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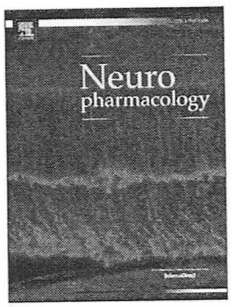


Targeting demyelination and virtual hypoxia with high-dose biotin as a treatment for progressive multiple sclerosis

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**Title:** Targeting demyelination and virtual hypoxia with high-dose biotin as a treatment for progressive multiple sclerosis

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Authors FS and DB are employees of MedDay Pharmaceuticals; author DM has served as a consultant for MedDay Pharmaceuticals; author AT has received in the last year, consulting and lecturing fees, travel grants and research support from Biogen Idec, Sanofi-Genzyme, Novartis, Merck Serono, Teva Pharma, and Roche.

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## Abstract

Progressive multiple sclerosis (MS) is a severely disabling neurological condition, and an effective treatment is urgently needed. Recently, high-dose biotin has emerged as a promising therapy for affected individuals. Initial clinical data have shown that daily doses of biotin of up to 300 mg can improve objective measures of MS-related disability. In this article, we review the biology of biotin and explore the properties of this ubiquitous coenzyme that may explain the encouraging responses seen in patients with progressive MS. The gradual worsening of neurological disability in patients with progressive MS is caused by progressive axonal loss or damage. The triggers for axonal loss in MS likely include both inflammatory demyelination of the myelin sheath and primary neurodegeneration caused by a state of virtual hypoxia within the neuron. Accordingly, targeting both these pathological processes could be effective in the treatment of progressive MS. Biotin is an essential co-factor for five carboxylases involved in fatty acid synthesis and energy production. We hypothesize that high-dose biotin is exerting a therapeutic effect in patients with progressive MS through two different and complementary mechanisms: by promoting axonal remyelination by enhancing myelin production and by reducing axonal hypoxia through enhanced energy production.

**Keywords:**

multiple sclerosis, biotin, promyelinogenic agent, virtual hypoxia

## 1. Introduction

Multiple sclerosis (MS) affects an estimated 2.3 million people worldwide (Browne et al., 2014). MS is the most common disabling neurological disease of young adults, with first symptoms typically manifesting between 20 and 40 years of age (Browne et al., 2014). The condition is more common in women than men, and prevalence generally increases with latitude (Simpson S Jr et al., 2011). In Europe, the highest prevalence of MS is seen in Nordic countries and the British Isles (Kingwell et al., 2013). A diagnosis of MS places a high burden on the affected individual, both economically and with respect to MS-associated disability (Pike et al., 2012).

In the majority (85%) of cases, patients experience an initial phase of relapsing-remitting neurological dysfunction (RRMS), which typically evolves into a secondary progressive disease at a later point in the clinical course (SPMS) (Confavreux et al., 2000). Once MS is in the progressive phase, individuals experience a gradual worsening of neurological disability leading to problems with vision, walking, balance, incontinence, cognitive changes, fatigue, and pain (Gibson and Frank, 2002). Primary progressive MS (PPMS), characterized by disease progression from onset, is less common, affecting 10–15% of patients (Confavreux et al., 2000; Koch et al., 2009). Despite these different initial clinical phenotypes, the time to reach certain disability milestones and the ages at which the milestones are reached are similar for all patients with progressive MS (Confavreux and Vukusic, 2006).

Treatment options for MS remain inadequate. Most currently approved therapies for MS target inflammatory processes and aim to reduce the frequency of exacerbations in patients with RRMS. These include β-interferons, dimethyl fumarate, glatiramer acetate, fingolimod, teriflunomide, and natalizumab (Wingerchuk and Carter, 2014). β-interferon (Trojano et al., 2007), teriflunomide (Confavreux et al., 2014), fingolimod (Kappos et al., 2010), alemtuzumab (Coles et al., 2012), natalizumab (Polman et al., 2006), and mitoxantrone

(Martinelli et al., 2013) may also delay or reduce the risk of disability progression. However, currently available treatments have little to no efficacy in patients with progressive MS, particularly if superimposed relapses are absent, and there is no pharmacological therapy capable of arresting or reversing MS-related disability (Comi, 2013). Encouraging results have recently been reported with the use of nonmyeloablative hematopoietic stem cell transplantation in patients with RRMS (Burt et al., 2015). This technique achieved a striking improvement in disability (as measured by the Expanded Disability Status Scale; EDSS) in approximately half of the patients at 2-year follow-up; moreover, 80% of patients remained relapse free at 4-year follow-up. However, no benefit was seen in patients who had progressive MS at the time of transplant. For these individuals, an effective treatment remains a significant unmet medical need.

