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MR Spectroscopy in Multiple Sclerosis - A New Piece of the Puzzle or Just a New Puzzle

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1. Introduction

In the late ‘80s magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) revolutionized the care and monitoring of patients with multiple sclerosis (MS). For the first time detailed, high resolution images of the brain, spinal cord and lesions could be made by MRI in vivo, and followed over time. However, the changes detectable on T1- or T2-weighted magnetic resonance images are non-specific for the underlying pathology, especially in MS patients, and slow down the enthusiasm. They may reflect edema, demyelination, axonal loss, inflammation, gliosis, remyelination, or Wallerian degeneration in MS.

Similar it is with MRS. Early on it was suggested that metabolites within the tissue may reflect certain cellular compartments and tissue conditions in health and disease. First reports about in vivo detection of brain metabolites in humans were published in 1989 (Bruhn et al., 1989; Frahm et al., 1989a; 1989b). Combined with conventional MRI technique spatial anatomical and chemical information about the analysed tissue may be achieved. MRS was approved by the FDA in 1996. However, only few metabolites are detectable (table 1), limiting the main applications of MRS yet to (1) brain tumors (low grade vs. high grade gliomas, metastasis, lymphoma, meningioma), (2) follow-up of tumors (under chemotherapy and radiotherapy), (2) infectious, mostly focal CNS processes such as pyogenic abscesses, toxoplasmosis, tuberculosis, cryptococcosis, and (3) hepatic encephalopathy, other metabolic disorders, inborn errors of metabolism or hypoxic encephalopathy. In some selected MS cases MRS might be useful to differentiate tumor-like MS brain lesions or Balo-like MS lesions from tumor lesions (table 1) or confluent MS lesions from leucodystrophies or leucoencephalopathies.

Well characterized peaks obtained at long echo times (more than TE 135ms, highlighted in grey) are: (1) at 2.0 ppm (‘NAA-peak’; N-acetyl-aspartate (NAA)), (2) at 3.0 ppm (‘Cr-peak’; creatine (Cr)), (3) at 3.2 ppm (‘Cho-peak’; choline (cho)), (4) at 1.3 ppm (‘Lac-peak’; lactate (Lac)), (5) at 1.48ppm (‘Alanine Peak’; alanine (Ala)),

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1. Claude Bernard (French physiologist, 1813 – 1878)
| marker (metabolite) | ppm     | [mM] | indicates                                                                 | possible to find in...                        |
|-------------------|---------|------|---------------------------------------------------------------------------|----------------------------------------------|
| lipids            | 0.9-1.2 | -    | tissue necrosis (highly specific)                                          | brain tumors, abscesses, tissue necrosis     |
| cytosolic amino acids (valine, leucine, and iso-leucine) | 0.9     | -    | products of proteolysis (neutrophil cells)                                | abscesses, neurocysticercosis (but not in neoplasms!) |
| lactate (Lac)     | 1.2     | -    | anaerobic glycolysis; inverted double peak (TE136ms)                      | Balo like MS lesions, malignant tumors, infarcts, abscesses, mitochondrial disorders |
| alanine (Ala)     | 1.48    | -    | inverted double peak (TE 136ms)                                           | meningiomas brain abscesses                 |
| acetate           | 1.5     | -    | product of propionic acid fermentation and mixed acid fermentation (anaerobic bacteria) | abscesses, neurocysticercosis              |
| N-acetylaspartate (NAA) | 2.0 | 7-17 | neurons, axons                                                             | decreased in tumors, and any process with tissue destruction |
| glutamate and glutamine (glx) | 2.2-2.7 | 6-12 and 3-12 | excitatory neurotransmitter                                                | increased in stroke, lymphoma, hepatic encephalopathy, metabolic disorders |
| succinate         | 2.4     | -    | product of propionic acid fermentation and mixed acid fermentation         | abscesses, neurocysticercosis              |
| Creatine (Cr)     | 3.0     | 4.5-10.5 | cell energy/metabolism                                                    | mostly stable, used as reference peak tumors, lymphomas, stroke, infectious processes, MS |
| Choline (Cho)     | 3.2     | 0.5-3.0 | cell membranes (cell turnover, cell destruction)                          |                                             |
| myo-Inositol (mIns) | 3.5 | 4.0-9.0 | glucose metabolism mainly in astrocytes                                   | gliosis, hepatic encephalopathy, pontine myelinolysis, MS |

Table 1. Brain metabolites

And at short echo times (less than TE 30ms) are: (6) at 3.5 ppm (‘mIns-Peak’; myo-inositol, (mIns)), (7) at 0.9-1.2 ppm (‘free lipids peak’).

Normal peak levels are given in [mM].
Brain metabolites were suggested as markers for (1) neurons (NAA), (2) energy metabolism (Cr), (3) cell membranes or cell membrane turnover (Cho), (4) anaerobic glycolysis, i.e. tissue necrosis (Lac, lipids, cytosolic amino acids, Ala, acetate, succinate), (5) astrogliosis (mIns), and (6) myelin break down (lipids, cytosolic amino acids). Abnormally strong or weak peaks or certain patterns of several peaks may be indicative for various pathological processes, and may help to interpret changes found by conventional MRI.

