Anterior visual system imaging to investigate energy failure in multiple sclerosis

Iris Kleerekooper*[a,b], Axel Petzold[a,b,c], S Anand Trip[a]

Affiliations:

[a] Department of Neuroinflammation, UCL Institute of Neurology, Queen Square, London, UK; The National Hospital for Neurology and Neurosurgery

[b] Moorfields Eye Hospital, City Road, London

[c] Dutch Expertise Centre Neuro-ophthalmology and MS Centre Amsterdam, Amsterdam University Medical Centre, Amsterdam, The Netherlands

Abstract

Mitochondrial failure and hypoxia are key contributors to multiple sclerosis pathophysiology. Importantly, improving mitochondrial function holds promise as a new therapeutic strategy in multiple sclerosis. Currently, studying mitochondrial changes in multiple sclerosis is hampered by a paucity of non-invasive techniques to investigate mitochondrial function of the central nervous system *in vivo*. It is against this backdrop that the anterior visual system provides new avenues for monitoring of mitochondrial changes. The retina and optic nerve are among the metabolically most active structures in the human body and are almost always affected to some degree in multiple sclerosis. Here, we provide an update on emerging technologies which have the potential to indirectly monitor changes of metabolism and mitochondrial function. We will report on the promising work with optical coherence tomography, showing structural changes in outer retinal mitochondrial signal bands, and with optical coherence angiography, quantifying retinal perfusion at the microcapillary level. We show that adaptive optics scanning laser ophthalmoscopy can visualise live perfusion through microcapillaries and structural changes at the level of single photoreceptors and neurons. Advantages and limitations of these techniques will be summarised with regard to future research into the pathology of the disease and as trial outcome measures.

*Corresponding author ORCID: 000-0001-6456-9034 E-mail: iris.kleerekooper.18@ucl.ac.uk

Introduction

Anita Harding's landmark paper, describing a group of patients with the mitochondrial disease Leber's hereditary optic neuropathy (LHON) that developed demyelinating disease of the CNS, indicated that mitochondrial gene mutations may contribute to multiple sclerosis susceptibility and poor outcome (Harding *et al*., 1992). Her work has prompted substantial expansion of research on mitochondrial failure in multiple sclerosis. However, the exact role of energy failure in the disease pathophysiology remains an issue of debate. Retinal imaging may provide valuable new tools for future investigations in this field. To show this, we will first summarise the current knowledge regarding the energy failure paradigm in multiple sclerosis. Subsequently, we will describe the unique cellular processes that account for the extremely high metabolic demand of the anterior visual system, which make retinal imaging an excellent tool for investigating energy failure. And finally, we will review the exciting prospects that the evolving field of retinal imaging offer to evaluate metabolic status.

The anterior visual system is one of the most metabolically active structures in the human body and is often affected by multiple sclerosis (Joyal *et al*., 2018; Wong-Riley, 2010, Okawa *et al*., 2008; Beck *et al*., 2003). The prevalence of symptomatic multiple sclerosis associated optic neuritis (MSON) increases from 20% to 80% in early compared with later disease (Shams and Plant, 2009; Optic Neuritis Study Group, 1991). Post-mortem evidence even shows anterior visual pathway involvement in 90% of examined cases (Toussaint *et al*., 1983). Optical coherence tomography (OCT) reveals retinal thinning independent of clinical MSON (Coric *et al*., 2018; Petzold *et al*., 2017; Trip *et al.*, 2006), suggesting that progressive features of multiple sclerosis, such as atrophy, can be captured in the retina. Furthermore, the pathology of multiple sclerosis involves all retinal layers in histological studies (Evangelou and Alrawashdeh, 2016; Green *et al*., 2010). This is relevant because retinal imaging permits imaging of a variety of retinal pathologic processes in multiple sclerosis, such as primary pathology to ganglion cells, anterograde degeneration (Saidha *et al*., 2011; Petzold *et al*., 2010) and inflammation in the nuclear layers (Balk *et al.* 2019; Petzold *et al*., 2017; Gelfand *et al*., 2012).

Currently, there are no validated quantitative methods for non-invasive, longitudinal, *in vivo* assessment of CNS mitochondrial function and metabolism. Such methods are key to a better

understanding of the role of energy failure in patients suffering from multiple sclerosis. All existing methods for human *in vivo* studies rely on indirect measures. It is against this backdrop that we update on the potential of cutting-edge multimodal imaging tools of the anterior visual system.

In summary, the eye provides an easily accessible window to the CNS, and retinal imaging tools, which in recent years have rapidly become smaller, faster and capable of extremely high resolutions, provide exciting new prospects for non-invasive investigations into local metabolic function (Koustenis Jr *et al*., 2017; Godara *et al*., 2010).

