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Multiple Sclerosis and Related Disorders (J Graves, Section Editor)

Lipoic Acid and Other Antioxidants as Therapies for Multiple Sclerosis

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Abstract

Oxidative stress (OS), when oxidative forces outweigh endogenous and nutritional antioxidant defenses, contributes to the pathophysiology of multiple sclerosis (MS). Evidence of OS is found during acute relapses, in active inflammatory lesions, and in chronic, longstanding plaques. OS results in both ongoing inflammation and neurodegeneration. Antioxidant therapies are a rational strategy for people with MS with all phenotypes and disease durations.
Purpose of review To understand the function of OS in health and disease, to examine the contributions of OS to MS pathophysiology, and to review current evidence for the effects of selected antioxidant therapies in people with MS (PwMS) with a focus on lipoic acid (LA).
Recent findings Studies of antioxidant interventions in both animal and in vivo models result in reductions in serum markers of OS and increases in levels and activity of antioxidant enzymes. Antioxidant trials in PwMS, while generally underpowered, detect short-term improvements in markers of OS and antioxidant defenses, and to a lesser extent, in clinical symptoms (fatigue, depression). The best evidence to date is a 2-year trial of LA in secondary progressive MS which demonstrated a significant reduction of whole-brain atrophy and trend toward improvement in walking speed.
Summary Antioxidant therapy is a promising approach to treat MS across the spectrum and duration of disease. Rigorous and well-powered trials are needed to determine their therapeutic benefits.

Introduction

Multiple sclerosis (MS) is a presumed autoimmune disorder of the central nervous system (CNS) characterized pathologically by immune-mediated demyelination along with progressive atrophy caused by axon and glial loss [1]. Clinical symptoms relate to overt inflammation of eloquent regions of the brain, optic nerves, and spinal cord resulting in vision loss, weakness, sensory disturbance, diplopia, other bulbar dysfunction, incoordination, gait disturbance, and bladder dysfunction. Additional non-localizing symptoms are thought related to a more global neurodegenerative process with axonal loss and manifests as impaired memory, slowed cognitive processing, fatigue, and mood changes.

Often MS pathology and phenotype are paired, with inflammatory demyelination matched to early and relapsing forms of MS (RMS), and neurodegeneration with later and progressive forms of MS (PMS). However, it is now clear that neurodegeneration and gray matter loss occur early in disease, and in all phenotypes, RMS is characterized clinically by episodic bouts of disability and recovery corresponding to initially contrast-enhancing areas of focal damage seen on MRI [2]. Relapses can also occur radiographically without overt clinical correlate. In contrast, PMS is characterized clinically by gradual worsening of disability not attributable to either clinical or radiographic relapses, although both can happen alongside progression. PMS is associated with brain volume loss on serial MRIs which is thought to reflect neurodegenerative processes including genetic derangements, mitochondrial dysfunction, energy failure, and eventually cell apoptosis [1]. The simplistic dichotomy of inflammation/RMS versus neurodegeneration/PMS ignores evidence for neurodegenerative processes in RMS and immune-mediated damage in PMS and thus may help explain the incomplete benefit of current disease-modifying therapies (DMT) [3]. Targeting common pathophysiological mechanisms across the MS phenotypic spectrum and duration of disease is a rational approach for developing effective therapies.

Oxidative stress (OS) is one such common mechanism of injury in MS [4]. Oxidation occurs when free radicals, unstable chemical species containing one or more unpaired electrons, steal electrons from other molecules. If unchecked, oxidation can lead to downstream effects including excitotoxicity, alterations in transcriptional factors and gene regulation, cellular dysfunction, and tissue death. While free radicals serve as critical cellular signaling molecules, they cause damage when in excess. Normal cellular and nutritional antioxidant defenses prevent injury by accepting (reducing) free radicals. OS occurs when oxidative forces outweigh the resident antioxidant mechanisms available [5]. In this article, we review the role of antioxidants in health and disease, the role of OS in MS pathophysiology, the mechanisms by which antioxidant therapies could benefit MS, and current evidence for the effects of selected antioxidant therapies with a focus on lipoic acid (LA).

Background

Oxidative stress

Free radicals, byproducts of chemical and enzymatic reactions, consist of reactive oxygen species (ROS) and reactive nitrogen species (RNS), although they are generally grouped under the term ROS [6]. Examples of ROS are superoxide (O_2–), peroxide (H_2O_2) peroxynitrite (ONOO–), and hydroperoxyl radical (HO_2–). At physiological levels, ROS are essential parts of normal cell-signaling mechanisms. Higher local concentrations produced by immune cells kill pathogens. Mitochondria, which produce 90% of the energy supply necessary for living cells, also generate 95% of human ROS, thus becoming both the source and target of free radicals [7]. Approximately 1–3% of oxygen is converted to ROS by mitochondrial citric acid cycle enzymes, pyruvate dehydrogenase, dihydroorotate dehydrogenase, glycerol-3-phosphate dehydrogenase, and methemoglobin reductase as well as complexes I and III of the electron transport chain driving the oxidative phosphorylation required for efficient

adenosine triphosphate (ATP) production [8, 9]. Other endogenous pathways that result in free radical formation involve cyclooxygenases and lipoxygenases. Reactive metals within the cell, particularly iron and copper, are also closely tied to free radicals generation including the Fenton reaction [10]. Exogenous environmental influences like UV radiation, microbes, cigarette smoking, and ozone all amplify ROS generation in the body [11].

