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Review

Mitochondrial respiration as a target for neuroprotection and cognitive enhancement

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ABSTRACT

This paper focuses on brain mitochondrial respiration as a therapeutic target for neuroprotection and cognitive enhancement. We propose that improving brain mitochondrial respiration is an important future direction in research and treatment of Alzheimer's disease (AD) and other conditions associated with cognitive impairment and neurodegeneration. The central thesis is that supporting and improving brain mitochondrial respiration constitutes a promising neurotherapeutic principle, with potential applications in AD as well as in a wide variety of neuropsychological conditions. We propose three different interventional approaches to improve brain mitochondrial respiration based on (a) pharmacology, (b) photobiomodulation and (c) nutrition interventions, and provide detailed examples for each type of intervention. First, low-dose USP methylene blue is described as a pharmacological intervention that can successfully increase mitochondrial respiration and result in memory enhancement and neuroprotection. Second, transcranial low-level light/laser therapy with near-infrared light is used to illustrate a photobiomodulation intervention with similar neurometabolic mechanisms of action as low-dose methylene blue. Finally, a nutrition intervention to improve mitochondrial respiration is proposed by increasing ketone bodies in the diet. The evidence discussed for each intervention supports a fundamental neurotherapeutic strategy based on improving oxidative energy metabolism while at the same time reducing the pro-oxidant tendencies of the nervous system. Targeting brain mitochondrial respiration with these three types of interventions is proposed as part of a holistic neurotherapeutic approach to improve brain energy metabolism and antioxidant defenses. This strategy represents a promising new bioenergetics direction for treatment of AD and other neuropsychological disorders featuring cognitive impairment and neurodegeneration.

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1. Introduction

Brain oxidative energy metabolism is a target to which attention has only indirectly been devoted in neurotherapeutic interventions, but it is suspected to have a large potential for the implementation of effective treatments of brain diseases and for enhancing normal cognitive functions. The paucity of neurotherapeutic strategies targeting brain energy metabolism may be explained not only by a lack of technology and pharmaceutical resources to specifically enhance brain oxidative metabolism, but also by failure to identify energy metabolism as one of the most important processes in neuronal physiology. The brain has one of the highest rates of energy demand in the body. Neurons have a unique oxidative potential and heavily rely on an adequate supply of oxygen and glucose to survive and maintain normal function. Of all neuronal functions, active transport of ions against their concentration and electrical gradients is by far the largest energy consuming function of neurons [1]. Active ion transport restores the plasma membrane potential after depolarization by activation of the Na + K + ATPase pump [2]. In consequence, neuronal activity and energy metabolism are tightly coupled [3]. In other words, highly active neurons display high energy consumption and formation. To achieve this, the intracellular metabolic machinery that supports the generation of energy from oxygen and glucose is abundantly expressed. The core of this metabolic machinery resides in mitochondria, and within it, key components for energy demand/production coupling are those in the electron transport chain in the inner mitochondrial membrane, including cytochrome oxidase. Mitochondria are central organelles in neuronal physiology. They coherently integrate cell respiration, energy metabolism, and calcium ion balance to support cell survival. Remarkably, a single neuron is not metabolically homogeneous, but the neuronal metabolic capacity, represented by mitochondrial content, is highest in dendrites, intermediate in cell bodies and lowest in axon trunks [3]. This subcellular compartmentalization of energy reflects an adaptation to maximize efficiency in energy utilization, so that energy is generated only when and where energy is needed.

Recent progress in our understanding of brain oxidative metabolism has revealed discrete potential mitochondrial molecular targets that may be used for neurotherapeutic purposes. Effective cognitive enhancement and neuroprotection are two clinical desirable outcomes that may be achievable by targeting brain energy metabolism. Both seem a crucial unmet need in the treatment, for example, of neurodegenerative diseases. Neurodegenerative disorders are heterogeneous, but they all feature progressive neuronal atrophy and loss. The etiology of neurodegeneration in most cases is unknown, but it has been hypothesized to be multifactorial, with both genetic and environmental contributing factors. Whereas differential regional vulnerability and distinct types and patterns of protein aggregation seem to distinguish between neurodegenerative entities, universal features of neurodegeneration include chronic and progressive cell loss, atrophy and loss of function in specific brain systems. In addition, mitochondrial failure has gained attention as a major pathogenic event common to the broad spectrum of neurodegenerative disorders. Leber's hereditary optic neuropathy (LHON) appears as a model neurodegenerative disease caused by mitochondrial failure. This relatively rare condition is produced by specific mutations in NADH dehydrogenase, the entry enzyme of the respiratory chain in mitochondria. As it is classical of mitochondrial disorders, LHON follows a particular inheritance pattern, affecting mainly young adult males. Nevertheless, its expressivity is variable and its onset can occur during childhood or in elder individuals [4]. On the other extreme of the neurodegeneration spectrum, Alzheimer's disease (AD) appears as the most common neurodegenerative disorder. It is mostly sporadic, and associated with advanced age and β-amyloid and tau accumulation in the brain. In contrast to LOHN, in which the role of mitochondrial dysfunction is widely acknowledged, the mainstream hypothesis on the cause of AD puts little emphasis on the potential role of mitochondrial dysfunction. While many believe that the amyloid/tau pathogenic hypothesis will further our ability to understand and treat AD, this view is not universal and alternate pathogenic hypotheses exist.