Tissue energy failure in multiple sclerosis

Mitochondrial failure

Mitochondria are the key facilitators of oxidative phosphorylation, the most efficient metabolic pathway for producing energy in the form of ATP in aerobic organisms (Suomalainen and Battersby, 2018). Mitochondria also contribute to Ca^{2+} homeostasis, failure of which may lead to intracellular Ca^{2+} overload and apoptosis (Giorgi *et al*., 2018). The close functional and structural association of glia and the neuro-axonal complex is an important factor in multiple sclerosis pathophysiology. Glia are a major source of inflammatory mediators such as reactive oxygen species (ROS) (Fischer et al., 2012; Nave, 2010), which in increased concentrations aggravate demyelination and metabolic dysfunction by damaging vulnerable oligodendrocytes and mitochondria (Smith *et al.*, 1999). Also, glia play important roles in adapting to variable energy demands by regulating glucose availability in multiple sclerosis lesions (Saab and Nave, 2017; Nijland *et al.*, 2014).

Mitochondrial dysfunction, particularly the accumulation of dysmorphic and swollen mitochondria, is among the first histopathological manifestations of the murine multiple sclerosis model experimental autoimmune encephalomyelitis (EAE) (Nikić *et al*., 2011). Mitochondrial changes in EAE are related to clinical disease activity in time and severity (Sadeghian *et al*., 2016).

Chronic multiple sclerosis lesions, chronically demyelinated axons and even remyelinated axons typically contain increased numbers of mitochondria (Zambonin *et al*., 2011; Mahad *et al*., 2009) and marked upregulation of respiratory chain complex IV activity (Campbell *et al.*, 2011; Witte *et al*., 2009; Mahad *et al*., 2008).

Multiple sclerosis plaques contain high levels of oxidative damage, particularly to the vulnerable mitochondrial DNA (mtDNA) (Vladimirova *et al*., 1998). One study found that respiratory deficient neurons affected by multiple sclerosis lack an mtDNA-encoded catalytic subunit of respiratory chain complex IV, which could be brought back to high levels of clonally expanded mtDNA deletions on a single-neuron level (Campbell *et al*., 2011). Furthermore, multiple sclerosis is associated with high levels of mtHSP70, a marker for mitochondrial stress (Witte *et al*., 2009), and a significant decrease in messenger RNA of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), an important regulator of metabolism and mitochondrial function (Witte *et al*., 2013).

Exposing neurons to cerebrospinal fluid (CSF) from individuals affected by multiple sclerosis *in vitro* leads to mitochondrial elongation and dysfunction of respiratory chain complexes I, III and IV. Interestingly, this neurotoxic effect could be reversed if extra glucose and lactate was supplied (Wentling *et al.*, 2019).

Studies investigating mitochondrial failure in multiple sclerosis in humans are relatively rare. However, patients with multiple sclerosis were found to have higher concentrations of extra-mitochondrial glucose metabolites, such as lactate, as well as ATP metabolites (such as purines and oxypurines) in their CSF and serum compared with controls (Lazzarino *et al*., 2017; Albanese *et al*., 2016; Amorini *et al*., 2014). A recent study identified decreased respiratory chain complex IV activity in serum mononuclear cells of multiple sclerosis patients compared with controls (Hargreaves et al., 2018).

Finally, lower concentrations of N-acetyl-acetate (NAA), a molecule believed to reflect mitochondrial function, have been shown to predict a worse clinical outcome in multiple sclerosis (Van Horssen *et al*., 2012). However, as NAA is thought to reflect neuronal cell loss

as well as dysfunction of neuronal mitochondria, these results might be partly confounded by CNS atrophy.

Hypoxia

Cerebral type-III multiple sclerosis lesions have histological characteristics similar to hypoxic insult and multiple sclerosis lesions tend to form in watershed areas of the brain, suggesting a role for hypoxia in its pathophysiology (Yang and Dunn, 2018; Martinez Sosa and Smith, 2017).

Indeed, several studies identified severe hypoxia of the brain and spinal cord in EAE affected animals (Johnson *et al*., 2016), which was related in time and severity to the neurologic deficit (Davies *et al*., 2013). In a model multiple sclerosis lesion in the rat spinal cord, demyelination could be considerably reduced by breathing oxygen in high concentrations (Desai *et al*, 2016).

In humans, multiple studies demonstrated that multiple sclerosis is associated with reduced cerebral blood flow compared with healthy controls, also in areas of normal appearing white matter (Marshall *et al*., 2016; Law *et al.*, 2004). A study which used near-infrared spectroscopy (NIRS) showed that almost half of multiple sclerosis patients had haemoglobin saturation values that were significantly reduced compared with healthy controls (Yang and Dunn, 2015).

In addition to genuine hypoxia, the increased energy demands of impulse conduction along demyelinated axons in multiple sclerosis may result in mitochondrial upregulation, causing increased numbers of mitochondria to be a continuous source of deleterious ROS (Trapp and Stys, 2009). This situation, referred to as ‘virtual hypoxia’, initiates cellular signalling pathways affiliated with hypoxic and ischaemic conditions, resulting in oxidative stress, further mitochondrial dysfunction and increased intracellular Ca^{2+} concentrations through release of toxic Ca^{2+} from the axoplasmic reticulum (Desai and Smith, 2017; Trapp and Stys, 2009).

Hypoxia and mitochondrial dysfunction can cause a vicious circle inducing axonal failure and neurodegeneration in multiple sclerosis, with one process impairing neuronal resilience to the other.

