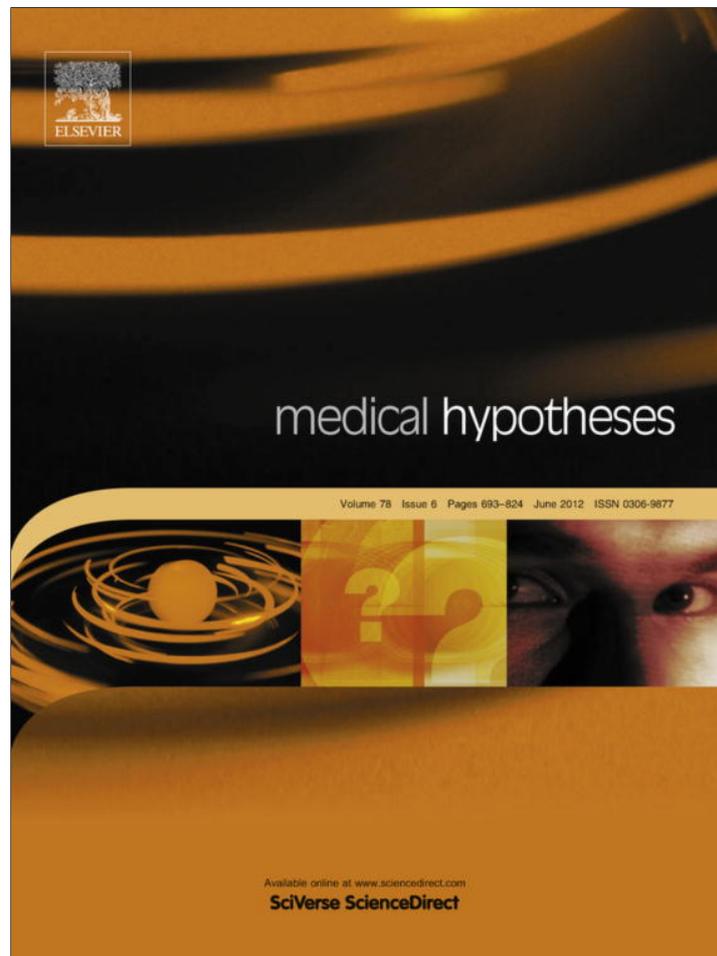


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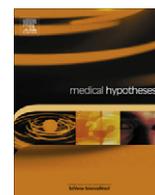
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Impairment of heme synthesis in myelin as potential trigger of multiple sclerosis

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ABSTRACT

The pathogenesis of multiple sclerosis (MS), a disease characterized by demyelination and subsequent axonal degeneration, is as yet unknown. Also, the nature of the disease is as yet not established, since doubts have been cast on its autoimmune origin. Genetic and environmental factors have been implied in MS, leading to the idea of an overall multifactorial origin. An unexpected role in energizing the axon has been reported for myelin, supposed to be the site of consumption of most of oxygen in brain. Myelin would be able to perform oxidative phosphorylation to supply the axons with ATP, thanks to the expression therein of mitochondrial F_0F_1 -ATP synthase, and respiratory chains. Interestingly, myelin expresses the pathway of heme synthesis, hence of cytochromes, that rely on heme group, in turn depending on Fe availability. Poisoning by these pollutants shares the common characteristic to bring about demyelination both in animal models and in man. Carbon monoxide (CO) and lead poisoning which cause functional imbalance of the heme group, as well as of heme synthesis, cause myelin damage. On the other hand, a lack of essential metals such as iron and copper, produces dramatic myelin decrease. Myelin is a primary target, of iron shortage, indicating that in myelin Fe-dependent processes are more active than in other tissues. The predominant spread of MS in industrialized countries where pollution by heavy metals, and CO poisoning is widespread, suggests a relationship among toxic action of metal pollutants and MS.

According to the present hypothesis, MS can be primarily triggered by environmental factors acting on a genetic susceptibility, while the immune response may be a consequence of a primary oxidative damage due to reactive oxygen species produced consequently to an imbalance of cytochromes and respiratory chains in the sheath.

