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Proton MR spectroscopy to assess axonal damage in multiple sclerosis and other white matter disorders

Nicola De Stefano*[,1], Sridar Narayanan[2], Paul M Matthews[3], Marzia Mortilla[1], Maria Teresa Dotti[1], Antonio Federico[1] and Douglas L Arnold[2]

[1]Neurometabolic Unit, Institute of Neurological Sciences, University of Siena, Italy; [2]Department of Neurology and Neurosurgery, Montreal Neurological Institute and Hospital, Quebec, Canada and [3]Department of Clinical Neurology, Centre for Functional Magnetic Resonance Imaging of the Brain, University of Oxford, Oxford, UK

Proton MR spectroscopy allows *in vivo* measurement of N-acetylaspartate in white matter, providing a biochemical index of axonal integrity. Several recent studies of patients with multiple sclerosis and other white matter disorders have shown both transient and sustained decreases in N-acetylaspartate in white matter lesions and in brain regions appearing normal on conventional MRI. These data have emphasised that a substantial amount of axonal damage or loss (presumably secondary to myelin pathology) is consistently present in most of these disorders. Recent post-mortem studies support these results. In contrast to changes seen with conventional MR imaging, decreases in N-acetylaspartate have shown a close correlation with changes in neurological status. This suggests that axonal damage may be more relevant than demyelination for determining chronic functional impairments in primary white matter diseases. Thus, serial measurement of brain N-acetylaspartate with proton MR spectroscopy can provide a reliable and clinically-relevant monitor of disease evolution. As pathological changes responsible for long-term morbidity are logically important targets for therapeutic agents, early treatment directed at axonal protection should be useful in these disorders. *Journal of NeuroVirology* (2000) **6,** S121–S129.

Keywords: N-acetylaspartate; white matter; multiple sclerosis; leukodystrophy; magnetic resonance spectroscopy

Introduction

Magnetic resonance (MR) imaging (MRI) has provided important new insights into the understanding of multiple sclerosis (MS) (Arnold *et al*, 1997) and has proven useful in an increasing number of conditions with brain white matter (WM) pathology (van der Knaap *et al*, 1991; De Stefano *et al*, 1997). However, although conventional MRI detects brain lesions with great sensitivity, it does not provide specific information about the pathology underlying the detected WM lesions and is not a good predictor of functional impairment or disability (Arnold *et al*, 1997).

Recent years have seen the development of new MR techniques that complement conventional MRI and are able to overcome some of its limitations. Proton MR spectroscopy (MRS) is one of these promising techniques and has been used recently in a number of clinical studies to supplement conventional structural neuroimaging with spatially-localised biochemical information (Arnold and Matthews, 1996). Proton MRS can provide non-invasive quantitative measurements of brain metabolites, including N-acetylaspartate (NAA), a metabolite localised almost exclusively in neurons and neuronal processes in the mature brain (Moffett *et al*, 1991; Simmons *et al*, 1991). The resonance intensity of NAA therefore provides a reliable index of neuronal integrity (Birken and Oldendorf, 1989; De Stefano *et al*, 1995b).

A number of research groups have used proton MRS and MR spectroscopic imaging (MRSI) to study patients with MS and other types of WM diseases (Arnold *et al*, 1990; Wolinsky *et al*, 1990;

*Correspondence: N De Stefano, Neurometabolic Unit, Institute of Neurological Sciences, University of Siena, Viale Bracci 2, 53100 Siena, Italy

Hanefeld *et al*, 1991; Miller *et al*, 1991; van der Knaap *et al*, 1992; Kruse *et al*, 1993; Davie *et al*, 1994; Schiffmann *et al*, 1997). These MR studies have provided important insights into these disorders by emphasising axonal pathology. Here we review MRS studies assessing axonal pathology *in vivo* and present evidence indicating that axonal injury and loss may be the main link between pathological changes and the development of chronic disability.

Introduction to proton MRS

MRS utilises the same hardware and computers as conventional MRI. However, while conventional MRI provides structural information based on signal from water, proton MRS provides measures of chemical compounds present at concentrations in the vicinity of 10^{-5} that of water. MR spectra can be obtained with either single voxel (Figure 1) or spectroscopic imaging (Figure 2) methods. In both approaches, conventional MRI is performed first and used to plan the spectroscopy acquisition.

In a single voxel study one MR spectrum is obtained from a volume of interest chosen to include specifically the pathology under study. In the spectroscopic imaging approach, phase-encoding gradients are used to measure the distribution of metabolites throughout a larger volume of interest. Low-resolution images then can be generated for each metabolite by integration of the MR signals from each voxel. As spatial resolution is limited by signal intensity, the low-concentrations of brain metabolites makes the spatial resolution of the spectroscopic methods much lower than that achieved with conventional imaging based on water (70 M concentration). However, statistical inferences from the combination of the two types of MR acquisition can provide sufficiently well resolved descriptions of pathology to be useful for a broad range of applications.