For instance, lac is normally only detectable if the lac concentration in the CNS parenchyma has reached at least the lac concentration in the cerebrospinal fluid (CSF). Lac is the end product of anaerobic glycolysis and may occur in severe tissue necrosis or abscesses (table 1).

The most discussed MRS peaks (or brain metabolites) yet are, NAA, Cho, Crea and mIns.

1.1.1 N-acetyl-aspartate (NAA), at 2.0 ppm
In normal CNS tissue the highest peak in the proton spectrum is found at 2.0 ppm, resembling the sharp resonance signal from NAA, largely composed of NAA itself, and to a lesser proportion out of N-acetyl-aspartate-glutamate. NAA is an amino-acid derivative synthesized from L-aspartate and acetyl-CoA. NAA was found to be mainly expressed in the mitochondria of neurons, their cell bodies, dendrites and axons, and thus was suggested to indicate either the structural or functional integrity of neurons or axons. However, its function remains unknown. In principal three scenarios, where a reduction of the NAA peak might occur, are discussed: (1) if neurons or axons are damaged irreversible, and degenerate, (2) if their density is reduced relatively in the tissue due to oedema, or (3) if their function is impaired, only.

1.1.2 Choline (Cho), at 3.2 ppm
Cho and Cho-containing phospholipids hardly resonate under normal conditions because they are mainly insoluble and immobile as they constitute all cell membranes and myelin. In tumors, inflammation, infarcts, leukencephalopathy, leucodystrophies or in certain MS lesions, high cell membrane turnover due to cellular mitosis and cell death, or myelin break down may lead to higher concentration of soluble, freely mobile Cho, which may be detected at 3.2 ppm on the frequency scale. If freely mobile Cho molecules are abundant and resonate, an increased ‘Cho-peak’ may be found.

Note that severe tissue destruction, i.e. necrosis, where cell membranes are heavily damaged, torn asunder and single cell membrane compounds released into the necrosis, may produce other resonances or peaks (such as free lipids, lac, acetate, succinate).

1.1.3 Creatine (Cr), at 3.0 ppm
The ‘Cr-peak’ is composed of Cr and phosphor-creatine, and lies at 3.0 ppm on the frequency scale, representing a marker for cellular energy metabolism. As the Cr concentration was found relatively stable throughout the CNS, and found relatively resistant to change, Cr is often used as internal standard, and thus the signals strength of the other metabolites are expressed as ratio to Cr. However, under certain conditions such as higher age, tumors, infarcts or trauma Cr is found decreased. (Sometimes Cr was also found increased in the NAWM of MS patients, see below).
1.1.4 Myo-Inositol (mINS), at 3.5 ppm

The ‘mIns-peak’ is believed to resemble an ‘activated’ state of higher cell metabolism or proliferation of astrocytes. mIns is a sugar-like molecule which may be crucial for the osmotic regulation in the CNS parenchyma. An elevated ‘mIns-peak’ may be interpreted as higher astroglial activity and proliferation or gliosis.

But MRS allows a limited view only. From a scientific point of view, it is hardly conceivable that a few metabolites really allow the complete representation of complex biological processes of individual cells within the spectroscoped CNS tissue. Note that the function of the metabolites and that their (resonance) characteristics under certain conditions are still largely unknown.

One of the most important and controversially discussed hypotheses today is that ‘early axonal damage already exists in very early stages of (all) MS patients’. This view may open the way for ‘an (very) early treatment’ of MS patients, also if clinical symptoms are minor or even absent (Miller et al., 2008; Gilmore et al., 2010). This hypothesis comes mainly from MRI and MRS findings reporting reduced NAA levels in the normal appearing white matter (NAWM). NAA levels are suggested as marker for neuronal integrity and function and should reflect the burden of disease in MS patients (figure 1).

Fig. 1. A hypothesis, nothing else. a, It remains unclear, simply hypothetically, whether progressive, i.e. steadily ongoing ‘silent’, axonal damage exists in all MS patients, or not and if so, whether it can be reliably detected and monitored over time. It was suggested that NAA levels in the NAWM decline in some MS patients continuously. This NAA decline would be expected more than naturally occurring declines by age. b, Objective and neutral interpretation of the data allows also a second scenario, where brain volume (and NAA concentration?) only slightly decreases with age, or secondary progression. And during relapses temporarily a decrease of NAA can be found, that may recover fully.

However, due to the most often relapsing-remitting course of MS patients, with remission over years and sometimes over decades, early treatment per se must be carefully considered and risks and benefits carefully weighed.
1.2 Recently the pivotal role of advanced MR technique was emphasized

1.2.1 ‘Differential diagnosis of suspected multiple sclerosis: A consensus approach’

Miller DH, Weinshenker BG, Filippi M, Banwell BL, Cohen JA, Freedman MS, Galetta SL, Hutchinson M, Johnson RT, Kappos L, Kira J, Lublin FD, McFarland HF, Montalban X, Panitch H, Richert JR, Reingold SC, Polman CH.