Two promyelinogenic agents are currently in development as treatment for MS. LINGO1 (leucine-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein 1) inhibits the differentiation of oligodendrocyte precursor cells (OPCs) and consequently inhibits myelination (Jepson et al., 2012). Upregulation of this receptor is seen in many neurological disorders, including in OPCs found within areas of demyelination from patients with MS (Mi et al., 2013). Inhibition of LINGO1 in animal models of autoimmune encephalomyelitis results in the formation of new myelin sheaths (Mi et al., 2007). Phase I data from healthy volunteers and patients with MS indicate that inhibition of LINGO1 with the monoclonal antibody BIIB033 (Biogen Idec) appears to be well tolerated and a phase II trial in patients with RRMS is ongoing (EUDRACT #: 2011-006262-40). First results have been presented at the American Academy of Neurology. In the per-protocol population, the anti-LINGO-1 group showed significantly improved optic nerve conduction latency vs placebo at Week 32. No statistical difference was observed in the ITT population or for the secondary endpoints including visual acuity at low contrast (Cadavid et al., 2015). In addition, a recombinant form of a human IgM (rHIgM22; Acorda Therapeutics) that binds to myelin and the surface of oligodendrocytes has been shown to promote remyelination in murine models

of MS and other demyelinating diseases (Mitsunaga et al., 2002; Warrington et al., 2007). The safety and preliminary efficacy of rHIgM22 are currently being investigated in patients with all forms of MS in a phase I trial (NCT01803867).

In this paper, we review recently published data that suggest that high-dose biotin is an effective treatment for patients with progressive MS and propose two distinct (and complementary) mechanisms of action to explain this efficacy.

## *2. Etiology and pathogenesis of MS*

Despite decades of research into the biology of MS, the etiology of this progressive neurological disease remains incompletely understood. Multiple sclerosis is classically considered to be an autoimmune demyelination disorder in which activated T-cells migrate across the blood brain barrier and attack the insulating myelin sheath that surrounds the axons (Compston and Coles, 2008). This leads to a progressive demyelination of the axons that ultimately culminates in degeneration of the denuded neurons. Indeed, there is abundant evidence supporting the inflammatory component of MS. Acute inflammatory lesions are characterized by the presence of infiltrating activated T-cells (both CD4+ and CD8+) (Denic et al., 2013; Traugott et al., 1983), and the frequency of axonal damage is related to the degree of inflammation within lesions (Trapp et al., 1998). Inflammation is initially transient and remyelination of axons occurs (Olsen and Akirav, 2015), which may explain the relapsing-remitting nature of the signs and symptoms of RRMS. If demyelination persists, denuded axons are vulnerable to damage and these immune-mediated attacks lead to axonal injury and neuronal death (Brugarolas and Popko, 2014; Podbielska et al., 2013).

However, autoimmunity alone fails to fully explain the pathophysiology of MS. In particular, axonal degradation can occur in normal-appearing white matter that has no evidence of myelin loss (Bjartmar et al., 2001; Lovas et al., 2000) or inflammation (Bitsch et al., 2000; Dutta and Trapp, 2011), and axonal degradation can occur at all stages of MS disease progression (De Stefano N. et al., 2001; Levin et al., 2014; Trapp et al., 1998). An alternative

theory proposes that MS is primarily a neurodegenerative disorder in which the inflammatory responses observed in patients with MS arise in response to highly antigenic components that are released during degradation of the neurons (Bruck, 2005; Stys et al., 2012; Trapp and Nave, 2008). Regardless of whether neurodegeneration occurs secondary to demyelination or whether primary neurodegeneration is the trigger for inflammatory demyelination, axonal loss or damage is undeniably the cause for the progressive neurological disability seen in MS (Bjartmar et al., 2000; Bjartmar et al., 2003; De Stefano N. et al., 1998; Dutta and Trapp, 2007). The exact mechanisms underlying neurodegeneration remain poorly understood but are believed to include a combination of energy imbalance, CD8+ cells, glutamate, nitric oxide, and loss of trophic interaction with oligodendrocytes (Bitsch et al., 2000; Bjartmar et al., 2003; Bruck, 2005).