The anterior visual system as a model for metabolic failure

Energy demand

The CNS has a very high energy demand, representing only 2% of bodyweight, but 20% of all resting state energy consumption (Lax *et al*., 2017). The retina consumes even more energy, with its metabolic rate generally reported to exceed that of the brain (Joyal *et al*., 2018, Wong-Riley, 2010, Warrant, 2009; Okawa *et al*., 2008) (Fig. 1). Accordingly, the choroid has the highest perfusion rate of any other structure within the human body and can rapidly adapt to changes in energy demands (Yu *et al*., 2019; Joyal *et al*., 2018).

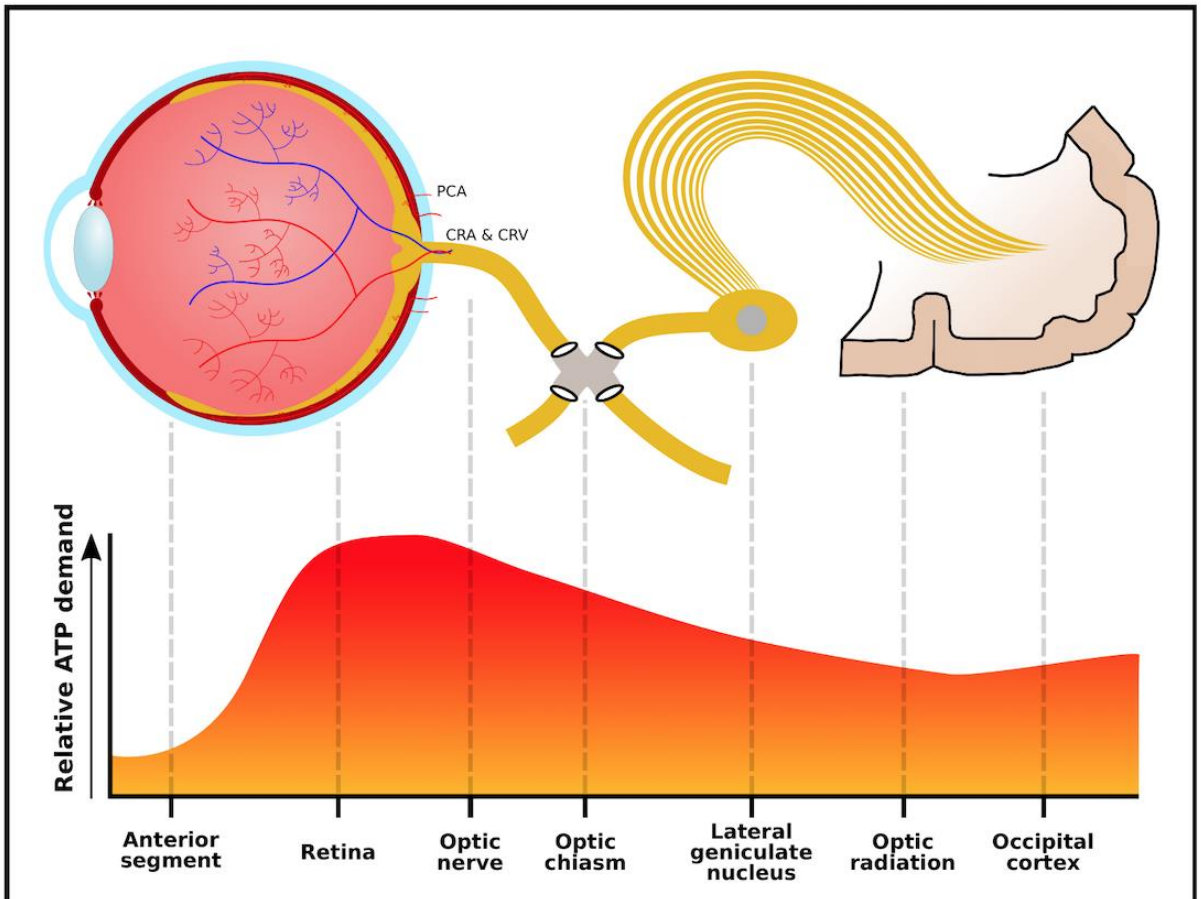

Figure 1. Schematic representation of the relative ATP demand along the length of visual stimulus processing in the visual pathway, showing the relatively high ATP demand of the anterior visual system. *PCA: posterior cilliary artery. CRA & CRV: central retinal artery and central retinal vein.* Original work.

The main function of the retina is the process of converting photons into an electrical retinal signal, called phototransduction. Counter-intuitively, in daylight, rods use approximately 75% less energy than in scotopic conditions. In contrast, cones use a similar amount of energy as dark-adapted rods in scotopic as well as photopic conditions. So, cones are more metabolically costly than rods on a cellular level, but because rods greatly outnumber cones, net retinal energy demand still decreases in daylight (Okawa *et al.*, 2008). In the dark, ion channels in the cell membrane of photoreceptors are open and allow influx of ions (Warrant, 2009). To maintain a relative depolarisation of -40 mV, a state called the 'dark current', Na^+ and Ca^{2+} ions are actively pumped out extracellularly against an ion-concentration gradient using two solute pumps, Na^+/K^+-ATPase and Ca^{2+}-ATPase. Together, these two pumps account for virtually all expended energy in dark-adapted photoreceptors. The energy consumption of a dark-adapted photoreceptor ranks top of all mammalian cells (Wong-Riley, 2010; Warrant, 2009).