Multiple Sclerosis, Vol. 14, No. 9, pp. 1157-1174.

P 1164, […] Although symptoms and signs of a monophasic illness have been an essential prerequisite for diagnosis of CIS, there is an exceptional scenario that the Panel feels warrants inclusion as CIS Type 5 (Table 2): patients who have no symptoms or only non-specific symptoms (e.g., headache, dizziness), but have MRI evidence for multifocal abnormalities typical for demyelination. Such patients are increasingly identified using MRI for incidental indications (e.g., headache) especially with high-field strength magnets with greater sensitivity for such lesions [17]. Current criteria preclude a diagnosis of MS without objective clinical evidence for CNS abnormality and the ability to establish a confident diagnosis of MS in such individuals and their natural history should be addressed through prospective studies [18]. […]

P 1172, […] Disease biomarkers will aid enormously in differential diagnosis. Accurate and sensitive disease markers – imaging or laboratory based – may provide non-invasive aids to differential diagnosis. The discovery of NMO-IgG that helps to distinguish NMO from MS is a good example. Relevant imaging advances may include non-conventional MR techniques to quantitate change in normal appearing white matter that may be relatively specific for MS, and high-field MRI to better visualize Dawson’s fingers [17] or cortical lesions that may be specific to MS. […]

Interestingly, the reference No. 17 (title ‘Cerebral Cortical Lesions in Multiple Sclerosis Detected by MR Imaging at 8 Tesla’) is a case report where the post mortem/ex vivo findings of a single patient, a male aged 42 years, were described (Kangarlu et al., 2007). Coronal slices of the patient’s formalin fixed brain were examined using MRI at 8T. Details about the disease course, or how the diagnosis ‘MS’ were made was not specified, p262, […]

‘Cerebral cortical lesions in multiple sclerosis detected by MR imaging at 8 Tesla.’ Kangarlu A, Bourekas EC, Ray-Chaudhury A, Rammohan KW.

AJNR American Journal of Neuroradiology, Vol. 28, No. 2, pp. 262-266.

A 42-year-old man with severe disabilities secondary to MS died of aspiration pneumonia. In the year before his death, he had been seen at our medical center on 2 occasions, mostly for palliative care. He was bed-bound and very dependent for all activities of daily living. He was paraplegic with additional severe weakness of the upper extremities, which were also severely ataxic. The ataxia also affected his trunk and prevented him from sitting unassisted in a standard wheelchair. He had complete bilateral internuclear ophthalmoplegia, vertical nystagmus, and severe

2 Claude Bernard (French physiologist, 1813 – 1878)
titubation of his head. His ability to communicate was intact, though his speech was barely intelligible. His mentation seemed intact; he remained completely oriented and participated in all decisions of his care, including his desire to have his brain evaluated for scientific endeavors after death. He needed to be fed through a percutaneous gastrostomy tube. Cognitive status could not be formally evaluated because of the severity of his disabilities. The duration of his disease was estimated to be approximately 12 years. At the time of his demise, he was considered to be in the advanced stages of the secondary-progressive form of MS. His general health was otherwise excellent, and he had no known cardiovascular, cerebrovascular, hypertensive, or diabetes-related disabilities. […]

For reference No. 18 see, Lebrun at al., 2008 and the comment by Chattaway, 2008.

As critical readers we are aware that we must read more than the headlines and abstracts of the papers and that the hypothesis that ‘early axonal damage already exists in very early stages of (all) MS patients’ has yet to be proven (Figure 1), and that the very controversial discussion is still ongoing (e.g. Chattaway, 2008 and 2010; Gilmore et al., 2010; Lebrun et al., 2008; Miller et al., 2008). In any case, we must keep in mind that direct scientific evidence is still lacking.

I consider the hospital as the antechamber of medicine.
It is the first place where the physician makes his observations.
But the laboratory is the temple of the science of medicine. 3

2. Multiple sclerosis

Multiple sclerosis (MS) is a chronic idiopathic disease of the central nervous system (CNS). Inflammation, demyelination and axonal injury are most typical pathological features, but their underlying pathogenetic mechanisms are still unclear (Barnett et al., 2009; Hibberd, 1994; Lassmann et al., 2007). They are probably complex and heterogeneous, and may be triggered outside the CNS or conversely, may be triggered within the CNS. And they may initiate focally and further disperse, or they may affect the whole CNS diffusely at once. In either case, blood brain barrier (BBB) alterations are conceivable, focally or diffusely, visible or invisible (Figure 2). But at least the primary cause of MS, and whether BBB alterations are the initial detectable pathological event in the evolution of the MS lesions, or why and whatever, the MS lesions are caused by, remains unknown (Aboul-Enein & Lassmann, 2006; Allen & McKeown, 1979; Allen et al., 1989; Barnes et al., 1991; Barnett et al., 2004; Barnett & Prineas, 2009; Henderson et al., 2009; Höftberger et al., 2004; Lassmann et al., 2007).

