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

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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
Hypoxia. Oligodendrocytes. Demyelination. Vasculature. Watershed. Therapy.

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, Poiseuielle'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 venular 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 lenticulostriate 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 auto-regulatory 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 arteriolosclerotic" 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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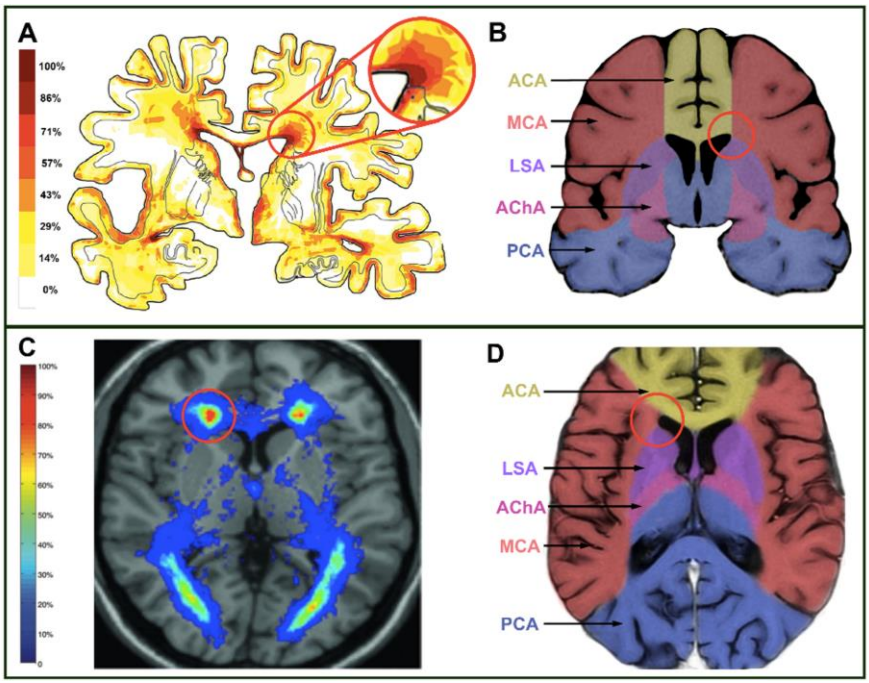

Figure 1. Heat maps showing lesion distribution in MS, and their relationship with arterial watersheds. (A) Coronal map of demyelinating MS lesions in postmortem brain, superimposed on a virtual brain section. Upper inset: magnification showing the high frequency of lesions at the periventricular margin. 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.

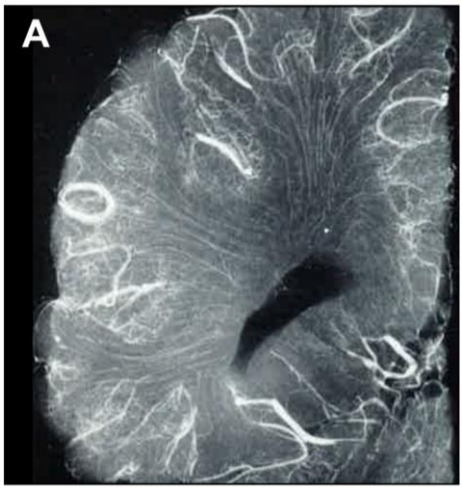

Figure 2. Periventricular vasculature. Coronal radiograph of a hemisphere of the human brain vasculature filled with a radio-opaque marker (microplaque). The larger vessels are veins, but note the long, fine end-arteries/arterioles penetrating from the brain surface towards the periventricular white matter. Adapted from [23].

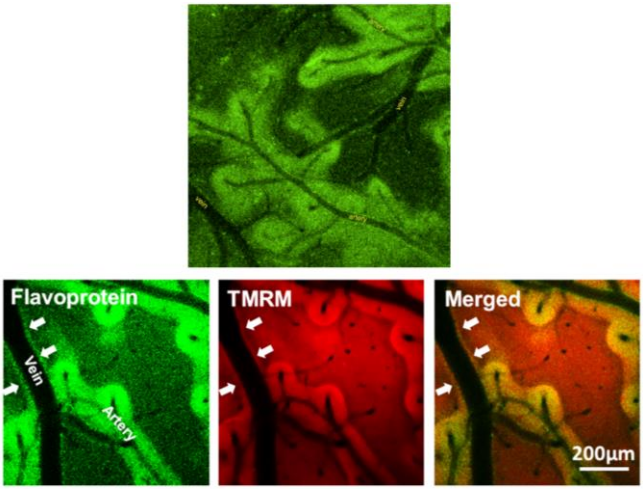

Figure 3. Oxygen diffusion across arteries, arterioles and venules. Confocal *in vivo* image of the cerebral cortex of an anaesthetized mouse showing the surface arteries and veins. 3A. The inherent flavoprotein fluorescence (green) shows the distribution of functioning mitochondria a few minutes after 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 potentiometic 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.

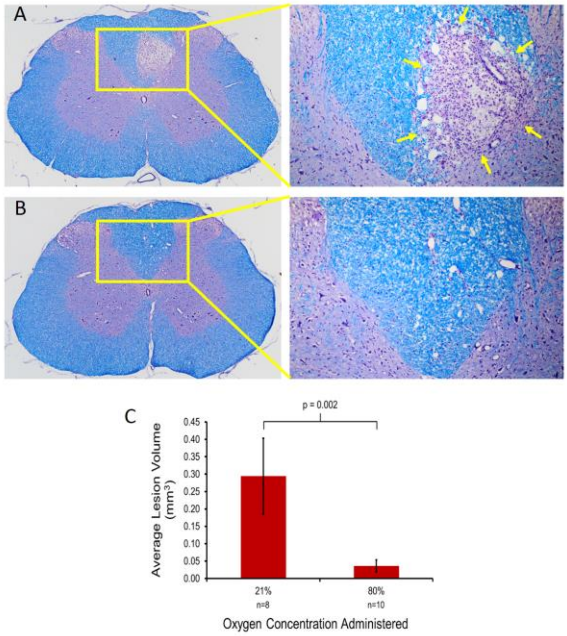

Figure 4. Prevention of Pattern III demyelination by raised inspiratory oxygen. **(A & B)** Transverse sections through the spinal cord of two rats, taken at the level of an intraspinal (dorsal column) injection of lipopolysaccharide two weeks previously (stained for myelin, luxol fast blue/periodic 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].

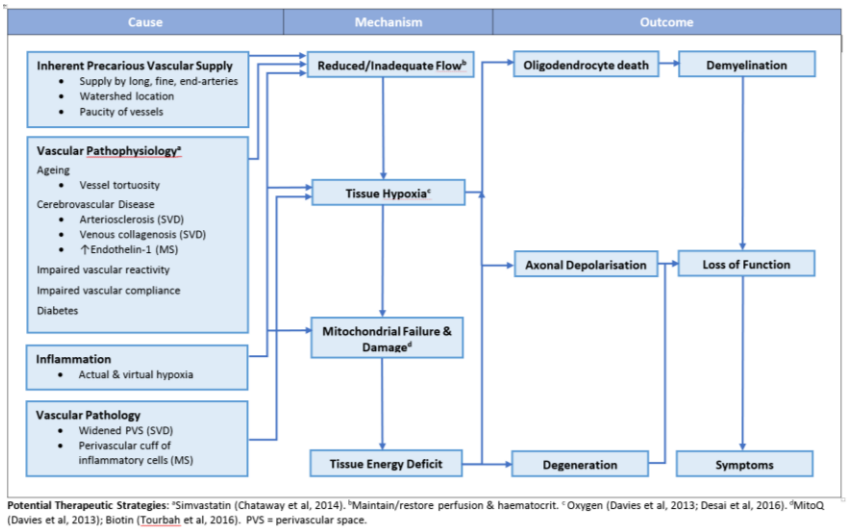

Figure 5. Diagram showing the relationships of some of the pathophysiological phenomena described in this review.

Reference List

1. Desai,R.A., Davies,A.L., Tachrount,M. et al. (2016) Cause and prevention of demyelination in a model multiple sclerosis lesion. Ann.Neurol. **79**, 591-604.

2. Geraldes,R., Esiri,M.M., DeLuca,G.C. and Palace,J. (2016) Age-related small vessel disease: a potential contributor to neurodegeneration in multiple sclerosis. Brain Pathol.