Water-suppressed localised proton MRS and MRSI of normal human brain acquired at relatively long echo times (TE 136 or 272 ms) show four main peaks: (i) a large signal from N-acetyl groups (mainly NAA (Ross *et al*, 1992; Arnold and Matthews, 1996)), (ii) a smaller but still easily

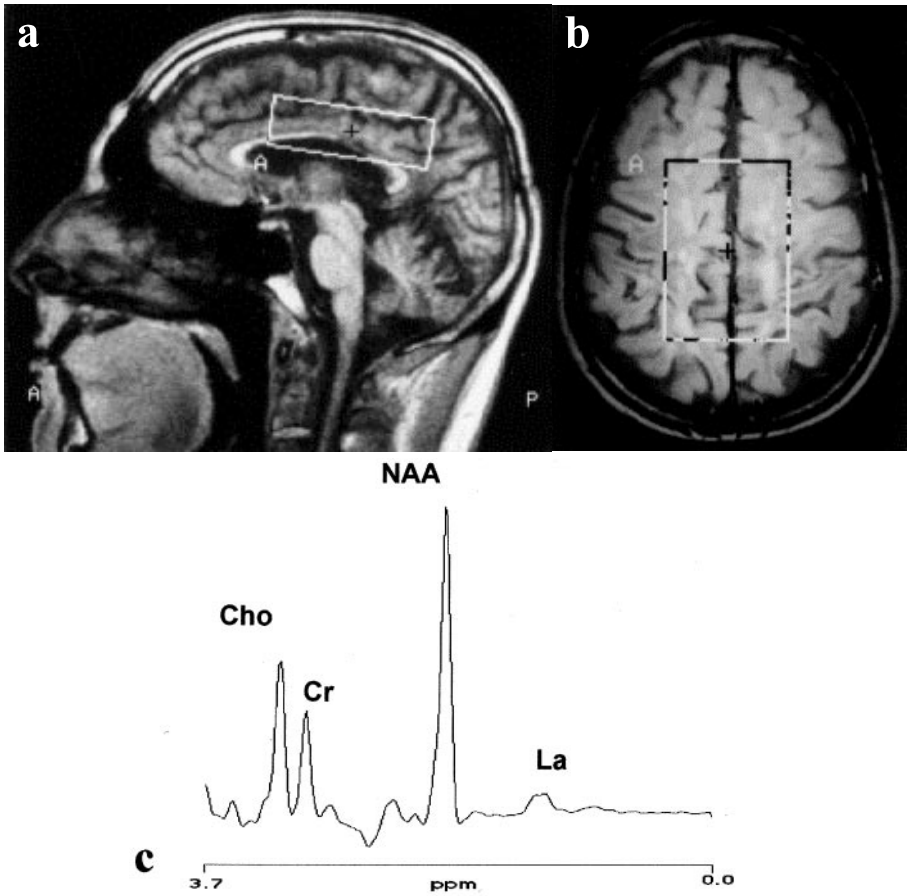

Figure 1 MRI scan in sagittal orientation (**a**) illustrating the volume of interest used for spectroscopy, (**b**) axial view of the same volume of interest and (**c**) proton MR spectrum from that volume of interest.

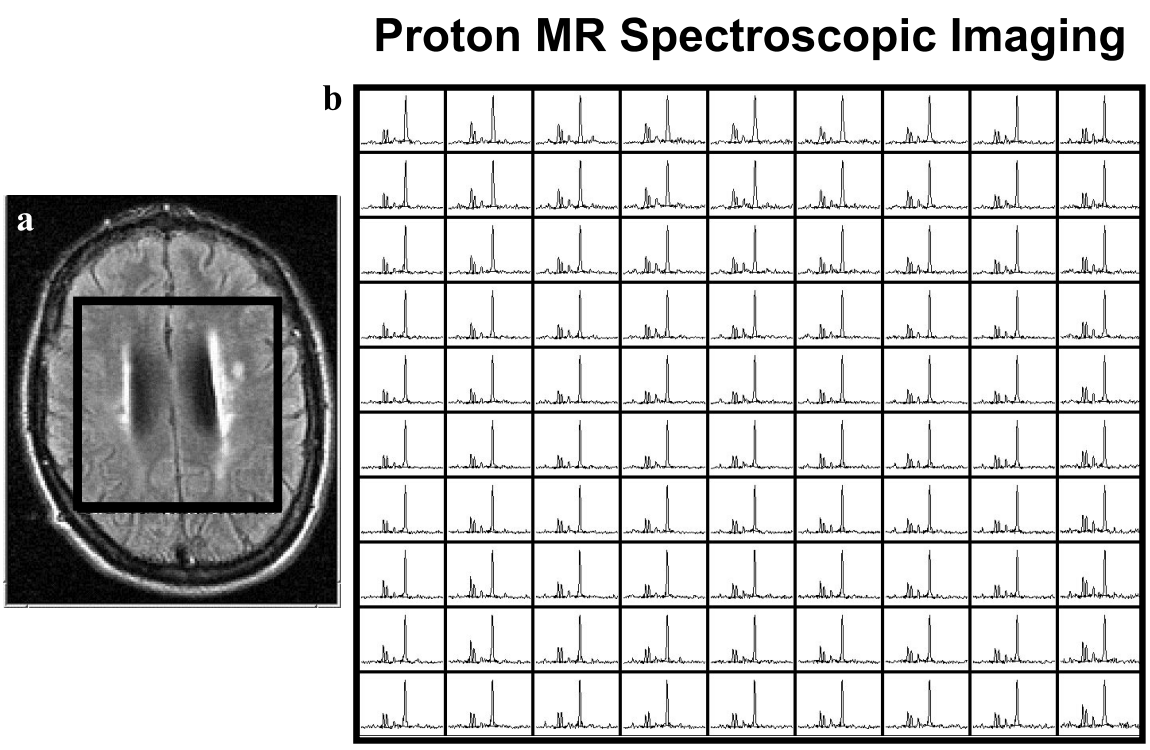

Figure 2 Conventional MR imaging of a patient with multiple sclerosis in transversal orientation illustrating the volume of interest used for spectroscopy (**a**) and the proton spectra obtained inside this volume (**b**).

