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Review

Nutritional and exercise-based interventions in the treatment of amyotrophic lateral sclerosis

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SUMMARY

Background & aims: Disease pathogenesis in amyotrophic lateral sclerosis (ALS) involves a number of interconnected mechanisms all resulting in the rapid deterioration of motor neurons. The main mechanisms include enhanced free radical production, protein misfolding, aberrant protein aggregation, excitotoxicity, mitochondrial dysfunction, neuroinflammation and apoptosis. The aim of this review is to assess the efficacy of using nutrition- and exercise-related interventions to improve disease outcomes in ALS.
Methods: Studies involving nutrition or exercise in human and animal models of ALS were reviewed.
Results: Treatments conducted in animal models of ALS have not consistently translated into beneficial results in clinical trials due to poor design, lack of power and short study duration, as well as differences in the genetic backgrounds, treatment dosages and disease pathology between animals and humans. However, vitamin E, folic acid, alpha lipoic acid, lyophilized red wine, coenzyme Q10, epigallocatechin gallate, *Ginkgo biloba*, melatonin, Cu chelators, and regular low and moderate intensity exercise, as well as treatments with catalase and L-carnitine, hold promise to mitigating the effects of ALS, whereas caloric restriction, malnutrition and high-intensity exercise are contraindicated in this disease model.
Conclusions: Improved nutritional status is of utmost importance in mitigating the detrimental effects of ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most frequent adult-onset motor neuron disease.[1] Approximately 85–90% of cases are sporadic (SALS), the remaining 10–5% categorized as familial (FALS). SALS and FALS are clinically and pathologically similar.[2] The pathological hallmarks of the disease are the selective and progressive atrophy of neurons in the corticospinal tract, swelling of perikarya and proximal axons and the presence of Bunina bodies, axonal spheroids and inclusions.[1,3] These pathological features result from the degeneration of upper motor neurons in the cerebral cortex and lower motor neurons in the brainstem and spinal cord. In the end stages, there is considerable loss of large myelinated fibres in the corticospinal tract and ventral roots.[4] Pulmonary muscles weaken leading to respiratory failure within 1–5 y of onset. However, when aggressive non-nutritional interventions such as percutaneous endoscopic gastrostomy (PEG) feeding and BiPAP non-invasive ventilation are initiated early in the course of the disease, survival can be improved.[5]

Separate molecular pathways may converge to elicit motor neuron death, and nutritional and exercise interventions have been

Abbreviations: ALA, alpha lipoic acid; BCAAs, branched chain amino acids; CAT, catalase; CoQ10, coenzyme Q10; CR, caloric restriction; DR, dietary restriction; EGCG, Epigallocatechin gallate; FA, folic acid; FFD, fast-food diet; GSH, glutathione; HFD, high-fat diet; KD, ketogenic diet; NAC, *N*-acetylcysteine; TCR, transient caloric restriction.

Non-standard abbreviations: SALS, sporadic ALS; FALS, familial ALS; mSOD1, mutant SOD1; WT, wild type; BBB, blood brain barrier; CAT, catalase; PUT-CAT, putrescine catalase; CR, caloric restriction; AL, ad libitum; DR, Dietary restriction; GS, grip strength; HED, human equivalent dose; HRQoL, Health-Related Quality of Life; SF-36, Short-Form 36-Item Health Survey; SEIQol, Schedule for the Evaluation of Individual Quality of Life-Direct Weighting; ALS FRS, ALS functional rating scale; GAE, Gallic acid equivalent; TCR, transient caloric restriction; KD, ketogenic diet; HFD, high-fat diet; FFD, fast-food diet; MUNE, motor unit number estimation; AUC, area under the curve; CD, chronic denervating process; SL, stride length; TRT, tight rope test; FIM, Functional Independent Mobility; RFT, Respiratory Function Test; MMT, Manual muscle testing.

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assessed in pre-clinical and clinical trials with the hope of slowing the deterioration of neurons. In this review, we present proposed mechanisms in the pathogenesis of ALS and nutritional and exercise-based therapies used to target these pathways.

2. Mutated SOD1

The most widely researched mutation leading to ALS is the cytosolic antioxidant enzyme Cu/Zn-SOD (SOD1).[6] 20–30% of FALS cases are caused by dominantly inherited mutations in the SOD1 gene.[6] The mutations occur outside the active site of the SOD1 enzyme, modify the stability of the protein backbone,[7,8] and lessen the enzyme's affinity to Zn.[9] G93A mice have an enhanced free radical-generating function, but a similar dismutation function, as compared with normal human SOD1.[10–12] This animal model displays the ALS phenotype even in the presence of normal SOD1,[13] suggesting that FALS mutations in SOD1 may operate through a toxic gain of function, as opposed to a loss of function.[8,13–16]

3. Proposed pathogenic mechanisms in ALS

3.1. Oxidative stress

This gain of function mutation may lead to oxidative damage through mutant SOD1's (mSOD1) ability to generate hydroxyl radicals ($^{\bullet}OH$).[17,18] FALS mutations in mSOD1 may increase the openness of the enzyme's active site,[7,19] and the accessibility of H_2O_2 and other oxidizable anionic substrates such as glutamate.[17,20] Wild type (WT) SOD1 can also function as a peroxidase, generating $^{\bullet}OH$ from the increased access of H_2O_2 to the active site of the enzyme.[21] H_2O_2 is reduced by interacting with copper ions (Cu^{2+}) released from mSOD1, leading to $^{\bullet}OH$ production.[22] The increase in mSOD1's free radical-generating function also promotes its inactivation, possibly leading to the release of Cu and Zn from the inactivated protein, further inducing damage.[20] Moreover, mSOD1 may use peroxynitrite ($ONOO^-$) as an enzyme substrate, instead of O_2^- or H_2O_2.[23] $ONOO^-$ is produced nonenzymatically by the reaction between O_2^- and nitric oxide (NO), which occurs at a greater rate than that of dismutation by SOD1.[20] Also, mSOD1 catalyzes the reaction between $ONOO^-$ and tyrosine residues, promoted by the depletion of Zn from the inactived SOD1.[20] Increased 3-nitrotyrosine levels, a product of this reaction, is evident in the spinal cords of two transgenic mouse lines expressing the G37R SOD1 mutation.[24]