3. Craggs,L.J., Yamamoto,Y., Deramecourt,V. and Kalaria,R.N. (2014) Microvascular pathology and morphometrics of sporadic and hereditary small vessel diseases of the brain. Brain Pathol. **24**, 495-509.

4. Lucchinetti,C., Bruck,W., Parisi,J., Scheithauer,B., Rodriguez,M. and Lassmann,H. (2000) Heterogeneity of multiple sclerosis lesions: Implications for the pathogenesis of demyelination. Ann.Neurol. **47**, 707-717.

5. Pantoni,L. (2010) Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol. **9**, 689-701.

6. Wardlaw,J.M., Sandercock,P.A., Dennis,M.S. and Starr,J. (2003) Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? Stroke **34**, 806-812.

7. Tomimoto,H., Akiguchi,I., Wakita,H., Lin,J.X. and Budka,H. (2000) Cyclooxygenase-2 is induced in microglia during chronic cerebral ischemia in humans. Acta Neuropathol. **99**, 26-30.

8. Rincon,F. and Wright,C.B. (2014) Current pathophysiological concepts in cerebral small vessel disease. Front Aging Neurosci. **6**, 24.

9. Smith,E.E. (2010) Leukoaraiosis and stroke. Stroke **41**, S139-S143.

10. DeCarli,C., Fletcher,E., Ramey,V., Harvey,D. and Jagust,W.J. (2005) Anatomical mapping of white matter hyperintensities (WMH): exploring the relationships between periventricular WMH, deep WMH, and total WMH burden. Stroke **36**, 50-55.

11. Wharton,S.B., Simpson,J.E., Brayne,C. and Ince,P.G. (2015) Age-associated white matter lesions: the MRC Cognitive Function and Ageing Study. Brain Pathol. **25**, 35-43.

12. Brownell,B. and Hughes,J.T. (1962) The distribution of plaques in the cerebrum in multiple sclerosis. J.Neurol.Neurosurg.Psychiatry **25**, 315-320.

13. Lumsden,C.E. (1970) The neuropathology of multiple sclerosis. In Handbook of clinical neurology (Vinken,P.J. and Bruyn,G.W., eds.), pp. 217-309, Elsevier, Amsterdam

14. DeLuca,G.C., Williams,K., Evangelou,N., Ebers,G.C. and Esiri,M.M. (2006) The contribution of demyelination to axonal loss in multiple sclerosis. Brain **129**, 1507-1516.

15. Evangelou,N., DeLuca,G.C., Owens,T. and Esiri,M.M. (2005) Pathological study of spinal cord atrophy in multiple sclerosis suggests limited role of local lesions. Brain **128**, 29-34.

16. Evangelou,N., Konz,D., Esiri,M.M., Smith,S., Palace,J. and Matthews,P.M. (2001) Size-selective neuronal changes in the anterior optic pathways suggest a differential susceptibility to injury in multiple sclerosis. Brain **124**, 1813-1820.

17. Toosy,A.T., Mason,D.F. and Miller,D.H. (2014) Optic neuritis. Lancet Neurol. **13**, 83-99.

18. Holland,C.M., Charil,A., Csapo,I. et al. (2012) The relationship between normal cerebral perfusion patterns and white matter lesion distribution in 1,249 patients with multiple sclerosis. J Neuroimaging **22**, 129-136.

19. Haider,L., Zrzavy,T., Hametner,S. et al. (2016) The topography of demyelination and neurodegeneration in the multiple sclerosis brain. Brain **139**, 807-815.

20. Narayana,P.A., Zhou,Y., Hasan,K.M., Datta,S., Sun,X. and Wolinsky,J.S. (2014) Hypoperfusion and T1-hypointense lesions in white matter in multiple sclerosis. Mult.Scler. **20**, 365-373.

21. Roman,G.C. (1987) Senile dementia of the Binswanger type. A vascular form of dementia in the elderly. JAMA **258**, 1782-1788.

22. Moody,D.M., Bell,M.A. and Challa,V.R. (1990) Features of the cerebral vascular pattern that predict vulnerability to perfusion or oxygenation deficiency: an anatomic study. AJNR Am.J.Neuroradiol. **11**, 431-439.

23. ROWBOTHAM,G.F. and LITTLE,E. (1965) Circulations of the cerebral hemispheres. Br.J.Surg. **52**, 8-21.

24. Lazorthes,G. (1961) Vascularisation et circulation cérébrales , Masson, Paris,

25. Brown,W.R. and Thore,C.R. (2011) Review: cerebral microvascular pathology in ageing and neurodegeneration. Neuropathol.Appl.Neurobiol. **37**, 56-74.

26. Moody,D.M., Santamore,W.P. and Bell,M.A. (1991) Does tortuosity in cerebral arterioles impair down-autoregulation in hypertensives and elderly normotensives? A hypothesis and computer model. Clin.Neurosurg. **37**, 372-387.

27. Vovenko,E.P. and Chuikin,A.E. (2010) Tissue oxygen tension profiles close to brain arterioles and venules in the rat cerebral cortex during the development of acute anemia. Neurosci.Behav.Physiol **40**, 723-731.

28. Vovenko,E.P. (2009) Transmural oxygen tension gradients in rat cerebral cortex arterioles. Neurosci.Behav.Physiol **39**, 363-370.

29. Ivanov,K.P., Sokolova,I.B. and Vovenko,E.P. (1999) Oxygen transport in the rat brain cortex at normobaric hyperoxia. Eur.J Appl.Physiol Occup.Physiol **80**, 582-587.

30. Vovenko,E. (1999) Distribution of oxygen tension on the surface of arterioles, capillaries and venules of brain cortex and in tissue in normoxia: an experimental study on rats. Pflugers Arch. **437**, 617-623.

31. Ivanov,K.P., Derry,A.N., Vovenko,E.P., Samoilov,M.O. and Semionov,D.G. (1982) Direct measurements of oxygen tension at the surface of arterioles, capillaries and venules of the cerebral cortex. Pflugers Arch. **393**, 118-120.

32. Ivanov,K.P. (2001) [Current views on oxygen transport from blood to tissues]. Usp.Fiziol.Nauk **32**, 3-22.

33. Chisholm,K.I., Ida,K.K., Davies,A.L. et al. (2016) In Vivo Imaging of Flavoprotein Fluorescence During Hypoxia Reveals the Importance of Direct Arterial Oxygen Supply to Cerebral Cortex Tissue. Adv.Exp.Med.Biol. **876**, 233-239.

34. Kasischke,K.A., Lambert,E.M., Panepento,B. et al. (2011) Two-photon NADH imaging exposes boundaries of oxygen diffusion in cortical vascular supply regions. J.Cereb.Blood Flow Metab **31**, 68-81.

35. Ndubuizu,O. and LaManna,J.C. (2007) Brain tissue oxygen concentration measurements. Antioxid.Redox Signal. **9**, 1207-1219.

36. Dow,R.S. and Berglund,G. (1942) Vascular pattern of lesions of multiple sclerosis. Archives of Neurology and Psychiatry **47**, 1-18.

37. Rindfleisch,E. (1863) Histologisches Detail zur grauen Degeneration von Gehirn und Rückenmark. Arch Pathol Anat Physiol Klin Med (Virchow) **26**, 474-483.

38. Rae-Grant,A.D., Wong,C., Bernatowicz,R. and Fox,R.J. (2014) Observations on the brain vasculature in multiple sclerosis: A historical perspective. Mult.Scler.Relat Disord. **3**, 156-162.

39. Dawson,J.W. (1916) XVIII.—The Histology of Disseminated Sclerosis. Transactions of the Royal Society of Edinburgh **50**, 517-740.

40. Grabner,G., Dal-Bianco,A., Hametner,S., Lassmann,H. and Trattnig,S. (2014) Group specific vein-atlasing: an application for analyzing the venous system under normal and multiple sclerosis conditions. J.Magn Reson.Imaging **40**, 655-661.

41. Adams,C.W.M. (1977) Pathology of multiple sclerosis: progression of the lesion. Br.Med.Bull. **33**, 15-20.

42. Lassmann,H. and Wekerle,H. (2006) The pathology of multiple sclerosis. In McAlpine's Multiple Sclerosis (Compston,A., Confavreux,C., Lassmann,H. et al., eds.), pp. 557-599, Churchill Livingstone, London

43. Wakefield,A.J., More,L.J., Difford,J. and McLaughlin,J.E. (1994) Immunohistochemical study of vascular injury in acute multiple sclerosis. J.Clin.Pathol. **47**, 129-133.