A major alternate hypothesis supported by us [5] and others [6–8] proposes mitochondrial dysfunction as a key pathogenic step, not only in AD but also in other neurodegenerative conditions. The mitochondrial hypothesis of neurodegeneration derives from the observed relationship between mitochondrial durability (*e.g.* efficiency, accumulation of mitochondrial DNA mutations) and aging, which is believed by some groups to be causal [9]. Hence, whereas the baseline mitochondrial function is determined by gene inheritance, exposure to environmental factors, in turn proportional to age, determine the rate of mitochondrial decline [10]. The mitochondrial hypothesis of neurodegeneration also predicts that mitochondrial failure precedes synaptic dysfunction, protein aggregation, atrophy and loss of function. Mitochondrial failure has been linked to known major pathogenic aspects of neuronal dysfunction associated with neurodegeneration, including excitotoxicity [11], abnormal protein aggregation [12], neuroinflammation [13] and oxidative stress [14]. Specific evidence supporting the primordial role of mitochondrial dysfunction in AD include (1) decreased ATP, reduced basal oxygen consumption, decreased NAD+/NADH ratios, increased oxidative stress, pervasive mitochondrial depolarization, altered calcium homeostasis and increased β-amyloid production in cell cultures after AD and mild cognitive impairment (MCI) subject mitochondrial transfer [15]; (2) reduced cytochrome oxidase activity in AD subject platelets and brains [16,17]; (3) correlation between disease duration and cytochrome oxidase activity in the posterior cingulate cortex, a region showing hypometabolic changes in preclinical stages of dementia [18]; and (4) consistent early selective brain hypometabolism that precedes cognitive decline in AD, underlies synaptic dysfunction and occurs in brain regions with higher synaptic activity, including multimodal cortical network hubs [19]. Defects in energy metabolism are a constant in the pre-clinical stages of dementia. For example, cognitively normal individuals with a family history of late onset AD, in particular individuals with a maternal history of AD, have a progressive reduction in glucose metabolism on FDG-PET in the posterior cingulate, parieto-temporal, and medial temporal regions. These regions are affected in patients with clinical AD and such changes are more significant than those seen in individuals with a paternal or negative family history of AD [20]. Notably, this evidence has led to a recent revision of a popular pathogenic model for AD to now include energy metabolic failure as one of the earliest steps in the natural history of the disease [21]. Similarly, early metabolic changes preceding neuronal atrophy have been observed in patients with parkinsonism [22,23] and Huntington's disease [24]. Since mitochondrial bioenergetics plays a central role in neuronal function and survival, it can be hypothesized that the putative heterogeneous etiologic factors of neurodegeneration may find in mitochondria points of vulnerability for structural and functional neuronal integrity.

Based on a growing body of data discussed below, targeted manipulations of mitochondrial respiratory function seem to be the next logical step in attempts to design effective therapeutic interventions against neurodegeneration, including AD. Nevertheless, as more is learned about the metabolism of the nervous system, it becomes evident that the oxidative bioenergetics' particularities of the brain would demand consideration of so far non-conventional strategies of neuroprotection and cognitive

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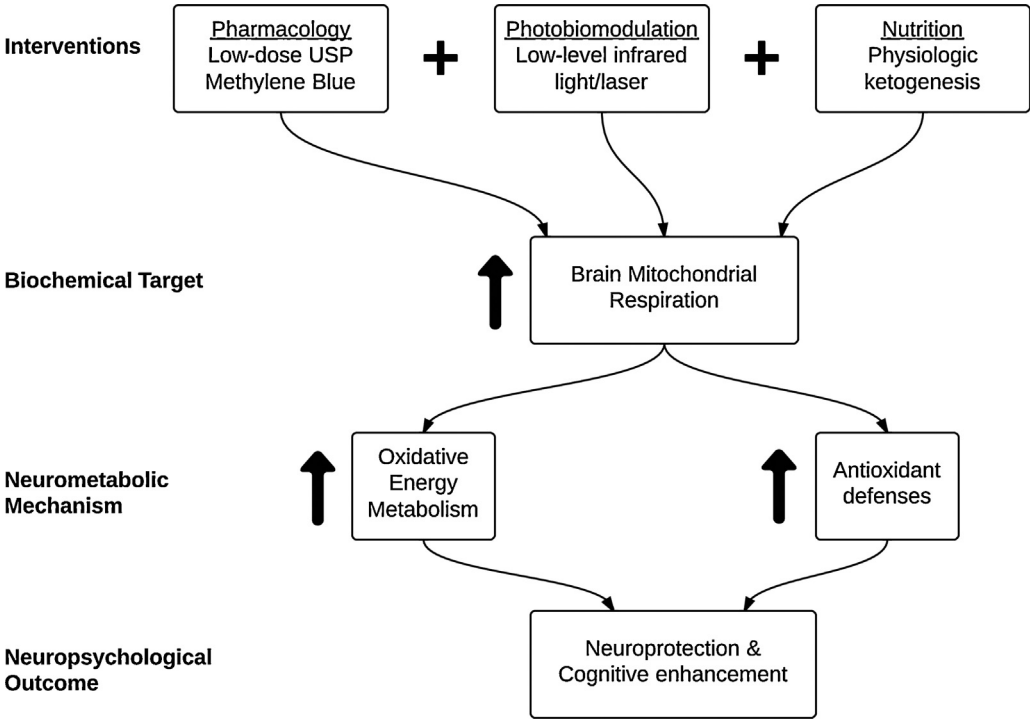