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Introduction

Myelin sheath, the proteolipid wrap surrounding some axons both in CNS and PNS, has been supposed for decades to augment the speed of conduction [1] by an insulating and mechanical support role [2]. However, myelin may also play a trophic role for the axonal functioning [3–6]. In fact, its loss in demyelinating diseases does not only cause a slowdown of conduction velocity, but also a degeneration of the axons they wrap [7,8]. For example, Multiple Sclerosis (MS), the major cause of nontraumatic neurological disability in young adults in North America and Europe [9–11], is a demyelinating disease of the human central nervous system (CNS), characterized by focal areas of demyelination of the brain and spinal cord white matter [12]. The clinical course is variable, with about 85% of MS patients beginning with a course of recurrent and reversible neurological deficits in the third decade of life (Relapsing–Remitting MS) [12]. Subsequently, a phase characterized by continuous and irreversible neurological decline follows that reduces lifespan by 7–8 years, on average [13]. The major cause of neurological disability in MS patients is neurodegeneration, in

the form of a progressive loss of axons, dendrites, and neurons, subsequent to demyelination [13]. It has been supposed that chronically demyelinated axons degenerate due to the lack of myelin-derived trophic support [3,14] and energy depletion [12,15,16]. Demyelination increases the energy demand of nerve conduction, and compromises axoplasmic ATP production [16]. The metabolic interaction between neurons and glia has been investigated [17]. In the giant axon of the crayfish, Hargittai and Lieberman [18] observed that myelin contributes approximately for 70% of the O_2 consumption. Consistently with this observation, we have reported data suggesting that myelin sheath is a site of aerobic ATP production [3,19]. An ATP synthesis dependent on a transmembrane electrochemical proton gradient generated by an ectopic electron transfer chain (ETC) was observed in isolated myelin vesicles (IMV) [3,19]. Microscopy techniques identified ETC and F_0F_1 -ATP synthase in IMV and in optic nerve sections [3]. The inhibitor of F_1 (IF1), a small protein that binds the F_1 moiety of ATP synthase in low pH (a kind of “crash-barrier” against ischemia damage), controls IMV F_0F_1 -ATP synthase [19]. These biochemical data are confirmed by several proteomic studies conducted on myelin [20–23], reporting the expression therein of ETC, F_0F_1 -ATP synthase proteins and tricarboxylic acid cycle enzymes. A conceivable hypothesis of an energetic function for myelin has been proposed [24].

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Neurons recruit mitochondria in the energetically compromised chronically demyelinated axoplasm [25,26]. It is tempting to conclude that this mechanism is intended to compensate for a lost mitochondrial function. Moreover, mitochondria that reach demyelinated axoplasm in turn get imbalanced and have a reduced capacity for ATP production [25]. An imbalanced respiration in myelin [3] would produce reactive oxygen species (ROS) that would in turn damage the mitochondria reaching the redox-imbalanced axoplasm. Indeed, oxidative stress plays a major role in the pathogenesis of MS [25]. In fact, ROS, have been implied as mediators of demyelination and axonal damage in both MS and experimental autoimmune encephalomyelitis (EAE) [27]. In addition, weakened cellular antioxidant defense systems in the central nervous system (CNS) in MS, and its vulnerability to ROS effects may increase damage [28].

Even though MS is considered an autoimmune disease, several Authors have recently hypothesized that the triggering mechanism may not only be immune [12,13]. In fact, anti-inflammatory therapies although effective in slowing the progression of MS, have not reached the goal of stopping or preventing the disease process [12]. MS is also considered a complex genetic disorder in which multiple interacting polymorphic genes with low penetrance, each exerting a small effect on the overall disease risk [12]. Risk assessment studies show increased MS incidence in families with a history for the disease [29]. However, even though no single environmental agent has by now been associated with the disease, many studies support a role for the environment in MS disease susceptibility [30]. Studies show a relatively higher risk in northern Europeans and lower risk in Africans, Asians, and American Indians [31]. MS has the lowest prevalence in countries with low pollution. Interestingly, England, where industrial revolution took place was the first country where MS was first described, [32].