44. Dal-Bianco,A., Hametner,S., Grabner,G. et al. (2015) Veins in plaques of multiple sclerosis patients - a longitudinal magnetic resonance imaging study at 7 Tesla. Eur.Radiol. **25**, 2913-2920.

45. Ge,Y., Zohrabian,V.M. and Grossman,R.I. (2008) Seven-Tesla magnetic resonance imaging: new vision of microvascular abnormalities in multiple sclerosis. Arch.Neurol. **65**, 812-816.

46. Tan,I.L., Van Schijndel,R.A., Pouwels,P.J. et al. (2000) MR venography of multiple sclerosis. AJNR Am.J Neuroradiol. **21**, 1039-1042.

47. Tallantyre,E.C., Dixon,J.E., Donaldson,I. et al. (2011) Ultra-high-field imaging distinguishes MS lesions from asymptomatic white matter lesions. Neurol. **76**, 534-539.

48. Grabner,G., Dal-Bianco,A., Schernthaner,M., Vass,K., Lassmann,H. and Trattnig,S. (2011) Analysis of multiple sclerosis lesions using a fusion of 3.0 T FLAIR and 7.0 T SWI phase: FLAIR SWI. J.Magn Reson.Imaging **33**, 543-549.

49. Prineas,J.W. and Parratt,J.D. (2012) Oligodendrocytes and the early multiple sclerosis lesion. Ann.Neurol. **72**, 18-31.

50. Brown,W.R., Moody,D.M., Thore,C.R., Anstrom,J.A. and Challa,V.R. (2009) Microvascular changes in the white mater in dementia. J.neurol.Sci. **283**, 28-31.

51. Mayer,P.L. and Kier,E.L. (1991) The controversy of the periventricular white matter circulation: a review of the anatomic literature. AJNR Am.J.Neuroradiol. **12**, 223-228.

52. Hayreh,S.S. (2004) Posterior ischaemic optic neuropathy: clinical features, pathogenesis, and management. Eye (Lond) **18**, 1188-1206.

53. Hayreh,S.S. (2011) Blood supply of the optic nerve. Ischemic Optic Neuropathies 35-78.

54. Shamji,M.F., Maziak,D.E., Shamji,F.M., Ginsberg,R.J. and Pon,R. (2003) Circulation of the spinal cord: an important consideration for thoracic surgeons. Ann.Thorac.Surg. **76**, 315-321.

55. Cheshire,W.P., Santos,C.C., Massey,E.W. and Howard,J.F., Jr. (1996) Spinal cord infarction: etiology and outcome. Neurol. **47**, 321-330.

56. Zulch,K.J. and Kurth-Schumacher,R. (1970) The pathogenesis of "intermittent spinovascular insufficiency" ("spinal claudication of Dejerine") and other vascular syndromes of the spinal cord. Vasc.Surg. **4**, 116-136.

57. Hendrikse,J., Petersen,E.T., van Laar,P.J. and Golay,X. (2008) Cerebral border zones between distal end branches of intracranial arteries: MR imaging. Radiology **246**, 572-580.

58. Petersen,E.T., Mouridsen,K. and Golay,X. (2010) The QUASAR reproducibility study, Part II: Results from a multi-center Arterial Spin Labeling test-retest study. Neuroimage **49**, 104-113.

59. Momjian-Mayor,I. and Baron,J.C. (2005) The pathophysiology of watershed infarction in internal carotid artery disease: review of cerebral perfusion studies. Stroke **36**, 567-577.

60. Patrikios,P., Stadelmann,C., Kutzelnigg,A. et al. (2006) Remyelination is extensive in a subset of multiple sclerosis patients. Brain **129**, 3165-3172.

61. Husain,J. and Juurlink,B.H. (1995) Oligodendroglial precursor cell susceptibility to hypoxia is related to poor ability to cope with reactive oxygen species. Brain Res. **698**, 86-94.

62. Penderis,J., Shields,S.A. and Franklin,R.J. (2003) Impaired remyelination and depletion of oligodendrocyte progenitors does not occur following repeated episodes of focal demyelination in the rat central nervous system. Brain **126**, 1382-1391.

63. Sharma,R., Fischer,M.T., Bauer,J. et al. (2010) Inflammation induced by innate immunity in the central nervous system leads to primary astrocyte dysfunction followed by demyelination. Acta Neuropathologica **120**, 223-236.

64. Marik,C., Felts,P.A., Bauer,J., Lassmann,H. and Smith,K.J. (2007) Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? Brain **130**, 2800-2815.

65. Felts,P.A., Woolston,A.M., Fernando,H.B. et al. (2005) Inflammation and primary demyelination induced by the intraspinal injection of lipopolysaccharide. Brain **128**, 1649-1666.

66. Blakemore,W.F. (1982) Ethidium bromide induced demyelination in the spinal cord of the cat. Neuropathol.Appl.Neurobiol. **8**, 365-375.

67. Blakemore,W.F., Eames,R.A., Smith,K.J. and McDonald,W.I. (1977) Remyelination in the spinal cord of the cat following intraspinal injections of lysolecithin. J.neurol.Sci. **33**, 31-43.

68. Smith,K.J. and Hall,S.M. (1994) Central demyelination induced in vivo by the calcium ionophore ionomycin. Brain **117**, 1351-1356.

69. Tveten,L. (1976) Spinal cord vascularity. IV. The spinal cord arteries in the rat. Acta Radiol.Diagn. **17**, 385-398.

70. Koyanagi,I., Tator,C.H. and Lea,P.J. (1993) Three-dimensional analysis of the vascular system in the rat spinal cord with scanning electron microscopy of vascular corrosion casts. Part 2: Acute spinal cord injury. Neurosurgery **33**, 285-291.

71. Zulch,K.J. (1976) Pathogenetic and clinical observations in spinovascular insufficiency. Zentralbl.Neurochir. **37**, 1-13.

72. Fennema,M., Wessel,J.N., Faithful,N.S. and Erdmann,W. (1989) Tissue oxygen tension in the cerebral cortex of the rabbit. Adv.Exp.Med.Biol. **248**, 451-460.

73. Nair,P., Whalen,W.J. and Buerk,D. (1975) PO2 of cat cerebral cortex: response to breathing N2 and 100 per cent O21. Microvasc.Res. **9**, 158-165.

74. Stokes,B.T., Garwood,M. and Walters,P. (1981) Oxygen fields in specific spinal loci of the canine spinal cord. Am.J.Physiol **240**, H761-H766.

75. Leniger-Follert,E., Lubbers,D.W. and Wrabetz,W. (1975) Regulation of local tissue PO2 of the brain cortex at different arterial O2 pressures. Pflugers Arch. **359**, 81-95.

76. Murr,R., Berger,S., Schurer,L., Peter,K. and Baethmann,A. (1994) A novel, remote-controlled suspension device for brain tissue PO2 measurements with multiwire surface electrodes. Pflugers Arch. **426**, 348-350.

77. Pries,A.R., Secomb,T.W., Gaehtgens,P. and Gross,J.F. (1990) Blood flow in microvascular networks. Experiments and simulation. Circ.Res. **67**, 826-834.

78. Chebbi,R. (2015) Dynamics of blood flow: modeling of the Fahraeus-Lindqvist effect. J.Biol.Phys. **41**, 313-326.

79. Ostergaard,L., Engedal,T.S., Moreton,F. et al. (2016) Cerebral small vessel disease: Capillary pathways to stroke and cognitive decline. J.Cereb.Blood Flow Metab **36**, 302-325.

80. Kozinska,D., Holland,C.M., Krissian,K., Westin,C.F. and Guttmann,C.R. (2004) A method for the analysis of the geometrical relationship between white matter pathology and the vascular architecture of the brain. Neuroimage. **22**, 1671-1678.

81. Aboul-Enein,F., Rauschka,H., Kornek,B. et al. (2003) Preferential loss of myelin-associated glycoprotein reflects hypoxia- like white matter damage in stroke and inflammatory brain diseases. J.Neuropathol.Exp.Neurol **62**, 25-33.