The downstream effects of OS are particularly deleterious in the brain and are considered a driving factor in aging [12]. The brain, consuming a fifth of all glucose and oxygen supplied to the body, has the highest mitochondrial content and therefore greatest ROS production. Moreover, the brain is particularly susceptible to oxidative damage due to its high fatty acid content prone to peroxidation, lower catalase activity than other organs (10% of liver activity), and higher levels of iron and ascorbate (involved in the Haber-Weiss reaction) thus creating an even more pro-oxidant environment [13, 14]. The ROS damage to cell membranes, DNA, RNA, metabolic apparatus, antioxidant enzymes, and DNA and protein repair enzymes all lead to genetic mutations, mitochondrial dysfunction, necrosis, and apoptosis. Dysfunctional mitochondria have less buffering capacity which can tip the calcium balance toward neuronal death.

Antioxidant defenses against oxidative stress

Cellular defense mechanisms against ROS damage include non-enzymatic and enzymatic antioxidants. Non-enzymatic direct antioxidants are typically small molecule scavengers that bind ROS such as endogenous uric acid, glutathione (GSH), LA, NADPH, coenzyme Q, albumin, and bilirubin, as well as exogenous (natural or synthetic) vitamin C (ascorbate), vitamin E (alpha tocopherol), carotenoids, polyphenols, flavonoids (e.g., quercetin), selenium, and others derived principally from dietary grains, fruits, and vegetables [11]. Non-enzymatic indirect antioxidants include chelating agents that bind to redox metals to prevent free radical generation. Enzymatic antioxidants chemically convert ROS into less reactive species. Examples include catalase, SOD, heme oxygenase, peroxiredoxin, glutathione peroxidase, glutathione reductase, and glutathione S-transferase. The expression of antioxidant enzymes is under the control of transcription factor nuclear factor-E2-related factor (Nrf2). NrF2 regulates the expression of over 250 antioxidant proteins in a feedback loop responsive to OS as well as regulating the transcription of proinflammatory cytokines [15, 16••]. Nrf2 knockout mice fare worse in animal models of inflammatory diseases [17]. While dimethyl fumarate, an FDA-approved for treatment of RMS, activates the Nrf2 pathway, yet unknown are the relative contributions to clinical efficacy of its antioxidant versus anti-inflammatory effects [18]. There are numerous exogenous compounds, some of which are also direct antioxidants (e.g., vitamin E, quercetin), that are able to stabilize or activate the Nrf2 pathway. Mitochondria themselves have resident antioxidant enzymes (iron/manganese superoxide dismutase, thioredoxin, glutathione glutaredoxin) that buffer the free radical production from normal ATP production [19•].

Oxidative stress in MS

Evidence for OS in MS is found in the animal model and experimental autoimmune encephalomyelitis (EAE) as well as pathologic specimens from people with MS (PwMS). Lesions in EAE mice demonstrate nitrotyrosine, a marker of

peroxynitrite-induced damage and inducible isoform nitric oxide (NO) synthase [20]. Subsequently, nitrotyrosine was found by immunohistochemistry within autopsied CNS tissues of MS patients, particularly the sections displaying inflammation. CSF levels of NO and peroxynitrite were found elevated in PwMS during acute relapses compared to controls [21]. CNS tissue samples from PwMS demonstrate various biomarkers of OS in both acute and chronic lesions. MS plaque specimens exhibit increased free radical activity and decreased levels of glutathione, vitamin E (alpha tocopherol), and uric acid [22]. Increased mitochondrial density found in MS lesions is a presumed response to maintain the Na+/K+ gradient in demyelinated and disrupted neurons. This density is associated with increased mitochondrial heat shock protein 70, a marker of mitochondrial stress [23]. Four-hydroxy-2-neonal (4-HNE), a highly reactive aldehyde toxic to CNS cells and marker of OS, accumulates within phagocytic macrophages and large hypertrophic astrocytes in MS lesions [24]. Immunohistochemistry demonstrates oxidized lipid and nucleotide species in highly active plaques while chronic MS lesions demonstrate NO and NADPH markers of OS [25, 26].

Activated inflammatory cells in MS are a large source of ROS that both damage CNS tissues and self-perpetuate the cells' activation. ROS produced by activated leukocytes facilitate their transmigration into the brain by altering endothelial tight junctions via stimulation of the second messenger molecule vascular endothelial marker-1 (VCAM-1) and endothelial NAPDH oxidase [27]. ROS produced by infiltrative leukocytes induce subsequent myelin phagocytosis by macrophages, oligodendroglial damage, and neuron and axon injury [28]. Activated microglia and macrophages, seen in both active inflammatory RMS lesions and slowly expanding PMS lesions, produce high levels of NO and H_2O_2 as found in homogenates of white matter, deep gray matter, and cortex, and feed additional cycles of lipid peroxidation [29]. The oxidative burst from activated microglia and macrophages also produces reactive aldehydes which, with their longer half-life of 1–2 min, can diffuse and propagate damage to new areas; this capability for delayed and distant damage has important implications for MS pathogenesis [30]. Extracellular glutamate, released by activated immune cells, inhibits the cellular availability of cysteine, depletes glutathione, and results in a form of injury known as oxidative glutamate toxicity [31]. Given this excitotoxic and oxidative milieu, endogenous antioxidant capabilities may provide substantial clinical benefits in MS. In one study, glutathione S-transferase supergene family polymorphisms that confer lesser antioxidant protection are associated with increased disability and more gadolinium enhancing lesions than normal variants in PwMS [32]. Exogenous antioxidant therapy may do the same.