quantifiable resonance from choline-containing phospholipids (Cho, mainly resulting from membrane phosphocholine and glycerol-phosphocholine), (iii) creatine and phosphocreatine (Cr), which are relatively homogeneously distributed in normal brain and relatively resistant to changes (Ross *et al*, 1992; Arnold and Matthews, 1996), and (iv) lactate (Lac), which increases in concentration when oxidative metabolism cannot meet energy requirements or, acutely, in conditions associated with inflammation (Prichard, 1991; Ross *et al*, 1992). Excellent spectra can also be obtained with short echo time measurements. A greater number of metabolites (lipids, *myo*-inositol, GABA, glutamate etc.) can be detected with this method, but short echo time spectra are also much more demanding in terms of hardware, more susceptible to artifact and more difficult to quantify (Ross *et al*, 1992; Arnold and Matthews, 1996).

With both short and long echo times, the proton brain MR spectrum is dominated by the signal of NAA. Brain NAA is synthesised in neuronal mitochondria from L-aspartate and acetyl-CoA and hydrolyzed by an N-acetyl-L-aspartate amidohydrolase that is widely distributed in glial cells of the brain and in other human tissues (Birken and Oldendorf, 1989; De Stefano *et al*, 1995b). Although a number of possible functions have been suggested for NAA, its physiological role is still not known (Birken and Oldendorf, 1989; De Stefano *et al*, 1995b). However, the fact that NAA is present almost exclusively in neurons in mature brain (Moffett *et al*, 1991; Simmons *et al*, 1991) makes it a powerful surrogate marker of neuronal damage or loss.

NAA as marker of axonal damage in multiple sclerosis

Decreases in NAA have been observed in proton MRS studies in many pathologies associated with neuronal damage or loss (Arnold and Matthews, 1996). However, studies of NAA in patients with MS have been particularly illuminating because the extent of axonal injury associated with WM inflammation and demyelination had not been well-appreciated from classical pathology studies (McDonald, 1994; Arnold *et al*, 1997). Large decreases in brain NAA were reported in the earliest single voxel MRS studies of patients with MS (Arnold *et al*, 1990; Miller *et al*, 1991; Wolinsky *et al*, 1990; Bruhn *et al*, 1992) and confirmed in a number of more recent reports (Davie *et al*, 1994,1997; De Stefano *et al*, 1995a; Narayanan *et al*, 1997; Fu *et al*, 1998; Narayana *et al*, 1998; Sarchielli *et al*, 1999). Initial work (Arnold *et al*, 1990) also showed that greater decreases in brain

NAA were found in patients with clinically more severe disease. Since axons in the central nervous system have an extremely limited capacity for regrowth, the decreases in NAA initially were interpreted as a measure of irreversible neuronal loss. However, recovery of NAA has been reported in several conditions (Arnold *et al*, 1992; De Stefano *et al*, 1993; Lee *et al*, 1994; Vion-Dury *et al*, 1995; Matthews *et al*, 1995). Longitudinal studies of patients with demyelinating disease have emphasised that a substantial proportion of the decreases in NAA are transient in the acute phase of demyelination (Arnold *et al*, 1992; De Stefano *et al*, 1995a). Although matrix volume changes from edema may contribute to reversible decreases of NAA, the magnitude of bulk volume change is insufficient to account for the effects observed (De Stefano *et al*, 1995b). Experimental models have demonstrated clearly reversible decreases of NAA in different conditions that are not associated with volume loss. In an *in vitro* study of a neuronal cell line, a decrease in NAA was caused by serum deprivation with recovery after subsequent replacement of serum (Matthews *et al*, 1995). Reversible decreases in intracellular NAA also were shown in an *in vivo* ischaemic rat brain model (Sager *et al*, 1999). This suggests that either reversible metabolic dysfunction or considerable axonal shrinkage (as is observed immunohistochemically (Trapp *et al*, 1998)) associated with hypophosphorylation of neural filaments contribute to the observed brain NAA changes.

Early single voxel MRS studies were focused mainly on MRI-defined lesions (Arnold *et al*, 1990; Wolinsky *et al*, 1990; Miller *et al*, 1991). More recently, studies exploiting the greater coverage and resolution of MRSI have shown that the observed decreases in NAA in MS patients are not restricted to lesions, but are present both adjacent to and distant from the lesions. Group metabolite maps averaged in a standardised coordinate space have shown loss of NAA extending beyond regions of high lesion probability into surrounding regions of low lesion probability (Narayanan *et al*, 1997). Other studies have demonstrated both transient and chronic decreases in NAA in WM appearing normal on MRI (Husted *et al*, 1994; Narayanan *et al*, 1997; Davie *et al*, 1997; Fu *et al*, 1998; Narayana *et al*, 1998; Sarchielli *et al*, 1999). As axons generally project through lesions, any axonal interruption that occurs will be associated with anterograde and retrograde axonal degeneration. Therefore, it should not be surprising that axonal dysfunction or volume loss can be observed beyond the borders of MS lesions as defined by T_2-weighted MRI.