82. Fazekas,F., Kleinert,R., Offenbacher,H. et al. (1993) Pathologic correlates of incidental MRI white matter signal hyperintensities. Neurol. **43**, 1683-1689.

83. Shi,Y., Thrippleton,M.J., Makin,S.D. et al. (2016) Cerebral blood flow in small vessel disease: A systematic review and meta-analysis. J.Cereb.Blood Flow Metab **36**, 1653-1667.

84. ten Dam,V.H., van den Heuvel,D.M., de Craen,A.J. et al. (2007) Decline in total cerebral blood flow is linked with increase in periventricular but not deep white matter hyperintensities. Radiology **243**, 198-203.

85. O'Sullivan,M., Lythgoe,D.J., Pereira,A.C. et al. (2002) Patterns of cerebral blood flow reduction in patients with ischemic leukoaraiosis. Neurol. **59**, 321-326.

86. Nezu,T., Hosomi,N., Aoki,S. et al. (2015) Endothelial dysfunction is associated with the severity of cerebral small vessel disease. Hypertens.Res. **38**, 291-297.

87. Hoth,K.F., Tate,D.F., Poppas,A. et al. (2007) Endothelial function and white matter hyperintensities in older adults with cardiovascular disease. Stroke **38**, 308-312.

88. Bakker,S.L., de Leeuw,F.E., de Groot,J.C., Hofman,A., Koudstaal,P.J. and Breteler,M.M. (1999) Cerebral vasomotor reactivity and cerebral white matter lesions in the elderly. Neurol. **52**, 578-583.

89. Isaka,Y., Okamoto,M., Ashida,K. and Imaizumi,M. (1994) Decreased cerebrovascular dilatory capacity in subjects with asymptomatic periventricular hyperintensities. Stroke **25**, 375-381.

90. Moody,D.M., Brown,W.R., Challa,V.R. and Anderson,R.L. (1995) Periventricular venous collagenosis: association with leukoaraiosis. Radiology **194**, 469-476.

91. D'Haeseleer,M., Hostenbach,S., Peeters,I. et al. (2015) Cerebral hypoperfusion: a new pathophysiologic concept in multiple sclerosis? J.Cereb.Blood Flow Metab **35**, 1406-1410.

92. Juurlink,B.H. (2013) The evidence for hypoperfusion as a factor in multiple sclerosis lesion development. Mult.Scler.Int. **2013**, 598093.

93. Ingrisch,M., Sourbron,S., Herberich,S. et al. (2016) Dynamic Contrast-Enhanced Magnetic Resonance Imaging Suggests Normal Perfusion in Normal-Appearing White Matter in Multiple Sclerosis. Invest Radiol. **52**, 135-141.

94. Paling,D., Thade,P.E., Tozer,D.J. et al. (2014) Cerebral arterial bolus arrival time is prolonged in multiple sclerosis and associated with disability. J.Cereb.Blood Flow Metab **34**, 34-42.

95. Rashid,W., Parkes,L.M., Ingle,G.T. et al. (2004) Abnormalities of cerebral perfusion in multiple sclerosis. J.Neurol.Neurosurg.Psychiatry **75**, 1288-1293.

96. Papadaki,E.Z., Mastorodemos,V.C., Amanakis,E.Z. et al. (2012) White matter and deep gray matter hemodynamic changes in multiple sclerosis patients with clinically isolated syndrome. Magn Reson.Med. **68**, 1932-1942.

97. Sun,X., Tanaka,M., Kondo,S., Okamoto,K. and Hirai,S. (1998) Clinical significance of reduced cerebral metabolism in multiple sclerosis: a combined PET and MRI study. Ann.Nucl.Med. **12**, 89-94.

98. Lycke,J., Wikkelso,C., Bergh,A.C., Jacobsson,L. and Andersen,O. (1993) Regional cerebral blood flow in multiple sclerosis measured by single photon emission tomography with technetium-99m hexamethylpropyleneamine oxime. Eur.Neurol. **33**, 163-167.

99. Ge,Y., Law,M., Johnson,G. et al. (2005) Dynamic susceptibility contrast perfusion MR imaging of multiple sclerosis lesions: characterizing hemodynamic impairment and inflammatory activity. AJNR Am.J.Neuroradiol. **26**, 1539-1547.

100. Varga,A.W., Johnson,G., Babb,J.S., Herbert,J., Grossman,R.I. and Inglese,M. (2009) White matter hemodynamic abnormalities precede sub-cortical gray matter changes in multiple sclerosis. J.neurol.Sci. **282**, 28-33.

101. Law,M., Saindane,A.M., Ge,Y. et al. (2004) Microvascular abnormality in relapsing-remitting multiple sclerosis: perfusion MR imaging findings in normal-appearing white matter. Radiology **231**, 645-652.

102. Inglese,M., Adhya,S., Johnson,G. et al. (2008) Perfusion magnetic resonance imaging correlates of neuropsychological impairment in multiple sclerosis. J Cereb.Blood Flow Metab **28**, 164-171.

103. Adhya,S., Johnson,G., Herbert,J. et al. (2006) Pattern of hemodynamic impairment in multiple sclerosis: dynamic susceptibility contrast perfusion MR imaging at 3.0 T. Neuroimage **33**, 1029-1035.

104. Zamboni,P., Menegatti,E., Weinstock-Guttman,B. et al. (2011) Hypoperfusion of brain parenchyma is associated with the severity of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis: a cross-sectional preliminary report. BMC Med. **9**, 22.

105. Inglese,M., Park,S.J., Johnson,G. et al. (2007) Deep gray matter perfusion in multiple sclerosis: dynamic susceptibility contrast perfusion magnetic resonance imaging at 3 T. Arch.Neurol. **64**, 196-202.

106. Doche,E., Lecocq,A., Maarouf,A. et al. (2016) Hypoperfusion of the thalamus is associated with disability in relapsing remitting multiple sclerosis. J.Neuroradiol.

107. Ota,M., Sato,N., Nakata,Y. et al. (2013) Abnormalities of cerebral blood flow in multiple sclerosis: a pseudocontinuous arterial spin labeling MRI study. Magn Reson.Imaging **31**, 990-995.

108. Hojjat,S.P., Cantrell,C.G., Vitorino,R. et al. (2016) Regional reduction in cortical blood flow among cognitively impaired adults with relapsing-remitting multiple sclerosis patients. Mult.Scler. **22**, 1421-1428.

109. Hojjat,S.P., Cantrell,C.G., Carroll,T.J. et al. (2016) Perfusion reduction in the absence of structural differences in cognitively impaired versus unimpaired RRMS patients. Mult.Scler. **22**, 1685-1694.

110. Saindane,A.M., Law,M., Ge,Y., Johnson,G., Babb,J.S. and Grossman,R.I. (2007) Correlation of diffusion tensor and dynamic perfusion MR imaging metrics in normal-appearing corpus callosum: support for primary hypoperfusion in multiple sclerosis. AJNR Am.J Neuroradiol. **28**, 767-772.

111. Rovira,A., Pericot,I., Alonso,J., Rio,J., Grive,E. and Montalban,X. (2002) Serial Diffusion-Weighted MR Imaging and Proton MR Spectroscopy of Acute Large Demyelinating Brain Lesions: Case Report. A.J.N.R. **23**, 989-994.

112. Papadaki,E.Z., Simos,P.G., Mastorodemos,V.C. et al. (2014) Regional MRI perfusion measures predict motor/executive function in patients with clinically isolated syndrome. Behav.Neurol. **2014**, 252419.

113. Wuerfel,J., Bellmann-Strobl,J., Brunecker,P. et al. (2004) Changes in cerebral perfusion precede plaque formation in multiple sclerosis: a longitudinal perfusion MRI study. Brain **127**, 111-119.

114. Haselhorst,R., Kappos,L., Bilecen,D. et al. (2000) Dynamic susceptibility contrast MR imaging of plaque development in multiple sclerosis: application of an extended blood-brain barrier leakage correction. J.Magn Reson.Imaging **11**, 495-505.

115. Monti,L., Donati,D., Menci,E. et al. (2015) Cerebral circulation time is prolonged and not correlated with EDSS in multiple sclerosis patients: a study using digital subtracted angiography. PLoS.One. **10**, e0116681.

116. Mancini,M., Morra,V.B., Di,D.O. et al. (2012) Multiple sclerosis: cerebral circulation time. Radiology **262**, 947-955.