Fig. 1. Pharmacology, photobiomodulation and nutrition interventions proposed to improve neuropsychological outcome by increasing brain mitochondrial respiration. The first intervention uses a pharmacological approach with low-dose USP methylene blue. The second intervention involves transcranial low-level light/laser therapy with near-infrared light as a photobiomodulation approach with similar neurometabolic mechanisms of action as low-dose methylene blue. Finally, a nutrition intervention producing ketogenesis is proposed by increasing ketone bodies in the diet. The three different interventions converge biochemically on the same target by successfully increasing mitochondrial respiration. The neurometabolic mechanisms mediating the increase in mitochondrial respiration involve elevations in oxidative energy metabolism and antioxidant defenses. These neurometabolic actions will in turn result in neuroprotection and cognitive enhancement. These interventions are readily bioavailable to the brain, but at the same time selective at affecting those neuronal networks that require metabolic support. Detailed mechanistic rationale and evidence-based applications for each type of intervention are described in the text.

enhancement. The ideal intervention should target the mitochondrial energetic machinery, be readily bioavailable to the brain, but at the same time selective at affecting those neurons or networks that require support, thus contributing to minimization of undesired side effects. We propose here three such interventional approaches based on specific *pharmacology*, *photobiomodulation* and *nutrition* interventions (Fig. 1), and provide below detailed mechanistic rationale and evidence-based applications for each type of specific intervention.

2. Pharmacology intervention with low-dose USP methylene blue

There is a growing consensus that the energy failure and oxidative stress produced by dysfunction of mitochondrial respiration play a key role in the pathogenesis of neurodegenerative illnesses [25]. Hence interventions that are aimed at preventing these early mitochondrial events may efficaciously treat neurodegeneration. Numerous animal models have been used successfully to demonstrate the efficacy of pharmaceutical grade (USP) methylene blue (MB) as a mitochondrial neuroprotective intervention (reviewed in Rojas et al.) [26]. MB (IUPAC name: [7-(dimethylamino)phenothiazin-3-ylidene]-dimethylazanium chloride; CAS number: 97130-83-1; common synonyms: methylthioninium chloride, chromosmon, basic blue 9) is a potent redox diaminophenothiazine with high bioavailability to mitochondria and autoxidizable properties that supplement the action of ubiquinone as an electron carrier in the respiratory chain. Therefore, MB's potent redox action and unique auto-oxidazible properties make it suitable for improving mitochondrial respiration and for use as an intervention against neurodegeneration. MB is already an FDA-approved drug routinely prescribed as an antidote for the treatment of poison-induced methemoglobinemia due to its powerful antioxidant properties [27]. MB readily crosses the blood–brain barrier and has great affinity for mitochondria, a property that has allowed the use of MB as a redox indicator and supravital stain of nervous tissue [28,29].

2.1. Neurometabolic mechanisms of low-dose USP methylene blue

Low-dose MB can act in mitochondria as a radical-scavenging antioxidant, by trapping radicals produced by dysfunctional respiratory chain components before they reach other cellular targets. But low-dose MB is not only a potent antioxidant. Due to its auto-oxidizable activity, low-dose MB can also act as a metabolic enhancer by bypassing blocked points of electron flow in the respiratory chain and thus improving mitochondrial respiration [30,31]. MB's mitochondrial action is unique because its neurobiological effects are not determined by regular drug–receptor interactions or drug–response paradigms. MB shows a hormetic dose–response, with opposite effects at low and high doses [32]. At low doses of 0.5–4 mg/kg, MB is an electron cycler in the mitochondrial electron transport chain, with unparalleled antioxidant and cell respiration-enhancing properties that affect neural function in a versatile manner. The unique auto-oxidizing property of MB and its pleiotropic effects on a number of tissue oxidases explain its potent neuroprotective and metabolic effects at low doses. A major role of the mitochondrial respiratory enzyme cytochrome oxidase on the neuroprotective and cognitive-enhancing effects of MB is supported by available data [26].

Low-dose MB does not affect the nitric oxide-guanylyl cyclase system as high-dose MB, but low-dose MB's auto-oxidizing

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property acts as an electron cycler that allows MB to redirect electrons to the mitochondrial electron transport chain, thereby enhancing ATP production and promoting cell survival [26]. In bypassing complex I–III activity to generate ATP, low-dose MB reduces reactive oxygen species (ROS) production from the mitochondrial electron transport chain, which has the potential to minimize AD physiopathology [5]. Low-dose MB's antioxidant property is thus unique. Therefore, we hypothesized that MB's mechanism of neuroprotection is mediated by support of mitochondrial function *via* its powerful *in situ* antioxidant effects and its mitochondrial respiration-enhancing properties. For example, in a model of mitochondrial optic neuropathy, rotenone produced a noticeable loss of ganglion cell bodies and optic nerve fibers (reviewed in Rojas and Gonzalez-Lima) [4]. But MB co-administration prevented these changes [33,34]. MB's neuroprotective effects in this model were also demonstrated at the functional level, inducing preservation of visual function, as measured by neurometabolic and behavioral parameters. Our research with MB in the model of optic neuropathy supports the concept that in the presence of complex I inhibition, stimulation of the electron transport chain paired with free radical-scavenging is highly efficacious at preventing the neurodegenerative effects of mitochondrial dysfunction. Numerous *in vitro* and *in vivo* studies spanning over 50 years have firmly established that low-dose MB enhances cytochrome c oxidase (complex IV) activity, oxygen consumption, cerebral blood flow, brain glucose utilization and ATP production in cells while simultaneously reducing oxidative stress [33,35–37].