Rationale for antioxidant therapy in MS

The sources of ROS and their inflammatory and neurodegenerative effects in MS are summarized in Fig. 1. Antioxidant strategies to combat OS is a rational and feasible approach to treat all phenotypes and durations of MS to boost defenses against OS and reduce production of ROS. Defensive strategies include supplementing endogenous (e.g., LA) and exogenous (e.g., vitamins C and E) direct and indirect antioxidants, and stimulating antioxidant enzyme production (via Nrf2 pathway). Healthy lifestyle behaviors also exert antioxidant

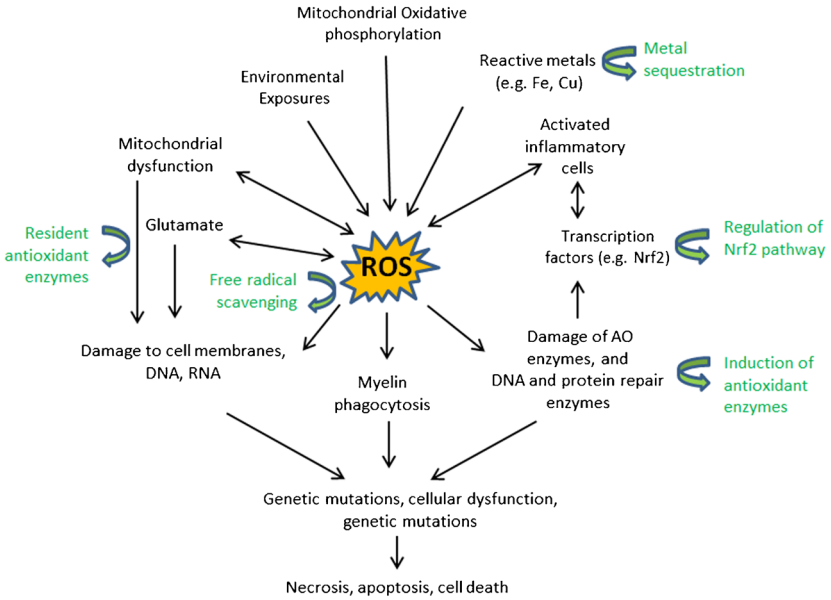

Fig. 1. Schematic of the generation and effects of reactive oxygen species (ROS) and reactive nitrogen species (ROS) in multiple sclerosis, along with targets of antioxidant therapies (in green).

properties such as exercise and a plant-based diet [33]. Reducing ROS production includes treatment with DMT to inhibit inflammation, and avoidance of environmental pollutants (e.g., cigarette smoking). While antioxidant diets, supplements, and healthy lifestyles have long been advocated, the rigorous study is more recent [34, 35]. The remainder of this review explores the evidence for supplemental antioxidants as therapy for MS with a special focus on LA.

Evidence for lipoic acid as antioxidant therapy for MS

Lipoic acid background

LA, an endogenous antioxidant with multiple biological functions, has the most robust evidence for efficacy in EAE and MS. LA, also termed α-lipoic acid, pyruvate oxidation factor, thioctic acid and lipoate, is a fatty acid containing 8 carbons and 2 sulfur molecules in a dithiolane ring [36]. Initially discovered as a bacterial growth factor in potato extract, LA was later isolated and characterized from bovine liver [37, 38]. LA's antioxidant properties were identified in 1959 when Rosenberg and Culik found that the administration of LA to vitamin C-deficient guinea pigs relieved scurvy symptoms [39]. LA has been used for decades in Germany to treat diabetic neuropathy, cirrhosis, and mushroom and heavy metal poisonings [40]. More recently, LA is under investigation as treatment for various neurodegenerative diseases, stroke, metabolic syndrome, diabetes, renal failure, and others [41–43].

An organosulfur compound synthesized endogenously in small amounts from octanoic acid, LA is also absorbed from selected vegetables (spinach, broccoli, and tomato) and organ meat. Endogenous LA occurs in the R chiral conformation (R-LA) at the C6 carbon atom. Manufacturing LA produces racemic LA, an even mix of the R-LA and S-LA chiral conformations. The bioavailability and bioactivity of racemic vs R-LA is debated [43, 44]. Unless

Table 1. Key in vitro and short-term lipoic acid human investigations relevant to multiple sclerosis

Study	Sample	Questions and interventions	Results (only significant differences reported)
[58•]	RRMS & HC PBMCs *N* = 19	Question being addressed: Does LA decrease monocyte and B cell migration? In vitro study: Monocyte and B cell–enriched PBMCs from RRMS and HC with and without exposure to LA 100 μg/ml LA; migrated monocytes and B cells counted	1. Monocyte migration was significantly greater among RRMS than HC at baseline. 2. LA treatment significantly reduced monocyte and B cell migration in RRMS and HC exposure groups.
[59]	HC PBMCs	Question being addressed: Does lipoic acid stimulation cAMP in T cells? In vitro study: T cell–enriched human PBMCs exposed to LA 25–75 μg/ml; cAMP measured	1. LA increased cAMP production in T cells. 2. cAMP increase is dependent on adenylyl cyclase activity. 3. LA also increased cAMP production in NK cells.
[60]	PwMS PBMCs *N* = 29	Questions being addressed: 1. Does LA alter T cell activation and production of inflammatory cytokines in vitro, and 2. Does orally administered LA increase cAMP in T cells in PwMS? 1. Oral LA 1200 mg given to PwMS (3 formulations); T cell–enriched PBMCs isolated, and pre-treated with LA 0, 50, or 100 mg/ml for 5 min to determine IL-6, IL-10, and IL-17 levels. 2. PBMCs also incubated with LA 10 or 50 μg/ml, stimulated with anti-CD3 and anti-CD28, and measured T cell proliferation.	1. PBMCs pre-treated with 50 and 100 mg/ml of LA had significantly reduced IL-6 and IL-17 levels. 2. LA inhibits T cell proliferation and activation. 3. Oral LA 1200 mg increased cAMP levels in T cell–enriched PBMCs.
[61]	RRMS, SPMS, HC Plasma, PBMCs *N* = 57	Questions being addressed: 1. Does MS subtype alter bioavailability of oral LA, and 2. Does LA increase cAMP in PBMCs from RRMS, SPMS, and HC? PK and in vitro study: RRMS, SPMS, and HC given 1200-mg LA. Peak LA levels measured hourly for 4 h, 24 h, and 48 h. Levels of cAMP from PBMCs at each timepoint measured.	1. MS disease state does not alter LA bioavailability or PK parameters. 2. Compared to baseline, LA treatment increased cAMP in SPMS and HC subjects at 2 and 4 h, but decreased cAMP in RRMS subjects.
[62]	PwMS *N* = 30	Questions being addressed: 1. What is the LA dose and frequency best absorbed and tolerated in PwMS, and 2. What re the effects of LA on serum markers of inflammation? Comparison of LA serum levels 600-mg BID, 1200-mg QD, 1200-mg BID, and placebo BID for 2 weeks.	1. LA 1200 mg/day was best tolerated and detectable in serum 2. Significant negative correlations between peak LA and mean change of MMP-9 and ICAM-1.