3.2. Protein misfolding and aggregation

When SOD1 is demetallated, its protein dimers destabilize and tend to form hydrophobic monomers,[25] which may self-aggregate.[26] Free Cu ions released from misfolded proteins increase the cellular concentration of Cu and lead to toxicity.[27] Free cupric ions can be reduced by O_2^- to form cuprous ions, then react with H_2O_2 to produce $^{\bullet}OH$.[28] Additionally, mSOD1 has a decreased affinity for Zn.[9] Loss of Zn induces the disorganization of the Zn-binding loops, which protect against self-aggregation.[29] Deficiencies in Zn lead to a toxic SOD1 protein that is able to incite antioxidants, like ascorbate, to form O_2^-.[30] Protein misfolding may then lead to protein accumulation, alterations in axonal transport and mitochondrial/proteasome dysfunctions. These events may indirectly induce free radical generation and activation of the apoptotic pathway.[31–33]

3.3. Skeletal muscle dysfunction

Skeletal muscle dysfunction in G93A and G86R mice was reported before disease onset.[34–37] Elevated uncoupling protein 3 (UCP3) expression in skeletal muscle of SALS patients and G86R mice coincide with a decrease in ATP levels in the mitochondria.[35] UCPs mediate controlled proton leak from the intermembrane space into the mitochondrial matrix, reducing the electrochemical membrane potential within the intermembrane space.[38] An increase in the membrane potential elevates free radical production through the transfer of electrons from complexes of the electron transport chain (ETC) to O_2. UCP3 is upregulated in response to O_2^- as a protective response to increased oxidative stress.[35]

3.4. Glutamate excitotoxicity

Classical excitotoxicity occurs when an increase in glutamate (GLU) concentration ($\sim$2–5 μM) leads to neuronal degeneration.[39] Continuous stimulation of GLU receptors, increases in GLU release into the cleft or insufficient re-uptake by the EAAT2/GLT1 transporters lead to an increased intracellular concentration of Na^+ and Ca^{2+}, resulting in neuronal death. Decreased levels of EAAT2/GLT1 have been identified in some post-mortem ALS-afflicted brains, signifying a selective loss of function.[40] As well, GLU levels are elevated in the cerebrospinal fluid (CSF) of ALS patients.[41,42] *Slow onset excitotoxicity* occurs when damaged postsynaptic neurons slowly die in the presence of normal synaptic GLU levels,[43] possibly caused by an increased sensitivity of the GLU receptor to GLU stimulation, which may be induced by structural changes in the receptors or mitochondrial dysfunction.[43] Mitochondrial defects due to the colocalization of mSOD1 affect Ca^{2+} buffering capacity and may lead to increases in cytoplasmic Ca^{2+} levels.[44] Ca^{2+} influx through NMDA receptors, AMPA receptors or voltage-gated Ca^{2+} channels mediate neuronal injury[45] by activating several enzymes, such as lipases, phospolipases, proteases, endonucleases and NO synthase, as well as exacerbating free radical production.[43] Moreover, mSOD1 may increase the sensitivity of motor neurons to GLU, while stimulation of GLU receptors may induce mSOD1 to generate more radicals.[46]

3.5. Mitochondrial dysfunction

A prominent pathological feature in FALS mice is the distortion of mitochondria, including morphological abnormalities and vacuolar degeneration in the spinal cord.[47,48] mSOD1 colocalizes with the mitochondrial intermembrane space and matrix to directly induce damage,[10,11,49] including mitochondrial fragmentation,[50] and can lead to the inhibition of protein import channels[51] or the diminution of Bcl-2, an anti-apoptotic protein.[52] The mSOD1 in mitochondria may trigger the release of cytochrome *c*, a critical intermediate during apoptotis.[53]

3.6. Neuroinflammation and apoptosis

Activation of non-neuronal cells (e.g. glial cells) may orchestrate cell death through the production of inflammatory cytokines, microglial proliferation and neurotoxicity.[54] TNF-α, a proinflammatory cytokine may elicit apoptosis[55] and contribute to neurotoxic stress through the activation of microglial cells.[56] TNF-α is upregulated in G93A spinal cord,[57] and its expression coincides with disease progression.[56] Circulating TNF-α and its receptors are increased in the serum of ALS patients.[58] Increased TNF-α, as well as microglial proliferation,[56] suggests that neuroinflammation may play a role in the pathogenesis of ALS, leading to apoptosis.[59]

Many apoptotic features (cell shrinkage, condensing of the cytoplasm and nucleus, DNA fragmentation) are evident in post-mortem spinal cords and motor cortex from ALS patients.[3,60]

In ALS, apoptosis can be initiated by activation of cell-surface receptors of the TNF-family (extrinsic pathway), cytochrome *c* release from the mitochondria (intrinsic pathway) and stress to the endoplasmic reticulum (ER).[3] More than one mechanism most likely contributes to motor neuron death through activation of the apoptotic pathway (Fig. 1). As the complexity of the disease and its multiple mechanisms make it difficult to establish an effective treatment when targeting one mechanism, a combination of treatments or a cocktail will help target separate pathways at once.

4. Potential therapeutic interventions in ALS

The following section highlights the dietary and physical activity interventions conducted in animal models and in clinical trials, with an analysis of why some interventions may have failed and which treatments warrant further investigation. To do that, we conducted literature searches in Medline and Scopus, using the following keywords, ALS, SOD1, G93A mice, nutrition, exercise, antioxidants, caloric restriction, dietary restriction, diet, motor neuron disease, enzymes, oxidative stress, mitochondrial dysfunction and interventions.