2.2. Neurotherapeutic applications of low-dose USP methylene blue

MB – widely used to treat methemoglobinemia and cyanide poisoning – has recently been shown to have multiple positive therapeutic effects in reducing neurological impairment and enhancing cognitive measures [38]. Low-dose MB has important implications as a new treatment to improve cognitive outcome and neurodegeneration associated with AD. Low doses of MB have also been used for neuroprotection against mitochondrial dysfunction in humans and experimental models of disease, including metabolic encephalopathy, optic neuropathy, cardiac arrest-induced brain damage, striatal neurodegeneration, and stroke [26]. For example, MB has been shown to reduce neurobehavioral impairment in animal models of mitochondrial optic neuropathy [33,34], AD [39–41], Parkinson's disease (PD) [38,42], Huntington's disease (HD) [43] and nerve injury [44]. The evidence supports a mechanistic role of low-dose MB as a promising and safe intervention for improving the cognitive rehabilitation of conditions characterized by increased oxidative stress, neurometabolic compromise and behavioral impairment.

In addition to neuroprotection, MB's effects have been associated with improvement of memory and behavior in a network-specific and practice-dependent fashion. Specifically, low-dose MB has shown cognitive-enhancing effects in a considerable number of learning and memory paradigms, including: inhibitory avoidance, spatial memory, fear extinction, object recognition, open-field habituation and discrimination learning [26]. MB also rescues memory function in models of amnestic MCI induced by mitochondrial dysfunction [45], anticholinergics [46], systemic cytochrome oxidase inhibition [47] and transgenic mouse models of Tau and amyloid-associated pathologies [41,40]. Moreover, a single 4 mg/kg and daily 1 mg/kg intraperitoneal MB doses improve learning and memory in animals by long-lasting upregulation of brain cytochrome c oxidase activity [26,35,48–50]. In particular, animals treated with low-dose MB showed larger fMRI responses and cerebral O_2 consumption changes compared to vehicle-treated rats, under normoxia and hypoxia conditions [51]. These findings support the notion that low-dose MB is a brain metabolic energy enhancer *in vivo* [52].

MB is an FDA-grandfathered drug that has already been rigorously studied and used in humans for over 120 years. PubMed lists 4908 human studies of MB (searched 2013). MB's pharmacokinetics, side effect profile, and contraindications are well-known and most importantly, minimal in humans [28,53]. MB has been used in parathyroid surgery to aid in lymphatic mapping since the early 1970s at doses of 3.5–10 mg/kg. A safety announcement from the FDA warned physicians about possible serious serotonin reactions in patients who received intravenous MB during parathyroid surgery if taking serotonergic psychiatric drugs. A subsequent report by Mayo Clinic surgeons and pharmacologists summarized the FDA evidence and literature and concluded "that the use of methylene blue dye at low doses for lymphatic mapping likely carries very little risk for serotonin neurotoxicity" [54]. Furthermore, none of the FDA cases are based on oral MB. Daily 300 mg oral MB (4.28 mg/kg/day based on a body weight of 70 kg) has been used safely for one year in clinical trials [55]. Thus, low-dose MB has a long history of safe usage that supports its translational relevance to human populations.

Especially important is the burden of cognitive decline in the aging population, including those at risk for developing MCI and AD, since these conditions are expected to reach unparalleled endemic proportions. Interestingly, the brains and peripheral tissues of patients affected by MCI and AD display a prominent cytochrome oxidase inhibition within the mitochondrial respiratory chain [56,57]. Cytochrome oxidase has a key role in neuronal activity as a rate-limiting enzyme for oxidative energy production in the mitochondrial electron transport chain [58,59] and it also can catalyze the production of nitric oxide under hypoxic conditions [60]. Since memory functions are extremely sensitive to oxidative energy deficits, cytochrome oxidase inhibition linked to aging and impairment in cerebral perfusion [61–63] has long been recognized as a major pathophysiological mechanism underlying memory dysfunction and neurodegeneration in AD that is present prior to AD onset [5]. Fortunately, brain cytochrome oxidase activity can be modulated by MB in a hormetic dose-response manner [32]. MB's hormetic dose-response consists of an increase in beneficial effects at low doses, followed by opposite (detrimental) effects at high doses, while at intermediate doses the effect is equal to a control-type effect. Therefore, high MB doses should be avoided because low-dose MB interventions induce the maximal pharmacologic beneficial effects on mitochondrial respiration and cytochrome oxidase activity, which correspond to 30–60% increases compared to control [32].