The hypothesis

We hypothesize that any factors unbalancing heme synthesis or functioning or Fe or Cu ion supply to myelin, such as environmental pollutants, would cause damage to the sheath and be environmental “*primum movens*” in the context of a genetic susceptibility in a multifactorial ethiopathogenesis of MS. In fact, the cytochromes, possessing a prosthetic heme group, play a pivotal role in the ETC electron transport. The idea of myelin supplying the axons with aerobically synthesized ATP, implies a sustained oxidative phosphorylation (OXPHOS), i.e. a great flux of Oxygen, in the sheath. This in turn would expose it to potential damage by ROS. Also, a continual intense heme synthesis is necessary for the cytochromes operating in the OXPHOS [34], as well as a convenient supply of iron.

Evaluation and discussion of the hypothesis

Interestingly, myelin is a site of intense heme synthesis [34]. Cultures of mouse dorsal root ganglia (DRG) incubated for 48 h with delta-aminolevulinic acid (ALA), a precursor of heme group, showed intense porphyrin fluorescence localized in myelin sheath but not in axons, or neuronal somata neither in demyelinated axons [34]. The same Authors observed that lead poisoning (saturnism) causes a progressive demyelination in myelinated DRG cultures treated with lead. This effect was counteracted by the addition of exogenous ALA, suggesting that the lead-induced effect may be due to toxic effects of the metal on the heme biosynthetic pathway in myelin [34]. Similar conclusions were obtained on brain myelin in rat subjected to prolonged lead poisoning: Pb accumulation in myelin induced a modification in the sheath structure,

associated with changes in myelin membrane fluidity [35]. Electron microscopy analyses showed a loss of the ordered layers in the Pb-intoxicated sample with respect to the control experiments [35] [36] so the Authors conclude that Pb may be considered as a one of the factors contributing to demyelinating diseases. Porphyrins, a set of rare genetic diseases that share a common feature, i.e. an imbalance in heme synthesis, cause demyelination and severe neural dysfunctions, such as schizophrenia e seizures [37].

CO poisoning is primarily associated with brain injury, and causes demyelination in a high percentage of cases. Brain injury after acute CO poisoning has been related to oxidative stress, ROS production, and consequent inflammatory responses. However, unless myelin is active in OXPHOS it would be unclear why ROS should affect it. Some patients who develop brain damage following severe CO poisoning do so due to an autoimmune reaction [38] [39]. This damage seems to depend on a change in myelin basic protein (MBP) structure, induced by CO poisoning, similar to that seen in MS [40], that in turn, sets an autoimmune response [39]. Hydrogen inhalation, exerting antioxidant effect was proposed as a simple, and economic therapy in the treatment of acute CO poisoning, and also to protect brain against ischemia-reperfusion injury [38].

OXPHOS functioning relies on copper (Cu) and iron (Fe) ions. Cu is contained in the ETC complex IV, contributing to reduce oxygen to water, while Fe is a component of cytochrome heme group and the Fe-S proteins involved in electron transport. Zimmerman et al. [41] observed that Cu deficiency induced by a low copper diet in rats is associated with a substantial reduction in myelin content (56%) and brain weight (11%), and suggested that Cu is essential for myelin formation and growth during critical periods in development. Moreover, Cu deficiency is always associated with neurological disorders [42]. Brain injury associated to Cu lack is also observed in Menkes disease, an X-linked recessive neurodegenerative disorder, caused by mutations in a copper-transporting p-type ATPase (ATP7A) that delivers copper to the CNS [43] and in mouse exposed to the copper chelator, cuprizone (bis(cyclohexanone)oxalylidihydrazone) [44]. Komoly et al. showed that before demyelination development [45] carbonic anhydrase II (CA II) activity falls in the brain of cuprizone treated mice.

A consistent Fe content was reported in oligodendrocytes and myelin sheath [46]. Iron content can be directly measured in myelin by MRI [47]. Activity of Fe-requiring enzymes involved in metabolic activity is elevated in oligodendrocytes, that are the principal cells that stain for iron in the CNS under normal conditions [48]. Early hypomyelination is associated to a decreased availability of Fe in the diet [48]. Also, iron deficiency has been associated with irreversible alterations in brain myelination, in early development [49]. These data suggest that Fe ions play a role in myelin, setting up a link among Fe and MS [50]. An iron dysregulation in the pathogenesis of MS has been given attention [51]. Interesting is the high content in serum ferritin in MS chronic patients [51].