117. ElSankari,S., Baledent,O., van,P., V, Sindic,C., de,B.Q. and Duprez,T. (2013) Concomitant analysis of arterial, venous, and CSF flows using phase-contrast MRI: a quantitative comparison between MS patients and healthy controls. J Cereb.Blood Flow Metab. **33**, 1314-1321.

118. D'Haeseleer,M., Beelen,R., Fierens,Y. et al. (2013) Cerebral hypoperfusion in multiple sclerosis is reversible and mediated by endothelin-1. Proc.Natl.Acad.Sci.U.S.A. **110**, 5654-5658.

119. De Keyser,J., Steen,C., Mostert,J.P. and Koch,M.W. (2008) Hypoperfusion of the cerebral white matter in multiple sclerosis: possible mechanisms and pathophysiological significance. J Cereb.Blood Flow Metab **28**, 1645-1651.

120. Pache,M., Kaiser,H.J., Akhalbedashvili,N. et al. (2003) Extraocular blood flow and endothelin-1 plasma levels in patients with multiple sclerosis. Eur.Neurol. **49**, 164-168.

121. Haufschild,T., Shaw,S.G., Kaiser,H.J. and Flammer,J. (2003) Transient raise of endothelin-1 plasma level and reduction of ocular blood flow in a patient with optic neuritis. Ophthalmologica **217**, 451-453.

122. Haufschild,T., Shaw,S.G., Kesselring,J. and Flammer,J. (2001) Increased endothelin-1 plasma levels in patients with multiple sclerosis. J.Neuroophthalmol. **21**, 37-38.

123. Speciale,L., Sarasella,M., Ruzzante,S. et al. (2000) Endothelin and nitric oxide levels in cerebrospinal fluid of patients with multiple sclerosis. J Neurovirol. **6**, S62-S66.

124. Jankowska-Lech,I., Terelak-Borys,B., Grabska-Liberek,I., Palasik,W., Bik,W. and Wolinska-Witort,E. (2015) Decreased endothelin-1 plasma levels in multiple sclerosis patients: a possible factor of vascular dysregulation? Med.Sci.Monit. **21**, 1066-1071.

125. Fjeldstad,C., Frederiksen,C., Fjeldstad,A.S., Bemben,M. and Pardo,G. (2010) Arterial compliance in multiple sclerosis: a pilot study. Angiology **61**, 31-36.

126. Huang,X., Kang,X., Xue,J., Kang,C., Lv,H. and Li,Z. (2015) Evaluation of carotid artery elasticity changes in patients with cerebral small vessel disease. Int.J.Clin.Exp.Med. **8**, 18825-18830.

127. Marshall,O., Lu,H., Brisset,J.C. et al. (2014) Impaired cerebrovascular reactivity in multiple sclerosis. JAMA Neurol. **71**, 1275-1281.

128. Marshall,O., Chawla,S., Lu,H., Pape,L. and Ge,Y. (2016) Cerebral blood flow modulation insufficiency in brain networks in multiple sclerosis: A hypercapnia MRI study. J.Cereb.Blood Flow Metab **36**, 2087-2095.

129. Jiang,H., Delgado,S., Tan,J. et al. (2016) Impaired retinal microcirculation in multiple sclerosis. Mult.Scler. **22**, 1812-1820.

130. Modrzejewska,M., Karczewicz,D. and Wilk,G. (2007) Assessment of blood flow velocity in eyeball arteries in multiple sclerosis patients with past retrobulbar optic neuritis in color Doppler ultrasonography. Klinika Oczna **109**, 183-186.

131. Gugleta,K., Kochkorov,A., Kavroulaki,D. et al. (2009) Retinal vessels in patients with multiple sclerosis: baseline diameter and response to flicker light stimulation. Klin.Monbl.Augenheilkd. **226**, 272-275.

132. Lightman,S., McDonald,W.I., Bird,A.C. et al. (1987) Retinal venous sheathing in optic neuritis. Its significance for the pathogenesis of multiple sclerosis. Brain **110**, 405-414.

133. Kominsky,D.J., Campbell,E.L. and Colgan,S.P. (2010) Metabolic shifts in immunity and inflammation. J.Immunol. **184**, 4062-4068.

134. El-Benna,J., Hurtado-Nedelec,M., Marzaioli,V., Marie,J.C., Gougerot-Pocidalo,M.A. and Dang,P.M. (2016) Priming of the neutrophil respiratory burst: role in host defense and inflammation. Immunol.Rev. **273**, 180-193.

135. Sierra,A., Abiega,O., Shahraz,A. and Neumann,H. (2013) Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. Front Cell Neurosci. **7**, 6.

136. Granger,D.N. and Senchenkova,E. (2010) Inflammation and the microcirculation. Colloquium Series on Integrated Systems Physiology: From Molecule to Function to Disease.

137. Schuh,C., Wimmer,I., Hametner,S. et al. (2014) Oxidative tissue injury in multiple sclerosis is only partly reflected in experimental disease models. Acta Neuropathol. **128**, 247-266.

138. Ibitoye,R., Kemp,K., Rice,C., Hares,K., Scolding,N. and Wilkins,A. (2016) Oxidative stress-related biomarkers in multiple sclerosis: a review. Biomark.Med. **10**, 889-902.

139. Fischer,M.T., Sharma,R., Lim,J.L. et al. (2012) NADPH oxidase expression in active multiple sclerosis lesions in relation to oxidative tissue damage and mitochondrial injury. Brain **135**, 886-899.

140. Fischer,M.T., Wimmer,I., Hoftberger,R. et al. (2013) Disease-specific molecular events in cortical multiple sclerosis lesions. Brain **136**, 1799-1815.

141. Haider,L., Fischer,M.T., Frischer,J.M. et al. (2011) Oxidative damage in multiple sclerosis lesions. Brain **134**, 1914-1924.

142. Bolanos,J.P., Peuchen,S., Heales,S.J., Land,J.M. and Clark,J.B. (1994) Nitric oxide-mediated inhibition of the mitochondrial respiratory chain in cultured astrocytes. J.Neurochem. **63**, 910-916.

143. Brorson,J.R., Schumacker,P.T. and Zhang,H. (1999) Nitric oxide acutely inhibits neuronal energy production. J.Neurosci. **19**, 147-158.

144. Jekabsone,A., Neher,J.J., Borutaite,V. and Brown,G.C. (2007) Nitric oxide from neuronal nitric oxide synthase sensitises neurons to hypoxia-induced death via competitive inhibition of cytochrome oxidase. J.Neurochem. **103**, 346-356.

145. Brookes,P.S., Kraus,D.W., Shiva,S. et al. (2003) Control of mitochondrial respiration by NO*, effects of low oxygen and respiratory state. J.Biol.Chem. **278**, 31603-31609.

146. Brown,G.C. and Cooper,C.E. (1994) Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Letters **356**, 295-298.

147. Brown,G.C. (2000) Nitric oxide as a competitive inhibitor of oxygen consumption in the mitochondrial respiratory chain. Acta Physiol.Scand. **168**, 667-674.

148. Trapp,B.D. and Stys,P.K. (2009) Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. Lancet neurol **8**, 280-291.

149. Mander,P., Borutaite,V., Moncada,S. and Brown,G.C. (2005) Nitric oxide from inflammatory-activated glia synergizes with hypoxia to induce neuronal death. J.Neurosci.Res. **79**, 208-215.

150. Ge,Y., Zhang,Z., Lu,H. et al. (2012) Characterizing brain oxygen metabolism in patients with multiple sclerosis with T2-relaxation-under-spin-tagging MRI. J Cereb.Blood Flow Metab. **32**, 403-412.

151. Brown,G.C. and Neher,J.J. (2010) Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. Mol.Neurobiol. **41**, 242-247.

152. Chung,K.K. and David,K.K. (2010) Emerging roles of nitric oxide in neurodegeneration. Nitric Oxide **22**, 290-295.

153. Herz,J., Zipp,F. and Siffrin,V. (2010) Neurodegeneration in autoimmune CNS inflammation. Exp.Neurol. **225**, 9-17.

154. Calabrese,V., Mancuso,C., Calvani,M., Rizzarelli,E., Butterfield,D.A. and Stella,A.M. (2007) Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. Nature Reviews Neuroscience **8**, 766-775.