Whether a beneficial use of low-dose MB in aging populations may be generalized to treat MCI and early AD patients should be investigated. For example, a research group garnered a great deal of media attention after a 2008 Alzheimer's Association international conference where they presented preliminary data using MB in a study showing that low-dose MB prevented cognitive deterioration in AD patients. Using the trade name of Rember™, and the MB synonym methylthioninium chloride, MB was given orally at 60 mg three times a day (a low dose of 2.57 mg/kg/day based on a body weight of 70 kg) for 24 months to patients with mild to moderate AD [64]. There was an 81% reduction in the rate of cognitive decline compared to controls after 50 weeks. This is an effect that, if reproducible, would be without precedent in pharmacological interventions against AD. But data from this abstract and meeting presentation should be regarded as preliminary since it has not been published after a peer-reviewed process. The authors suggested that the cognitive benefit of MB on AD patients was linked to prevention of Tau aggregation, based on prior *in vitro* studies with high-dose MB [65]. Since MB improves cognition only at low doses, while it prevents Tau aggregation only

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at high doses, it is more likely that low-dose MB is acting *via* mitochondrial respiration mechanisms for neuroprotection and cognitive enhancement, as proposed here (Fig. 1). An important point that the research with the animal model of optic neuropathy demonstrated is that MB is able to prevent neuronal loss in a model of degeneration *not mediated by* Tau or β-amyloid deposition [4]. Therefore, these animal studies suggest that the neuroprotective mechanism of action of low-dose MB is related to enhancement of mitochondrial respiration and to its powerful antioxidant effect in the presence of a mitochondrial respiration inhibitor. This further suggests that prevention of oxidative stress and enhancement of mitochondrial respiration *in vivo* may be used to design new metabolic drugs against neurodegenerative disorders. For example, a number of phenothiazinic MB derivatives may share some of its neurochemical and neuroprotective effects. MB derivatives such as azure A, azure B or thionin vary in structure from MB by the absence of one, two or three methyl groups [29]. But little if any research data are available on the neuroprotective or cognitive enhancing effects of these MB derivatives. These derivatives should be screened for neuroprotective and cognitive effects because they also share MB's imino chemical group, which could support similar neurometabolic effects on mitochondrial respiration [66].

3. Photobiomodulation intervention with low-level light/laser therapy

In recent years, transcranial low-level light/laser therapy (LLLT) has emerged as an intervention with potential to modulate neuroprotective and cognitive functions. Transcranial LLLT can be defined as the use of directional low-power and high-fluence monochromatic or quasimonochromatic light from lasers or light-emitting diodes (LEDs) in the red to near-infrared wavelengths (λ = 600–1100 nm) to modulate a neurobiological function or induce a neurotherapeutic effect in a nondestructive and nonthermal manner *via* stimulation of the respiratory enzyme cytochrome oxidase [67]. LLLT is based on the principle that certain molecules in living systems are able to absorb photons and trigger signaling pathways in response to light. This process is termed energy conversion, and implies that the molecule targeted by light reaches an electronically excited state that temporarily changes its conformation and function. In turn, this induces activation of signaling pathways that affect cellular metabolism.

3.1. Neurometabolic mechanisms of low-level light/laser therapy

Molecules that can absorb light are called photoacceptors. A chromophore is a particular moiety within a photoreceptor or photoacceptor molecule that is responsible for the absorption of light. Chromophores are usually organic cofactors or metal ions within a protein structure and contain electrons that can be excited from ground state to excited state. Excitation induces a molecular conformational change that is linked to changes in molecular function and intracellular metabolism. This redox reactivity of chromophores may be structurally coupled to protein distortion, allowing chromophore-containing enzymes to catalyze a very efficient coupling of electromagnetic free energy flow to the chemical energy flow of substrate conversion [68,69]. In the case of LLLT for neurotherapeutic applications, the photoacceptor targeted by near-infrared light is the mitochondrial enzyme cytochrome oxidase, which contains four metal centers relevant for electron transfer that act as chromophores. A wavelength range in the red to near-infrared spectrum has been shown to be the most effective at inducing beneficial biological effects. A "therapeutic window" for clinical effects has been established since lower wavelengths such as violet and ultraviolet appear to have poor tissue penetration, are scattered in biological tissues and tend to be absorbed by melanin. Similarly, water significantly absorbs energy at wavelengths higher than 1150 nm [70]. This therapeutic window is also important because it contains the maximal peaks of absorbance of cytochrome oxidase [71].

Transcranial energy doses delivered by LLLT are too low to cause concerns about heating and tissue destruction, yet they are high enough to modulate cortical cell functions. The effects of LLLT implicate conversion of luminous energy to metabolic energy with a subsequent modulation of the biological functioning of cells. An evident requirement for a beneficial effect of LLLT is that the target brain tissue should have an intact molecular substrate to support or facilitate energy conversion. In addition, the effect of LLLT is expected to be more biologically meaningful if it targets tissues with an energetic balance inclined toward energy consumption as opposed to energy production. The mechanism of action of LLLT consists of primary effects and secondary effects. Primary effects occur with light on, are immediate and depend on light absorption by cytochrome oxidase. LLLT may act as an exogenous source of highly energized electrons to the respiratory chain. Thus, LLLT facilitates the catalytic activity of cytochrome oxidase, accelerates the electron transfer in the inner mitochondrial membrane and boosts cell respiration and energy production [67]. In turn, secondary effects may occur with light off. Secondary effects are always preceded by primary effects and they rely on the presence of the intact intracellular molecular machinery. Secondary effects are pleiotropic and depend on activation of enzymatic pathways that affect metabolic capacity, gene expression for mitogenic and repair signaling, cytoskeleton processing and protein expression and translocation. Such secondary effects are triggered due to the central role of mitochondria as integrators of energy metabolism, cellular homeostasis and cell survival signaling [72]. For example, LLLT has been shown to accelerate metabolism, DNA synthesis and the replication rate in cultured fibroblasts [73]. Cells exposed to LLLT *in vitro* demonstrate profiles of gene expression that support improved cell metabolism, growth and survival [74].