If myelin is an active site of OXPHOS, and for the considerations exposed, any factor inhibiting this function would concur towards MS triggering. The hypothesis of myelin as a mitochondria-like membrane [3,4,24] implies that any impairment in cytochrome functioning or in oxygen delivery to the ETC would cause ROS production and a damage to the sheath. Damaging agents may be pollutants, particularly heavy metals inhibiting myelin heme synthesis, or other environmental poisons, such as CO. A massive ROS production is expected when the ETC is imbalanced. Oxidative stress would represent a major cause of myelin damage. This would also adequately explain why MS symptoms start in the second decade of life. This is the moment when myelin synthesis is complete, and its turnover slows down. Oxidative stress of the

lipids in the sheath would be clinically silent as long as myelin is rapidly renewed. When myelin turnover slows down damage increases to a point of no return. The challenging hypothesis of a trophic role for myelin and its involvement in sleep mechanism [33] can also explain the known sleep disturbances typical of MS subjects.

Conclusions

In line with studies on the symbiotic relationship of the axon and its sheath, we have focused our attention on the implications of the new role of myelin as a respiring wrap. We hypothesize that environmental pollution may impair at various levels the OXPHOS in myelin sheath. In this respect, the *primum movens* in MS, would be an oxidative myelin damage, possibly determining a susceptibility to the disease, in the context of a genetic polymorphism.

Interestingly, epidemiologic studies indicate that MS is more diffused in North America and in Europe, with its incidence decreasing with the closeness to equator [32], i.e. MS is prevalent in the industrial countries, where environmental pollution is consistent.

The importance of the present hypothesis would lay in its representing a common molecular explanation for a number of well characterized processes all of which otherwise lack an explanation. This is the case for the alterations induced on myelin by essential metals as Fe, Cu poisoning or lack, as well as by nonessential heavy metal poisoning and by pollutants as CO, that are chemically different but related to OXPHOS functionality. Rare is such a case for a single hypothesis. Trapp and Nave [12] suggested that a multi-disciplinary approach is necessary to be able to look for novel aspects of MS pathogenesis to develop therapies that may delay or prevent the irreversible and progressive neurological decline. A new view on a primary damage to myelin and a subsequent immune-mediated injury may represent a fresh point of view about the ethio-pathogenesis of MS to start with.

Conflicts of interest statement

Authors declare no conflicts of interest.