5. Therapies targeting oxidative stress

5.1. Vitamin E (alpha-tocopherol)

Vitamin E (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) is a lipid soluble antioxidant, essential in protecting biomembranes against lipid peroxidation.[61] However, it does not cross the blood brain barrier (BBB) readily.[62] An age-dependent increase in vitamin E in the brain and spinal cord is absent in G93A mice.[63] Supplementation with vitamin E delayed disease onset, but had no effect on survival in G93A mice.[63] In ALS patients, vitamin E and Riluzole had no effect on the rate of motor function deterioration or survival versus patients supplemented with placebo and Riluzole.[64] Another ALS clinical study found that a high dose of vitamin E plus Riluzole showed a trend for a lower vital capacity versus placebo-supplemented patients, despite less patients on vitamin E requiring intermittent assisted ventilation.[65] An observational study showed no improvement in quality of life scores between ALS patients supplemented with vitamin E plus Riluzole or Riluzole separately.[66] Clinical trials in humans reveal that the inability of this antioxidant to extend lifespan may be due to its limited capacity to cross the BBB and thus sufficiently increase human brain vitamin E levels.[65] (Please refer to the Discussion section for a more detailed discussion of the limitations inherent in human clinical studies.)

Despite these negative results, one epidemiological study analyzing individuals who regularly consume vitamin E showed that habitual use was associated with a lower risk of ALS.[61] A separate case–control study discovered that a high intake of vitamin E (>22 mg/day versus <18 mg/day; RDA is 15 mg/day) was associated with a 50% reduction in the risk of developing ALS in patients who had definite, probable or possible ALS.[67] Since there is no clear contraindication, and vitamin E is readily available, it is still widely prescribed for ALS patients.

5.2. Vitamin C (ascorbate)

See under Copper chelators alongside trientine.

5.3. Folic acid and vitamin B12

The demethylation of methionine generates the cytotoxic amino acid homocysteine (Hcy).[68] Elevated levels of Hcy are observed in the plasma of ALS patients and in patients who had a shorter onset-diagnosis interval (i.e. fast progressors).[69] Folate and vitamin B12 (B12) (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) remethylate Hcy to methionine and help to maintain low levels of Hcy.[70] Mice administered FA or FA + B12, exhibited a significant delay in the onset of symptoms and in disease onset, compared to control SOD1 mice. No significant delay in either outcome measure was observed in the B12

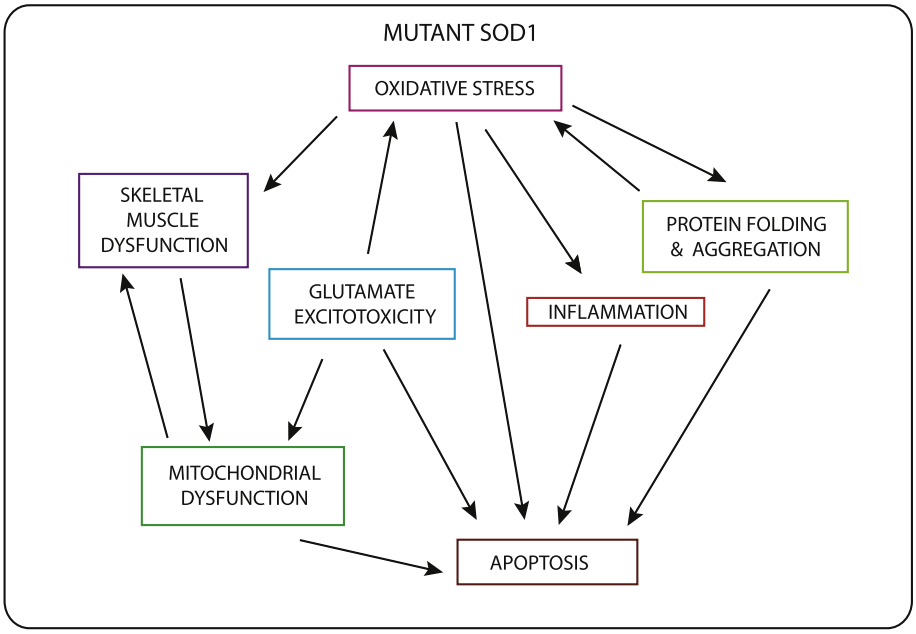

Fig. 1. A summary showing the interconnectivity of mechanisms in ALS, and their relationship to mutant SOD1. This diagram illustrates how more than one pathway is most likely involved in the pathogenesis of ALS. Certain mechanisms, such as oxidative stress, are linked to other mechanisms, and these multiple associations lend to the complexity of the disease pathology.

only group, possibly due to its partial role in the methylation process in comparison to FA.[71] Both treatments with FA elicited a decrease in plasma Hcy levels, signifying the need for FA in the attenuation of this cytotoxic amino acid.[72] Oral administration of FA and FA + B12 extended disease onset and survival through decreases in neuronal death, while preventing apoptosis and neuroinflammation, thus making it a potential therapeutic agent in a clinical trial.

5.4. Enzymes

Catalase (CAT) (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) functions to reduce H_2O_2 into H_2O[73] and prevent the formation of NO.[74] Putrescine-modified catalase (PUT-CAT) (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) readily crosses the BBB[75] and maintains its enzyme activity in the spinal cord of rats when delivered to the CNS.[76] PUT (1,4-diaminobutane) is a polyamine that is attached to the carboxylic acid groups of CAT with a covalent bond.[77] PUT-CAT and CAT delayed disease onset in high and low copy G93A mice, with PUT-CAT displaying a more pronounced effect. However, there was no significant extension in lifespan in the high-copy mice treated with PUT-CAT or CAT, but a trend towards a 10 d extension in the low copy group with PUT-CAT.[77] The treatment may not have extended survival in the high-copy mice due to the smaller time frame and rapid progression of the disease in this model (average lifespan of 130 d). Furthermore, the greater benefit of PUT-CAT to that of CAT is most likely due to its greater BBB permeability and the ability to deliver it systemically.[75,76] Follow-up analysis examined whether subcutaneous osmotic pump delivery of PUT-CAT in low copy mice may have a greater effect on survival.[78] Treatment with PUT-CAT delayed disease onset and extended survival. Therefore, the enzymatic activity of PUT-CAT and CAT at depleting H_2O_2 and catalyzing NO breakdown is essential to disease pathogenesis/progression and makes it a good candidate for treating ALS.