## *3. High-dose biotin as a treatment for MS*

MD1003 (MedDay Pharmaceuticals, Paris, France) is an oral formulation of high-dose pharmaceutical-grade biotin currently in clinical development as a treatment for progressive MS and adrenomyeloneuropathy (AMN). The daily dose of MD1003 currently being investigated in phase III trials (300 mg biotin) is 10,000-fold higher than the Adequate Intake (AI) – the daily dietary intake level of biotin that is considered sufficient for the maintenance of health by the Food and Nutrition Board of the Institute of Medicine is 30 µg/day for adults (IOM Standing Committee on the Scientific Evaluation of Dietary Reference Intervals, 1998). At 300 mg per day, biotin is considered by regulators to be an active pharmaceutical agent. Therefore, the efficacy and safety of high-dose biotin as a therapeutic option in MS require careful investigation prior to being made available for clinical use.

Clinical data from the first patients treated with MD1003 have recently been published (Sedel et al., 2015). This open-label pilot trial investigated daily administration of high-dose biotin (100–300 mg/day) in 23 patients with primary or secondary progressive MS who received MD1003 for a mean duration of 9.2 months (range 2–36 months). Over 90% of the patients

(21/23) exhibited some degree of qualitative or quantitative clinical improvement following MD1003 therapy including reduced MS-related disability. A beneficial effect was seen in all forms of progressive MS. Improvements in walking distance, EDSS values, and time to walk 25 feet (TW25) were observed in patients with prominent spinal cord involvement, and improvements in visual acuity and the progressive reappearance of visual evoked potentials with normal latencies were recorded in patients with visual impairment related to optic nerve injury (Sedel et al., 2015). The promising efficacy observed in this pilot study suggests that high-dose biotin is effective in both reversing disease progression and reducing chronic disability. The study has important methodological limitations including the small number of patients, the small number of centers, the lack of a placebo or control group, the heterogeneity of patients enrolled, the heterogeneity of evaluation criteria, and the fact that treating and assessing physicians were the same. Results of a double-blind, placebo-controlled, multisite study were presented in April at the American Academy of Neurology (Tourbah et al., 2015a). 154 patients were randomized, (103 received 300 mg of biotin per day and 51 received a placebo). Patients had secondary or primary progressive MS with EDSS between 4.5 and 7 and evidence of EDSS progression within the past two years. Treatment duration was 48 weeks. The primary endpoint was the proportion of patients who improved at month 9 and confirmed at month 12, defined as decreased EDSS (by at least 1 point for EDSS ≤5.5 and 0.5 point for EDSS ≥6) or improved TW25 of at least 20%. A significant proportion of biotin-treated patients achieved the primary endpoint versus none of 51 placebo-treated patients (p=0.0051). The primary endpoint was confirmed by a significant decrease in the mean change in EDSS and the Clinical Global Impression Scale assessed by the investigator and subject in the biotin group (Tourbah et al., 2015b). We are hopeful that these results ultimately represent a major breakthrough in the treatment of MS because no therapeutic options have yet been validated to treat the progressive (primary or secondary) forms of the disease and its sequelae-related symptoms. In each patient treated with MD1003, there was a delay between the onset of treatment and the onset of objective clinical improvement; delays ranged from 2 to 8 months. This tantalizing observation

suggests that MD1003 is triggering some form of slowly progressive repair and furthermore may act as a disease-modifying therapy in patients with progressive disease. Both the compassionate use open-label study and the placebo-controlled trial after 12 months' follow-up are however of insufficient duration to answer this question. Data from the 12-month extension phase of this trial (during which all participants receive high-dose biotin) are expected in January 2016 and will be important in assessing the impact of the drug on long-term disability progression.