If an idea presents itself to us, we must not reject it simply, because it does not agree with the logical deductions of a reigning theory. 4
It is what we know already that often prevents us from learning. 5

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3 Claude Bernard (French physiologist, 1813 – 1878)
4 Claude Bernard (French physiologist, 1813 – 1878)
5 Claude Bernard (French physiologist, 1813 – 1878)
Fig. 2. ‘The chicken or the egg causality dilemma’ in MS lesion evolution.

**Red**, enhancing (active) MR lesions; **blue**, old(er), at least not enhancing lesions; **green**, in MR invisible inflammation or an underlying, yet not determined, pathogenetic mechanism around focal MS lesions; **lime**, hypothesized invisible, yet not determined, compartmentalized focal or diffuse inflammation or yet not determined pathogenetic process. **a-e**, the disease might be triggered from outside the CNS. **a**, normal brain without any lesion or invisible disease activity; **b**, the naïve brain with two enhancing lesions (red) only; **c**, brain with typical periventricular and subcortical old (not enhancing) white matter (WM) lesions only; **d**, same scenario as in **c**, but with new enhancing lesions (red). **e**, older non enhancing WM lesions (blue) of which one enhances in its right anterior margin (red margin) with ongoing (invisible) activity (green) beyond the in MR visible lesion border and two new enhancing (red) with invisible activity in the left hemisphere. **f-o**, the disease might be already compartmentalized within the CNS from the very beginning, but invisible with MRI (lime): **f-j**, focally, but widely dispersed; **k-o**, diffusely within the CNS. **f-o**, show the same enhancing (red) and old/er (blue) in MRI detectable lesions as presented in **a-d**. In both scenarios, the in MRI detectable lesions may resemble only the ‘tip of the iceberg’, leading to over time and space disseminated focal lesions with BBB alterations. In other words, the hypothesized underlying, invisible disease activity may lead from inside out to focal ‘eruptions’ with BBB alterations and influx of inflammation, or might attract inflammatory cells which lead to the BBB breakdown. The infiltrating inflammatory cells might be primarily harmful to the CNS, or otherwise, they might be primarily beneficial but may cause substantial bystander tissue damage, or both.
2.1 Diagnostic criteria and the disease course of MS

The diagnosis ‘MS’ is a clinical one, if stringent diagnostic criteria are fulfilled and if there is no better explanation for the clinical presentation (McDonald et al., 2001; Polman et al. 2005, 2010; Poser et al., 1983; Schuhmacher et al., 1965). The diagnosis ‘MS’ is based on the clinical evidence for typical disseminated CNS lesions, and may be supported by cerebrospinal fluid (CSF) analysis, magnetic resonance imaging (MRI), and evoked potentials. However, all findings are non-specific by themselves, but overall, they might be typical for the diagnosis ‘MS’. Up till now, a reliable specific para-clinical marker neither for the diagnosis nor for monitoring disease activity of MS could yet be established (e.g. Aboul-Enein et al., 2010; Gilmore et al., 2010; Kuhle et al., 2007; Serbecic et al., 2010, 2011).

In most of the cases MS starts between the age of 20 to 40 years and follows a relapsing-remitting course with clear defined relapses with no apparent clinical deterioration between the relapses. Each MS patient follows his/her individual disease course (Gilmore et al., 2010). It is unclear why MS sometimes follows a benign disease course with clinical remission over decades, or changes after an uncertain period of time into secondary progression with or without superimposed relapses (SPMS, secondary progressive MS), or follows rarely a progressive disease course right from the disease onset (PPMS, primary progressive MS) (Lublin & Reingold, 1996).

To sum up, no clinical or paraclinical parameter could yet be found, which allows a reliable prognosis of the ‘relapse-free intervals’, the relapses, the sequelae and the possible ‘conversion into secondary progression’. In particular, neither the frequency nor severity of relapses nor disability in the first years after onset nor the lesion load in magnetic resonance images (MRI) correlates strictly with the disease activity or its impact on the individual clinical disease course (e.g. Brex et al., 2002; Fisniku et al., 2008; Gilmore et al., 2010; Weinschenker et al., 1989a, 1989b). And axonal alterations or axonal loss, which is yet suggested as main pathological substrate, unfortunately, provides only some explanation.

In teaching man, experimental science results in lessening his pride more and more by proving to him every day that primary causes, like the objective reality of things, will be hidden from him forever and that he can only know relations.  