Another enzyme which reduces H_2O_2 into H_2O is glutathione peroxidase (GPx) (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf).[73] Since GPx uses reduced glutathione (GSH) as a substrate, increasing amounts of this substrate may increase GPx's function.[79] Although GSH can readily cross the BBB,[80] it failed to significantly delay disease progression in ALS patients.[79] However, the trial was only 6 months long and the dosage was based on its efficacy in treating hepatic intoxications.[79] While CAT seemed promising, GPx did not prove beneficial in this one clinical trial. Further investigation is needed to elucidate the impact of GSH supplementation on ALS pathology.

5.5. N-acetylcysteine (NAC)

NAC (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) is a precursor for GSH and has a similar action to GPx in that it acts as an antioxidant.[81] The use of NAC is clinically safe and can readily pass the BBB.[82] However NAC did not delay disease onset or death in low copy G93A mice when this intervention was initiated at 120 d, well before clinical onset.[83] In contrast, high-copy G93A mice revealed that NAC in drinking water at a similar dosage to the previous study significantly increased survival compared to controls and preserved motor performance.[84] In ALS patients, a subcutaneous injection of NAC for 12 months revealed that disease progression was similar between treatment and control groups.[82] Despite evidence that free radical damage is essential in ALS pathogenesis, human studies failed to show a strong association between NAC and an increase in survival.[82]

5.6. Alpha lipoic acid

Alpha lipoic acid (ALA) (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) is reduced in the mitochondria to form dihydrolipoic acid (DHLA).[85] Administration of ALA to aging rats reduces lipid peroxidation and enhances antioxidant function.[86] ALA-treated G93A mice had improved rotarod performance (a measure of motor performance), less weight loss and increased survival compared to unsupplemented G93A mice.[87] No clinical trials have been conducted in ALS patients to date, although a 600 mg/day dose of ALA in healthy humans decreased markers of oxidative stress, including urinary isoprostanes.[88] This potent antioxidant may provide a benefit in the treatment of ALS.

5.7. Lyophilized red wine

Lyophilized red wine (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) is an antioxidant-rich extract that is proposed to block glutamate-induced apoptosis in neurons.[89] There was a significant extension in lifespan in G93A mice treated with red wine compared to unsupplemented G93A mice.[90] The experiment was repeated with the treatment commencing from age 30–40 d instead of the more variable time frame used in the previous study. In this experiment, red wine significantly increased mean survival time.[89] The authors postulated that red wine may prevent apoptosis by inhibiting caspase 3 activity,[89,91] thus exhibiting an anti-apoptotic as well as an antioxidant effect.

5.8. Coenzyme Q10

Coenzyme Q10 (CoQ10) (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) is a mobile carrier located in the inner mitochondrial membrane and transfers electrons from complexes 1 and 2 to complex 3 of the ETC.[38] CoQ10 readily crosses the BBB,[92] and is a free radical scavenger in mitochondrial and lipid membranes.[93] CoQ10 levels diminish with age in both human and rat tissues.[94] G93A mice supplemented with CoQ10 resulted in a significant extension in lifespan.[95] Doses as high as 3000 mg/day were well-tolerated in ALS patients treated for 8 months[92] and a phase II clinical trial using CoQ10 has been completed and the results are awaiting publication (NCT00243932). The evidence that CoQ10 functions to decrease oxidative stress and can defend against glutamate excitotoxicity in cerebellar neurons[96] lends support to the usefulness of this intervention in ALS.

5.9. Epigallocatechin gallate (EGCG)

EGCG (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf), an active constituent of green tea, has been known to have anti-apoptotic, anti-cancer, anti-mutagenic and anti-neurodegenerative effects.[97] Green tea consumption may enhance the activity of enzymes involved in antioxidant defense and carbohydrate metabolism.[98] Dosages of 2.9 and 5.8 μg/g of body weight significantly delayed disease onset, rotarod failure and endpoint.[97] The duration from disease onset to endpoint was also prolonged in supplemented versus unsupplemented G93A mice.[97] In a subsequent study, G93A mice supplemented with a higher dose of EGCG displayed a delay in disease onset, an extension in lifespan, an improvement in motor performance and a reduction in markers of inflammation and apoptosis as compared to unsupplemented G93A mice.[99] Evidently, presymptomatic treatment with EGCG could be a potential neuroprotective strategy for humans with ALS.

5.10. Ginseng

Ginsenosides, steroid-like compounds in ginseng, are proposed to be the active ingredients behind the efficacy of the herb.[100] Ginseng (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) had a protective effect in transient forebrain ischemia, possibly through antioxidant and ·OH scavenging activity.[101,102] Ginseng may be able to alter NO release or synthesis,[103] and reduce Ca^{2+} influx into neurons.[104] In one study, 40 mg/kg and 80 mg/kg of crude American ginseng powder significantly prolonged disease onset and extended survival in G93A mice.[100] However, it is not certain that the effects of ginseng are neuroprotective, and the authors explained that ginseng may have provoked a symptomatic effect.[100] Also, the results involve only one type of ginseng and do not allow us to generalize the advantages of this herbal remedy. Although Jiang et al. presented a novel finding, further research is needed to clarify the possible protective role of ginseng in treating ALS patients.

5.11. Ginkgo biloba

EGb761 is an extract of green *G. biloba* (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) leaves and is both a platelet activating factor[105] and a NO scavenger.[106] EGb761 exhibits neuroprotective effects against mitochondrial dysfunction and oxidative stress.[107] EGb761 supplemented male G93A mice showed a significant extension in lifespan as compared to control G93A male mice,[108] but was not able to significantly increase survival in females. Despite this sexual dimorphism, the neuroprotective properties associated with this extract appear to be beneficial in decreasing oxidative damage within this model.

