lactate suggests that insufficient oxygenation requires increased glycolysis. Most convincingly, Yang and Dunn used frequency domain near-infrared spectroscopy to measure microvascular haemoglobin saturation in the brains of awake MS patients. They found a significant relationship between saturation and clinical disability, and that 42% of oxygen saturation values were more than two times the standard deviation lower than the control mean [168].

**Vulnerability of oligodendrocytes to hypoxia**

Selective white matter damage is a common consequence of hypoxia [169-171], ischaemia [172-174], and inhibitors of mitochondrial oxidative phosphorylation [175-190]. Surviving energy limitation due to tissue hypoxia is a challenge for many cells, but in the white matter the major cell types faced with the problem are the axons, oligodendrocytes, astrocytes, microglia and endothelial cells. At first thought, the most vulnerable components may be suspected to be the axons, but in fact Desai et al. [1] found that sustained hypoxia in an inflammatory environment selectively kills oligodendrocytes, resulting in primary Pattern III, hypoxia-like demyelination, as occurs in MS [4;81] (see also [191]). Although the inflammatory environment included several toxic mediators such as superoxide and nitric oxide, the authors found that it was the hypoxia that was decisive in causing the demyelination: treatment with 80% oxygen during the 24 hours when the lesion was otherwise hypoxic either reduced the size of the lesion, or, in some cases, prevented the lesion from forming at all (figure 4) [1]. Perhaps oligodendrocytes succumb to hypoxia in preference to axons because they reside wholly within a hypoxic focus, whereas axons can be sustained by portions of their structure that lie in more hospitable locations. Axons may also survive if they are selflessly sustained by the energy provided by oligodendrocytes [192-199] to their own fatal detriment. Aside from providing metabolic support for axons, oligodendrocytes are also stressed by the substantial metabolic load of having to maintain numerous internodes of myelin [189;200]. Oligodendrocytes may also suffer, even at the earliest stages of demyelination, from some of the mitochondrial impairments that have been described in established multiple sclerosis lesions [201-205]. The special vulnerability of oligodendrocytes to hypoxic and metabolic stress is also illustrated by the fact that carbon monoxide poisoning, which systemically impairs both oxygen delivery (by reacting with haemoglobin and inducing hypotension) and mitochondrial function, selectively kills oligodendrocytes, resulting in Grinker’s myelinopathy [184;206] a week or more after exposure. Astrocytes are less vulnerable to hypoxia because they have a robust capability for
anaerobic glycolysis [207;208], and, together with microglia, do not consume as much oxygen as oligodendrocytes in hypoxic tissue [1]. Endothelial cells face less of a challenge due to their proximity to blood.

Perhaps the simplest explanation for the susceptibility of oligodendrocytes to hypoxia is that their mitochondria are unable to maintain sufficient oxidative phosphorylation and ATP production under such conditions, but this possibility is undermined by the observation that adult myelinating oligodendrocytes can survive even when their normal mitochondrial function is impaired, at least if this change occurs progressively over time [193]. Furthermore, adult human oligodendrocytes maintained in vitro are able to use aerobic glycolysis for the majority of their ATP production, an adaptation which should protect them from the deleterious effects of hypoxia [209]. Interestingly, in vitro observations suggest that oligodendrocytes are actually more vulnerable to a slowly developing hypoxia, as will occur in disease, than they are to anoxia [61]. This, and evidence of free radical-mediated lipid peroxidation [61], suggests that oxidative damage, more than failure of aerobic respiration, is the lethal underlying mechanism. Alternatively, oligodendrocytes might also perish under hypoxic conditions following lethal H⁺-gated increases in intracellular calcium via TRPA1 channels [210]. Oligodendrocytes are therefore particularly vulnerable to succumb to any of several factors [211], especially oxidative stress, and this vulnerability is attributable to their poor capacity to scavenge free radicals effectively and their radical-promoting large iron stores [212-218]. At first glance, it may appear surprising that oxidative stress should be a problem under hypoxic conditions, but oxidative damage can indeed result from hypoxic conditions [219], as well as from the more commonly recognized hyperoxic ones.

The very common detection of MS lesions in the frontal and occipital horns of the lateral ventricles [18] could, at least in part, indicate that lesions at these locations fail to repair as well on MRI examination as lesions at other locations [18;20]. If so, the failure could be due to the especially high vulnerability to hypoxia of oligodendrocyte precursor cells [61;209]. The demise of such cells at hypoxic foci would result in a shortage that would impede the repair of demyelination by remyelination.