155. Moncada,S. and Bolanos,J.P. (2006) Nitric oxide, cell bioenergetics and neurodegeneration. J.Neurochem. **97**, 1676-1689.

156. Smith,K.J. and Lassmann,H. (2002) The role of nitric oxide in multiple sclerosis. Lancet neurol **1**, 232-241.

157. Lassmann,H., Reindl,M., Rauschka,H. et al. (2003) A new paraclinical CSF marker for hypoxia-like tissue damage in multiple sclerosis lesions. Brain **126**, 1347-1357.

158. Mhaille,A.N., McQuaid,S., Windebank,A. et al. (2008) Increased expression of endoplasmic reticulum stress-related signaling pathway molecules in multiple sclerosis lesions. J Neuropathol.Exp.Neurol. **67**, 200-211.

159. Cunnea,P., Ni,M.A., McQuaid,S., Farrell,M., McMahon,J. and FitzGerald,U. (2011) Expression profiles of endoplasmic reticulum stress-related molecules in demyelinating lesions and multiple sclerosis. Mult.Scler. **17**, 808-818.

160. McMahon,J.M., McQuaid,S., Reynolds,R. and FitzGerald,U.F. (2012) Increased expression of ER stress- and hypoxia-associated molecules in grey matter lesions in multiple sclerosis. Mult.Scler. **18**, 1437-1447.

161. Stadelmann,C., Ludwin,S., Tabira,T. et al. (2005) Tissue preconditioning may explain concentric lesions in Balo's type of multiple sclerosis. Brain **128**, 979-987.

162. Aboul-Enein,F. and Lassmann,H. (2005) Mitochondrial damage and histotoxic hypoxia: a pathway of tissue injury in inflammatory brain disease? Acta Neuropathol.(Berl) **109**, 49-55.

163. Graumann,U., Reynolds,R., Steck,A.J. and Schaeren-Wiemers,N. (2003) Molecular changes in normal appearing white matter in multiple sclerosis are characteristic of neuroprotective mechanisms against hypoxic insult. Brain Pathol. **13**, 554-573.

164. Zeis,T., Graumann,U., Reynolds,R. and Schaeren-Wiemers,N. (2008) Normal-appearing white matter in multiple sclerosis is in a subtle balance between inflammation and neuroprotection. Brain **131**, 288-303.

165. Davie,C.A., Hawkins,C.P., Barker,G.J. et al. (1994) Serial proton magnetic resonance spectroscopy in acute multiple sclerosis lesions. Brain **117**, 49-58.

166. Regenold,W.T., Phatak,P., Makley,M.J., Stone,R.D. and Kling,M.A. (2008) Cerebrospinal fluid evidence of increased extra-mitochondrial glucose metabolism implicates mitochondrial dysfunction in multiple sclerosis disease progression. J Neurol.Sci. **275**, 106-112.

167. Albanese,M., Zagaglia,S., Landi,D. et al. (2016) Cerebrospinal fluid lactate is associated with multiple sclerosis disease progression. J.Neuroinflammation. **13**, 36.

168. Yang,R. and Dunn,J.F. (2015) Reduced cortical microvascular oxygenation in multiple sclerosis: a blinded, case-controlled study using a novel quantitative near-infrared spectroscopy method. Sci.Rep. **5**, 16477.

169. Lyons,S.A. and Kettenmann,H. (1998) Oligodendrocytes and microglia are selectively vulnerable to combined hypoxia and hypoglycemia injury in vitro. J.Cereb.Blood Flow Metab **18**, 521-530.

170. Okeda,R., Funata,N., Song,S.J., Higashino,F., Takano,T. and Yokoyama,K. (1982) Comparative study on pathogenesis of selective cerebral lesions in carbon monoxide poisoning and nitrogen hypoxia in cats. Acta Neuropathol.(Berl) **56**, 265-272.

171. Ginsberg,M.D., Hedley-Whyte,E.T. and Richardson,E.P., Jr. (1976) Hypoxic-ischemic leukoencephalopathy in man. Arch.Neurol. **33**, 5-14.

172. Petito,C.K., Olarte,J.P., Roberts,B., Nowak,T.S., Jr. and Pulsinelli,W.A. (1998) Selective glial vulnerability following transient global ischemia in rat brain. J Neuropathol.Exp.Neurol. **57**, 231-238.

173. Putnam,T.J. (1935) Studies in multiple sclerosis IV. "Encephalitis" and sclerotic plaques produced by venular obstruction. Archives of Neurology and Psychiatry **33**, 929-940.

174. James,P.B. (1982) Evidence for subacute fat embolism as the cause of multiple sclerosis. Lancet **1**, 380-386.

175. Jack,C., Antel,J., Bruck,W. and Kuhlmann,T. (2007) Contrasting potential of nitric oxide and peroxynitrite to mediate oligodendrocyte injury in multiple sclerosis. Glia **55**, 926-934.

176. Druzhyna,N.M., Musiyenko,S.I., Wilson,G.L. and LeDoux,S.P. (2005) Cytokines induce nitric oxide-mediated mtDNA damage and apoptosis in oligodendrocytes. Protective role of targeting 8-oxoguanine glycosylase to mitochondria. J.Biol.Chem. **280**, 21673-21679.

177. Scott,G.S., Virag,L., Szabo,C. and Hooper,D.C. (2003) Peroxynitrite-induced oligodendrocyte toxicity is not dependent on poly(ADP-ribose) polymerase activation. Glia **41**, 105-116.

178. Mitrovic,B., Ignarro,L.J., Montestruque,S., Smoll,A. and Merrill,J.E. (1994) Nitric oxide as a potential pathological mechanism in demyelination: its differential effects on primary glial cells in vitro. Neuroscience **61**, 575-585.

179. Brierley,J.B., Brown,A.W. and Calverley,J. (1976) Cyanide intoxication in the rat: physiological and neuropathological aspects. J.Neurol.Neurosurg.Psychiatry **39**, 129-140.

180. Ferraro,A. (1933) Experimental toxic encephalopathy. Diffuse sclerosis following subcutaneous injection of potassium cyanide. Psychiatry Quarterly **7**, 267-283.

181. Ibrahim,M.Z., Briscoe,P.B., Jr., Bayliss,O.B. and Dams,C.W. (1963) The relationship between enzyme activity and neuroglia in the prodromal and demyelinating stages of cyanide encephalopathy in the rat. J Neurol.Neurosurg.Psychiatry **26**, 479-486.

182. Lessell,S. (1971) Experimental cyanide optic neuropathy. Arch.Ophthalmol. **86**, 194-204.

183. Philbrick,D.J., Hopkins,J.B., Hill,D.C., Alexander,J.C. and Thomson,R.G. (1979) Effects of prolonged cyanide and thiocyanate feeding in rats. J.Toxicol.Environ.Health **5**, 579-592.

184. Okeda,R., Song,S.Y., Funta,N. and Higashino,F. (1983) An experimental study of the pathogenesis of Grinker's myelinopathy in carbon monoxide intoxication. Acta Neuropathol.(Berl) **59**, 200-206.

185. SCHWEDENBERG,T.H. (1959) Leukoencephalopathy following carbon monoxide asphyxia. J Neuropathol.Exp.Neurol. **18**, 597-608.

186. Hsu,Y.K. and Cheng,Y.L. (1938) Cerebral subcortical myelinopathy in caron monoxide poisoning. Brain **61**, 384-392.

187. Foncin,J.F. and Le,B.J. (1978) [Myelinopathy due to carbon monoxyde poisoning. A study in ultrastructural neuropathology (author's transl)]. Acta Neuropathol. **43**, 153-159.

188. Ginsberg,M.D., Myers,R.E. and McDonagh,B.F. (1974) Experimental carbon monoxide encephalopathy in the primate. II. Clinical aspects, neuropathology, and physiologic correlation. Arch.Neurol. **30**, 209-216.

189. Zeis,T. and Schaeren-Wiemers,N. (2008) Lame ducks or fierce creatures? The role of oligodendrocytes in multiple sclerosis. Journal of Molecular Neuroscience **35**, 91-100.