2.2 The clinical-pathological paradox of MS

Pathologists and neurologists have known for a long time that a considerable amount of CNS lesions with demyelination, axonal changes, inflammation and gliosis might be found, although clinical signs and symptoms were absent or should have never occurred (Lumdsen, 1970). A plausible explanation could be that in such cases the lesions were small, or that they occurred in non-eloquent CNS areas, but in any way, it means that recovery of function is possible. Axonal loss of function may be caused either by structural damage such as demyelination and (secondary) axonal injury (McDonald and Sears, 1970) or may be caused without any detectable structural damage by certain immune mediators only, which might interfere with axonal conductance (Aboul-Enein et al., 2006; Smith & Lassmann, 2002). Block of conduction is in principal reversible as axons may be remyelinated and the axonal

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* Claude Bernard (French physiologist, 1813 – 1878)
sodium channels may be restored, or the conduction block causing mediators may be removed (Smith, 2007). Of course, other mediators derived from any other tissue or incorporated from outside, and being not associated with the body’s immune system might be worth considering. But neither remyelination nor gliosis, both can be extensive in autopsies, correlate to permanent functional loss, if at all, only the irreversible axonal loss was found to correlate. And pathologists have only a very limited point of view (as neurologists and radiologists, too!). They can merely try to reconstruct the in vivo situation in most of the cases after long disease courses with very specific markers which however, allow mostly no further statement about the in vivo integrity and function over the patients’ lifetime.

2.3 The clinical-radiological paradox of MS

On a more philosophical - someone might argue - on a very ‘realistic’ level, we must remember that we merely know nothing about the disease’s origin or the disease’s specific processes. Direct scientific evidence is lacking for almost every little so far identified experimental, pathological, clinical, radiological or epidemiological fragment. We even do not know whether MS is one disease or a whole spectrum of disorders, or vice versa, whether the same pathogenetic mechanisms may have different effects in different individuals, or at different times in one individual MS patient. Otherwise not a specific primary cause itself, but the lack of specific resistant factor(s) or repair mechanisms might be responsible that some individuals become ill (and diagnosed), whereas all the others remain healthy. In other words, how many healthy people (Also you, dear readers!) could have a considerable lesion load or brain atrophy in MRI suggestive for MS, but without any further consequence as they feel healthy (and never see a doctor). Patients diagnosed with MS may be that ones with lesions mainly in eloquent areas. Thus they may resemble only a very small portion, the so called ‘tip of the iceberg’, but of course, they shape our view about MS.

However, by occasion some patients, who receive a MRI of their brain (because of a wide range of ‘unspecific’ symptoms, and because MRI is now widely available), show asymptomatic lesions suggestive for MS (Chataway, 2010). Recently, these ‘patients’ were classified as radiological isolated syndrome (RIS) or clinical isolated syndrome (CIS) type 5 (Miller et al., 2008). It is controversially discussed, whether these ‘patients’ should be followed closely, or even treated (Chataway, 2008, 2010; Gilmore et al., 2010; Lebrun et al., 2008; Miller et al., 2008; Sellner et al., 2010; Siva et al., 2009).

The link between disease onset and possible irreversible end-stage parameters such as glial scar tissue formation and brain and spinal cord atrophy is still missing. MRI and magnetic resonance spectroscopy (MRS) allows to visualize changes during individual disease courses in vivo, but they provide at least information from magnetic fields of certain molecules, mostly hydrogen nuclei (protons, or $^1$H) of water molecules, nothing else.

_A fact in itself is nothing. It is valuable only for the idea attached to it, or for the proof which it furnishes._

*Claude Bernard* (French physiologist, 1813 – 1878)
3. Of what we believe about MRI lesion evolution

3.1 Acute enhancing lesions

It is believed, that the alteration of the BBB may be the earliest event in formation of a focal MS lesion which may be visualized by gadolinium enhancement in T1-weighted MRI images with high sensitivity. Prerequisite for this is that gadolinium must be able to reach the specific region and that the BBB alterations are rather gross (‘BBB leakage’, in a strict sense). Gadolinium enhancement was suggested to be more sensitive in detecting disease activity than either clinical examination or T2 weighted MRI (Miller et al., 1993). Theoretically, all relapses should be accompanied by new, active enhancing MRI lesions (Figure 1b, d, e, g, i, j, l, n o). But, enhancement is dependent on the dose and the technique used, and dependent on the lesion size and location. This must be borne in mind when different studies are compared.

Higher doses of gadolinium (triple dose, 0.3mmol/kg body weight), longer delay between injection and the MRI scan (30-60 minutes), and the combination with a magnetization transfer pulse which suppresses the normal brain tissue, but spares areas with enhancement, can increase the sensitivity of MRI, i.e. make an higher number of enhancing MS lesions visualized (Filippi et al., 1996; Miller et al., 1998). Caveat is the increase of false positive results. However it remains unclear, if all lesions, in particular, those with only subtle BBB alterations can be detected hereby (Barnes et al., 1991; Kwon & Prineas et al., 1994, Waubant, 2006). Very often neurological symptoms may occur without any (correlating) acute enhancing MRI lesions, especially in the spinal cord. And most confusing is that acute enhancing MRI lesions that are clinically silent, may be found between relapses, even though they are located in eloquent areas (Davie et al., 1994; Schubert et al., 2002).

Put off your imagination as you take off your overcoat, when you enter the laboratory.  