Table 1. (Continued)

Study	Sample	Questions and interventions	Results (only significant differences reported)
[51]	EAE Mice, PwMS, *N* = 24	Question being addressed: 1. What are the peak LA plasma concentrations at the most effective dose for EAE, and 2. What formulation of 1200-mg LA provides analogous serum levels and AUC in PwMS? Dose escalation of LA administered parenterally to mice for maximal therapeutic benefit. Associated peak plasma LA concentrations measured. Three formulations of LA 1200 mg administered to PwMS. Peak plasma concentrations and AUC of LA measured.	1. LA 50 mg/kg/day highly effective at suppressing EAE. 2. LA 1200 mg in PwMS from 2 of the 3 LA formulations resulted in peak serum concentrations and AUC comparable to that of mice receiving 50 mg/kg of LA.

AUC, area under the curve; *cAMP*, cyclic adenosine monophosphate; *HC*, Health controls; *I MMP-9*, matrix metalloproteinase-9; *NK*, natural killer cells; *PBMC*, peripheral blood mononuclear cells; *PK*, pharmacokinetic; *RRMS*, relapsing–remitting multiple sclerosis; *SPMS*, secondary progressive multiple sclerosis

otherwise specified, LA refers to the racemic form throughout this review including figures and tables. The functional activity of LA derives from the disulfide bond between the 2 thiol groups that cycles between the oxidized (LA) and reduced conformations (dihydrolipoic acid, DHLA).

Oral LA is quickly absorbed by the gastrointestinal system via an energy-dependent process mediated by a monocarboxylic acid transporter [45]. Absorption in is additionally likely both from passive transfer due to the small size of the molecule (206.32 g mol^{-1}) and active transport by the Na+-dependent multivitamin transporter [2, 46]. LA is either distributed throughout the body, or filtered through the kidneys and excreted [43, 47]. Whether or not LA crosses the blood brain barrier is debated with conflicting findings in studies of rats given oral LA [48, 49]. Within cells, LA brakes down into metabolites or reduced to DHLA. Animal models demonstrate that within 1 day of taking oral LA, up to 98% of LA, LA metabolites, and DHLA are rapidly excreted in the urine [43, 50]. Human PK studies demonstrate variable peak absorption times of 60–90 min followed by undetectable serum levels by approximately 4 h [51, 52•].

Antioxidant properties of LA

Although LA exhibits other potentially therapeutic effects in MS, the antioxidant mechanisms are primarily discussed here [53]. LA and more so DHLA are highly reactive to free radicals, preventing oxidative damage by directly scavenging and neutralizing ROS [36, 54]. LA and DHLA bind to Cu and Fe preventing their production of free radicals. The LA/DHLA redox couple serves to regenerate other antioxidants including glutathione, vitamins C and E, and coenzyme Q10 [36, 40, 54]. In mitochondria, the LA/DHLA redox couple serves as a key cofactor of the pyruvate dehydrogenase complex and α-ketoglutarate dehydrogenase of

the Krebs cycle, and aids synthesis of nucleic acids, thereby improving mitochondrial efficiency and reducing ROS generation [40, 54]. LA modulates the PKB/Akt signaling pathway important for vascular endothelial integrity and the redox-sensitive transcription factor Nrf2 and NF-κB pathways, thereby indirectly promoting an antioxidant environment [53, 55–57].

Effects of LA in pre-clinical and short-term MS studies

Both animal and human pre-clinical and in vitro studies provide evidence for LA's anti-inflammatory and neuroprotective effects (key trials presented in Table 1). In EAE, LA limits the transmigration of inflammatory T cells and monocytes into the CNS [44, 63•]. These results were recently confirmed by a study demonstrating reduced migratory capacity peripheral blood mononuclear cells and B cells in the plasma of RMS participants treated with LA [58•]. LA reduces inflammatory mediators in peripheral blood from PwMS including ICAM-1 and VCAM-1, MMP-9, sICAM-1, INF-y and IL-4 [44, 62, 64–66]. Levels of the small molecule cyclic adenosine monophosphate (cAMP) in human lymphocytes and other cell types are altered by LA, which may contribute to its anti-inflammatory and neuroprotective effects [59–61].

LA effectively treats EAE. In pre-symptomatic mice, subcutaneous doses of LA 10-100 mg/kg/day significantly suppressed EAE development (cumulative disease scores) after 10 days [44, 57, 67]. Mice treated with intraperitoneal LA 50 mg/kg after the onset of EAE clinical symptoms demonstrated slowed EAE progression (clinical and cumulative disease scores) [64]. Reversal of the clinical benefits of LA has also been demonstrated when LA treatment was stopped indicating an ongoing treatment effect [44, 68].