Human vision starts with photon absorption by visual pigments in the photoreceptor outer segments, of which rhodopsin is the most abundant. Captured photons activate rhodopsin, after which rhodopsin binds to the G-protein transducin. As a result, the GDP molecule bound to the transducin α-subunit (T-α) is replaced by GTP, and the T-α GTP complex is released. The T-α GTP complex subsequently removes one ɣ-subunit from inactive phosphodiesterase (PDE). A total of two ɣ-subunits need to be removed to fully activate PDE. Activated PDE decreases the intracellular concentration of cyclic guanine monophosphate (cGMP) by hydrolysing cGMP to GMP. As a result of the lower cGMP concentration, ion channels in the cell membrane of the photoreceptor close, halting ion influx which leads to subsequent hyperpolarisation of the neuron.

The hyperpolarised photoreceptor stops releasing excitotoxic neurotransmitter glutamate, resulting in the generation of an electrical potential in one or more bipolar cells (Wong-Riley *et al*, 2010; Okawa *et al*., 2008). Most energy is used in the rod outer segments, while the majority of energy is created in the inner segments, where 60-65% of all retinal mitochondria accumulate.

Axons in the nerve fibre layer conducting this information are unmyelinated before they pass through the lamina cribrosa. This unmyelinated portion has slower conduction velocities and a markedly higher energy demand, as signified by marked cytochrome c oxidase (COX) staining locally (Carelli *et al.,* 2004).

Mitochondrial optic neuropathies

The combination of LHON and multiple sclerosis (LHON-MS) occurs approximately 50 times more often than is expected by chance (Matthews *et al.,* 2015). Both LHON-MS and multiple sclerosis predominantly occur in women, even though LHON is more prevalent in men, and have a relapsing and a progressive course (Palace, 2009; Harding *et al.,* 1992). MRI images of traditional multiple sclerosis and LHON-MS patients have been found to be radiologically indistinguishable (Matthews *et al.,* 2015) and a post-mortem case report found multiple sclerosis-like neuropathological features, such as axonal damage and demyelination, in the motor cortex of a patient with LHON-MS (Kovács *et al.,* 2005). From clinical experience we recognise that patients with LHON-MS have more severe visual loss and poorer recovery of optic neuropathy compared with multiple sclerosis, implying that impaired metabolism related to LHON mtDNA mutations aggravates the inflammatory insult and impairs recovery. Interestingly, both multiple sclerosis and mitochondrial optic neuropathies such as LHON predominantly affect the thinner retinal ganglion cell fibres ending in the parvocellular layers of the lateral geniculate nucleus (Carelli *et al.,* 2004; Evangelou *et al.,* 2001). One possible explanation is that the thin diameter of these parvocellular fibres poses anatomical constraints on the transport of mitochondria through the axon (Carelli *et al.,* 2004).

Furthermore, another mitochondrial optic neuropathy, 'dominant optic atrophy' can cause extra-ocular neurologic manifestations such as ataxia and spasticity, and some patients show MRI lesions resembling multiple sclerosis (Yu-Wai-Man *et al*., 2010).

Retinal imaging of metabolism

Structural changes

Besides the use of OCT for imaging of retinal layer thickness as a marker of atrophy and microcystic macular oedema as a marker of inflammation (Gelfand *et al*., 2012), novel OCT modalities, focusing on the metabolically active photoreceptors, could indirectly provide insight into mitochondrial function. Mitochondria have a high reflectivity index on OCT (Litts *et al*., 2018). Both the second and the fourth hyperreflective band seen on OCT relate to local mitochondrial accumulation, with the second band corresponding to the ellipsoid zone of the photoreceptor inner segments, containing the majority of retinal mitochondria (Cuenca *et al*., 2018). Reduced relative intensity or structural integrity of the ellipsoid zone may reflect decreased numbers or function of mitochondria. In age-related macular degeneration (AMD), a decreased relative intensity of this ellipsoid zone has indeed been reported (Tao *et al*., 2016). This is an interesting observation as mitochondrial complex failure has been implicated in AMD (Nag and Wadhwa, 2016).

Furthermore, OCT allows functional imaging of retinal dynamics during dark-adaptation. Photoreceptor outer segments increase in length in dark-adapted compared with photopic conditions (Lu *et al.*, 2017). This lengthening of retinal outer segments during dark adaptation was found to be reduced in vitelliform macular dystrophy (synonymous Best disease) (Abràmoff *et al*., 2013). Taken into account the discussed very high energy demands of dark adaptation and the close relationship of severity with well characterised genetic mutations in Best disease, this observation warrants further exploration as it may be a valid indirect quantitative measure for metabolic failure in the retina.