5.12. Tomato carotenoids

Tomatoes are comprised of a number of potent antioxidants, including carotenoids (including lycopene), polyphenols and vitamins (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf).[109] Lyophilized tomato powder had no impact on survival or disease onset in G93A mice.[109] Given that clinical onset was defined as the first day the mouse could not stay on the rotarod for 5 min at 20 rpm, there may have been a discrepancy in determining this measure between the treatment and control groups, since other studies have presented alternate ways in which to characterize disease onset.[100,110,111]

5.13. L-Carnitine

L-Carnitine (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) transports long-chain fatty acids from the cytosol to the mitochondrial matrix during beta-oxidation[112] and inhibits mitochondrial-dependent apoptosis and oxidative stress-induced mitochondrial damage.[113–115] Due to the increase in free radical production and elevation in lipid peroxidation in ALS, free fatty acid levels increase[116] and can induce the release of cytochrome *c* or activation of caspases.[117] Treatment with L-carnitine significantly delayed disease onset and progression and also suppressed hind limb muscle and spinal cord injuries, while decreasing markers of oxidative stress in male G93A.[118] L-carnitine prevented apoptosis in muscle and extended lifespan. In a second experiment, treatment with L-carnitine in low copy G93A mice increased lifespan in both males and females. L-carnitine levels were decreased in non-supplemented G93A mice, but when given this intervention, this decrease was prevented.[118] Reductions in L-carnitine may be implicated in heightened lipid peroxidation in ALS, and further research into this treatment would clarify its potential role as a treatment in this disease.

5.14. Genistein

Choi et al. reported that female sex hormones play a strong role in extending lifespan in G93A mice, with 17beta-estradiol moderating disease progression in ovariectomized females.[119] Genistein (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) is a phytoestrogen displaying mild estrogenic effects,[120–122] which may include neuroprotection in conditions of oxidative stress.[123] Treatment with genistein delayed clinical onset and extended survival in male, but not female, G93A mice.[123] The lack of protection observed in treated female G93A mice suggests that supraphysiological amounts of this estrogen do not impart any further benefit.[123]

5.15. Melatonin

Melatonin (Table 1 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) may have potential in the treatment of ALS due to its antioxidant properties and ability to readily cross the BBB.[124] Melatonin is derived from the amino acid tryptophan and plays a role in scavenging free radicals, while stimulating GPx and suppressing NO synthase.[125] It can interfere with NO metabolism and consequently protect against $ONOO^-$-induced damage and Ca^{2+}-dependent excitoxicity.[125,126] High dose melatonin produced a significant extension in lifespan and lengthened disease progression in G93A mice.[127] Although no clinical trial has been conducted in ALS patients to date, 300 mg of melatonin/d was well-tolerated and produced a significant reduction in protein carbonyls (a marker of oxidative damage to protein) in patients at 4 months versus at baseline (before treatment).[127] These positive findings of melatonin as a potential candidate justify conducting an intervention study in the treatment of ALS.

5.16. Caloric restriction

Caloric restriction (CR) (Table 2 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf), without nutrient deprivation prolongs lifespan due to a decrease in oxidative stress.[128–132] Tissues in aged mice reveal diminished antioxidant activity, and CR has been shown to maintain the function of antioxidant enzymes.[133] Furthermore, metabolic rate has been associated with oxidative stress.[134] However, it is still debatable as to whether a reduction in metabolic rate has a role in extending lifespan through CR, independent of a reduction in oxidative stress.[132,135,136] Despite the life-extending benefits observed with CR, malnutrition in ALS patients is indicative of reduced survival and has been implicated in ALS due to difficulties in mastication, prolonged meal times and dysphagia.[137] Although a reduction in lean body mass is associated with malnutrition, ALS patients exhibit hypermetabolism and an increase in energy expenditure.[138] Malnutrition aggravates respiratory muscles and an inadequate diet may lead to a weakened immune response and infection.[139] A study examining the diets of ALS patients discovered that 70% had dietary intakes below what is normally recommended and 25% had a 10% reduction in body weight.[140] Rio and Cawadias recommended that patients adopt a high energy/high-fat diet at the time of diagnosis to counteract the repercussions of malnutrition.[141]

Evidence that malnutrition is a predictor of death in ALS patients coincides with the results observed in G93A mice. In contrast to the extension in lifespan and delay in disease onset observed across phyla, CR exacerbated disease onset in this animal model.[110] When G93A mice assigned to the CR group were fed a diet

equal to 60% of the average ad libitum diet (AL) of the G93A control group, they reached clinical onset 10% faster versus AL.[110] These findings suggest that decreases in mitochondrial oxidant production due to CR fail to counteract the increase in free radicals in this animal model.[110] It is probable that the majority of free radical production stems from mSOD1, an enzyme not responsive to changes in diet.[130] In a subsequent study, transient CR (TCR) (Table 2 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) in G93A mice was found to have an adverse effect in males only, suggesting that even short-term CR is detrimental, especially in male ALS rodents.[142] Intermittent dietary restriction (Table 2 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) (DR, 60–70% of AL) shortened disease progression in low copy G93A mice.[143] However, there was no significant difference in clinical onset between the DR and AL groups, despite DR mice reaching clinical onset 33 d sooner than the AL mice (a 12% difference).[143] The lack of significance may be attributed to a type 2 error, whereby Hamadeh et al. calculated statistical power to be less than 50%.[110] In addition to this, the intermittent feeding and starvation regimen may have increased oxidative stress, an effect similar to what is observed with ischemia-reperfusion.[144–146] Nevertheless, the outcomes of these three studies suggest that a calorically dense diet may be more beneficial. Mice fed a diet rich in ketones (Table 2 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf), which promote mitochondrial energy production and membrane stabilization, had improved motor performance and altered mitochondrial function.[147] Similarly, G86R mice administered a high-fat diet (Table 2 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) had an extension in mean survival compared to control-fed mice.[148] In G93A mice, a high-fat/high sugar fast-food diet (Table 2 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) extended both disease onset and survival.[149] The results of these studies indicate that increases in energy intake prevent the energy deficit observed in mSOD1 models.[148] Nutritional status in ALS patients is predictive of survival,[150] thus adequate energy intake should be maintained in this population and higher calorie diets may in fact help retard the progression of disease.