In a 2 week RCT, Yadav et al. compared daily 600 mg twice daily, 1200 mg daily (with evening placebo), 1200 mg twice daily doses of LA, and placebo in 30 PwMS [62]. They found that the 1200-mg dose achieved significantly higher peak serum levels than the 600-mg dose, although wide variability in peak levels existed at both doses. Peak serum levels correlated with decreases in serum MMP-9 and sICAM-1 levels, but differences between 1200 mg daily and twice daily were not significant. The greatest side effects (in order of frequency: nausea, malodorous urine, headache, weakness, pain/spams, and rash) were higher for both 1200-mg and 600-mg doses. Yadav et al. subsequently compared serum LA levels following intake of three different formulations of a 1200-mg dose of LA in PwMS ($N = 24$) to those from mice at three different subcutaneous doses [51]. They found that peak serum concentrations and area under the curve levels for two of the three formulations of LA were comparable to the highly therapeutically efficacious 50 mg/kg dose in EAE. These key studies determined the 1200 mg daily dose of LA used in their subsequent clinical trials.

Effects of LA in randomized clinical trials

Table 2 provides the published results from selected key longitudinal randomized clinical trials (RCT) of LA in PwMS. Khalili et al. treated RRMS subjects ($n = 50$) with 1200 mg daily LA or placebo for 3 months but found no changes in fatigue, disability, or MRI outcomes [69]. The

same group compared 1200 mg daily LA to placebo ($n = 52$) for 3 months and demonstrated improved total antioxidant capacity (TAC) and reduced inflammatory markers INF-y, TGF-β, and IL-4 in the LA cohort [65, 70]. The group also examined a subset of the cohort ($n = 24$) and reported significantly reduced plasma asymmetric dimethylarginine (ADMA) at 3 months in the LA cohort, but again failed to demonstrate a decrease in EDSS [71]. A 6-month RCT for people with RRMS and primary progressive (PPMS, $n = 43$) consisting of usual diet, Mediterranean diet alone, or Mediterranean diet with supplements, that included daily 300 mg LA, found a benefit of the diet, with or without supplements, in reducing serum markers of inflammation [72].

Table 2. Key randomized controlled trials of lipoic acid in people with multiple sclerosis

Study	Sample	Questions and interventions	Results
[65, 69–71]	RRMS *N* = 24–52	Questions being addressed: Does LA affect the cytokine profile, improve fatigue and disability, affect changes on MRI, increase TAC, and affect serum markers in PwMS? Protocol: LA 600-mg BID vs placebo for 3 months	1. LA significantly reduced inflammatory markers INF-y, TGF-β, and IL-4, but not TNF-α, IL-6, or MMP-9. 2. No significant changes from baseline in fatigue, disability, or enhancing plaques on MRI in either group. 3. LA significantly improved TAC, but not other oxidative stress markers. 4. LA significantly reduced serum ADMA, an inhibitor of NO.
[72]	RRMS PPMS *N* = 43	Questions being addressed: What are the effects of nutritional intervention on the wellness and inflammatory status of PwMS? Protocol: Multi-arm study with combinations Mediterranean diet, 300-mg LA, multi-supplements Duration: 6 months	1. Diet, with or without supplements, resulted in increased serum PUFA concentrations, while diet with supplements reduced serum MMP-9 levels, indicating lower inflammation. 2. No significant change in disability, fatigue, or depression scores in any group.
[73••]	SPMS *N* = 51	Questions being addressed: 1. Does LA reduce brain atrophy rate, 2. Does LA reduce disability and improve quality of life, and 3. Is LA safe? Protocol: LA 1200 mg/day vs placebo for 2 years. MRI at baseline, and months 12 and 24.	Primary outcome: LA treatment resulted in a significant 68% reduction in annualized PCBV on MRI. Secondary outcomes: 1) LA treatment group had near significant improvement in walking speed ($p = 0.06$). 2) LA treatment was safe, well-tolerated, and had high compliance (> 90%).
[52•]	SPMS *N* = 27	Questions being addressed: What are the peak serum levels of LA achieved at baseline and after 1 year of treatment? Protocol: Pharmacokinetic levels drawn on day 1 and after 1 year of treatment with 1200 mg daily LA.	1. Peak serum levels occurred between 90 and 120 min after ingestion. 2. Variability was high at both time points, and within subject variation was also discrepant between years.

ADMA, asymmetric dimethylarginine; *BID*, twice daily dosing; *IL-4*, interleukin-4; *IL-6*, interleukin-6; *MMP-9*, matrix metallopeptidase-9; *NO*, nitrous oxide; *PCBV*, percent change brain volume; *PPMS*, primary progressive multiple sclerosis; *RRMS*, relapse-remitting multiple sclerosis; *TAC*, total antioxidant capacity; *TNF-α*, tumor necrosis factor-alpha; *TGF-β*, transforming growth factor-beta

In the longest trial to date, Spain et al. conducted a single-center, 2-year, double-blind, phase 2 RCT ($n = 51$) of daily oral LA vs placebo in secondary PMS subjects. The LA cohort demonstrated a 68% reduction in the primary outcome of annualized rate of whole-brain atrophy (outcome = 0.002, Fig. 2) [73••]. Analysis of the subset of participants who were ambulatory at baseline ($n = 42$) nearly, but did not significantly, maintained walking speed over placebo ($p = 0.06$). Adherence to LA was high (> 90%) despite the known side effects of gastrointestinal intolerance, headache, malodourous urine, and rash [62, 73••]. A multisite follow-up trial is in progress (NCT03161028). Bittner et al. analyzed the pharmacokinetic data from baseline and year 1 of study, again demonstrating high inter-subject variability, but newly reporting high intra-subject variability between evaluations [52•]. Notably, peak serum levels did not correspond to side effects or study drop-outs.

Evidence for other antioxidant therapies for MS

There are many antioxidant therapies under evaluation for both disease-modifying and symptomatic treatment of MS with prior excellent reviews [74••]. Herein, we review selected clinical trials of otherwise naturally occurring endogenous and dietary antioxidants with the most compelling recent evidence (Table 3).