Adaptive optics uses an adaptive mirror to reduce wavefront distortions and can thereby improve the resolution of imaging techniques. The combination of adaptive optics and scanning laser ophthalmoscopy (AOSLO) has a particularly high spatial resolution and is capable of imaging the individual cone photoreceptor mosaic within the retina (Godara *et al*., 2010) (Fig. 2). Using AOSLO, cones of patients with the mitochondrial syndrome of neurogenic weakness, ataxia and retinitis pigmentosa (NARP) have been shown to contain various levels of photoreceptor degeneration and significant variability in spacing and packing within individual eyes (Yoon *et al*., 2009). This might be the first non-invasive and *in vivo* measurement

photographing the effects of mitochondrial dysfunction on cone structure at a single cellular level.

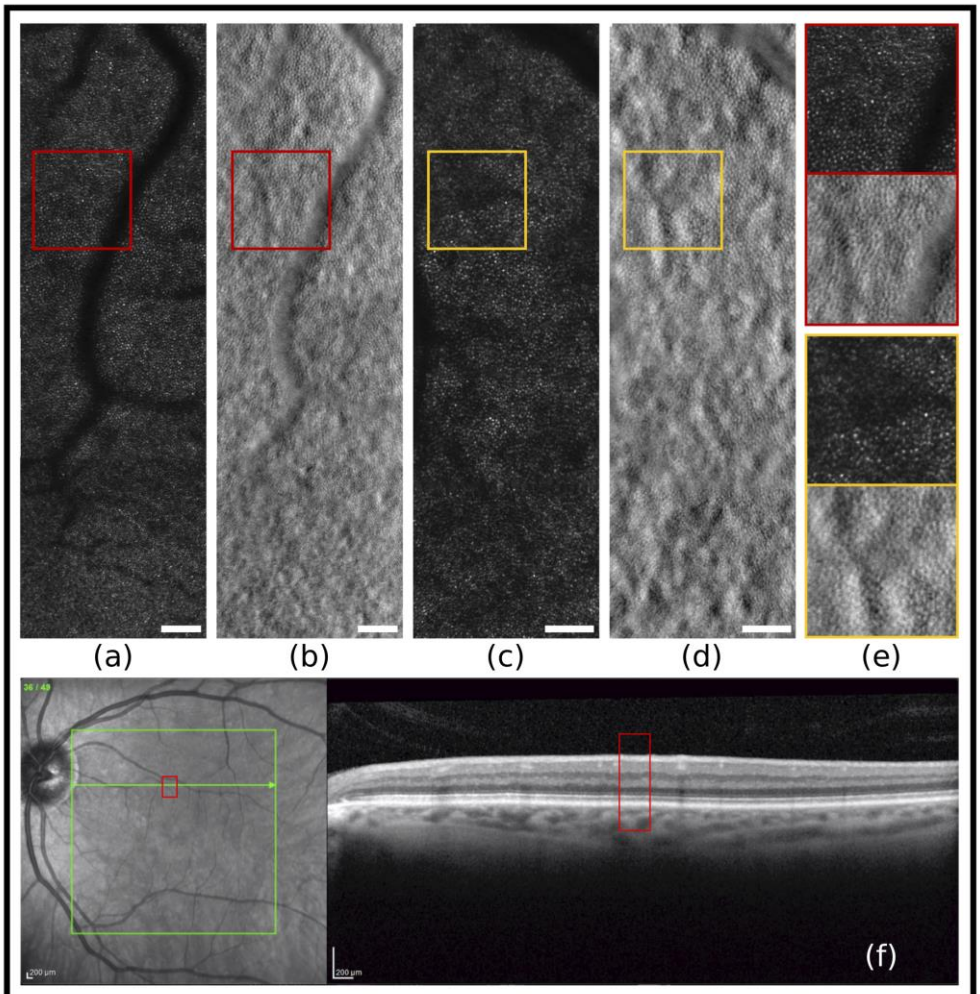

Figure 2. AOSLO cone mosaic imaging. (a) Confocal AOSLO image of a normal cone mosaic. (b) Split detector AOSLO image of normal cone mosaic, each dimple representing an individual cone. (c) Confocal AOSLO cone mosaic image in an MS patient showing darker patches with reduced cone reflectance, see yellow circle inset for an example of such an area. (d) Split detector AOSLO image of the same MS patient as in previous figure showing similar cone density in these areas of reduced cone reflectance, suggesting MS may be related to a change in cone composition of unknown etiology that warrants further investigation. (e) enlarged insets of a normal cone mosaic with reduced reflectance on confocal AOSLO image reflecting a vessel (in red) and enlarged insets of abnormal area of reduced cone reflectance (in yellow). (f) OCT image of same patient as in images (c) and (d), showing no explanatory abnormalities, such as cysts, for the shadowing effects in the AOSLO images. Red inset represents area imaged with AOSLO. Figures courtesy of Dr. Adam Dubis and Ms. Sarah Houston (UCL Institute of Ophthalmology, London). *Scale bar: 100 µm.*

In the evolving field of retinal imaging, multiple approaches to *en face* imaging techniques are promising alternatives to regular OCT, which uses an axial B-scan approach. Dynamic full field OCT (FFOCT) achieves resolutions of less than 1µm *ex vivo*, allowing for visualising dynamic properties of subcellular structures such as mitochondria (Leitgeb, 2019; Dubois *et al.*, 2002). Acquisition of *in vivo* images of similar resolution is still hampered by challenges, mainly related to ocular movement (Leitgeb, 2019). However, provided that similar resolutions could be achieved, this would allow quantification of retinal mitochondria and potentially evaluation of their structural integrity. If done longitudinally, these evaluations could provide valuable insights into the timing of mitochondrial failure in multiple sclerosis.