## *4. The role of biotin in brain chemistry*

Biotin (vitamin H) is a ubiquitous B-complex vitamin that acts as an essential coenzyme for five essential carboxylases: pyruvate carboxylase (PC) 3-methylcrotonyl-CoA carboxylase (MCC), propionyl-CoA carboxylase (PCC), and the two isoforms of acetyl-CoA carboxylase (ACC1 and ACC2) (Tong, 2013; Zempleni et al., 2009). Biotin is covalently bound to specific lysine residues on apocarboxylases; the biotinylation reaction is catalyzed by holocarboxylase synthetase (HLCS), an ATP-dependent enzyme encoded by the HLCS gene (Chapman-Smith and Cronan, Jr., 1999). Once bound, biotin acts as a transporter of an activated carboxyl moiety in a variety of carboxylation reactions involved in energy metabolism, fatty acid synthesis, and amino acid catabolism (Tong, 2013). The central role of biotin in intermediary metabolism is likely responsible for the observation that biotin is required by all tissues of the body; however, biotin appears to play a particularly important role in brain chemistry. In contrast to liver, kidney, and brain, biotin is present in relatively small amounts in many tissues (a fact on which many (strept-)avidin-biotin histochemical techniques rely); however, studies in the rat have demonstrated that significant levels of covalently bound biotin are found in the brain, particularly in the cerebellar motor system and the brainstem auditory system (McKay et al., 2004). Biotin is actively transported across the human blood-brain barrier (BBB) predominantly via the $Na^+$-dependent multivitamin transporter (SLC5A6/SMVT; (Uchida et al., 2015)). This transporter has an estimated half-saturation concentration ($K_m$) of roughly 100 μM in rats (Spector and Mock, 1987), 35 μM in

mice (Park and Sinko, 2005) and 20 µM in rabbits (Spector and Mock, 1988a). By extrapolation of data obtained with smaller doses (Mock and Mock, 1997; Zempleni et al., 2001), assuming dose proportionality, high doses of biotin in the 300 mg/day range would result in plasma concentrations of biotin of about 5 µM, which theoretically would not be saturating the biotin transporter (Spector and Johanson, 2007). Consistent with these data, biotin concentration was shown to increase from about 0.1 to 6 nmol/g of brain tissue in juvenile rats given increasing oral doses of biotin corresponding to about 0.3 µg to 3 mg/kg body weight in humans, the highest dose being closest to the human therapeutic dose given in progressive MS (Sawamura et al., 2007). Biotin concentration also increased in other tissues evaluated, namely skeletal muscle, heart, kidney, lung, spleen and testis. Within animal brain, limited data indicate that biotin is localized to the oligodendrocytes (LeVine and Macklin, 1988; Wang and Pevsner, 1999) and in some neurons (McKay et al., 2004). Interestingly, levels of covalently bound biotin and the expression of HCLS, biotin-dependent carboxylases, and genes involved in biotin transport and biotin recycling are maintained in the brain during periods of biotin deficiency at the expense of other tissues such as liver (Chiang and Mistry, 1974; Pacheco-Alvarez et al., 2004; Rodriguez-Melendez et al., 2001; Sander et al., 1982).

Further evidence for the vital role of biotin in neuron function comes from the observation that neurological dysfunction is prominent in patients with biotin deficiency and inborn errors of biotin metabolism (Wolf, 2011; Wolf and Feldman, 1982). Biotin has a vital role in the treatment of the orphan neurological disease biotin-thiamine responsive basal ganglia disease (BTBGD). This autosomal recessive disorder is caused by inherited mutations in the gene encoding a transmembrane thiamine transporter that was originally termed solute carrier family 19 member 3 [SLC19A3] but now more commonly known as thiamine transporter 2 (THTR2) (Said et al., 2004; Zeng et al., 2005). The symptoms of BTBGD are severe and are characterized by recurrent subacute onset of encephalopathy that manifest as confusion, seizures, dysarthria, ataxia, dystonia, supranuclear facial palsy and even coma

(Ozand et al., 1998; Tabarki et al., 2013). Despite the fact that THTR2 is not a biotin transporter in studies in hepatic, intestinal, or neuronal cell lines (Subramanian et al., 2006), the condition is effectively and rapidly treated with high-dose biotin (5–10 mg/kg/day) (Bindu et al., 2009; Ozand et al., 1998).

## *5. Hypothesized modes of action of high-dose biotin in the treatment of progressive MS*

We hypothesize that high-dose biotin exerts its therapeutic effect in progressive MS through two primary mechanisms: 1) promotion of remyelination through enhanced myelin formation in oligodendrocytes, and 2) enhancement of brain energy production, thereby protecting demyelinated axons from degradation.