190. Tsutsui,S. and Stys,P.K. (2013) Metabolic injury to axons and myelin. Exp.Neurol. **246**, 26-34.

191. d'Anglemont,d.T., X, Sirerol-Piquer,M.S., Gomez-Pinedo,U. et al. (2015) Resistance of subventricular neural stem cells to chronic hypoxemia despite structural disorganization of the germinal center and impairment of neuronal and oligodendrocyte survival. Hypoxia.(Auckl.) **3**, 15-33.

192. Lee,Y., Morrison,B.M., Li,Y. et al. (2012) Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature **487**, 443-448.

193. Funfschilling,U., Supplie,L.M., Mahad,D. et al. (2012) Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. Nature **485**, 517-521.

194. Nave,K.A. (2010) Myelination and the trophic support of long axons. Nat.Rev.Neurosci. **11**, 275-283.

195. Nave,K.A. (2010) Myelination and support of axonal integrity by glia. Nature **468**, 244-252.

196. Saab,A.S., Tzvetavona,I.D., Trevisiol,A. et al. (2016) Oligodendroglial NMDA Receptors Regulate Glucose Import and Axonal Energy Metabolism. Neuron **91**, 119-132.

197. Simons,M. and Nave,K.A. (2015) Oligodendrocytes: Myelination and Axonal Support. Cold Spring Harb.Perspect.Biol. **8**, a020479.

198. Nave,K.A. and Werner,H.B. (2014) Myelination of the nervous system: mechanisms and functions. Annu.Rev.Cell Dev.Biol. **30**, 503-533.

199. Saab,A.S., Tzvetanova,I.D. and Nave,K.A. (2013) The role of myelin and oligodendrocytes in axonal energy metabolism. Curr.Opin.Neurobiol. **23**, 1065-1072.

200. Matthews,M.A. and Duncan,D. (1971) A quantitative study of morphological changes accompanying the initiation and progress of myelin production in the dorsal funiculus of the rat spinal cord. J.Comp.Neurol. **142**, 1-22.

201. Witte,M.E., Mahad,D.J., Lassmann,H. and van,H.J. (2013) Mitochondrial dysfunction contributes to neurodegeneration in multiple sclerosis. Trends Mol.Med.

202. Dutta,R., McDonough,J., Yin,X. et al. (2006) Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. Ann.Neurol. **59**, 478-489.

203. Campbell,G.R. and Mahad,D.J. (2012) Mitochondrial changes associated with demyelination: consequences for axonal integrity. Mitochondrion **12**, 173-179.

204. Ziabreva,I., Campbell,G., Rist,J. et al. (2010) Injury and differentiation following inhibition of mitochondrial respiratory chain complex IV in rat oligodendrocytes. Glia **58**, 1827-1837.

205. Mahad,D., Lassmann,H. and Turnbull,D. (2008) Review: Mitochondria and disease progression in multiple sclerosis. Neuropathol.Appl.Neurobiol. **34**, 577-589.

206. COURVILLE,C.B. (1957) The process of demyelination in the central nervous system. IV. Demyelination as a delayed residual of carbon monoxide asphyxia. J Nerv.Ment.Dis. **125**, 534-546.

207. Bouzier-Sore,A.K. and Pellerin,L. (2013) Unraveling the complex metabolic nature of astrocytes. Front Cell Neurosci. **7**, 179.

208. Falkowska,A., Gutowska,I., Goschorska,M., Nowacki,P., Chlubek,D. and Baranowska-Bosiacka,I. (2015) Energy Metabolism of the Brain, Including the

Cooperation between Astrocytes and Neurons, Especially in the Context of Glycogen Metabolism. Int.J.Mol.Sci. **16**, 25959-25981.

209. Rone,M.B., Cui,Q.L., Fang,J. et al. (2016) Oligodendrogliopathy in Multiple Sclerosis: Low Glycolytic Metabolic Rate Promotes Oligodendrocyte Survival. J.Neurosci. **36**, 4698-4707.

210. Hamilton,N.B., Kolodziejczyk,K., Kougioumtzidou,E. and Attwell,D. (2016) Proton-gated Ca(2+)-permeable TRP channels damage myelin in conditions mimicking ischaemia. Nature **529**, 523-527.

211. Mifsud,G., Zammit,C., Muscat,R., Di,G.G. and Valentino,M. (2014) Oligodendrocyte pathophysiology and treatment strategies in cerebral ischemia. CNS.Neurosci.Ther. **20**, 603-612.

212. Juurlink,B.H., Thorburne,S.K. and Hertz,L. (1998) Peroxide-scavenging deficit underlies oligodendrocyte susceptibility to oxidative stress. Glia **22**, 371-378.

213. Thorburne,S.K. and Juurlink,B.H.J. (1996) Low glutathione and high iron govern the susceptibility of oligodendroglial precursors to oxidative stress. J.Neurochem. **67**, 1014-1022.

214. McDonald,J.W., Althomsons,S.P., Hyrc,K.L., Choi,D.W. and Goldberg,M.P. (1998) Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. Nat.Med. **4**, 291-297.

215. Juurlink,B.H. (1997) Response of glial cells to ischemia: roles of reactive oxygen species and glutathione. Neuroscience & Biobehavioral Reviews **21**, 151-166.

216. Connor,J.R. (1994) Iron acquisition and expression of iron regulatory proteins in the developing brain: Manipulation by ethanol exposure, iron deprivation and cellular dysfunction. Dev.Neurosci. **16**, 233-247.

217. Roth,A.D. and Nunez,M.T. (2016) Oligodendrocytes: Functioning in a Delicate Balance Between High Metabolic Requirements and Oxidative Damage. Adv.Exp.Med.Biol. **949**, 167-181.

218. Hametner,S., Wimmer,I., Haider,L., Pfeifenbring,S., Bruck,W. and Lassmann,H. (2013) Iron and neurodegeneration in the multiple sclerosis brain. Ann.Neurol. **74**, 848-861.

219. Murphy,M.P. (2009) How mitochondria produce reactive oxygen species. Biochem.J **417**, 1-13.

220. Gay,F. (2007) Activated microglia in primary MS lesions: defenders or aggressors? International Ms Journal **14**, 78-83.

221. Gay,F.W., Drye,T.J., Dick,G.W. and Esiri,M.M. (1997) The application of multifactorial cluster analysis in the staging of plaques in early multiple sclerosis. Identification and characterization of the primary demyelinating lesion. Brain **120**, 1461-1483.

222. Barnett,M.H. and Prineas,J.W. (2004) Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann.Neurol. **55**, 458-468.

223. Gay,F.W. (2006) Early cellular events in multiple sclerosis. Intimations of an extrinsic myelinolytic antigen. Clin.Neurol.Neurosurg. **108**, 234-240.

224. Prineas,J.W. (1985) The neuropathology of multiple sclerosis. In: Handbook of Clinical Neurology Vol.3(47): Demyelinating diseases 213-257.

225. Henderson,A.P., Barnett,M.H., Parratt,J.D. and Prineas,J.W. (2009) Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. Ann.Neurol. **66**, 739-753.

226. Lassmann,H. (2007) Experimental models of multiple sclerosis. Rev.Neurol. **163**, 651-655.

227. Barnett,M.H., Henderson,A.P. and Prineas,J.W. (2006) The macrophage in MS: just a scavenger after all? Pathology and pathogenesis of the acute MS lesion. Multiple Sclerosis **12**, 121-132.

228. Traka,M., Podojil,J.R., McCarthy,D.P., Miller,S.D. and Popko,B. (2016) Oligodendrocyte death results in immune-mediated CNS demyelination. Nat.Neurosci. **19**, 65-74.

229. Davies,A.L., Desai,R.A., Bloomfield,P.S. et al. (2013) Neurological deficits caused by tissue hypoxia in neuroinflammatory disease. Ann.Neurol. **74**, 815-825.

230. Bennett,M. and Heard,R. (2010) Hyperbaric oxygen therapy for multiple sclerosis. CNS.Neurosci.Ther. **16**, 115-124.

231. Bennett,M. and Heard,R. (2004) Hyperbaric oxygen therapy for multiple sclerosis. Cochrane.Database.Syst.Rev. CD003057.

232. Novak,V., Last,D., Alsop,D.C. et al. (2006) Cerebral blood flow velocity and periventricular white matter hyperintensities in type 2 diabetes. Diabetes Care **29**, 1529-1534.

233. Yau,P.L., Hempel,R., Tirsi,A. and Convit,A. (2013) Cerebral white matter and retinal arterial health in hypertension and type 2 diabetes mellitus. Int.J.Hypertens. **2013**, 329602.