Evidence of axonal damage both in lesions and in the normal-appearing WM of MS patients also has been found in recent post-mortem studies (Davies *et al*, 1995; Trapp *et al*, 1998). Although significant axonal damage was reported in several early pathology studies, axonal loss had been difficult to quantify with histopathology and, because of the relative prominence of demyelination, was rather neglected (McDonald, 1994). More recent pathology studies have emphasised that axonal damage does occur in MS. Axons in lesions can change in size, shape and morphology as a result of inflammation and demyelination (Prineas *et al*, 1993). Loss of axons varies considerably between lesions, being less prominent in acute lesions than in chronic lesions, and can lead to a loss of over 50% of axons (Raine and Cross, 1989; Barnes *et al*, 1991; Rodriguez and Scheithauer, 1994; Ferguson *et al*, 1997). Moreover, alterations in neurofilament phosphorylation and substantial loss of axon density can be found well outside demyelinating lesions (Trapp *et al*, 1998).

Changes in NAA versus clinical disability

The nervous system dysfunction seen in patients with MS has generally been attributed to the electrical conduction block that can occur (at least acutely) after demyelination (McDonald, 1994). However, demyelination alone is not sufficient to explain chronic disability in MS (Matthews *et al*, 1998). A dramatic illustration of this comes in a recent study which elegantly demonstrated that mice deficient in class I major histocompatibility complex (MHC) infected with Theiler's murine encephalomyelitis virus develop demyelination without neurological impairment, while class II MHC-deficient mice develop demyelination and severe neurological deficits leading to premature death (Rivera-Quinones *et al*, 1998). Studies of patients with MS suggest that diffusible factors such as reactive oxygen species or membrane-channel directed antibodies may mediate acute conduction block with WM inflammation (Youl *et al*, 1991; Utzschneider *et al*, 1993). Remyelination surely plays an important role in recovery and in longer-term trophic maintenance of axons. However, other mechanisms such as reorganisation of axon membrane sodium channels to cover demyelinated internodal regions or downregulation of inhibitory channels may be also important (Waxman, 1998). Thus, axonal adaptations alone may lead to recovery of function in regions of chronic demyelination.

As the pathological changes primarily responsible for disability are logical targets of action for new therapeutic agents, understanding the changes responsible for functional impairments is a crucial issue. Proton MRS, by providing quantitative tools for non-invasive detection of axonal injury and loss in patients with MS, allows dynamic correlations between such changes and disability in life. Recent spectroscopic studies have demonstrated highly significant correlations between decreasing NAA and increasing clinical disability in patients with isolated acute demyelinating lesions (De Stefano *et*

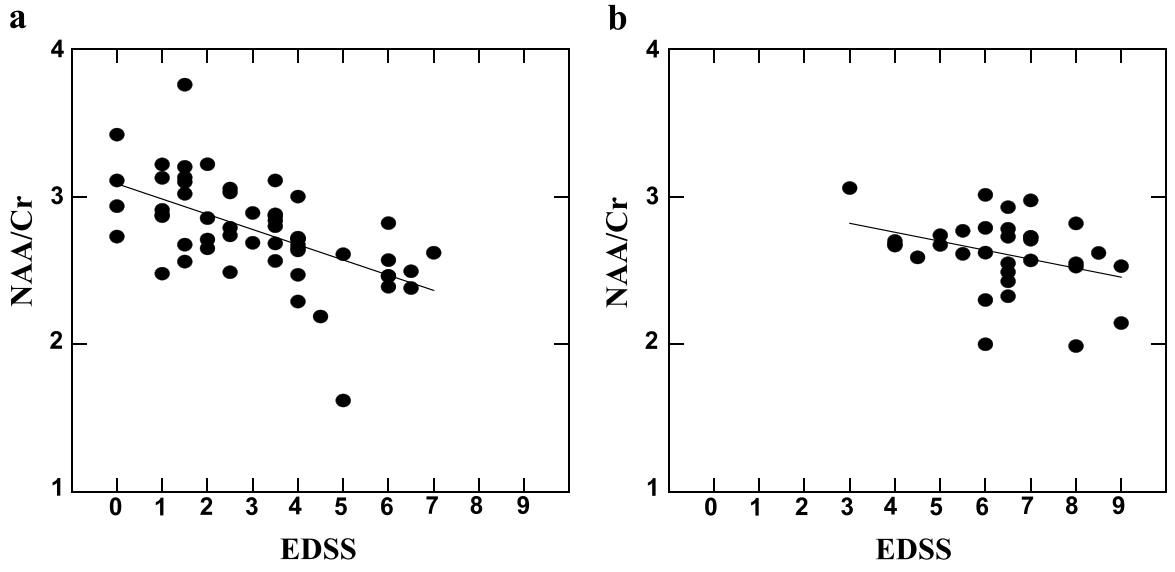

Figure 3 Data illustrating the significant relationship between clinical disability (EDSS) and NAA/Cr ratios for patients with the relapsing form of multiple sclerosis ($P<0.001$) (**a**). This relationship is weak in patients with the secondary progressive form of multiple sclerosis ($P>0.1$) (**b**).