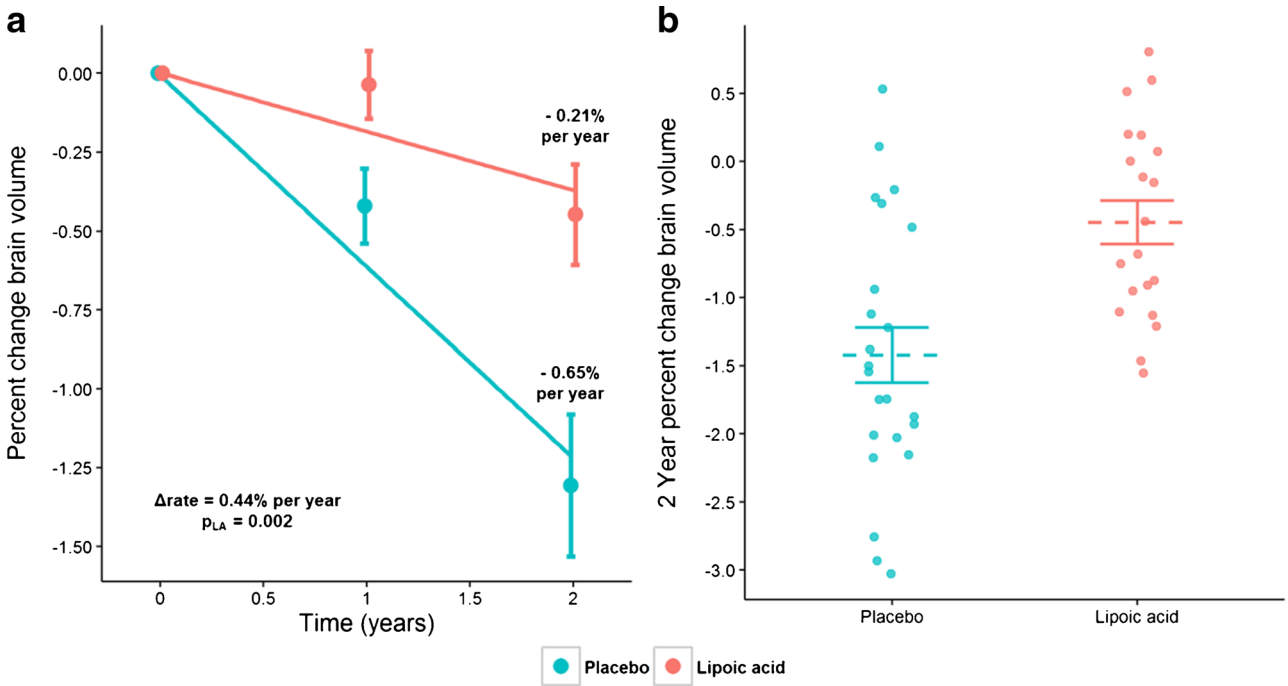

Fig. 2. Results from a RCT of daily oral LA vs placebo in secondary PMS subjects. LA group had significantly less percent change brain volume than controls at 2 years. **(Credit Line:** Spain R, Powers K, Murchison C, Heriza E, Winges K, Yadav V et al. Lipoic acid in secondary progressive MS. Neurology: Neuroimmunology and NeuroInflammation. 2017;4(5):1–9. https://nn.neurology.org/).

Table 3. Selected clinical trials of otherwise naturally occurring endogenous and dietary antioxidants

Antioxidant	Study	Sample	Intervention	Results
Vitamin A	[75]	PwMS, *N* = 39	Study arms: 25,000 IU/day vs placebo Duration: 6 months	Treatment group showed significant decreases in expression of inflammatory cytokine genes IFN-γ and T-bet in peripheral blood mononuclear cells.
	[76, 77•]	RMS, *N* = 101	Study arms: 25,000 IU/day retinyl palmitate for 6 months followed by 10,000 IU/day for 6 months vs placebo Duration: 1 year	Treatment group showed significantly improved disability (MS functional composite), fatigue, and depression scores, but not the EDSS disability score, relapse rate, or number of new/enhancing lesions.
Vitamin E	[78]	PwMS, *N* = 34 HC, *N* = 44	Study arms: 400 mg/day vitamin E vs placebo in MS arm Duration: 3 months	At baseline, PwMS have higher markers of urine oxidation (8-iso-PGF2α), serum lipid oxidation, and shorter telomere length. Treatment with vitamin E significantly reduced urine 8-iso-PGF2α only.
Coenzyme Q10 (CoQ10)	[79–81]	RMS, *N* = 48	Study arms: 500 mg/day CoQ10 vs placebo Duration: 12 weeks	Treatment group showed significant improvements in SOD activity, malondialdehyde (lipid peroxidation marker), and inflammatory markers of TNF-α, IL-6, and MMP-9. Treatment group had lower depression and fatigue scores.
Polyunsaturated fatty acids (PUFAs)	[82]	PwMS + depression, *N* = 39	Study arms: 6 g/day omega-3 fatty acids vs placebo Duration: 3 months	No significant differences in depression between groups
	[83]	RMS, *N* = 50	Study arms: 1 g/day fish oil vs placebo Duration: 12 months	No significant group differences in serum levels of TNF-α, IFN-γ, IL6, and IL-1β or EDSS.
	[84]	RMS, *N* = 50	Study arms: 4 g/day fish oil vs placebo (olive oil) Duration: 12 months	No changes in glutathione reductase activity, levels, or ratios of oxidized to reduced glutathione.
	[85]	RMS, *N* = 53	Study arms: combination of omega-3-fatty acids (500 mg DHA and 106 mg EPA/day) and vitamin D_3 (50,000 IU/biweekly) vs placebo Duration: 12 weeks	Treatment group showed significant improvements in EDSS, biomarkers of inflammation and OS, and metabolic profiles.