3.2. Neurotherapeutic applications of low-level light/laser therapy

A large body of evidence supports the relevant beneficial effects of LLLT in biological systems including the brain, and suggests that LLLT has many potential neurotherapeutic applications [67]. In fact, LLLT has been used to facilitate neurite outgrowth and there is evidence supporting a role in facilitation of nerve regeneration [75]. Similarly, LLLT was found to prevent the deleterious effects of neurotoxins *in vitro*, including mitochondrial inhibitors [76]. Several studies support that LLLT enhances cytochrome oxidase expression both *in vitro* [77] and *in vivo* [78], which is likely linked to a number of neuroprotective effects of LLLT against mitochondrial toxins observed *in vivo*. Preclinical studies support that LLLT may have a role in the management of conditions associated with mitochondrial failure such as methanol-induced retinopathy [79], Leber's optic neuropathy [78] and MPTP-induced toxicity model of PD [80]. Similarly, LLLT has been used to effectively reduce the functional deficits induced by ischemia in pre-clinical models of stroke [81,82]. A crucial observation is that the great majority of neuroprotective effects have been observed with transcranial delivery of LLLT. This supports the potential use of LLLT as a non-invasive way to treat neurological conditions, including AD. LLLT may be a convenient neuroprotective intervention since light sources including lasers and light emitting diodes have become portable, inexpensive and with potential to offer ergonomic features facilitating close delivery to the head. Regarding clinical effects in humans, it has been recently documented that LLLT increases cerebral blood flow when applied transcranially [83]. This effect was associated with no side effects and with improvements in mood scores. A recent randomized, placebo-controlled clinical trial provided proof-of-principle that

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transcranial LLLT is able to enhance cognitive functions in healthy human subjects [84]. Both of these human studies [83,84] used an LLLT irradiance and cumulative fluency of 250 mW/cm^2 and 60 J/cm^2, respectively. Rojas and Gonzalez-Lima recently summarized the LLLT treatments of 27 transcranial and brain stimulation studies which have shown effectiveness in providing neuroprotection and/or cognitive enhancement [67]. Current clinical trials are in progress for testing the role of LLLT in the management of depression (NCT00961454), Leber's optic neuropathy (NCT01389817) and memory deficits in patients with traumatic brain injury and Gulf War related illnesses (NCT01598532 and NCT01782378).

4. Nutrition intervention by increasing ketone bodies

Within the past decade the neurometabolic mechanisms and neuroprotective effects of ketogenic diets and ketone bodies supplementation have been revealed (for review Stafstrom and Rho) [85]. The state of ketosis is a normal physiologic state that occurs during fasting and carbohydrate restriction, and also normally occurs in newborns. It is beneficial because it derives energy from fatty acid oxidation that results in the formation of ketone bodies. Importantly, some of the beneficial neurometabolic effects of ketogenic diets may also be achieved without any significant dietary restriction, by adding ketone bodies to the diet. The main ketone bodies are beta hydroxybutyrate (BHB), acetoactetate, and propanone. Historically, a therapeutic ketogenic diet was formally constructed in 1921 as a way to achieve the beneficial effects of fasting to treat epilepsy but soon fell to the wayside when newer anticonvulsant drugs were discovered. However, recent large controlled clinical trials have confirmed the efficacy of ketogenic diets for the treatment of intractable epilepsy in both children and adults. Outside of epilepsy, ketogenic diets are now being investigated in a wide array of other neurological diseases, including neurodegenerative diseases such as AD [86]. We will focus here on the ability of ketone bodies to enhance mitochondrial respiration, briefly mention other pleiotropic mechanisms, and illustrate the neuroprotective effects of ketogenic diets and ketone bodies.

4.1. Neurometabolic mechanisms of physiological ketogenesis

The beneficial effects of ketogenesis are mediated by mechanisms that fall into three broad categories: augmentation of energy-dependent brain functions, decreased brain oxidative stress and anti-apoptotic and other neuroprotective effects. Models of normal physiology and aging serve to illustrate these mechanisms. One mechanism by which the ketogenic diet increases energy-dependent brain functions is by increasing energy substrate availability. A classic study by Owen et al. [87] showed that during prolonged fasting ketones can replace glucose as the predominate fuel for brain metabolism. More recently an animal study similarly showed that ketones provided by a ketogenic diet can proportionately spare glucose utilization in the central nervous system [88]. Ketogenic diets have also been shown to increase the monocarboxylate transporter (MCT1) in the endothelial lining of the blood–brain barrier, which resulted in an increase of central nervous uptake of ketones as well as glucose [89]. Another mechanism by which the ketogenic diet increases energy-dependent functions is an increase in metabolic capacity. Microarray analysis studies of animals on a ketogenic diet have shown an increase in the transcripts involved in mitochondrial respiration along with an increase in phosphocreatine and other energy metabolites [90,91]. However no significant changes in selected enzyme activities were found. Bough et al. [90] also found increased mitochondrial biogenesis in the hippocampus of animals placed on a ketogenic diet. These mechanisms are also seen in animal models of aging. Aged animals fed a ketogenic diet show an increased metabolic rate of acetoacetate and glucose as evidenced by dual tracer PET studies [92]. Balietti et al. [93] have shown that a medium-chain triglycerides supplemented ketogenic diet can counteract the aging-related decrease in mitochondria and increase mitochondrial metabolic efficiency.