The imaging methods described above may provide relevant outcome measures in trials investigating the effect of newly proposed metabolism targeted therapies in multiple sclerosis. For example, biotin (vitamin B7) is a co-enzyme involved in the TCA cycle and has been shown to improve ATP availability in a state of virtual hypoxia. Currently, a large trial investigating the effects of biotin in secondary progressive multiple sclerosis is under way, following a small trial that showed reduced progression rates in progressive multiple sclerosis patients (Tourbah *et al.*, 2016). Lipoic acid, an antioxidant and cofactor for key enzymes in the TCA cycle, has also been shown to be well tolerated while reducing brain atrophy rates in patients with secondary progressive multiple sclerosis (Spain *et al.*, 2017).

Vascular density

OCT angiography (OCTA) images the microvasculature in the retina and choroid by inferring information on blood flow through motion contrast between high-speed repeated B-scans (Koustenis Jr *et al*., 2017). Unlike old retinal vascular imaging techniques, such as fluorescein angiography, OCTA is non-invasive, quick and depth resolved (Yu *et al*, 2019; Leitgeb et al., 2014) (Fig. 3).

Already, OCTA has consistently shown that vessel density in the superficial and deep vascular plexuses is reduced in multiple sclerosis patients compared with healthy controls, and relates to visual function and disability scores (Feucht *et al.*, 2019; Murphy *et al.*, 2019; Wang *et al.*, 2018a). This decreased retinal vessel density, called ‘capillary dropout’, is mainly present

within the optic nerve head, and to a lesser degree in the macular area (Wang *et al.*, 2018a). Whether or not capillary dropout on OCTA imaging in acute MSON precedes later ganglion cell and inner plexiform layer loss on OCT is not known. If shown prospectively, this would lend further weight to the hypothesised role of hypoxia in multiple sclerosis lesion formation.