al., 1995a) and in patients with established MS followed through periods of relapse and remission (De Stefano *et al*, 1998b; Fu *et al*, 1998) (Figure 3a). Consistent with other evidence of widespread pathology in MS, a strong correlation also was found between decreases in NAA resonance intensity and increases in clinical disability in WM appearing normal on conventional MRI (Fu *et al*, 1998). Given that the normal-appearing WM represents by far the greatest bulk of WM, axonal loss in this brain region could be more relevant to functional impairment than axonal loss in lesions. In contrast, the correlation between NAA and clinical disability appears weak in patients with the secondary progressive form of the disease (Figure 3b), which is perhaps due to (i) the difficulty in demonstrating a relationship in this group because of a smaller slope between pathological change and disability; (ii) the greater contribution to disability of cerebral axonal pathology in the early, mild stages of MS with respect to the later, more severe stages of the disease; or (iii) the more prominent contribution of other pathological factors (i.e., spinal pathology) in the secondary progressive stage of the disease. Nonetheless, we believe that the so-called axonal hypothesis, 'axonal damage or loss in regions of myelin or glial cell destruction is required for chronic impairments and disability' (Matthews *et al*, 1998), remains a powerful way of synthesising our understanding of chronic disability in MS.

The link between axonal damage and neurological impairment has been explored indirectly by other studies using MR measurements of brain and spinal cord atrophy (Losseff *et al*, 1996; Filippi *et al*, 1997; Stevenson *et al*, 1998) or hypointense lesion volumes on T1-weighted MRI (Miller *et al*, 1998; van Walderveen *et al*, 1998). The relationship between brain WM atrophy and axonal rarefaction or dystrophy in WM has not been defined. Preliminary data showing that brain atrophy progresses most rapidly later in the disease, while the fastest NAA loss occurs in the early stages, suggests that the two measures may be providing complementary information. A combined NAA and atrophy measure may offer a highly sensitive, clinically-relevant surrogate of pathological change useful for studying patients progressing over a broad disability range.

NAA as marker of axonal damage in WM disorders other than MS

Brain WM disorders are a very heterogeneous group of diseases that can be due to a variety of pathological processes (autoimmune, metabolic, vascular, toxic, hereditary, etc.) and can be associated with many different types of myelin abnormality (de-myelination, hypo-myelination, myelin rarefaction, etc.) (Kolodny, 1993). The more frequent use of MRI in clinical evaluation has increased awareness for disorders involving brain WM (van der Knaap *et al*, 1991). However, the lack of pathological specificity of conventional MRI contributes to the inability to classify many patients with these diseases. Proton MRS and MRSI, by detecting chemical-pathological changes occurring in lesions and in WM appearing normal on MRI,

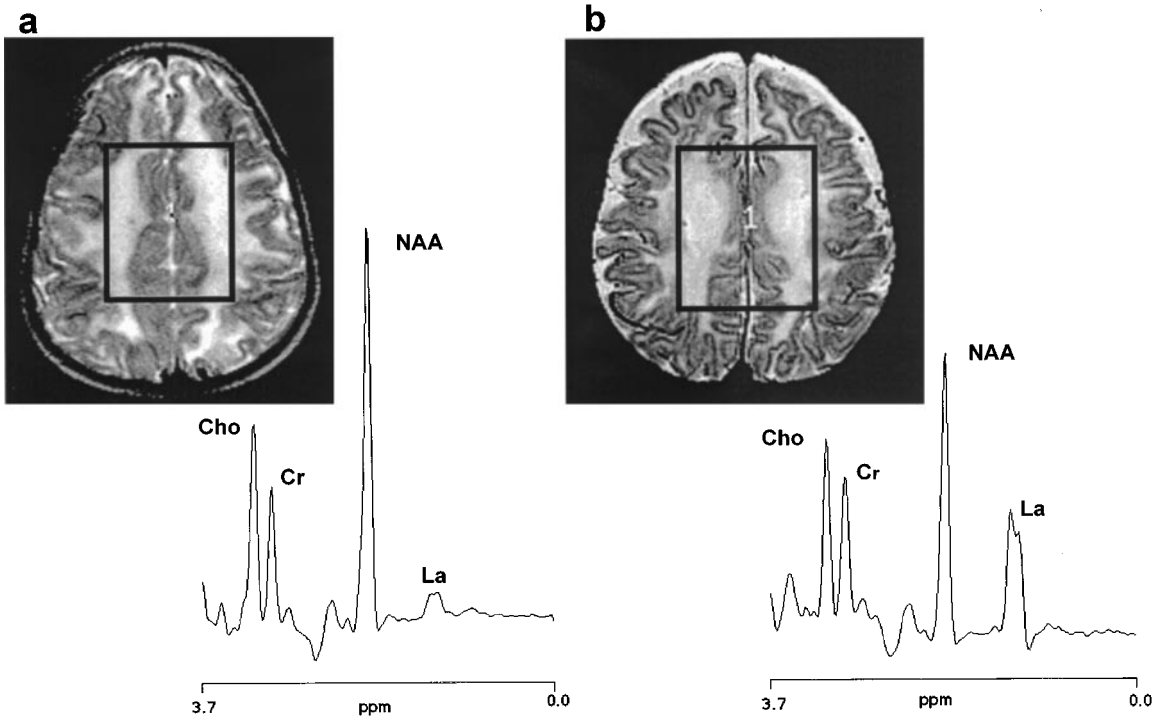

Figure 4 Conventional MRI in transversal orientation of a patient with congenital muscular dystrophy (**a**) and a patient with pyruvate dehydrogenase deficiency (**b**). MR images of both patients show similar, diffuse white matter hyperintensity. However, the MR spectrum is normal in the patient affected by congenital muscular dystrophy who did not have CNS impairment (bottom left quadrant), whereas there is a remarkable decrease in NAA and increase in Lac in the patient with pyruvate dehydrogenase deficiency who was severely neurologically impaired (bottom right quadrant).

can provide important additional information useful for diagnostic classification (van der Knaap *et al*, 1992; De Stefano *et al*, 1997).