Table 3. (Continued)

Antioxidant	Study	Sample	Intervention	Results
Melatonin	[86, 87]	PwMS by DMT treatment, *N* = 102 HC, *N* = 20	Study arms: 5 mg/day melatonin vs control Duration: 90 days	Treatment resulted in decreased plasma lipid hydroperoxides but not homocysteine levels. Some DMT-specific decreases in serum MDA, increases in SOD activity, and improvements in quality of life outcomes compared to HC.
	[88]	RMS + interferon, *N* = 26	Study arms: 3 mg/day melatonin vs placebo Duration: 12 months	No significant differences in disease activity, disability, cognition or fatigue between groups.
	[89]	PwMS + nocturia, *N* = 34	Study arms: 2 mg/day melatonin vs placebo Duration: crossover 6 weeks per treatment	No significant differences in nocturia between groups.
N-acetylcysteine (NAC)	[90]	RMS + glatiramer acetate, *N* = 7	Study arm: 5 mg/day NAC Duration: 36 weeks	Treatment trend toward more favorable erythrocyte GSSG:GSH ratios at 16 and 36 weeks, but no other redox chemistry or secondary MRI outcomes
Uric acid (inosine)	[91]	RMS + interferon, *N* = 159	Study arms: 3 g/day inosine vs placebo Duration: 2 years	No significant difference in accumulation of disability between groups.
	[92•]	RMS + interferon, *N* = 36	Study arms: 3 g/day inosine vs placebo Duration: 12 months	Treatment group was associated with hyperuricemia and renal colic with no additional effect on relapse rate or progression.
EGCG	[93]	PwMS, *N* = 10	Study arms: 800 mg/day EGCG vs placebo Duration: 6 months	Treatment group demonstrated a 10% increase in brain N-acetyl aspartate, but the study was halted for hepatotoxicity.
Antioxidant combinations	[94]	SPMS, *N* = 9	Study arms: low-fat diet and antioxidant supplement vs the low-fat diet alone. Duration: 42 days	The combined diet and supplement group demonstrated significantly lower 8-iso-PGF2α and inflammatory markers C-reactive protein and IL-6.
	[34]	PwMS, *N* = 250	Observational study arms: low-fat diet, vitamins A and D, vs high-fat diet Duration: 34 years	Both groups demonstrated decreased mortality and MS-related disability.

Antioxidant vitamins

Antioxidant vitamins are classified as either hydrophilic (vitamin C) or hydrophobic (vitamins A and E) providing insight as to their location of action once absorbed. Vitamin C aids in glutamate uptake reducing excitotoxicity and helps recycle the antioxidants vitamin E and glutathione [95]. Current evidence of vitamin C in MS is based upon limited epidemiologic data with no interventional trials of ascorbic acid in isolation in MS. Vitamin A is a group of compounds that includes retinol and beta-carotene and has many functions in human growth, development and ongoing health. Thirty-nine PwMS randomized to either retinyl palmitate 25,000 IU daily (RDA approximately 1600 IU daily) or placebo for 6 months and demonstrated significant decreases in expression of inflammatory cytokine genes IFN-γ and T-bet in peripheral blood mononuclear cells [75]. The same group randomized 101 people with RMS to 25,000 IU daily retinyl palmitate or placebo for 6 months followed by 10,000 IU daily for 6 months and found significantly improved MS function composite disability, fatigue, and depression scores, but not the EDSS disability score, relapse rate, or number of new/enhancing lesions [76, 77•]. Vitamin E, another well-known hydrophobic antioxidant vitamin, belongs to the family of tocotrienols and tocopherols. It helps recycle ascorbic acid and glutathione, and in conjunction with ascorbic acid decreases lipid peroxidation. A RCT of alpha tocopherol 400 mg/day in 34 PwMS showed reduced lipid serum oxidation and maintained telomere length, an evolving measure of OS [78, 96]. Finally vitamin D, among its many actions, acts as a membrane antioxidant by inhibiting iron-dependent liposomal lipid peroxidation. There are many potential roles of vitamin D in MS pathophysiology and treatment which are reviewed elsewhere [97, 98].

Coenzyme Q10

Coenzyme Q10 (CoQ10) is an endogenous free radical scavenger and cofactor in mitochondrial oxidative phosphorylation. Due to declining levels with age, it has been studied in a variety of neurodegenerative disorders and aging with mixed results; however, studies in MS have been limited [99]. Sanoobar et al. evaluated the effects of 500 mg/day vs placebo in 48 PwRMS over 12 weeks and found significant increases in SOD activity, decreases in malondialdehyde (MDA), a product of lipid peroxidation and marker of OS, and decreases in TNF-α, IL-6, and MMP-9 [79, 80]. These changes were accompanied by lower depression and fatigue scores [81]. New formulations of CoQ10 and its precursor idebenone are presently under investigation.

Polyunsaturated fatty acids

The polyunsaturated fatty acids (PUFAs) found in fish and plant oils (olive, flax, soy) are long-chain fatty acids that contain more than one carbon-carbon double bond. The parent compound to PUFAs eicosapentaenoic acid (EPA) and docosahexaemoic acid (DHA) is linolenic acid, an essential nutrient. While able to be oxidized themselves, PUFAs can act as indirect antioxidants, lowering lipid peroxidation products and ROS, and increasing SOD scavenging [100]. Most studies of PUFAs in MS evaluated their anti-inflammatory and symptomatic benefits [82, 83, 101]. Two studies addressed the antioxidant effects of PUFAs. Sorto-Gomez et al.

found no changes in glutathione reductase activity, levels or ratios of oxidized to reduced glutathione after 12 month supplementation with 4 g/day of fish oil (1.6 mg EPA, 1.6 mg DHA, and excipient containing tocopherol, canola oil, sunflower oil, flavors, others) or control (olive oil) in 50 participants with MS as add-on therapy to interferon beta-1b [84]. Of note, the authors did not discuss the potential antioxidant effects of olive oil in the comparator intervention. Kouchaki et al. investigated the effects of a combination of omega-3-fatty acids (500 mg DHA and 106 mg EPA daily) and vitamin D_3 supplementation (50,000 IU/biweekly) for 12 weeks [85]. Surprising for the size of the trial ($n = 53$), significant differences between groups in changes of disability levels, biomarkers of inflammation and OS, and metabolic profiles were found; however, DMT status of participants was not noted by the authors, which was a potential confounder.