**Pattern III Demyelination**

Different types, or patterns, of demyelination have been distinguished in MS lesions [4]. The type that characterizes the demyelination found in lesions described by different authors as the “initial”, “primary”, “primordial” or “prephagocytic” lesion [4;49;220-222] is the Pattern III type, also described as hypoxia-like demyelination [81]. Notably, Pattern III lesions form in the relative absence of T cells [49;220-225] although these cells are a prominent feature of autoimmune lesions, including those of the most commonly used animal model of MS, experimental autoimmune encephalomyelitis (EAE) [226]. An explanation for the lack of T cells may be found in the selective vulnerability of oligodendrocytes to hypoxia, and the widespread patchy distribution of hypoxia in the MS brain. Thus rather than succumb to autoimmune attack, oligodendrocytes in these lesions may die due to insufficient oxygen. This death and ensuing demyelination may incite the later accumulation of T cells observed after the original insult [221;225;227]. Indeed, the demyelination may trigger an autoimmune response against myelin [228]. Thus the importance of hypoxia in causing demyelination in MS may currently be underestimated. Large demyelinating lesions that are not centred on a vessel, such as Pattern III lesions [4], may be caused by hypoxia due to arterial inadequacy, whereas perivenular demyelination may be caused by hypoxia due to excessive venular oxygen desaturation.
It will be apparent from the observations in this review that the ingredients required to form Pattern III demyelinating lesions can arise without the participation of acquired, autoreactive immune cells, such as T or B cells. Whether Pattern III demyelinating lesions start to form in this way in MS, or whether the initial participation of acquired immune cells is essential in this disease, currently remains ambiguous, with observations favouring both interpretations. With either interpretation it seems likely that tissue hypoxia, promoted by the mechanisms outlined in this review, will play a key role in amplifying demyelination (and probably degeneration), whether or not it is responsible for initiating demyelination.

A diagram summarising how different pathophysiological events can conspire to cause tissue hypoxia, and thereby demyelination, degeneration and loss of function, is shown in figure 5.

**Hyperbaric oxygen therapy in MS**
The realisation (above) that the inflamed CNS can be quite profoundly hypoxic, and that breathing raised oxygen concentrations can restore function [229] and prevent demyelination [1], raises the long-running and controversial issue of the value of hyperbaric oxygen (HBO) as a therapy in MS. The therapy is conducted in pressure chambers, which may house several patients at a time, in which oxygen is breathed at raised pressure (e.g. 2 atmospheres), often for one hour. The therapy is favoured mainly by patients, but usually treated with scepticism by clinicians. The patients, who often pay for regular (e.g. weekly) sessions (“dives”), base their opinion on the benefit they experience in the days following a dive, often commenting on acute, temporary improvements in fatigue and bladder function in particular. Clinicians base their scepticism on a number of clinical trials, mainly conducted in the 1980s and now the subject of a Cochrane review [230;231] which noted “For example, the mean Expanded Disability Status Scale (EDSS) at 12 months was improved in the HBOT group (group mean reduction in EDSS compared to sham -0.85 of a point, 95% confidence interval -1.28 to -0.42, P = 0.0001).” but which concluded “At this time, the routine treatment of MS with [HBO] is not recommended.”, also commenting that the beneficial effects were “difficult to ascribe with biological plausibility”: in view of more recent knowledge, this opinion could be revised.

The former clinical trials were mostly not designed to detect acute, temporary changes in symptoms, which may account for the different opinions of the efficacy of HBO between patients and clinicians. From the perspective of current knowledge it is important to note that the clinical trials treated patients on an arbitrary timetable, rather than when the patients may have required oxygen to reverse ongoing hypoxia, and the trials also tended to include patients with long-standing disease, where marked improvements in response to therapy are perhaps less likely. These considerations will have reduced the likelihood of uncovering positive therapeutic effects, at least with regard to the recent findings of acute hypoxia at the onset of new inflammatory and demyelinating lesions. The new understanding is that it is patients with ongoing inflammatory lesions, such as occurs with relapsing remitting MS, who are most likely to benefit from oxygen, because it is these lesions that are likely to suffer from inadequate oxygenation.

Whether oxygen at hyperbaric pressure is better, or worse, than, say, breathing 70% oxygen at normobaric pressure, remains untested, and theoretically uncertain.

**Regional Hypoxic Vulnerability in Other Diseases**
Notably, the periventricular white matter is vulnerable not only in SVD and MS, but also in a range of other disorders where impaired blood flow, energy compromise, or frank hypoxia
are implicated [11]. Despite the varying aetiology and pathophysiological mechanisms involved, the similar anatomical involvement of the periventricular white matter suggests an inherent vulnerability of both this tissue, and the cells involved.