6. Therapies which stabilize mutant SOD1

6.1. Trientine and ascorbate

Triethylenetetramine dihydrochloride (trientine; a Cu chelator) (Table 3 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) decreases the formation of $^{\bullet}OH$ from reduced cupric ions,[151] while the antioxidant ascorbate (Table 3 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) scavenges free radicals in the CNS.[152] Cu chelators may inhibit the peroxidase activity of mSOD1 and possibly modulate its toxic gain of function.[153] The combination of trientine and ascorbate delayed disease onset, as well as endpoint, indicating that a reduction in Cu-mediated toxicity suppresses the degeneration of motor neurons in G93A mice.[28] A follow-up study by the same authors compared differences in survival between mice on a high dose of trientine and ascorbate separately, a low dose of trientine or a combination of the two.[154] The combined treatment group had less motor function deterioration compared to the control group. The combination of trientine/ascorbate or separate treatments administered after disease onset caused a significant increase in survival.[154] Another study in G93A mice revealed that trientine improved motor performance and increased survival.[87] Further research into the efficacy of Cu chelators is essential in determining the future role of this intervention in the treatment of ALS.

6.2. Zinc supplementation

Zn (Table 3 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) is connected with most of the major pathologies associated with the development of ALS, including oxidative stress, excitotoxicity and apoptosis.[29] Zn supplementation may act to increase the binding of Zn to the SOD1 enzyme.[155] A control group of G93A mice on a Zn-deficient diet died earlier than G93A mice supplemented with Zn.[155] A previous study used much higher amounts of zinc sulfate, resulting in decreased survival in G93A mice.[156] However, these amounts were 100–500 times in excess of the daily requirements for Zn in rodents.[155] As little as 18 mg/kg/day in mice on a Zn-deficient diet was enough to increase death in G93A mice.[155] Exceeding normal intakes of Zn can inhibit the absorption of copper, which is needed to prevent anemia.[157] These data suggest that moderate Zn supplementation may prevent the loss of Zn from the mSOD1 protein in ALS, and slow disease progression.[155] However, if Zn deficiency is rare in normally-fed mice, then will Zn supplementation prolong lifespan as compared to control-fed mice? Ermilova et al. proposed that the presence of mSOD1 made mice more susceptible to Zn deficiency compared to non-transgenic mice on a Zn-deficient diet. Thus, Zn supplementation may have compensated for the decrease in Zn concentrations observed in G93A mice and that further supplementation to elevate concentrations beyond what is found in normal mice has not been shown to be beneficial. Indeed, significantly increasing Zn concentrations higher than normal is detrimental.[155]

7. Therapies targeting mitochondrial dysfunction

7.1. Creatine

Mitochondrial dysfunction may lead to a decrease in the production of ATP and may be a factor in motor neuron death.[158] Creatine (Table 4 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) stabilizes mitochondrial creatine kinase, thus preventing the mitochondrial transition pore from opening, a process linked to excitotoxicity and apoptosis.[159,160] Phosphocreatine (PCr) may also serve as an energy source for glutamate uptake into synaptic vesicles.[161] It is also possible that a decrease in cellular glutamate levels may explain creatine's role in stimulating brain metabolism.[162]

Oral creatine supplementation extended lifespan in G93A mice and significantly improved motor performance compared to control G93A mice.[158] A similar extension in lifespan was observed in another study.[163] In contrast, creatine supplementation in G93A mice had no beneficial effect on motor performance, grip strength (GS), muscle ATP content and glycogen content.[164] In another study, both creatine and creatine plus Riluzole delayed disease onset in G93A mice by ~12 d compared to controls,[165] illustrating that creatine plus Riluzole does not have a synergistic effect in delaying symptom onset further than creatine alone.

One of the characteristics of ALS is muscle weakness and motor neuron loss, and creatine may help improve muscle strength.[166] Some ALS patients supplemented with 20 g/d of creatine showed a significant improvement in isometric muscle strength after 7 d compared to pre-treatment values.[167] After the 7 d loading phase, subjects participated in a 6-month follow-up involving a smaller dosage of creatine (3 g/d). Although the results seemed promising, the benefits of creatine supplementation were only temporary, with a 6-month follow-up showing a decline in all tested parameters. A 2003 study administered powdered creatine monohydrate or a placebo to ALS patients.[166] However, there was no significantly beneficial effect on survival, disease progression or vital capacity.[166] The authors attributed the positive effects observed in G93A mice

and a lack of effect observed in humans to a possible species-specific role that mitochondria exhibit in ALS pathogenesis.[166] Another difference between the benefit observed in animals [158,163,165] compared to this clinical trial in humans[166] is in the initiation of the intervention. Creatine was administered before disease onset in mice, but approximately 500 d (16.7 months) after symptom onset in humans.[166] Another confounding difference between the Mazzini et al. and Groeneveld et al. studies is the dosage of creatine used and the time frame. The Mazzini study used a larger dosage (20 g/d) for only 7 d, with a 6-month follow-up of 3 g/d, and showed an improvement in muscle strength only after the 7 d loading phase, whereas the Groeneveld study used 10 g/d for 16 months and reported no benefit. These results indicate that perhaps the dosage used in the Groeneveld study may have been too low to detect an improvement.[166,167] Furthermore, the mean duration of the disease prior to the start of the study was longer in the subjects in the Mazzini paper compared to the subjects in the Groeneveld paper. The improvement with 20 g of creatine/d was observed in subjects with a higher mean duration of disease (Mazzini, 22.5 months; versus Groeneveld, 16.7 months), adding further support that a larger dosage produces a more beneficial effect in ALS patients.