### *5.1 High-dose biotin as a promoter of myelination through enhanced myelin formation in oligodendrocytes*

Through its role as an essential cofactor for ACC1 and ACC2, high-dose biotin may be promoting remyelination by increasing the availability of the fundamental building blocks required for myelin membrane synthesis. ACC1 and ACC2 are encoded by two distinct genes (ACACA and ACACB) and have distinct subcellular locations and distinct metabolic roles. ACC1 catalyzes the rate limiting, committed step in fatty acid biosynthesis: the cytosolic synthesis of malonyl-CoA from acetyl-CoA (**Figure 1**) (Foster, 2012; Tong, 2013). A role for ACC in promoting myelination is supported by several observations. Within the CNS, ACC appears to be primarily expressed in oligodendrocytes, the cells specialized in myelin synthesis (Chakraborty and Ledeen, 2003; Tansey and Cammer, 1988); not surprisingly, a significant proportion of cytosolic ACC is detectable in purified myelin (Chakraborty and Ledeen, 2003) and the activity of ACC in oligodendrocytes in rats is highest during the neonatal myelinating period (DeWille and Horrocks, 1992; Tansey et al., 1988). The relative contribution of ACC1 and ACC2 to ACC activity in the brain is not known. However, in human adult brain (RNA pooled from multiple donors), ACC1 RNA was found to

be approximately twice as abundant as ACC2 RNA (Castle et al., 2009). Furthermore, levels of murine ACC are significantly reduced in the sciatic nerve of the trembler mouse model of peripheral nervous system dysmyelination compared with normal mice (Salles et al., 2003). Finally, malonyl-CoA, the product of the reaction catalyzed by ACC, is used as the two-carbon building block for fatty acid synthesis by the brain microsomal fatty acid elongating system for deposition in myelin (DeWille and Horrocks, 1992). Malonyl-CoA also coordinates the balance between fatty acid synthesis and fatty acid oxidation. As cytosolic levels of malonyl-CoA increase, the rate of fatty acid synthesis increases; as peri-mitochondrial levels of malonyl-CoA from the reaction catalyzed by ACC2 increase, carnitine palmitoyltransferase 1 (CPT1) is inhibited and the rate of fatty acid transport into mitochondria decreases leading to reduced fatty acid beta oxidation (Foster, 2012; McGarry et al., 1978). Therefore, high-dose biotin may be acting as a promyelinogenic agent through its role as a cofactor for ACC1 and ACC2.

The normalization of visual evoked potential latency observed in one MS patient with chronic visual loss after 9 months' treatment with MD1003 is consistent with myelin repair (Sedel et al., 2015). A progressive decrease in the choline/creatine ratio was observed with magnetic resonance spectroscopy in a second patient with prominent optic nerve involvement; the ratio normalized completely after 9 months of treatment with MD1003. Elevations of choline, the core component of the hydrophilic head of membrane phospholipids, are commonly observed in MS plaques and are thought to result from the breakdown of the phospholipid membrane that occurs during inflammation, gliosis, and demyelination (Arnold et al., 1992; Narayana, 2005). We speculate that normalization of the choline/creatine ratio overtime after treatment with MD1003 reflects progressive myelin repair accompanied by a decrease in free choline release from membranes. In addition, the delay in onset of clinical benefit (2–8 months) seen in MS patients treated with MD1003 is consistent with remodeling of the myelin sheath. Remodeling of the myelin sheath is believed to occur at a relatively slow rate.

MRI studies in patients with acute gadolinium (Gd)-enhancing lesions suggest that the process of remyelination takes an average of 7 months (Chen et al., 2008).

Remyelination of denuded axons is a logical and attractive strategy for the treatment of progressive MS (Franklin and Ffrench-Constant, 2008; Zawadzka and Franklin, 2007). Extensive regenerative remyelination occurs in some patients with early MS but is typically reduced as the disease progresses (Goldschmidt et al., 2009; Miron et al., 2011; Prineas et al., 1993). Remyelination has been documented in both active and inactive MS lesions (Miron et al., 2011), and remyelination strategies stimulate functional recovery in animal models of MS (Bai et al., 2012; Deshmukh et al., 2013; Duncan et al., 2009). The process underlying remyelination is complex and is thought to involve the maturation and migration of OPCs and numerous promoting and inhibitory factors (Munzel and Williams, 2013). However, by increasing the supply of the two carbon units required for fatty acid elongation (the fundamental building block of myelin), high-dose biotin acting through ACC is an attractive candidate for a novel promyelinogenic agent.