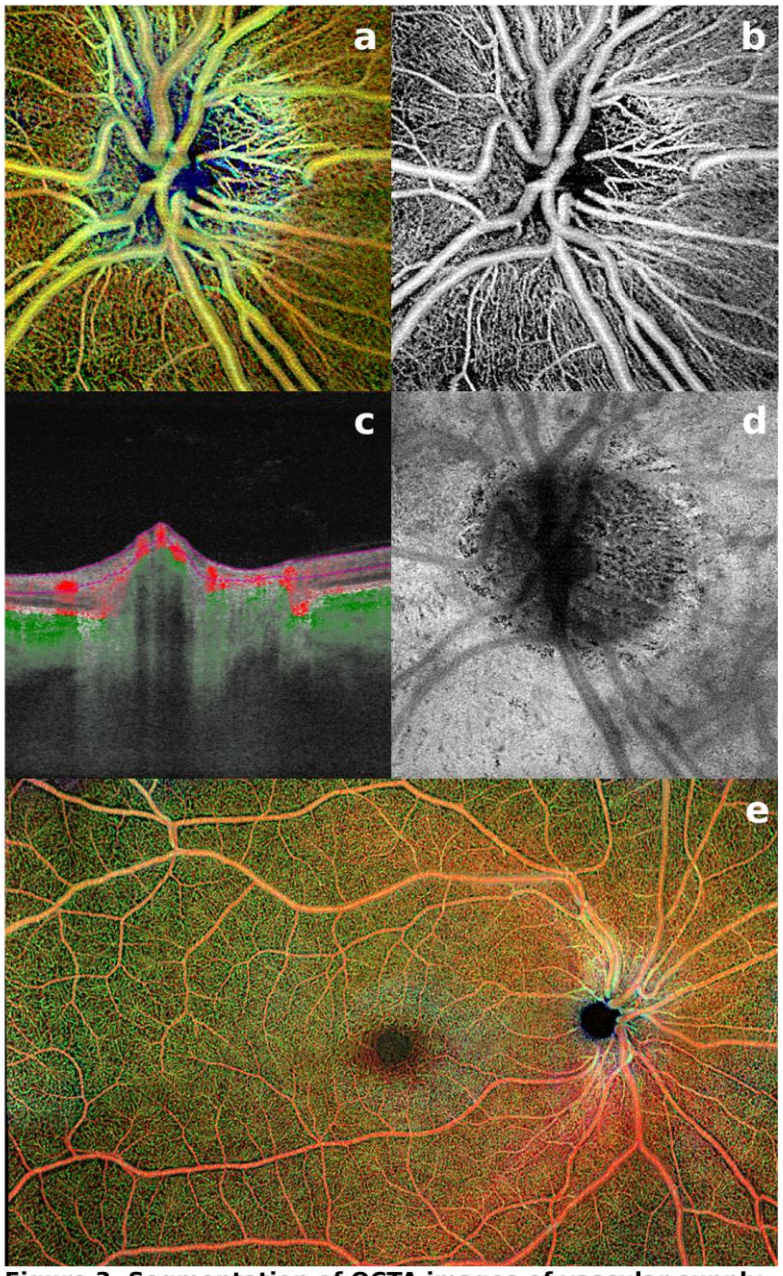

Figure 3. Segmentation of OCTA images of vascular supply in different layers. (a) (b) and (d) OCTA centered at the optic nerve showing depth encoded colour coding, segmentation of the superficial vascular plexus and the choriocapillary, respectively. (c) B-scan of optic nerve, colours representing different vascular layers. (e) composite of depth encoded vasculature whole retina. All images acquired with the PLEX Elite 9000 Swept-Source OCT. Images (a) to (d) represent a retinal segment of 3x3mm. All photo's from healthy individuals.

AOSLO can also image the retinal microvasculature, at even higher magnification. In diabetic retinopathy, small vasculopathies, such as neovascularisation and microaneurysms, that were not detectable with conventional methods such as OCT were readily detectable with AOSLO (Karst et al., 2018). Additionally, multiple microscopic inner-retinal phenotypes of unknown aetiology and relevance have been observed with AOSLO in multiple neurological diseases (Scoles *et al.*, 2014). Future research has to show if similar changes might also be of clinical importance in multiple sclerosis (Fig. 4).

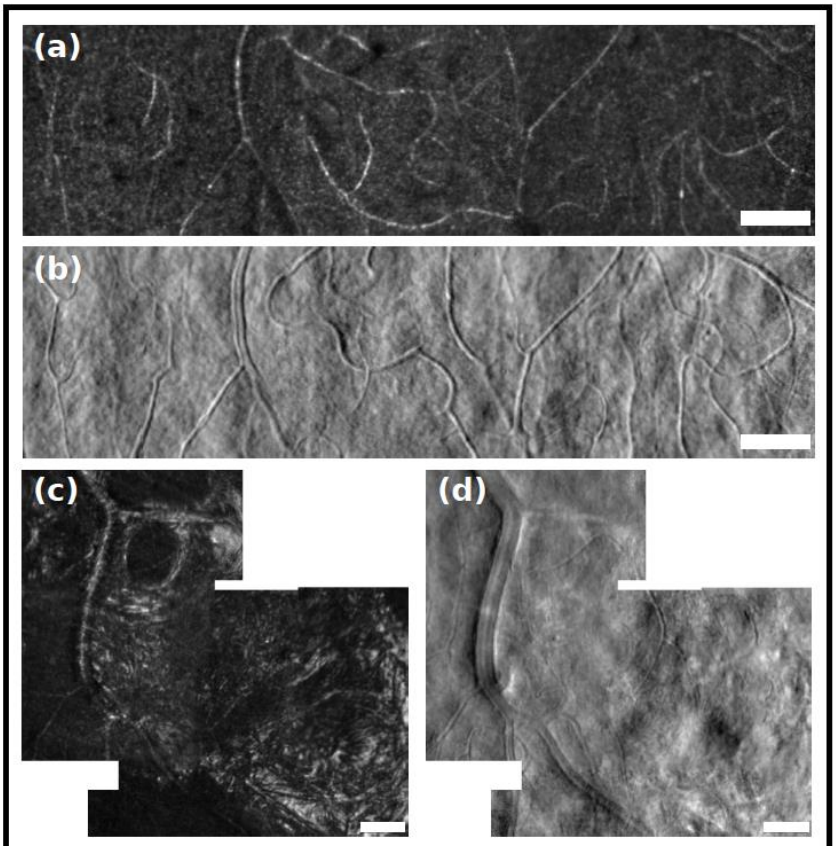

Figure 4. AOSLO microscopic inner retinal changes. (a) Confocal AOSLO image of normal inner retinal microvasculature. (b) Corresponding split detector AOSLO image of inner retinal microvasculature. (c) & (d) The magnification and resolution of AOSLO allows for imaging of structures that could previously not be observed. For example, figures (c) and (d), a confocal and split detector AOSLO image, respectively, depict a "waxy membrane" covering the retinal microvasculature of an MS patient. The pathophysiology of waxy membranes is currently unknown, and although they are likely not directly related to metabolic failure, future research is necessary to investigate its significance. These figures show the capabilities of AOSLO in visualising microscopic inner retinal changes around fundus vessels. Images courtesy of Dr. Adam Dubis and Ms. Sarah Houston (UCL, Institute of Ophthalmology, London). *Scale bar: 100 µm.*

Retinal blood flow and velocity

Several approaches have been developed to assess retinal blood flow and velocity, although quantification of total retinal blood flow remains challenging.

Currently, OCTA cannot provide highly accurate information on blood flow velocity, although constant improvements in hardware and algorithms mean this might change in the future (Ploner *et al*., 2017). Doppler OCT techniques, making use of the Doppler effect, have a great advantage in combining volumetric flow rate with blood flow velocity, which allows calculation of total retinal blood flow with relatively high reliability and reproducibility (Leitgeb *et al*., 2014). This technique is most suitable in larger retinal blood vessels (approximately >30μm), as it has insufficient sensitivity for low flow rates in smaller vessels. Doppler OCT has been used successfully to approximate total retinal blood flow *in vivo*, and recent advancements in *en face* Doppler OCT seem to provide even more accurate approximations of total retinal blood flow (Lee *et al.*, 2017).