There are several similarities between MRS findings in MS patients and in patients with other WM disorders. First, patients with acquired and hereditary WM disorders commonly show decreases in brain NAA (Kruse *et al*, 1993,1994; Tzika *et al*, 1993; van der Knaap *et al*, 1992; De Stefano *et al*, 1997). Second, in patients with WM disorders, decreases in brain NAA can be detected both in areas of hyperintense signal and in WM appearing normal on T2-weighted MRI (Kruse *et al*, 1994; De Stefano *et al*, 1997). The ubiquity of this finding suggests that some degree of axonal dysfunction and damage occurs. This is probably secondary to myelin disruption, causing a loss of trophic support for axons, and thus leading to a chronic neurodegenerative process. Third, brain NAA correlates well with the clinical status of patients affected by a variety of WM disorders. In patients with infectious disorders such as AIDS, brain NAA provided a reliable index of clinical impairment (Chong *et al*, 1993) and has been used to monitor improvements in neuronal and axonal integrity after therapeutic intervention (Vion-Dury *et al*, 1995). In a recent single voxel study performed in 12 patients with 12 different disorders presenting WM abnormalities on conventional MRI (De Stefano *et al*, 1998a), we found a very close relationship between NAA values from central brain regions and patients' CNS impairment (computed using the Functional Status Score (Kurtzke, 1983)). Data from this study showed that brain NAA and patients' CNS impairment may be correlated in circumstances in which there is no relationship between the clinical status and the degree of WM changes on conventional MRI (Figure 4).

Conclusions

There is enough MR and pathological evidence that progressive accumulation of disability in patients with MS and other WM disorders reflects chronic, progressive damage to axons. This has obvious implications for the development of new therapeutic approaches.

Proton MRS can provide *in vivo* measurement of brain NAA, an index of axonal integrity, allowing accurate monitoring of disease evolution and response to therapeutic intervention. Combining MRS with measures derived from conventional structural imaging (e.g., lesion volume, brain and

spinal cord atrophy measurements) can provide a more complete description of the dynamics responsible for pathological changes as well as a more accurate evaluation of disease progression and response to therapy.

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References

Arnold DL, Matthews PM, Francis G, Antel J (1990). Proton magnetic resonance spectroscopy of human brain in vivo in the evaluation of multiple sclerosis: assessment of the load of disease. *Magnet Reson Med* **14:** 154–159.

Arnold DL, Matthews PM, Francis GS, O'Connor J, Antel JP (1992). Proton magnetic resonance spectroscopic imaging for metabolic characterization of demyelinating plaques. *Ann Neurol* **31:** 235–241.

Arnold DL, Matthews PM (1996). Pratical aspects of clinical applications of MRS in the brain. In: *MR Spectroscopy: Clinical Applications and Techniques*. Young IR, Charles HC (eds). London: Martin Dunitz, pp 139–159.

Arnold DL, Matthews PM, De Stefano N (1997). MRI and proton MRS in the evaluation of multiple sclerosis. In: *Magnetic resonance spectroscopy and imaging in neurochemistry*. Bachelard (ed). Plenum Press, New York pp 267–288.

Barnes D, Munro PMG, Youl BD, Prineas JW, McDonald WI (1991). The longstanding MS lesion; a quantitative MRI and electron microscopic study. *Brain* **114:** 1271–1280.

Birken DL, Oldendorf WH (1989). N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain. *Neurosci Biobehav Rev* **13:** 23–31.

Bruhn H, Frahm J, Merboldt KD, Hxzynicke W, Hanefeld F, Christen HJ, Bauer HJ, Kruse B. (1992). Multiple sclerosis in children: Cerebral metabolic alterations monitored by localized proton magnetic resonance spectroscopy *in vivo*. *Ann Neurol* **32:** 140–150.

Chong WK, Sweeney B, Wilkinson ID, Paley M, Hall-Craggs MA, Kendall BE, Shepard JK, Beecham M, Miller RF, Weller IV. (1993). Proton spectroscopy of the brain in HIV infection: correlation with clinical, immunologic, and MR imaging findings. *Radiology* **188:** 119–124.

Davie CA, Hawkins CP, Barker GJ, Brennan A, Tofts PS, Miller DH, McDonald WI. (1994). Serial proton magnetic resonance spectroscopy in acute multiple sclerosis lesions. *Brain* **117:** 49–58.

Davie CA, Barker GJ, Thompson AJ, Tofts PS, McDonald WI, Miller DH (1997). 1H magnetic resonance spectroscopy of chronic cerebral white matter lesions and normal appearing white matter in multiple sclerosis. *J Neurol Neurosurg Psychiatry* **63:** 736–742.

Davies SE, Newcombe J, Williams SR, McDonald WI, Clark JB (1995). High resolution proton NMR spectroscopy of multiple sclerosis lesions. *J Neurochem* **64:** 742–748.