Ketogenic diets have been shown to decrease brain oxidative stress in normal animal models by multiple mechanisms. One mechanism is the increase in endogenous antioxidants. For example, Jarret et al. [94] showed that a ketogenic diet increased mitochondrial levels of glutathione and lipoic acid (a thiol antioxidant). The neurons in this study also showed increased resistance to hydrogen peroxide-induced mtDNA damage [94]. One possible driver for these effects is increased nrf2 pathway activation [95]. More recently, BHB has been shown to increase global histone acetylation and increase oxidative stress resistance factors FOXO3A and MT2, targets of which include superoxide dismutase and catalase [96]. In rats, ketogenic diets have been shown to increase uncoupling proteins which decrease ROS production [97]. Ketogenic diets also show anti-apoptotic and other neuroprotective effects *in vivo*. Obese rats fed a ketogenic diet showed a down-regulation of pro-apoptotic caspase 3 mRNA, in addition to decreased oxidative stress [98]. Ketogenic diets prevent age-related morphologic changes in the outer molecular layer of the dentate gyrus in rats, but have detrimental changes in CA1 [99]. Most of these changes are hypothesized to preserve synaptic function and metabolic energy supply.

Another emerging neuroprotective mechanism of the ketogenic diet is an increase in kynurenic acid, which itself has neuroprotective, anti-excitoxic, and anti-inflammatory properties [100]. Ketogenic diets increase brain concentration of kynurenic acid, which has implicated neuroprotective effects through NMDAR and neprilysin [100,101]. The mechanism through which this happens is not yet well understood. There is an increase in the actions of kynurenine aminotransferases in cultured neurons with BHB but this has not been confirmed *in vivo* [102]. Increased cellular metabolism seems to increase kynurenic acid concentration and this could be the mechanism by which ketogenic diets increase kynurenic acid. This story parallels that of other neuroprotective amino acid metabolites such as lanthionine ketimine, and its bioavailable derivatives [103,104]. It is unknown if the ketogenic diet has effects on these systems as well.

It is well established that in AD there are reductions in mitochondrial enzyme activities involved in energy metabolism including cytochrome oxidase, pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase [5,17,18,105–107]. These reductions have been found not only in brain tissue but also platelets, muscle and fibroblasts of AD and MCI patients [5,108–110,57]. Therefore, these reductions in mitochondrial respiratory enzymes in peripheral tissues cannot be explained as consequences of amyloid/tau pathology in the AD brain. One of the main actions of ketones is that they provide an energy substrate that bypasses the defects in cytochrome oxidase, pyruvate dehydrogenase and other electron transport chain enzymes. However a reduction in alpha-ketoglutarate dehydrogenase would seem to reduce the ability of a cell to utilize ketones because they are utilized in the tricarboxylic acid cycle. But no studies thus far have shown this inability. To the contrary, it seems that the ketogenic diet upregulates enzymes involved in energy metabolism, increasing the cell's ability to utilize energy substrates [90]. Furthermore, addition of substances such as triheptanoin oil increases the efficacy of the ketogenic diet by providing tricarboxylic acid cycle intermediates [111]. This again supports the assertion that increasing metabolic energy capacity would be neuroprotective.

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4.2. Neurotherapeutic applications of physiological ketogenesis

Ketogenic diets may enhance mitochondrial energy-dependent brain functions in AD and in a wide range of other neurological disorders [85]. In human studies using patients with probable AD and MCI, ketogenic interventions have shown improvements in cognitive scores, such as verbal memory, ADAS-cog score, and paragraph recall score [86,112–114]. These improvements correlated positively with blood levels of BHB. In animal models of AD, ketogenic interventions have shown improvements in learning and memory [111,115,116], decreased measures of anxiety [115] and improved mitochondrial function [117]. Zilberter et al. [118] showed that supplementation with oxidative energy substrates including BHB reverses early neuronal hyperexcitability in *ex vivo* slices, reduced amyloid-beta-induced abnormal neuronal activity, as well as improved metabolic energy deficiency. Ketogenic diets have also been shown to decrease brain oxidative stress in the aged dog, which is a model for AD. Studzinski et al. [117] found lower levels of oxidative damage markers in the mitochondrial fraction of parietal lobe tissue in aged dogs. Ketogenic diets have other neuroprotective effects in models of AD. Multiple animal studies using ketogenic diets and ketone esters have shown decreases in the brain levels of amyloid precursor protein, tau, and β-amyloid [115–117,119]. However, some studies find no changes in levels of these proteins [120,121]. Zhang et al. [116] also found decreases in brain atrophy and a decrease in brain expression of ApoE and caspase 3.