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References

- [1] Morell P, Norton WT. Myelin. *Sci Am* 242 (1980), pp. 88–90, 92, 96 passim.
- [2] Huxley AF, Stämpfli R. Evidence for saltatory conduction in peripheral myelinated nerve fibres. *J Physiol* 1949;108:315–39.
- [3] Ravera S, Panfoli I, Calzia D, et al. Evidence for aerobic ATP synthesis in isolated myelin vesicles. *Int J Biochem Cell Biol* 2009;41:1581–91.
- [4] Ravera S, Panfoli I, Aluigi MG, Calzia D, Morelli A. Characterization of myelin sheath F(o)F(1)-ATP synthase and its regulation by IF(1). *Cell Biochem Biophys* 2011;59:63–70.
- [5] Fields RD. White matter in learning, cognition and psychiatric disorders. *Trends Neurosci.* 2008;31:361–70.
- [6] Correale J, Mel F, Ysrraeli C. Neuronal injury in multiple sclerosis, vol. 66. B. Aires: Medicina; 2006 pp. 472–485.
- [7] Edgar JM, Nave KA. The role of CNS glia in preserving axon function. *Curr Opin Neurobiol* 2009;19:498–504.
- [8] Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. *Brain* 1997;120(Pt 3):393–9.
- [9] Weinschenker BG. Epidemiology of multiple sclerosis. *Neurol Clin* 1996;14:291–308.
- [10] Noseworthy JH, Lucchinetti C, Rodriguez M, Weinschenker BG. Multiple sclerosis. *New England J Med* 2000;343:938–52.
- [11] Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. *Neuron* 2006;52:61–76.
- [12] Trapp BD, Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder? *Annu Rev Neurosci* 2008;31:247–69.
- [13] Dutta R, Trapp BD. Mechanisms of neuronal dysfunction and degeneration in multiple sclerosis. *Prog Neurobiol* 2011;93:1–12.
- [14] Rammohan KW. Axonal injury in multiple sclerosis. *Curr Neurol Neurosci Rep* 2003;3:231–7.
- [15] Smith KJ. Axonal protection in multiple sclerosis—a particular need during remyelination? *Brain* 2006;129:3147–9.
- [16] Mahad D, Lassmann H, Turnbull D. Review: mitochondria and disease progression in multiple sclerosis. *Neuropathol Appl Neurobiol* 2008;34:577–89.
- [17] Hertz L, Peng L, Dienel GA. Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J Cereb Blood Flow Metab* 2007;27:219–49.
- [18] Hargittai PT, Lieberman EM. Axon-glia interactions in the crayfish: glial cell oxygen consumption is tightly coupled to axon metabolism. *Glia* 1991;4:417–23.
- [19] Ravera S, Panfoli I, Aluigi MG, Calzia D, Morelli A. Characterization of Myelin Sheath F(o)F(1)-ATP Synthase and its Regulation by IF(1). *Cell biochemistry and biophysics* 2010;59:63–70.
- [20] Taylor CM, Marta CB, Claycomb RJ, et al. Proteomic mapping provides powerful insights into functional myelin biology. *Proc. Nat. Acad. Sci. USA* 2004;101:4643–8.
- [21] Vanrobaeys F, Van Coster R, Dhondt G, Devreese B, Van Beeumen J. Profiling of myelin proteins by 2D-gel electrophoresis and multidimensional liquid chromatography coupled to MALDI TOF-TOF mass spectrometry. *J Proteome Res* 2005;4:2283–93.
- [22] Werner HB, Kuhlmann K, Shen S, et al. Proteolipid protein is required for transport of sirtuin 2 into CNS myelin. *J Neurosci* 2007;27:7717–30.
- [23] Yamaguchi Y, Miyagi Y, Baba H. Two-dimensional electrophoresis with cationic detergents: a powerful tool for the proteomic analysis of myelin proteins. Part 2: analytical aspects. *J Neurosci Res* 2008;86:766–75.
- [24] Morelli A, Ravera S, Panfoli I. Hypothesis of an Energetic Function for Myelin. *Cell biochemistry and biophysics* 2011;61:179–87.
- [25] Dutta R, Trapp BD. Pathology and definition of multiple sclerosis. *La Revue du Praticien* 2006;56:1293–8.
- [26] Mutsaers SE, Carroll WM. Focal accumulation of intra-axonal mitochondria in demyelination of the cat optic nerve. *Acta Neuropathol* 1998;96:139–43.
- [27] Penton-Rol G, Cervantes-Llanos M, Martinez-Sanchez G, et al. TNF-alpha and IL-10 downregulation and marked oxidative stress in neuromyelitis optica. *J Inflamm* 2009;6:18 (London, England).
- [28] Gilgun-Sherki Y, Melamed E, Offen D. The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. *J Neurol* 2004;251:261–8.
- [29] Ebers GC, Yee IM, Sadovnick AD, Duquette P, Canadian Collaborative Study Group. Conjugal multiple sclerosis: population-based prevalence and recurrence risks in offspring. *Ann Neurol* 2000;48:927–31.
- [30] Spirin NN, Kachura DA, Kachura AN, Boiko AN. [The influence of environmental factors on the incidence and prevalence of multiple sclerosis], *Zhurnal Nevrologii i Psikhatrii Imeni S.S* (2003), pp. 111–113.
- [31] Rosati G. The prevalence of multiple sclerosis in the world: an update. *Neurol Sci* 2001;22:117–39.
- [32] Kurtzke JF. Multiple sclerosis in time and space—geographic clues to cause. *J Neurovirol* 2000;6(Suppl 2):S134–140.
- [33] Morelli A, Ravera S, Panfoli I. Myelin sheath: a new possible role in sleep mechanism. *Sleep Med* 2011;1(2):199.
- [34] Whetsell Jr WO, Sassa S, Kappas A. Porphyrin-heme biosynthesis in organotypic cultures of mouse dorsal root ganglia. Effects of heme and lead on porphyrin synthesis and peripheral myelin. *J Clin Invest* 1984;74:600–7.
- [35] Dabrowska-Bouta B, Sulkowski G, Bartosz G, Walski M, Rafalowska U. Chronic lead intoxication affects the myelin membrane status in the central nervous system of adult rats. *J Mol Neurosci* 1999;13:127–39.
- [36] Dabrowska-Bouta B, Sulkowski G, Struzynska L, Rafalowska U. CNPase activity in myelin from adult rat brains after prolonged lead exposure in vivo. *Chem Biol Interact* 2004;150:171–8.
- [37] Gonzalez-Arriaza HL, Bostwick JM. Acute porphyrias: a case report and review. *Am J Psychiatry* 2003;160:450–9.
- [38] Shen M, He J, Cai J, Sun Q, Sun X, Huo Z. Hydrogen as a novel and effective treatment of acute carbon monoxide poisoning. *Med Hypotheses* 2010;75:235–7.
- [39] Thom SR, Bhopale VM, Fisher D, Zhang J, Gimotty P. Delayed neuropathology after carbon monoxide poisoning is immune-mediated. *Proc Nat Acad Sci USA* 2004;101:13660–5.
- [40] Miyagishi T, Suwa N. Electron microscopic studies on the cerebral lesions of rats in experimental carbon monoxide poisoning. *Acta Neuropathol* 1969;14:118–25.
- [41] Zimmerman AW, Matthieu JM, Quarles RH, Brady RO, Hsu JM. Hypomyelination in copper-deficient rats. Prenatal and postnatal copper replacement. *Arch Neurol* 1976;33:111–9.
- [42] Zatta P, Frank A. Copper deficiency and neurological disorders in man and animals. *Brain Res Rev* 2007;54:19–33.
- [43] Liu PC, Chen YW, Centeno JA, Quezado M, Lem K, Kaler SG. Downregulation of myelination, energy, and translational genes in Menkes disease brain. *Mol Genet Metab* 2005;85:291–300.
- [44] Matsumura GK, Morell P. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathol* 2001;11:107–16 (Zurich, Switzerland).