De Stefano N, Francis G, Antel JP, Arnold DL (1993). Reversible decreases of N-acetylaspartate in the brain of patients with relapsing remitting multiple sclerosis. *Proc Soc Magnet Reson Med* **3:** 1139 (Abstract).

De Stefano N, Matthews PM, Antel JP, Preul M, Francis G, Arnold DL (1995a). Chemical pathology of acute demyelinating lesions and its correlation with disability. *Ann Neurol* **38:** 901–909.

De Stefano N, Matthews PM, Arnold DL (1995b). Reversible decreases in N-acetylaspartate after acute brain injury. *Magnet Reson Med* **34:** 721–727.

De Stefano N, Federico A, Arnold DL (1997). Proton magnetic resonance spectroscopy in brain white matter disorders. *Ital J Neurol Sci* **18:** 331–339.

De Stefano N, Federico A, Mortilla M, Narayanan N, Dotti MT, Dal Pozzo G, Matthews PM, Arnold DL (1998a). Axonal Damage is a Proximate Cause of Central Nervous System Impairment in Patients with White Matter Disorders. *Neurology* **50:** A108 (Abstract).

De Stefano N, Matthews PM, Fu L, Narayanan S, Stanley J, Francis GS, Antel JP, Arnold DL (1998b). Axonal damage correlates with disability in patients with relapsing- remitting multiple sclerosis. Results of a longitudinal magnetic resonance spectroscopy study. *Brain* **121:** 1469–1477.

Ferguson B, Matyszak MK, Esiri MM, Perry VH (1997). Axonal damage in acute multiple sclerosis lesions. *Brain* **120:** 393–399.

Filippi M, Colombo B, Rovaris M, Pereira C, Martinelli V, Comi G (1997). A longitudinal magnetic resonance imaging study of the cervical cord in multiple sclerosis. *J Neuroimag* **7:** 78–80.

Fu L, Matthews PM, De Stefano N, Worsley KJ, Narayanan S, Francis GS, Antel JP, Wolfson C, Arnold DL (1998). Imaging axonal damage of normal-appearing white matter in multiple sclerosis. *Brain* **121:** 103–113.

Hanefeld F, Bauer HJ, Christen HJ, Kruse B, Bruhn H, Frahm J (1991). Multiple sclerosis in childhood: Report of 15 cases. *Brain Dev* **13(6)** 410–416.

Husted CA, Goodin DS, Hugg JW, Maudsley AA, Tsuruda JS, de Bie SH *et al*. (1994). Biochemical alterations in multiple sclerosis lesions and normal-appearing white matter detected by in vivo 31P and 1H spectroscopic imaging. *Ann Neurol* **36:** 157–165.

Kolodny EH (1993). Dysmyelinating and demyelinating conditions in infancy. [Review]. *Curr Opin Neurol Neurosurg* **6:** 379–386.

Kruse B, Hanefeld F, Christen HJ, Bruhn H, Michaelis T, Hanicke W, Frahm J (1993). Alterations of brain metabolites in metachromatic leukodystrophy as detected by localized proton magnetic resonance spectroscopy in vivo. *J Neurol* **241:** 68–74.

Kruse B, Barker PB, van Zijl PC, Duyn JH, Moonen CT, Moser HW (1994). Multislice proton magnetic resonance spectroscopic imaging in X-linked adrenoleukodystrophy. *Ann Neurol* **36:** 595–608.

Kurtzke JF (1983). Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* **33:** 1444–1452.

Lee JH, Arcinue E, Ross BD (1994). Brief report: organic osmolytes in the brain of an infant with hypernatremia. *New Engl J Med* **331:** 439–442.

Losseff NA, Webb SL, O'Riordan JI, Page R, Wang L, Barker GJ, Tofts PS, McDonald WI, Miller DH, Thompson AJ (1996). Spinal cord atrophy and disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. *Brain* **119:** 701–708.

Matthews PM, Cianfaglia L, McLaurin J, Cashman N, Sherwin A, Arnold DL, Antel JP (1995). Demonstration of reversible decreases in N-acetylaspartate (NAA) in a neuronal cell line: NAA decreases as a marker of sublethal neuronal dysfunction. *Book Abstracts Soc Magnet Reson Med* **1:** 147 (Abstract).

Matthews PM, De Stefano N, Narayanan S, Francis GS, Wolinsky JS, Antel JP, Arnold DL. (1998). Putting magnetic resonance spectroscopy studies in context: axonal damage and disability in multiple sclerosis. *Semin Neurol* **18:** 327–336.

McDonald WI (1994). Rachelle Fishman-Matthew Moore Lecture. The pathological and clinical dynamics of multiple sclerosis. [Review]. *J Neuropathol Exp Neurol* **53:** 338–343.

Miller DH, Austin SJ, Connelly A, Youl BD, Gadian DG, McDonald WI (1991). Proton magnetic resonance spectroscopy of an acute and chronic lesion in multiple sclerosis [Letter]. *Lancet* **337:** 58–59.

Miller DH, Grossman RI, Reingold SC, McFarland HF (1998). The role of magnetic resonance techniques in understanding and managing multiple sclerosis. *Brain* **121:** 3–24.

Moffett JR, Namboodiri MAA, Cangro CB, Neale JH (1991). Immunohistochemical localization of N-acetylaspartate in rat brain. *NeuroReport* **2:** 131–134.