*5.2 High-dose biotin in the reversal of virtual hypoxia*

As well as promoting remyelination through its role as a cofactor for ACC, high-dose biotin may also be targeting cellular energy levels. Several lines of evidence suggest that cellular energy deprivation secondary to demyelination is responsible for the progressive irreversible neuronal degeneration observed in progressive MS (Luessi et al., 2012; Stys et al., 2012). In the normal, myelinated neuron, the nerve impulse is conducted along the length of the axon in discreet jumps spreading along the chain of nodes of Ranvier, which contain high densities of voltage-gated $Na^+$ and $K^+$ channels (**Figure 2**) (Rasband and Trimmer, 2001). This process, termed saltatory conduction, not only increases the speed at which the nerve impulse is propagated compared with nerve impulses in unmyelinated axons, but also reduces neuronal energy expenditure because ATP is only needed to restore the resting membrane potential at the nodes of Ranvier. Restoration of the resting membrane potential

is mainly achieved by the ATP-dependent $Na^+/K^+$ pump ($Na^+/K^+$ ATPase) (Krishnan et al., 2009).

Loss of the insulating myelin sheath causes the loss of saltatory conduction and thereby increases the energy required for nerve propagation. In denuded axons, voltage-gated $Na^+$ channels are redistributed along the length of the axon (**Figure 2**). This compensatory mechanism reflects a switch from saltatory to continuous conduction (Craner et al., 2004; Levin et al., 2014). This switch is accompanied by an increase in the number of mitochondria in the demyelinated axons as a result of the neuron's attempt to supply the increased energy demand required to maintain the intracellular/extracellular ion gradients (Witte et al., 2009). However, evidence suggests that, in MS patients, axonal ATP production is simultaneously compromised due to defects in their neuronal mitochondria (Dutta et al., 2006; Mahad et al., 2009; Su et al., 2009). The resulting imbalance between energy supply and energy demand causes a state of 'virtual hypoxia' which may be the trigger for neuron degeneration (Luessi et al., 2012; Trapp and Stys, 2009). If insufficient ATP is available for $Na^+/K^+$ ATPase to restore the membrane potential, the neuron will enter a state of depolarization characterized by a further influx of $Na^+$. This state can activate the $Na^+/Ca^{2+}$ exchanger in reverse mode, leading to an increase in intra-axonal $Ca^{2+}$ as $Na^+$ ions are removed from the neuron (Trapp and Stys, 2009). Excessive levels of $Ca^{2+}$ in the neuron can activate a number of $Ca^{2+}$-mediated degenerative pathways (Trapp and Stys, 2009). In chronic MS lesions, axonal levels of $Na^+/K^+$ ATPase are decreased, which may exacerbate the state of membrane depolarization (Young et al., 2008).

We hypothesize that treatment with high-dose biotin reverses this state of virtual hypoxia through its role as a cofactor for PC, MCC, and PCC. These three enzymes are central to aerobic energy production and generate intermediates for the tricarboxylic acid (TCA) cycle (**Figure 3**) (Tong, 2013). All three of these enzymes are known to be expressed in astrocytes and neurons (Ballhausen et al., 2009; Hassel, 2000). PC catalyzes the conversion of pyruvate to oxaloacetate, thus serving an essential anaplerotic role by

replenishing the 4-carbon "backbone" of the TCA cycle. PCC generates methylmalonyl-CoA from propionyl-CoA, which is then converted to succinyl-CoA by methylmalonyl-CoA mutase. MCC plays a role in the metabolism of leucine and ultimately leads to production of acetyl-CoA. Thus, these three biotin-dependent carboxylases feed the TCA cycle at three different entry points (oxaloacetate, succinate, and acetyl-CoA) and could be expected to increase the levels of cellular ATP at the rate of one molecule of acetyl-CoA used by the TCA cycle producing one molecule of ATP. Indeed, nutritional biotin deficiency and biotin deficiency induced by knocking out the recycling enzyme biotinidase cause severe ATP depletion (Hernandez-Vazquez et al., 2012; Hernandez-Vazquez et al., 2013; Velazquez-Arellano et al., 2011). By increasing the available intraneuronal pool of ATP, high-dose biotin may reduce demyelinated neural dysfunction and the adverse effects of hypoxia (Lazzarino et al., 2010; Trapp and Stys, 2009).