In contrast to usual CNS immune surveillance with regular, always occurring transmigration of only few immune cells (Aboul-Enein et al., 2004), it is supposed that the lesion evolution starts with large numbers of immune cells that cross the BBB into the CNS parenchyma, and thus may lead to BBB leakage (Barnes et al., 1991; Hawkins et al., 1991). This is mainly supported by animal models of experimental autoimmune encephalomyelitis (EAE) that however, must not be uncritically translated to humans. In EAE huge masses of CNS antigen specific immune cells or antibodies, or both, are injected, and initiate an acute, but mostly monophasic inflammatory disease in the CNS (Flugel et al., 2001, 2007; Floris et al., 2004; Hawkins et al., 1991; Kawakami et al., 2005). The situation in humans with MS remains unclear. It is even unknown whether the inflammation is primary, whether the infiltrating immune cells themselves cause the tissue damage, or whether some other primary event within the CNS leads to the actual lesion formation and attracts the inflammatory cells into the CNS. The inflammation may be beneficial or conversely, may cause some bystander damage (Barnett & Prineas, 2004; Henderson et al., 2009; Hohlfeld et al., 2006). Anyway, corticosteroids may decrease or may completely suppress the enhancement of MS lesions for several weeks (Barkhof et al., 1991, 1994; Burnham et al., 1991). Conceivable are various mechanisms of action such as apoptosis of circulating

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immune cells, down-regulation of adhesion molecules on immune cells or endothelial cells, or both, or another yet not determined mechanism, that seals the BBB (Engelhardt, 2006; Gelati et al., 2002; Leussink et al., 2001; Prat et al., 2002).

3.2 Others than acute enhancing lesions

Most of the acute gadolinium enhancing MS lesions change their MR characteristics in further follow up. Some convert to mildly or severely hypointense T1 lesions which might reflect severe underlying tissue damage with marked axonal loss and correlating reduced NAA levels (e.g. van Walderveen et al., 1998), others convert to persistent T2 lesions after several months. The latter may appear hyperintense in T1 weighted images or become undetectable in T1 weighted images. In persistent T2 lesions, no matter what behavior they had in T1, nearly all thinkable metabolite constellations were described (increase, decrease, no changes of NAA, Cr, Cho, mIns etc., e.g. Davie et al., 1997; van Walderveen et al., 1997.). Reduced NAA levels might reflect axonal injury, dysfunction or loss. They might be temporarily or permanently, within lesions or remote from lesions due to secondary axonal degeneration or Wallerian degeneration. (Increased NAA levels have yet been described in Canavan’s disease only). Elevated Cr, mIns levels were suggested to reflect ‘higher metabolism’ in astrocytes, and increased Cho was suggested to reflect higher cell membrane turnover or proliferation of astrocytes within MS lesions or NAWM. Decreased levels might be due to decreased cell numbers or lower ‘cellular energy state’. These divergent results may be explained by the very small and heterogeneous patient cohorts (e.g. 2 RRMS and 12 SPMS aged from 28 to 61 years, and 4 controls aged 33 to 48 years in van Walderveen et al., 1999), by the use of a 1.5 Tesla 1H-MRS and by the measurement of metabolite ratios instead of absolute metabolite concentrations.

Only single reports of histopathological correlations to in vivo MR data have been published (Bitsch et al., 1997) that are of most importance for our understanding, but must be interpreted very carefully. Biopsies are only very seldom performed in MS patients, mostly if tumor lesions must be ruled out or verified. Whether demyelinating tumor-like MS lesions have the same characteristics and behavior like common unspecific smaller MS lesions, is unclear. Moreover, it is still unknown, if MS is one disease or a syndrome of many disorders, or if the same trigger causes the same pathological changes in different MS patients or if the same trigger at different time points leads to different types of lesions in one individual MS patient. Therefore, any general interpretation must be done with caution.

Men who have excessive faith in their theories or ideas are not only ill prepared for making discoveries. They also make very poor observations.9

4. Of what we believe to see with MRT and MRS

MRI allows the detailed visualization of the anatomical and pathological structures based on different signals of freely mobile hydrogen protons between different tissues or anatomical compartments.

9 Claude Bernard (French physiologist, 1813 – 1878)
The signals from freely mobile hydrogen protons are up to 1000 fold more than the resonances from other brain metabolites. The selective suppression of the signal from hydrogen protons ‘unmasks’ the spectra of various specific metabolites within the magnetic resonance frequency spectrum, and thus allows to detect and separate them, as each brain metabolite resonates at a characteristic position along the frequency scale expressed in parts per million (ppm). The sharpness and the amplitude of a specific peak along the frequency scale is determined by the specific metabolite itself, its concentration and most importantly, by its mobility. The brain metabolites must be freely mobile to produce well detectable peaks. In general, the more freely mobile specific metabolites in a specific volume of interest (VOI) resonate the higher their resulting peak is. If the specific peak is low, or even absent, this could mean that the protons in the examined VOI are either not occurring or not freely mobile or might require specific MR technique/parameters to be detected.

For instance, lipids are abundant within the brain, but under normal conditions immobile, as they constitute mainly highly compacted myelin. If myelin breaks down, they become mobile and may produce a ‘free lipid peak’ in the frequency spectrum at 0.9-1.2 ppm.