In type 2 diabetes, reduced blood flow velocity in the middle cerebral artery, increased cerebrovascular resistance, and impaired vascular reactivity are significantly related to the volume of WMH on MRI [232]. The additional co-morbidity of hypertension worsens white matter damage in diabetes, and both conditions are associated with decreased retinal arterial diameter, suggesting reduced oxygen supply is a feature of both [233]. Notably, treatment of hypertension prevents the progression of white matter damage [234]. Abnormal blood flow (perhaps with a dysfunction of neurovascular coupling [235]), is also an important feature of migraine [236;237], and, again, migraine-associated lesions are prominent in the periventricular white matter [238;239].

The leukodystrophy Krabbe’s disease is associated with a psychosine-mediated inhibition of mitochondrial complex IV [240-242] as well as extensive white matter pathology on MRI [243-245], and spectroscopic evidence of axonal degeneration and myelin loss [244]. Periventricular white matter lesions are also prominent in several disorders associated with mitochondrial impairment. Leber’s hereditary optic neuropathy (LHON) is caused by a mitochondrial mutation [246], and the distribution of the lesions is similar to that in MS, with a preponderance in the periventricular white matter [247].

Given the evidence presented above, an interesting consideration is whether exposure to hypoxia might induce white matter lesions in healthy and physically fit people, such as mountaineers. Remarkably, climbing to over 7000 metres without supplemental oxygen is indeed associated with the presence of periventricular WMH [248], with new lesions emerging near the posterior horns of the lateral ventricles [249] or at other metabolically-demanding watershed zones [250]. People who normally reside at high altitude have adaptations that limit their vulnerability to hypoxia.

**Therapeutic implications**
The observations discussed in this review suggest that parts of the periventricular white matter habitually rely on an oxygen supply that is only marginally higher than required to sustain normal function. It follows that reductions in oxygen supply, or increases in demand, can erode this slim safety margin, rendering the tissue at risk of inadequate oxygenation. If, and when, this happens the most vulnerable cells, the oligodendrocytes, will die first. To prevent hypoxia, and thereby protect against the ensuing demyelination, imbalances between oxygen supply and demand must be avoided. For a chronic condition like SVD, which can continue for decades in people who may show few if any symptoms, the most realistic therapeutic approach may be heightened vigilance to guard against conditions which impair oxygen delivery to the brain. In MS, affected individuals are more easily distinguished, and vigilance to avoid hypoxia can be combined with existing therapies.

This vigilance may include greater attention to stringently maintaining haemoglobin levels within the normal range, and perhaps the upper part of the normal range, as haemoglobin is reported to be significantly decreased in patients with MS [251], and anaemia more than doubles the risk of developing MS [252], and the risk of relapse [253] (see also [254]). In this context, it is especially interesting that erythropoietin (which most notably boosts red cell production amongst a range of effects) improved outcome in both a pilot study in progressive MS, and a phase 2 trial in optic neuritis [255-257]. Common cardiovascular disorders, such
as occur in obesity and diabetes, should also be combated to avoid vasculopathy and cerebral ischaemia, especially noting the higher prevalence of insulin resistance in MS patients than in healthy controls, and the association of both insulin resistance and adiposity with disability in the disease [258]. In this respect, it is noteworthy that the protective effect of simvastatin [259] in secondary progressive MS may be due in part to its suspected effects in counteracting vascular pathology. The mechanism(s) underlying the beneficial effects of high dose biotin therapy in progressive MS [260] remain uncertain, but an enhancement of oxidative mitochondrial metabolism has been suggested [261].

Patients might also benefit from close management of their arterial oxygen saturations, and more aggressive treatment of any underlying respiratory conditions, such as chronic obstructive pulmonary disease. Lower arterial oxygen saturation, independent of confounders, correlates with more severe periventricular white matter lesions in non-demented patients, as does co-incident chronic obstructive pulmonary disease [262]. Similarly, infections, especially of the chest, should be promptly and effectively managed, as these can both impair normal gas exchange and promote the production of systemic pro-inflammatory cytokines that might contribute to the pathological processes of SVD and MS. Advice to cease cigarette smoking may be especially worthwhile, as smoking is associated with the progression of both SVD [79;263] and MS [264;265]. Although the detrimental effects of smoking are complex and multifactorial, it is readily evident how pulmonary, cardiac, vascular and haematological components (e.g. enhanced carbon monoxide levels) can act to impair tissue oxygenation.