Ketogenic diets have also been shown to enhance mitochondrial energy-dependent brain functions in other neurodegenerative diseases such as PD, HD and amyotrophic lateral sclerosis (ALS). A small pilot study of a ketogenic diet in PD patients showed an improvement in Unified Parkinson's Disease Rating Scores, with patients reporting improvement in symptoms including resting tremor, freezing, balance, gait, mood, and energy levels [122]. However placebo effects were not ruled out. In an animal model of ALS, a medium-chain triglycerides supplemented diet slowed the progression of weakness and decreased spinal cord motor neuron loss [123]. This study also showed an increased basal and maximal mitochondrial oxygen consumption rate, an indication of improvement in mitochondrial respiratory capacity [124]. A ketogenic diet in an animal model of HD showed improvement in working memory and delayed weight loss [125]. Infusion of BHB in an animal model of HD attenuated motor deficits, and extended lifespan [126]. The same study also found that the ketogenic diet reduced striatal lesions and microgliosis, and prevented histone deacetylation. Ketogenic diets decrease brain oxidative stress in other neurodegenerative disease models. In an animal model of PD a ketogenic diet promoted neuronal survival and increased levels of reduced glutathione [127]. Ketogenic diets show anti-apoptotic and other neuroprotective effects in other neurodegenerative diseases. Ketogenic diets in animal models of PD show improved neuronal and mitochondrial survival, decreased microglial activation and decreased inflammatory cytokines [127,128]. In an animal model of ALS, a ketogenic diet prevented the decrease in motor neuron count [123].

Ketogenic diets in animal models of traumatic brain injury (TBI) increase energy-dependent brain functions. In animal models of TBI, a ketogenic diet has shown beneficial motor effects in young animals, a restoration of brain ATP and increased brain levels of creatine and phosphocreatine [129,130]. An infusion of BHB in an animal model of TBI also restored brain ATP levels [131]. The ketogenic diet also appears to prevent apoptosis in animal models of TBI. A ketogenic diet reduced contusion volume [131,132] and brain edema [133,134]. In another animal model of TBI, a ketogenic diet increased mRNA and protein levels of BAX, an anti-apoptotic protein, and reduced release of cytochrome c [133,134].

Ketogenic diets have also been shown to increase energy-dependent brain functions in animal models of hypoxia/ischemia, glutamate toxicity, and multiple sclerosis (MS). In an animal model of hypoxia, BHB infusion maintained brain ATP levels and lowered lactate levels [135]. In an animal model of MS, a ketogenic diet has been shown to improve motor disability and spatial learning and memory [136]. Infusion of the ketone acetoacetate increased neuronal survival and increased brain levels of ATP in an animal model of glutamate toxicity [137]. Ketogenic diets have also been shown to reduce brain oxidative stress in animal models of glutamate toxicity and MS. BHB infusion in an animal model of glutamate toxicity showed increased neuronal survival and decreased lipid peroxidation [138]. In an animal model of MS, a ketogenic diet reversed hippocampal atrophy and periventricular lesions, decreased inflammatory cytokines and chemokines, and ROS production [136]. Ketogenic diets have also shown anti-apoptotic and other neuroprotective effects in animal models of hypoxia/ischemia and glutamate toxicity. Ketogenic diets and BHB infusion reduced the area of infarct in animal models of ischemia [139,140]. A ketogenic diet and BHB infusion upregulated HIF-1alpha and increased levels of the anti-apoptotic protein bcl-2 [139]. In a model of cardiac arrest-induced cerebral hypoxia/ischemia a ketogenic diet eliminated seizures, decreased myoclonic jerks, and completely prevented any neurodegenerative changes [141,142].

Together, these animal and human studies suggest that ketones can increase oxidative substrates, bypass mitochondrial respiration defects and upregulate mitochondrial biogenesis. Ketogenic interventions have the ability to reduce oxidative stress by decreasing the production of ROS, increasing cellular antioxidants, and upregulating stress response genes. Ketogenic diets prevent apoptosis by reducing pro-apoptotic proteins and increasing anti-apoptotic proteins. Ketogenic diets may also have many other pleiotropic neuroprotective effects such as the reduction of brain inflammation, which may be beneficial for the treatment of AD and a wide range of other neurological disorders.

5. Conclusion

We conclude that supporting and improving brain mitochondrial respiration constitutes a promising neurotherapeutic principle; and propose three different interventional approaches to improve brain mitochondrial respiration based on pharmacology (*e.g.* low-dose MB), photobiomodulation (*e.g.* transcranial LLLT) and nutrition (*e.g.* ketogenesis) interventions. The reviewed studies using these three specific interventions provide compelling evidence to suggest that redox-mediated bioenergetic improvement of brain mitochondrial respiration may be useful for further research and treatment of AD and other neuropsychological disorders. This targeted neurotherapeutic principle should be part of a more holistic treatment strategy that seeks to optimize (a) the context of the brain (*e.g.* aerobic exercise, rehabilitation, cognitive therapy), (b) the redox-energy equilibrium through increases of energy availability (*e.g.* cardiovascular risk factor reduction), and (c) improve mitochondrial respiration and reduce the pro-oxidant tendencies of neurobiological systems (*e.g.* low-dose MB, transcranial LLLT, ketogenic diet). Likewise, other exogenous or endogenous antioxidants and other ketogenic interventions such as medium-chain triglycerides or ketone esters should be therapeutic in a wide range of neurologic diseases because they can improve the energetic capacity of neural cells. The crossroads between pharmacological intervention with low-dose MB, modern photobiology with low-level lasers and LEDs, and nutritional ketogenic interventions may lead a new direction of bioenergetics research and treatment of AD and other neuropsychological disorders featuring cognitive impairment and neurodegeneration.

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