‘NAA-peak’ reduction or reduction of NAA absolute concentration in the spectroscopied tissue may occur due to axonal injury, damage or even loss, and might be irreversible or reversible. Infarcts, tumors, traumatic injury may lead to severe tissue destruction, heavy axonal damage and loss of axons, and thus may lead to irreversible NAA reduction. In the case of temporarily NAA reduction, various scenarios are conceivable: firstly, the NAA reduction correlates with structural axonal damage, i.e. axonal loss that in principle may be restored by remyelination and regain more or less the same NAA concentrations as before. Secondly, NAA reduction might reflect mitochondrial dysfunction in neurons or axons, whatever the underlying cause might be (e.g. inflammatory mediators, or any other molecule which might interfere with mitochondrial function), and might be restored when the interfering stimulus or agent is removed. The numbers of axons can be unchanged, or can be markedly reduced but have the same NAA levels, i.e. more ‘active mitochondria’ per axon or neuron. In other words, the loss of axons might be compensated with increase of numbers of mitochondria or increase of the NAA content in axonal mitochondria. (It was suggested that NAA might play a crucial role in the mitochondrial function).

A tumor or tumor-like brain lesion may either show profound reduction of NAA and relative high Cr concentrations in a first baseline scan. Several weeks later the NAA levels might be found still reduced or restored. In the first case, a tumor must be considered, whereas in the latter case a demyelinating tumor-like lesion with subsequent remyelination seems conceivable. (Tumors do not recover spontaneously).

A discovery is generally an unforeseen relation not included in theory, for otherwise it would be foreseen.10

5. Of what could be found with MRS in early stages of RRMS or even in CIS

Medline Database Research using following search term/key words ("multiple sclerosis" AND (NAWM OR "normal appearing white matter") AND ("N-acetyl-aspartate" OR NAA) and ("spectroscopy") and "absolute concentration") yielded 67 results (until March 2011).

10 Claude Bernard (French physiologist, 1813 – 1878)
Only 38 original articles providing MRS data (NAA/Cr ratios and/or NAA absolute concentrations) of RRMS or CIS patients could be further analysed. Other search results had to be excluded because they included MRS studies on SPMS or PPMS patients only (Leary et al., 1999; Cucurella et al., 2000; Sastre-Garriga et al., 2005), or did not compare the NAWM of matched controls (Hiehle et al., 1994), or were reviews, post mortem studies, MRS studies on patients with neuromyelitis optica, systemic lupus erythematos or relatives of MS patients who were not afflicted with MS.

A meta-analysis of the remaining 38 identified MRS studies could not be performed as they differ from study design (inclusion criteria and technical parameters), and do not provide all necessary data such as detailed demographic data and individual values for metabolite ratios and concentrations. However, following characteristics of the NAWM of CIS and RRMS patients could be identified (table 2, references are listed chronologically by publication date).

| Reference | TESLA | n | controls | CIS | NAA m f | SPMS | NAA m f | PPMS | NAA m f | CHO m f | NAA Cho m f |
|-----------|-------|---|----------|-----|---------|------|---------|------|---------|--------|------------|
| 1 Davie et al., 1994 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 2 Peters et al., 1993 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 3 Fu et al., 1993 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 4 Leary et al., 1999 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 5 Cucurella et al., 2000 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 6 Sastre-Garriga et al., 2005 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 7 Hiehle et al., 1994 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 8 Schiavo et al., 1997 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 9 Barone et al., 1999 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 10 Sambri et al., 1999 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 11 Roos et al., 1999 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 12 van Valkenhoef et al., 1999 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 13 Hiehle et al., 1994 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 14 Sastre-Garriga et al., 2005 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 15 Hiehle et al., 1994 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 16 Sartori et al., 1997 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 17 Cucurella et al., 2000 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 18 Sambri et al., 1999 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 19 Bredeler et al., 2002 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 20 Castaneda et al., 2003 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 21 Ingledew et al., 2004 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 22 Altmann et al., 2004 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 23 Fernando et al., 2004 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 24 Ruiz-Pena et al., 2004 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 25 Horvath et al., 2005 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 26 Vrenken et al., 2005 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 27 Mathiesen et al., 2005 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 28 Srinivasan et al., 2005 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 29 Sluyf et al., 2005 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 30 Khan et al., 2005 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 31 Tibero et al., 2006 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 32 Sijens et al., 2006 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 33 Marziali et al., 2007 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 34 Watanabe et al., 2007 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 35 Watanabe et al., 2007 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 36 Khan et al., 2005 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 37 Hallman-Jones et al., 2009 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| 38 About-Enin et al., 2009 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |

Table 2. Synopsis of literature research.
5.1 Reported NAA changes

5.1.1 Decreased NAA ratios or NAA concentrations

Twenty-four MRS studies were published with decreased ratios of NAA to Cr or Cho, or decreased NAA absolute concentrations in the NAWM in a total of 56 CIS or 418 RRMS patients (Davie et al., 1994; Husted et al., 1994; Peters et al., 1995; Fu et al., 1998; Schiepers et al., 1997; Foong J et al., 1999; Sarchielli et al., 1999; van Walderveen et al., 1999; Suhy et al., 2000; de Stefano et al., 2001; Tedeschi et al., 2001; Kapeller et al., 2001; Chard et al., 2002; de Stefano et al., 2002; Casanova et al. 2003; Inglese et al., 2004; Adalsteinsson et al., 2004; He et al., 2005; Staffen et al., 2005; Tiberio et al., 2006; Sijens et al., 2006; Pascual et al., 2007; Wattjes et al., 2008; Wattjes et al., 2008).