In vivo measurements of erythrocyte velocity has been accomplished with AOSLO by tracking erythrocytes in medium sized retinal arteries (Zhong *et al*., 2008). This allows for accurate flow measurements without the use of contrast dye (Supplementary Video 1). Additionally, AOSLO permits real-time visualisation of individual leukocytes. As leukocytes comprise approximately 1% of all blood cells, this technique is most accurate in small capillaries near the fovea where blood cells move in single file (Godara *et al*., 2010).

Whether cerebral hypoperfusion is a primary disease process or a result of cerebral atrophy in multiple sclerosis has long been an issue of debate. Two studies have investigated retinal microcirculation in multiple sclerosis patients using the Retinal Function Imager, a multimodal retinal imaging device which uses motion contrast to create structural capillary maps and measure blood flow velocity. Both studies found decreased retinal blood flow volume and velocities in multiple sclerosis compared with healthy controls. Importantly, these microcirculatory changes were not related to retinal nerve fibre layer thickness or clinical history of MSON, indicating these changes may be related to more global disease processes (Wang *et al.*, 2018b; Jian *et al.*, 2016). These results will need to be corroborated by larger

studies employing the described techniques to evaluate perfusion in multiple sclerosis longitudinally in concordance with retinal thickness and visual acuity measurements. The techniques may also provide valuable outcome measures for treatment trials aiming to improve cerebral perfusion in multiple sclerosis, such as currently being done with bosentan, an endothelin-1 antagonist (Hostenbach et al., 2019).

Retinal oxygen saturation

Dual-wavelength spectrophotometric retinal oximetry measures oxygen saturation of retinal blood vessels by comparing relative reflectance at 570 nm and 600 nm light. Oxidised and deoxidised haemoglobin absorb light equally at 570 nm light, while at 600 nm light deoxidised haemoglobin absorbs more light than oxidised haemoglobin. Retinal oximetry can distinguish venules from arteries and plot an oxygenation map of the retina. This is an improvement compared with cerebral NIRS, which calculates the average oxygenation in venules and arteries combined (Van Keer *et al*., 2018).

Pioneering work shows that retinal oxygen uptake is reduced in longstanding optic neuritis, but is increased in the acute stage in the absence of retinal atrophy (Einarsdottir *et al*., 2018; Svrčinová *et al.,* 2018). Future longitudinal data should explore if retinal oxygen uptake in acute optic neuritis predicts retinal thinning or visual recovery.

Challenges and limitations

Even though these retinal imaging techniques give an encouraging outlook on investigating metabolic function *in vivo* in multiple sclerosis, it is important to remember that prospective and longitudinal trials are necessary to validate most described methods. Also, like all current *in vivo* measures of mitochondrial function, these techniques mostly give indirect information on metabolic function. Furthermore, some techniques suffer from issues to ensure quality control, requiring further development to allow clinical implementation.

Conclusion

The anterior visual system holds great potential for indirect *in vivo* investigation of mitochondrial (dys)function in MSON as an accessible model for multiple sclerosis. Recent years have seen significant methodological developments in multiple fields which permit for

complementary assessment of structure and function. Validated body fluid biomarkers are already included as secondary outcome measures in clinical and experimental treatment trials. These data can now be complemented by non-invasive structural and function retinal imaging. Specifically, techniques such as OCT, OCTA and AOSLO can supply functional in addition to structural data to provide indirect assessment of mitochondrial failure in the human eye. Better understanding of mitochondrial changes in multiple sclerosis might provide new therapeutic options, particularly in the progressive stage.

Acknowledgements

We would like to thank Professor Kenneth Smith from the UCL Institute of Neurology, for encouraging discussions and feedback on this manuscript. Special thanks also goes to Dr Adam Dubis and Ms Sarah Houston from the UCL Institute of Ophthalmology for providing us with original high quality images of the retina to be included to this publication. Finally, we would like to thank the ECTRIMS postdoctoral research fellowship for supporting Ms Iris Kleerekooper.

Funding

This study was not funded. IK was supported through the ECTRIMS postdoctoral research fellowship programme. AP was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology. AT receives support from the University College London Hospitals Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Competing interests

IK and AT report no competing interests relating to this work. AP reports that the Amsterdam UMC (location VUmc) MS Centre Amsterdam and neuro-ophthalmology Expert Centre participated in the OCTIMS trial and the centre has received research support for OCT projects from the Dutch MS Society.

Supplementary data

Supplementary Video 1.

Video of parafoveal capillaries captured using a split detection adaptive optics scanning laser ophthalmoscope. Individual erythrocyte passage is observed. The video has been slowed from 16fps to 5fps to allow for easier visualisation. Video courtesy of Dr. Adam Dubis and Ms. Sarah Houston (UCL, Institute of Ophtalmology). *Scale bar = 50 micrometers.*

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