234. Fukuda,H. and Kitani,M. (1995) Differences between treated and untreated hypertensive subjects in the extent of periventricular hyperintensities observed on brain MRI. Stroke **26**, 1593-1597.

235. Fabjan,A., Zaletel,M. and Zvan,B. (2015) Is there a persistent dysfunction of neurovascular coupling in migraine? Biomed.Res.Int. **2015**, 574186.

236. Goadsby,P.J. (2013) Autonomic nervous system control of the cerebral circulation. Handb.Clin.Neurol. **117**, 193-201.

237. Tfelt-Hansen,P.C. and Koehler,P.J. (2011) One hundred years of migraine research: major clinical and scientific observations from 1910 to 2010. Headache **51**, 752-778.

238. Gaist,D., Garde,E., Blaabjerg,M. et al. (2016) Migraine with aura and risk of silent brain infarcts and white matter hyperintensities: an MRI study. Brain **139**, 2015-2023.

239. Yasuda,T., Kodera,Y., Iijima,K. et al. (2016) Characteristics of Cerebral White Matter Lesions on MRI in Juvenile Patients with Migraine. Tokai J.Exp.Clin.Med. **41**, 156-162.

240. Haq,E., Giri,S., Singh,I. and Singh,A.K. (2003) Molecular mechanism of psychosine-induced cell death in human oligodendrocyte cell line. J.Neurochem. **86**, 1428-1440.

241. Tapasi,S., Padma,P. and Setty,O.H. (1998) Effect of psychosine on mitochondrial function. Indian J.Biochem.Biophys. **35**, 161-165.

242. Cooper,C.E., Markus,M., Seetulsingh,S.P. and Wrigglesworth,J.M. (1993) Kinetics of inhibition of purified and mitochondrial cytochrome c oxidase by psychosine (beta-galactosylsphingosine). Biochem.J. **290**, 139-144.

243. Given,C.A., Santos,C.C. and Durden,D.D. (2001) Intracranial and spinal MR imaging findings associated with Krabbe's disease: case report. AJNR Am.J.Neuroradiol. **22**, 1782-1785.

244. Zarifi,M.K., Tzika,A.A., Astrakas,L.G., Poussaint,T.Y., Anthony,D.C. and Darras,B.T. (2001) Magnetic resonance spectroscopy and magnetic resonance imaging findings in Krabbe's disease. J.Child Neurol. **16**, 522-526.

245. Abdelhalim,A.N., Alberico,R.A., Barczykowski,A.L. and Duffner,P.K. (2014) Patterns of magnetic resonance imaging abnormalities in symptomatic patients with Krabbe disease correspond to phenotype. Pediatr.Neurol. **50**, 127-134.

246. Harding,A.E., Sweeney,M.G., Miller,D.H. et al. (1992) Occurrence of a multiple sclerosis-like illness in women who have a Leber's hereditary optic neuropathy mitochondrial DNA mutation. Brain **115**, 979-989.

247. Matthews,L., Enzinger,C., Fazekas,F. et al. (2015) MRI in Leber's hereditary optic neuropathy: the relationship to multiple sclerosis. J.Neurol.Neurosurg.Psychiatry **86**, 537-542.

248. Garrido,E., Castello,A., Ventura,J.L., Capdevila,A. and Rodriguez,F.A. (1993) Cortical atrophy and other brain magnetic resonance imaging (MRI) changes after extremely high-altitude climbs without oxygen. Int.J.Sports Med. **14**, 232-234.

249. Garrido,E., Segura,R., Capdevila,A. et al. (1995) New evidence from magnetic resonance imaging of brain changes after climbs at extreme altitude. Eur.J.Appl.Physiol Occup.Physiol **70**, 477-481.

250. Paola,M.D., Bozzali,M., Fadda,L., Musicco,M., Sabatini,U. and Caltagirone,C. (2008) Reduced oxygen due to high-altitude exposure relates to atrophy in motor-function brain areas. Eur.J.Neurol. **15**, 1050-1057.

251. Hon,G.M., Hassan,M.S., van Rensburg,S.J., Erasmus,R.T. and Matsha,T. (2012) The haematological profile of patients with multiple sclerosis. Open Journal of Modern Neurosurgery **2**, 36-44.

252. Koudriavtseva,T., Renna,R., Plantone,D., Mandoj,C., Piattella,M.C. and Giannarelli,D. (2015) Association between anemia and multiple sclerosis. Eur.Neurol. **73**, 233-237.

253. Tettey,P., Siejka,D., Simpson S Jr et al. (2016) Frequency of Comorbidities and Their Association with Clinical Disability and Relapse in Multiple Sclerosis. Neuroepidemiology **46**, 106-113.

254. Altinoz,M.A., Ozcan,E.M., Ince,B. and Guloksuz,S. (2016) Hemoglobins as new players in multiple sclerosis: metabolic and immune aspects. Metab Brain Dis. **31**, 983-992.

255. Suhs,K.W., Hein,K., Sattler,M.B. et al. (2012) A randomized, double-blind, phase 2 study of erythropoietin in optic neuritis. Ann.Neurol. **72**, 199-210.

256. Bartels,C., Spate,K., Krampe,H. and Ehrenreich,H. (2008) Recombinant Human Erythropoietin: Novel Strategies for Neuroprotective/Neuro-regenerative Treatment of Multiple Sclerosis. Ther.Adv.Neurol.Disord. **1**, 193-206.

257. Ehrenreich,H., Fischer,B., Norra,C. et al. (2007) Exploring recombinant human erythropoietin in chronic progressive multiple sclerosis. Brain **130**, 2577-2588.

258. Oliveira,S.R., Simao,A.N., Kallaur,A.P. et al. (2014) Disability in patients with multiple sclerosis: influence of insulin resistance, adiposity, and oxidative stress. Nutrition **30**, 268-273.

259. Chataway,J., Schuerer,N., Alsanousi,A. et al. (2014) Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. Lancet **383**, 2213-2221.

260. Tourbah,A., Lebrun-Frenay,C., Edan,G. et al. (2016) MD1003 (high-dose biotin) for the treatment of progressive multiple sclerosis: A randomised, double-blind, placebo-controlled study. Mult.Scler. **22**, 1719-1731.

261. Sedel,F., Bernard,D., Mock,D.M. and Tourbah,A. (2016) Targeting demyelination and virtual hypoxia with high-dose biotin as a treatment for progressive multiple sclerosis. Neuropharmacology **110**, 644-653.

262. van Dijk,E.J., Vermeer,S.E., de Groot,J.C. et al. (2004) Arterial oxygen saturation, COPD, and cerebral small vessel disease. J.Neurol.Neurosurg.Psychiatry **75**, 733-736.

263. van Dijk,E.J., Prins,N.D., Vrooman,H.A., Hofman,A., Koudstaal,P.J. and Breteler,M.M. (2008) Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan study. Stroke **39**, 2712-2719.

264. Ascherio,A. and Munger,K.L. (2016) Epidemiology of Multiple Sclerosis: From Risk Factors to Prevention-An Update. Semin.Neurol. **36**, 103-114.

265. Poorolajal,J., Bahrami,M., Karami,M. and Hooshmand,E. (2016) Effect of smoking on multiple sclerosis: a meta-analysis. J.Public Health (Oxf).

266. Conway,D.S., Thompson,N.R. and Cohen,J.A. (2016) Influence of hypertension, diabetes, hyperlipidemia, and obstructive lung disease on multiple sclerosis disease course. Mult.Scler.

267. van Dijk,E.J., Breteler,M.M., Schmidt,R. et al. (2004) The association between blood pressure, hypertension, and cerebral white matter lesions: cardiovascular determinants of dementia study. Hypertension **44**, 625-630.

268. Duschek,S. and Schandry,R. (2003) Functional transcranial Doppler sonography as a tool in psychophysiological research. Psychophysiology **40**, 436-454.

269. Salamon,G., Lazorthes,G. and Corbaz,J.M. (1971) Atlas de la Vascularisation Artérielle Du Cerveau Chez L'homme, Sandoz,

270. Chisholm,K.I., Ida,K.K., Davies,A.L. et al. (2015) Hypothermia protects brain mitochondrial function from hypoxemia in a murine model of sepsis. J.Cereb.Blood Flow Metab **36**, 1955-1964.