In progressive MS, the proportion of mutant mitochondria increases through clonal expansion of mitochondrial DNA deletions (Campbell et al., 2011). As a consequence, some neurons still possess functional mitochondria, while others have damaged mitochondria or a mixture of functional and damaged mitochondria (heteroplasmy; (Mahad et al., 2015)). In this latter case, the remaining pool of mitochondria with functional respiratory chain enzymes may not be sufficient to compensate for the increase in energy demand occurring in chronically demyelinated axons. It could be anticipated that only axons with some remaining healthy mitochondria would benefit from the treatment with high-dose biotin.

A similar hypothesis has been the basis for testing idebenone in progressive MS (Spindler et al., 2009) (NCT00950248 and NCT01854359). Increasing cellular energy production is also thought to be the mechanism by which idebenone (Catena®, Santhera Pharmaceuticals), a synthetic analog of coenzyme Q10 exerts its therapeutic activity. This compound is a substrate for reduction by two NAD(P)H:quinone oxidoreductases (NQO1 and NQO2) (Haefeli et al., 2011). The reduced form of idebenone is believed to pass electrons into the mitochondrial respiratory chain triggering an increase in cellular ATP levels. Of note,

experiments in the chronic experimental autoimmune encephalomyelitis EAE mouse model failed to demonstrate any effect of idebenone in preventing or attenuating disability (Fiebiger et al., 2013).

Several other mechanisms by which biotin may be exerting a therapeutic effect exist. Biotin is a key transcriptional regulator of several genes involved in glucose and lipid metabolism. *In vitro* and *in vivo* studies have shown that elevated biotin levels can upregulate the expression of hepatic and pancreatic glucokinase, insulin, and the insulin receptor, and downregulate hepatic phosphoenolpyruvate carboxykinase (Chauhan and Dakshinamurti, 1991; Dokusova and Krivoruchenko, 1972; Leon-Del-Rio, 2005; Marshall et al., 1980; Pacheco-Alvarez et al., 2002; Romero-Navarro et al., 1999). Interestingly, biotin also regulates the expression of two of the carboxylases for which it acts as a cofactor (PC and ACC1), and of HCLS (Pacheco-Alvarez et al., 2004). The mechanism by which biotin regulates gene expression remains elusive. Biotin was initially thought not to be a natural modifier of histones (Healy et al., 2009), However, biotinylation sites are known to be present on histones H2A, H3, and H4 (Zempleni et al., 2012b) and several studies have demonstrated that biotinylation of histones by HCLS induces chromatin remodeling that can regulate gene expression (Filenko et al., 2011; Gralla et al., 2008; Kuroishi et al., 2011; Wijeratne et al., 2010). However, more recent data suggests that HCLS itself may interact directly with chromatin as part of a multi-protein gene regulating complex (Liu and Zempleni, 2014; Zempleni et al., 2012a). Currently, an effect of biotin on MS through control of gene expression by modification of chromatin structure remains speculative.

## *6. Feasibility of high-dose biotin as a therapy for progressive MS*

Several characteristics of high-dose biotin make it an attractive therapeutic option in progressive MS. Biotin demonstrates high (~100%) bioavailability (Zempleni and Mock, 1999) and is able to cross the blood brain barrier (Spector and Mock, 1988b). The ability to dose orally will greatly facilitate treatment for individuals with MS and consequently a high

level of treatment compliance can be expected. While the body of preclinical toxicology data for high-dose biotin is limited, the available data indicates that only marginal adverse effects are observed in animals treated with biotin doses in the hundreds of milligrams per kilogram of body weight range (Hathcock, 2015). Adverse events in patients with progressive MS treated with MD1003 (100–300 mg/day) were infrequent; occurring in three of 23 (13%) patients treated in the compassionate use open-label trial (Sedel et al., 2015). Two patients experienced transient diarrhea and one patient died from cardiac failure 36 months after treatment onset. This patient exhibited mild aortic valvulopathy with dilatation of the ascending aorta together with a first-degree atrio-ventricular block which were found 18 months after treatment onset; no relation could be established between treatment onset, mild cardiac abnormalities and death. Whether these abnormalities existed before treatment is unknown.