- [45] Komoly S, Jeyasingham MD, Pratt OE, Lantos PL. Decrease in oligodendrocyte carbonic anhydrase activity preceding myelin degeneration in cuprizone induced demyelination. *J Neurol Sci* 1987;79:141–8.
- [46] Connor JR, Menzies SL. Relationship of iron to oligodendrocytes and myelination. *Glia* 1996;17:83–93.
- [47] Fukunaga M, Li TQ, van Gelderen P, et al. Layer-specific variation of iron content in cerebral cortex as a source of MRI contrast. *Proc Nat Acad Sci USA* 2010;107:3834–9.
- [48] Todorich B, Pasquini JM, Garcia CI, Paez PM, Connor JR. Oligodendrocytes and myelination: the role of iron. *Glia* 2009;57:467–78.
- [49] Wu LL, Zhang L, Shao J, Qin YF, Yang RW, Zhao ZY. Effect of perinatal iron deficiency on myelination and associated behaviors in rat pups. *Behav Brain Res* 2008;188:263–70.
- [50] Abo-Krysha N, Rashed L. The role of iron dysregulation in the pathogenesis of multiple sclerosis: an Egyptian study. *Multiple Scler* 2008;14:602–8 (Houndmills, Basingstoke, England).
- [51] Sfagos C, Makis AC, Chaidos A, et al. Serum ferritin, transferrin and soluble transferrin receptor levels in multiple sclerosis patients. *Multiple Scler* 2005;11:272–5 (Houndmills, Basingstoke, England).