While hypertension is a major risk factor for the progression of MS [266] and SVD [263], its counterpart, hypotension, is an important and often overlooked component of cerebral dysfunction that will nevertheless contribute to poor perfusion and hypoxia. Decreases in diastolic pressure over time (as well as increases) double the risk of severe periventricular lesions [267]. Despite the classical notion that cerebral autoregulation is able to compensate systemic falls in pressure up to 60mmHg, hypotensive patients with pressures above this cutoff exhibit significantly reduced blood flow in their middle cerebral arteries, as well as impaired adjustments in flow to cognitive demands [268], making them at increased risk of periventricular demyelination. Anti-hypertensive therapies, especially in patients with coincident SVD or MS, should therefore be managed carefully to avoid precipitous drops, with moderate and stable pressures becoming the end-goal of therapy: consideration should be given to treat even moderate hypotension in these patients, lest hypoperfusion should aggravate their pathology.

In patients at risk of suffering white matter disease, these (and other) considerations in their day-to-day medical care will help to maintain ideal cerebral oxygenation and prevent disease progression.

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Figure 1. Heat maps showing lesion distribution in MS, and their relationship with arterial watersheds. (A) Coronal map of demyelinating MS lesions in postmortem brain, superimposed on a virtual brain section. Upper inset: magnification showing the high frequency of lesions at the periventricular margin. Adapted from (Haider et al., 2016). Lower inset: map showing the territories of the major cerebral arteries, indicating that the most common location of lesions coincides with the watershed between three major arteries. (B) Axial map of lesions detected by MRI in secondary progressive MS, superimposed on a T1-MRI template. Adapted from Holland et al., 2012. Circled region in B and C shows the correlation between the most frequent lesion location and the watershed territory between three major arteries. ACA, anterior cerebral artery; LSA, lenticulostriate artery; MCA, middle cerebral artery; PCA, posterior cerebral artery. Diagrams in A & B copyright 2011 by the American Society of Neuroimaging.
Figure 2. Periventricular vasculature. Coronal radiograph of a hemisphere of the human brain vasculature filled with a radio-opaque marker (microplaque). The larger vessels are veins, but note the long, fine end-arteries/arterioles penetrating from the brain surface towards the periventricular white matter. Adapted from [23].
Figure 3. Oxygen diffusion across arteries, arterioles and venules. Confocal in vivo image of the cerebral cortex of an anaesthetized mouse showing the surface arteries and veins. 3A. The inherent flavoprotein fluorescence (green) shows the distribution of functioning mitochondria a few minutes after breathing 10% oxygen instead of room air (21% oxygen). It is only the mitochondria in a ‘halo’ around arteries that obtain sufficient oxygen for continued function, and the desaturated blood in the capillaries and veins is no longer able to support function: in animals breathing air the cortex shows a uniform green fluorescence. Adapted from [270]. 3B. Similar images illustrating the same field and showing inherent flavoprotein fluorescence (left, green), and the applied, red potentiometric dye TMRM, which is accumulated in polarised (functioning) mitochondria (middle), with the merged image (right). The mouse is again breathing 10% oxygen to make the brain hypoxic and thus reveal the provision of oxygen from the different vessels more clearly. As in 3A, the arteries are surrounded by a halo showing that the FAD-positive mitochondria (green) are also polarised (red) and so capable of making ATP: the FAD and TMRM fluorescence are precisely superimposed. The superimposition establishes that the FAD fluorescence is an accurate marker of functioning, polarised mitochondria. The arrows distinguish a vein which is surrounded by a narrow halo of FAD- and TMRM-positive mitochondria, indicating that veins can also supply oxygen to tissue.
Figure 4. Prevention of Pattern III demyelination by raised inspiratory oxygen. (A & B) Transverse sections through the spinal cord of two rats, taken at the level of an intraspinal (dorsal column) injection of lipopolysaccharide two weeks previously (stained for myelin, luxol fast blue/periodic acid–Schiff/hematoxylin): the area indicated at the base of the dorsal column is shown in more detail on the right. A Pattern III demyelinating lesion (outlined with arrows) is visible in A, but the lesion fails to form in the animal shown in B, which was treated with 80% oxygen during the first two days post-injection, namely the time when the lesion site is otherwise hypoxic. (C) Graph showing the area of demyelination in treated and untreated animals (**p<0.001). Adapted from [1].
Figure 5. Diagram showing the relationships of some of the pathophysiological phenomena described in this review.

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