A significant teratogenic effect on rabbit fetuses during the period of organogenesis was observed in pregnant rabbits treated with high-dose biotin (30 mg/kg/day; approximately twice the equivalent human dose currently under investigation based on the surface area conversion table; unpublished data, MedDay Pharmaceuticals). These effects included a higher incidence of malrotated paw (11.8%), domed head (4.1%), hydrocephaly (4.1%) and liquid content in the cranial cavity (4.7%) compared with control rabbits and those treated with 15 mg/kg/day biotin. Consequently, high-dose biotin may not be suitable for administration to women with MS who are pregnant or considering pregnancy. Fingolimod, teriflunomide and interferon beta-1a are also contraindicated in pregnant women (although pregnancy may start while the patient is receiving interferon beta-1a) (Biogen Idec Ltd., 2014; Novartis Europharm Ltd., 2011; Sanofi-Aventis Group, 2013). Control of pregnancy is recommended prior to each infusion of natalizumab and discontinuation of treatment should be considered if a women becomes pregnant while taking this drug (Biogen Idec Ltd., 2011).

Overall, high-dose biotin appears to be a practical and well-tolerated therapeutic option in patients with progressive MS, particularly when compared with currently approved

treatments for MS that have some significant safety and tolerability concerns. Patients receiving fingolimod require close monitoring due to the risk of serious cardiovascular complications, infections, and macular edema (Wingerchuk and Carter, 2014). Monoclonal antibodies such as natalizumab and alemtuzumab require parenteral administration which can be inconvenient for patients, and these agents have potentially serious side effects, such as the risk of developing progressive multifocal leukoencephalopathy with natalizumab (Bloomgren et al., 2012) and idiopathic thrombocytopenic purpura and antibody-mediated immune diseases for alemtuzumab (Cuker et al., 2011). While nonmyeloablative hematopoietic stem cell transplantation appears to be a promising treatment option for patients with RRMS (Burt et al., 2015), this procedure will not be suitable for all patients and is associated with significant risks.

## *7. Overview of ongoing studies with MD1003 and future directions*

In summary, we hypothesize that high-dose biotin treatment is targeting two key pathophysiological mechanisms in progressive MS: (1) by triggering myelin synthesis by oligodendrocytes and (2) by replenishing ATP in hypoxic neurons (**Figure 4**). Targeting both demyelination and neurodegeneration is likely to be important in the treatment of progressive MS. Although several other therapeutics that target one of these processes are currently under investigation in patients with MS, high-dose biotin might be simultaneously preventing the hypoxia-driven neurodegeneration of neurons and promoting the remyelination of axons.

The efficacy and safety of MD1003 is currently under investigation in three multicenter phase III placebo-controlled trials in patients with progressive MS involving either the spinal cord or chronic visual loss after optic neuritis, and in patients with AMN (**Table 1**). The study in patients with spinal progressive MS represents a particularly ambitious evaluation of high-dose biotin as a therapy for MS, because the patients recruited to this trial have relatively severe disease: PPMS or SPMS fulfilling revised McDonald criteria (Polman et al., 2011) and Lublin criteria (Lublin and Reingold, 1996) with clinical evidence of spastic paraparesis

and significant disability (EDSS scores 4.5–7). Furthermore, this is the first trial of an investigational MS therapy that has chosen a reduction in MS-related disability as the primary efficacy endpoint of the trial, as measured by one of two validated clinical assessment scales (the EDSS and the TW25). Results from all three trials are expected in 2015–2016. Several *in vivo* studies that will further investigate the pharmacodynamic effects of high-dose biotin are also currently underway.

The potential mechanism of action of high-dose biotin in progressive MS is still partly speculative and there is a striking lack of pre-clinical data on the exact mechanism of action. Some important gaps in knowledge remain to be elucidated experimentally:

(1) Are biotin or malonyl-CoA limiting factors for remyelination in chronic MS lesions? This might be addressed using *in vitro* models such as OPCs.

(2) Does biotin have an impact on myelination or remyelination *in vivo*? This could be assessed in relevant animal models with and without inflammation such as experimental auto-immune encephalomyelitis, cuprizone or toxin-induced demyelination.

(3) Does high-dose biotin increase ATP production by mitochondria in chronic MS lesions? This might be assessed in relevant *in vitro* and *in vivo* models.

## Author contributions

All authors contributed to the writing of this paper.

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