Melatonin

Melatonin, an indoleamine produced in the vertebrate pineal gland, functions as an antioxidant by direct free radical scavenging, stimulation of antioxidant enzymes, reducing electron leakage during mitochondrial oxidative phosphorylation, and augmenting other antioxidants [102]. While several recent publications establish the short-term metabolic changes (both antioxidant and anti-inflammatory) in response to melatonin in MS, sufficiently powered studies to establish clinical effects are lacking. Melatonin supplementation causes an immediate and differential increase in expression and activity of antioxidant genes in PwMS compared to controls [103]. A case control study of PwMS (separated into five DMT-treated groups) treated with 5-mg melatonin daily for 90 days demonstrated decreases in plasma lipid hydroperoxides, a marker of OS, but not homocysteine levels [86]. While not universal, the study also demonstrated DMT-specific decreases in serum MDA, increases in SOD activity, and improvements in quality of life outcomes compared to controls [87]. A 12-month RCT ($n = 26$) compared 3 mg/d melatonin vs placebo in PwRMS taking weekly interferon beta therapy did not show significant differences in disease activity, disability, cognition, or fatigue [88]. Drake et al. recently published the results of a crossover RCT ($n = 34$) of melatonin 2 mg/day vs placebo (6 weeks per treatment) but did not observe significant differences in nocturia [89].

N-acetylcysteine

N-acetylcysteine (NAC) is a multi-functional exogenous antioxidant that scavenges H_2O_2 hydroxyl radicals and hypochloric acid, acts as precursor for GSH synthesis, regenerates GSH by increasing intracellular cysteine, and more generally affects transcription factors, apoptosis, and cell survival. NAC inhibits acute EAE in mice and protects against EAE by scavenging ROS and reducing MMP-9 activity [104]. Schipper et al. examined the safety and tolerability of 5 mg daily NAC added to glatiramer acetate ($n = 7$) and found a trend toward more favorable erythrocyte GSSG:GSH ratios at 16 and 36 weeks, but no other redox chemistry or secondary MRI outcomes [90]. Other studies using NAC are ongoing. Uric acid is an

endogenous product of purine metabolism and a scavenger or peroxynitrite which is toxic to brain cells [105]. Evidence including low levels of uric acid in PwMS and amelioration of EAE with treatment led to several clinical trials; however, these RCTs of the uric acid precursor inosine have not shown efficacy and most recently, have been associated with hyperuricemia and renal colic without suggestion of benefit to MS [91, 92•, 106]. Polyphenols are an abundant group of dietary antioxidants found in plant-based foods such as fruits, vegetables, and cereals. Their antioxidant functions are free radical scavenging, metal sequestration, and interactions with antioxidant enzymes and transcriptional factors of antioxidant proteins [107]. Examples with sources include quercetin (flavonoid from fruits and vegetables), luteolin (celery, chamomile tea), curcumin (turmeric), resveratrol (grapes and red wine), and EGCG (green tea). Although a clinical trial of EGCG 800 mg/day demonstrated a 10% increase in brain N-acetyl aspartate, the study was halted for hepatotoxicity [93]. Several studies of EGCG in PwMS are ongoing.

Antioxidant combinations

Antioxidant combinations are frequently investigated with the rationale that similar to our diet, these nutrients work synergistically within the body. A scientific limitation to a combination trial is the inability to isolate the key drivers of the noted effects, including side effects. As proof of concept, Mauriz et al. reported a small ($n = 9$) RCT demonstrating significantly lower isoprostane 8-si-PGR2α-a marker of OS, as well as lower inflammatory markers C-reactive protein and IL-6 following a low-fat diet with a multiple antioxidant supplement versus the low fat diet alone [94]. Laudably, this study was completed in non-ambulatory PwMS, a group often overlooked in clinical trials. In the longest observational cohort study to date, adherents to a low-fat diet advocated by neurologist Roy Swank, MD, PhD, had both decreased mortality and MS-related disability at 34 years. This diet was supplemented by vitamins A and D, and permitted twice the amount of unsaturated vegetable and fish oils (PUFAs) than saturated fats [34].

Conclusions

As our understanding of the contributions of OS to the pathophysiology of MS grows, so does the potential of antioxidant therapies to complement immune-mediated DMTs. Pre-clinical and limited clinical trial evidence suggests benefits of antioxidant therapies to treat MS. Of these, LA is a leading candidate therapy. Specific trial considerations when using antioxidants for CNS disease include issues of gastrointestinal absorption, distribution, solubility, and ability to cross the BBB. As with other MS therapies, there is a need for meaningful biomarkers of immediate metabolic effects, intermediate alterations in gene expression and cellular function, and long-term outcomes capturing neuroprotection and neuro-repair. Comparing results across antioxidant studies is challenging without consensus regarding laboratory markers of OS. Still, the growing number of evaluations of antioxidant therapies in MS and other CNS diseases speaks to the commitment from scientific and patient advocate communities alike to pursue rigorous pre-clinical and clinical evaluation of this therapeutic approach.

Compliance with Ethical Standards

Conflict of Interest
The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent
All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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