5.1.2 No significant changes of NAA ratios or NAA concentrations

Fourteen MRS studies were published with no significant NAA changes in the NAWM in a total of 184 CIS or 263 RRMS patients (Davie et al., 1997; Sarchielli et al., 1998; Brex et al., 1999; Kapeller et al., 2002; Fernando et al., 2004; Ruiz-Pena et al., 2004; Vrenken et al., 2005; Mathiesen et al., 2005; Srinivasan et al., 2005; Khan et al., 2005; Khan et al., 2008; Kirov et al., 2008; Bellmann-Strobl et al., 2009; Aboul-Enein et al., 2010).

5.2 Reported Cho changes

5.2.1 No significant changes of Cho ratios or Cho concentrations

Twenty-nine MRS studies were published with no significant Cho changes in the NAWM in a total of 190 CIS or 513 RRMS patients (Husted et al., 1994; Peters et al., 1995; Schiepers et al., 1997; Davie et al., 1997; Sarchielli et al., 1999; Foong J et al., 1999; Sarchielli et al., 1999; Brex et al., 1999; van Walderveen et al., 1999; Suhy et al., 2000; de Stefano et al., 2001; Kapeller et al., 2001; Chard et al., 2002; de Stefano et al., 2002; Kapeller et al., 2002; Casanova et al. 2003; Inglese et al., 2004; Adalsteinsson et al., 2004; Fernando et al., 2004; Ruiz-Pena et al., 2004; Vrenken et al., 2005; Mathiesen et al., 2005; Khan et al., 2005; Sijens et al., 2006; Wattjes et al., 2008; Wattjes et al., 2008; Khan et al., 2008; Bellmann-Strobl et al., 2009; Aboul-Enein et al., 2010).

5.2.2 Significant changes of Cho ratios or Cho concentrations

In two MRS studies (8 and 19 RRMS patients) Cho was found decreased (Davie et al., 1994; Tedeschi et al., 2001) and in 4 MRS studies (9, 16, 43 and 21 RRMS patients) Cho levels were found increased (He et al., 2005; Srinivasan et al., 2005; Pascual et al., 2007; Kirov et al., 2008).

5.3 Reported mlns changes

5.3.1 No significant changes of mlns ratios or mlns concentrations

Five out of 12 MRS studies reported no significant mINS changes in the NAWM of in a total of 76 CIS or 17 RRMS patients (Brex et al., 1999; Sarchielli et al., 1998; Sijens et al., 2006; Wattjes et al., 2008a; Wattjes et al., 2008b).
5.3.2 Significant changes of mIns ratios or mIns concentrations

Seven out of 12 MRS studies reported increased levels of mIns in the NAWM of in a total of 96 CIS or 116 RRMS patients (Kapeller et al., 2001; Chard et al., 2002; Fernando et al., 2004; He et al., 2005; Vrenken et al., 2005; Srinivasan et al., 2005; Kirov et al., 2008).

Keep in mind, that in most of the cases statistical characteristics of groups of MS patients were reported. Only very rarely the presentation of the data allow the readers their own interpretation because data are summarized in tables (with means, standard errors or standard deviations, range) and bar charts, instead of tables and scatter plots presenting all data for each individual patients. That groups of patients differ significantly, does not necessarily mean that all patients differ significantly from age and sex matched normal controls. They even may lie within normal range. For instance, the NAA levels of RRMS patients and even a proportion of SPMS patients were found within the range of NAA absolute concentrations that were found in healthy controls (Aboul-Enein et al., 2010; fig.3 squares, RRMS patients (n=27); circles, SPMS patients (n=10); triangles, controls (n=8); bars, means). However the mean NAA levels of SPMS patients were found significantly decreased compared to controls and RRMS patients.

![Fig. 3. MRS absolute concentrations of brain metabolites in the NAWM of MS patients and controls (from Aboul-Enein F et al., 2010). White, NAA [mM]; grey, Cr [mM]; black, Cho [mM].](image)

6. Conclusion

Currently the potpourri of data allows no reliable general information or even evidence about metabolite changes in the NAWM that were hypothesized to occur in all CIS or RRMS patients. Sophisticated meta-analysis is yet not possible as different MR methods were used, and individual data such as age, sex, disease duration, disease activity, lesion load, therapy, and metabolites concentrations cannot be extracted. Moreover, it remains unclear, what the specific metabolites reflect in the tissue of individual MS patients. In any case, we must be
aware that the magnetic resonances of freely mobile hydrogen protons or certain other freely mobile metabolites are converted to images, and thus ‘are made visible for the human’s eye’.

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