A 2004 study did not show significant results in motor performance indices from creatine supplementation.[168] The authors proposed that since the study was powered only to detect a 50% or greater change in the rate of disease progression, the failure to show a significant positive effect may restrict the clinical significance of using this intervention.[168] Another issue raised in this study is that some patients in the control group failed to adhere to the experimental protocol due to a 4-fold increase in urinary creatine concentrations after 3 months.[168] This noncompliance may be a potential reason why there was no statistically significant difference between the experimental and treatments groups even when the noncompliant subjects were excluded from the statistical analysis, the low sample size presenting a potential type 2 error.[168]

Similarly, a recent 2008 study showed that there was no improvement in strength, respiratory function and quality of life scores, and no reduction in fatigue with creatine supplementation in ALS patients.[169] However, based on their data and using their values, for an $\alpha = 0.05$, our calculations showed they had less than 50% power to detect significant differences given the changes they observed. Based on these potential sources of error, it still may be of use to perform a randomized controlled trial (RCT) of creatine supplementation in ALS patients, paying specific attention to power calculations, length of supplementation, dosage, time of initiation of supplementation after disease onset and patient compliance.

7.2. Pyruvate

Pyruvate (Table 4 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) is an important biochemical energy substrate [170] with neuroprotective and anti-apotoptic properties.[171–173] Although there was no significant difference in disease onset between treatment and control groups, pyruvate extended survival in G93A mice.[174] A lack of difference in disease onset may be due to the treatment start time, infrequent injections (weekly) and definition of clinical onset using the rotarod test.

A follow-up study with pyruvate failed to significantly extend lifespan in this animal model, nor did it affect disease onset or motor performance.[109] One possible reason may be the more frequent and larger dose used in the study by Esposito et al., since high concentrations of pyruvate (5 mM) have been shown to revert this antioxidant into a potential pro-oxidant.[175] Another explanation may be the differences in the genetic background of the mice used in the two studies, with Park and colleagues using the B6SJLF1/J hybrid females and Esposito and colleagues using the C57BL/6 strain. The C57Bl/6J strain has a significantly longer mean survival than the B6/SJL strain, with no gender difference in survival.[176] Thus each strain may exhibit varying degrees of neuronal degradation, and this genetic difference plays a role in how a certain treatment may impart beneficial effects. Just as genetic variability within humans may determine the efficacy of an intervention, it is imperative to distinguish the susceptibility of certain strains of animal models of ALS to the treatment used and make note of these differences for future clinical studies.

8. Antiglutamatergic agents

8.1. Branched chain amino acids (L-leucine, L-valine, L-isoleucine) and L-threonine

Glutamate is partially metabolized by the enzyme glutamate dehydrogenase (GDH).[177] The branched chain amino acids (BCAAs) L-leucine, L-valine and L-isoleucine can activate GDH *in vitro* (Table 5 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf).[178] In the first RCT, BCAAs helped maintain muscle strength and the ability to walk in ALS patients.[179] However, two subsequent studies did not find favourable results with BCAAs.[180,181] Two studies analyzed the effect of BCAAs on glutamate concentrations in ALS patients, as opposed to clinical outcome measures.[177,182] Bastone et al. found that glutamic acid concentrations were elevated with BCAA treatment, while Gredal and Moller (1995) did not find an effect of BCAAs on glutamate metabolism in their study. Moreover, a double-blind RCT by Tandan et al. used BCAAs as well as L-threonine (Table 5 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf), an antiglutamatergic amino acid which is proposed to increase concentrations of glycine, an inhibitory neurotransmitter.[183] In one treatment group, ALS patients were administered L-leucine, L-isoleucine and L-valine powder daily.[184] In the other treatment group, patients received L-threonine powder and pyridoxyl-5-phosphate (P-5-P, which is involved in the conversion of L-threonine to glycine) daily.[184] Force vital capacity (FVC) was greater in the BCAA and L-threonine groups versus control, while body weight in the BCAA group was slightly increased compared to the L-threonine and control groups. Subsequent studies examing L-threonine supplementation in ALS patients found no significant change in the decline of clinical assessment score (as assessed by the Norris score) between treated and untreated patients.[185,186] Thus, BCAA and L-threonine do not seem worthy of further investigations in the treatment of ALS.

8.2. Magnesium

The magnesium ion (Mg) (Table 5 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) has been identified as a calcium antagonist/blocker through its ability to inhibit NMDA receptors,[187] as well as calcium channels,[188] thus reducing both calcium influx and, in turn, glutamate release.[189] There was no statistical difference between the onset of weakness in the Mg group compared to the control group, or in extending survival in G93A mice.[190] The authors proposed that concentrations of this ion may not have attained sufficient levels to properly inhibit current flow through the NMDA receptors.[190] As such, the researchers did not detect any significant increase in Mg levels in the CNS in accordance with the varying doses.[190] Another possible reason is that while NMDA receptors may have been blocked, Mg had no effect on AMPA receptors. Other studies have implicated calcium-permeable AMPA receptors as the primary source for motor neuron loss in ALS and that NMDA receptors were not predominantly involved in ALS glutamate excitotoxicity.[191,192] Thus, therapeutic involvement of this ion is not validated for use as a treatment in ALS patients.

9. Antioxidant, amino acid and mineral cocktail

9.1. Alsamin

Alsamin (Table 6 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) is a cocktail containing vitamin E, selenium, beta-carotene, L-arginine, L-methionine, L-leucine, L-isoleucine, L-valine and the pharmacological Ca^{2+} channel inhibitor nimodipine.[193] In a double-blind study, SALS patients were assigned either 1) Alsamin, 2) a cocktail of selenium, vitamin E and beta-carotene, 3) a cocktail of amino acids, 4) nimodipine or 5) a placebo.[193] The combination of antioxidants, selenium, amino acids and nimodipine (Alsamin group) enhanced the activity of GPx.[193] For the secondary outcome measure, the Alsamin group was reported to have some improvement in Norris score compared to the other groups, yet no statistical analysis was performed.[194] This study shows that selenium, which is required for the reduction of GPx, in combination with antioxidants and antiglutamatergic agents, enhances the activity of GPx and consequently antioxidant capacity. This clinical trial may provide insight into the efficacy of using a cocktail in nutritional interventions and to combat disease progression in ALS.