Narayana PA, Doyle TJ, Lai D, Wolinsky JS (1998). Serial proton magnetic resonance spectroscopic imaging, contrast-enhanced magnetic resonance imaging, and quantitative lesion volumetry in multiple sclerosis. *Ann Neuro* **43:** 56–71.

Narayanan S, Fu L, Pioro E, De Stefano N, Collins DL, Francis GS, Antel JP, Matthews PM, Arnold DL (1997). Imaging of axonal damage in multiple sclerosis: spatial distribution of magnetic resonance imaging lesions. *Ann Neurol* **41:** 385–391.

Prichard JW (1991). What the clinician can learn from MRS lactate measurements. *NMR Biomed* **4:** 99–102.

Prineas JW, Barnard RO, Revesz T, Kwon EE, Sharer L, Cho ES (1993). Multiple sclerosis. Pathology of recurrent lesions. *Brain* **116:** 681–693.

Raine CS, Cross AH (1989). Axonal dystrophy as a consequence of long-term demyelination. *Lab Invest* **60:** 714–725.

Rivera-Quinones C, McGavern D, Schmelzer JD, Hunter SF, Low PA, Rodriguez M (1998). Absence of neurological deficits following extensive demyelination in a class I-deficient murine model of multiple sclerosis. *Nat Med* **4:** 187–193.

Rodriguez M, Scheithauer B (1994). Ultrastructure of multiple sclerosis. *Ultrastruct Pathol* **18:** 3–13.

Ross B, Kreis R, Ernst T (1992). Clinical tools for the 90s: magnetic resonance spectroscopy and metabolite imaging. [Review]. *Eur J Radiol* **14:** 128–140.

Sager TN, Laursen H, Fink-Jensen A, Topp S, Stensgaard A, Hedehus M, Rosenbaum S, Valsborg JS, Hansen AJ (1999). N-Acetylaspartate distribution in rat brain striatum during acute brain ischemia. *J Cereb Blood Flow Metab* **19:** 164–172.

Sarchielli P, Presciutti O, Pelliccioli GP, Tarducci R, Gobbi G, Chiarini P, Alberti A, Vicinanza F, Gallai V (1999). Absolute quantification of brain metabolites by proton magnetic resonance spectroscopy in normal-appearing white matter of multiple sclerosis patients. *Brain* **122:** 513–521.

Schiffmann R, Tedeschi G, Kinkel RP, Trapp BD, Frank JA, Kaneski CR *et al*. (1997). Leukodystrophy in patients with ovarian dysgenesis. *Ann Neurol* **41:** 654–661.

Simmons ML, Frondoza CG, Coyle JT (1991). Immunocytochemical localization of N-acetyl-aspartate with monoclonal antibodies. *Neuroscience* **45:** 37–45.

Stevenson VL, Leary SM, Losseff NA, Parker GJ, Barker GJ, Husmani Y, Miller DH, Thompson AJ (1998). Spinal cord atrophy and disability in MS: a longitudinal study. *Neurology* **51:** 234–238.

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L (1998). Axonal transection in the lesions of multiple sclerosis [see comments]. *New Engl J Med* **338:** 278–285.

Tzika AA, Ball Jr WS, Vigneron DB, Dunn RS, Nelson SJ, Kirks DR (1993). Childhood adrenoleukodystrophy: assessment with proton MR spectroscopy. *Radiology* **189:** 467–480.

Utzschneider DA, Thio C, Sontheimer H, Ritchie JM, Waxman SG, Kocsis JD (1993). Action potential conduction and sodium channel content in the optic nerve of the myelin-deficient rat. *Proc R Soc Lond B Biol Sci* **254:** 245–250.

van der Knaap MS, Valk J, de Neeling N, Nauta JJ (1991). Pattern recognition in magnetic resonance imaging of white matter disorders in children and young adults. *Neuroradiology* **33:** 478–493.

van der Knaap MS, van der Grond J, Luyten PR, den Hollander JA, Nauta JJP, Valk J (1992). 1H and 31P magnetic resonance spectroscopy of the brain in degenerative cerebral disorders. *Ann Neurol* **31:** 202–211.

van Walderveen MA, Kamphorst W, Scheltens P, van WJ, Ravid R, Valk J, Polman CH, Barkhof F. (1998). Histopathologic correlate of hypointense lesions on T1-weighted spin- echo MRI in multiple sclerosis. *Neurology* **50:** 1282–1288.

Vion-Dury J, Nicoli F, Salvan AM, Confort-Gouny S, Dhiver C, Cozzone PJ (1995). Reversal of brain metabolic alterations with zidovudine detected by proton localised magnetic resonance spectroscopy [letter]. *Lancet* **345:** 60–61.

Waxman SG (1998). Demyelinating diseases – new pathological insights, new therapeutic targets [editorial; comment]. *New Engl J Med* **338:** 323 – 325.

Wolinsky JS, Narayana PA, Fenstermacher MJ (1990). Proton magnetic resonance spectroscopy in multiple sclerosis. *Neurology* **40:** 1764 – 1769.

Youl BD, Turano G, Miller DH, Towell AD, MacManus DG, Moore SG, Barrett G, Kendall BE, Moseley IF (1991). The pathophysiology of acute optic neuritis. An association of gadolinium leakage with clinical and electrophysiological deficits. *Brain* **114:** 2437 – 2450.