10. Physical activity

Physical activity (Table 7 http://www.yorku.ca/hamadeh/CLINNUTRTABLES2009.pdf) as an intervention in ALS has been controversial.[195,196] Previous epidemiological studies suggest that a heavily active lifestyle involving vigorous exercise and reduced body fat are associated with an increased incidence of ALS.[196,197] Vigorous exercise may amplify excitotoxic effects leading to increased Ca loading in motor neurons and subsequent death.[198] Alternatively, an increased production of free radicals via exercise may mediate damage at the molecular level.[199] Exercising muscle in ALS patients showed dysfunctional oxidative metabolism[200] and increased lipid peroxidation.[201] Despite this, exercise alters the balance between free radical production and radical scavenging systems.[202] Endurance exercise training induces adaptations by increasing the capacity of major antioxidant enzymes,[203] reducing oxidative stress following an acute bout of exercise[204] and increasing mitochondrial capacity in skeletal muscle.[205] Thus, regular exercise training may buffer energy deficits induced by mitochondrial dysfunction in ALS.[206]

Furthermore, exercise has neuroprotective effects, helping to alleviate motor deficit [207,208] and enhancing the formation of new neurons.[209] Exercise may elicit this neuroprotection via the upregulation of the neurotrophic hormone insulin-like growth factor (IGF-1).[210] A gene therapy approach which involved expressing IGF-1 led to significantly prolonged survival by 23% in G93A mice.[211] A combination of IGF-1 gene delivery and exercise had a synergistic effect on survival and function in G93A mice.[212]

Regular exercise extended lifespan in G93A males,[213] while another study found that exercise delayed disease onset in low copy female mSOD1 mice only.[214] The observed sexual differences between the two studies suggest the involvement of female and male hormones, which are neuroprotective during exercise.[213,214] Additionally, a lifetime of vigorous activity revealed a non-significant improvement in survival.[198] However, high-intensity endurance training in G93A mice did not affect disease onset, but hastened disease progression in male mice.[206] It is possible that high-intensity exercise overwhelmed the mouse's antioxidant system, leading to increased oxidative stress in skeletal muscle.[206] Again, female mice were protected from this stress possibly due to the neuroprotective effects of estrogen.[215] Thus, low and moderate intensity exercise protocols are not contraindicated in mouse models of ALS, while high-intensity endurance exercise imparts deleterious effects in male G93A mice.

Coinciding with the benefits observed in G93A mice, "moderate range of motion training" improved Spinal Norris score and the quality of life in ALS patients with respiratory insufficiency.[216] A resistance exercise program resulted in significantly higher scores on the ALS Functional Rating Scale, which measures decline in global function, and the SF-36 physical function subscale, as well as significantly less decline in leg strength.[217] This study was in line with a study from Drory et al.,[195] which also showed less deterioration in muscle function. This may set the framework for a larger clinical trial involving resistance exercise in ALS patients. The present studies show that exercise has promising benefits in improving the quality of life, thus engaging in moderate physical activity should be encouraged in these patients. Resistance exercise in ALS patients imparts some protective benefits despite the lack of a significant delay in disease progression. An improvement in function, less disuse atrophy, as well as a temporary improvement in strength and mobility may contribute to an enhanced standard of living for these patients.[217]

Ultimately, reviewing each of these nutrition- and exercise-based interventions has provided some insight into how particular mechanisms are targeted through these interventions. Animal and human studies reaffirm the likelihood that oxidative stress, Cu toxicity, glutamate excitotoxicity, and Zn deficiency are involved in the pathogenesis of ALS, due to the benefits observed through the administration of interventions targeting these pathways. Fig. 2 summarizes the nutritional and exercise-based interventions in the pathogenesis of ALS and the specific mechanisms they target.

11. Discussion/conclusion

Transgenic animal models provide insight into the etiology of the disease. Unfortunately, the success of therapeutic interventions in animal models of ALS has not always translated into effective treatments in human patients. The most commonly used model is the G93A mouse, whose reproducible and age-associated disease pattern make it a necessary component of studying the disease pathology.[54] A major challenge in ALS research is determining to which extent we can extrapolate the results of animal studies to humans. Most clinical trials have failed to show a significant improvement in their treatment group because their study protocols predicated a dosage in human subjects based on differing parameters of drug extrapolation from animal data.[166,168] Human Equivalent Dose (HED) calculations are usually based on either body surface area conversions (mg/m^2) or on body weight[218] and it is possible that the supplement/drug doses were insufficient. Hence dosages for humans should be calculated based on physiological plausibility and kinetics of the specific supplement/drug. Despite the differences between ALS patients and mouse models, several clinical reports have shown that 30–60% of the ALS population present with a hypermetabolic trait [150,219–221] reminiscent of what is observed in mSOD1 mice.[148] Evidently, ALS is not simply a motor neuron disease, but a systemic affliction involving the actions of a number of tissues and cells.[1] The hypermetabolism observed in SALS patients suggests that at least a subset of ALS patients may present with a systemic form of the disease, even in the absence of SOD1 mutations. While certain pathological features such as motor neuron loss with gliosis are shared between ALS patients and mSOD1 animal models,[222] it has been suggested that these models only truly mimic the familial form of the disease.[223] Specifically, while Bunina bodies are considered a feature of most patients suffering from ALS they are not evident in SOD1-mutated FALS or transgenic rodents with mSOD1.[222] Although both sporadic and familial forms of the disease appear to be clinically and

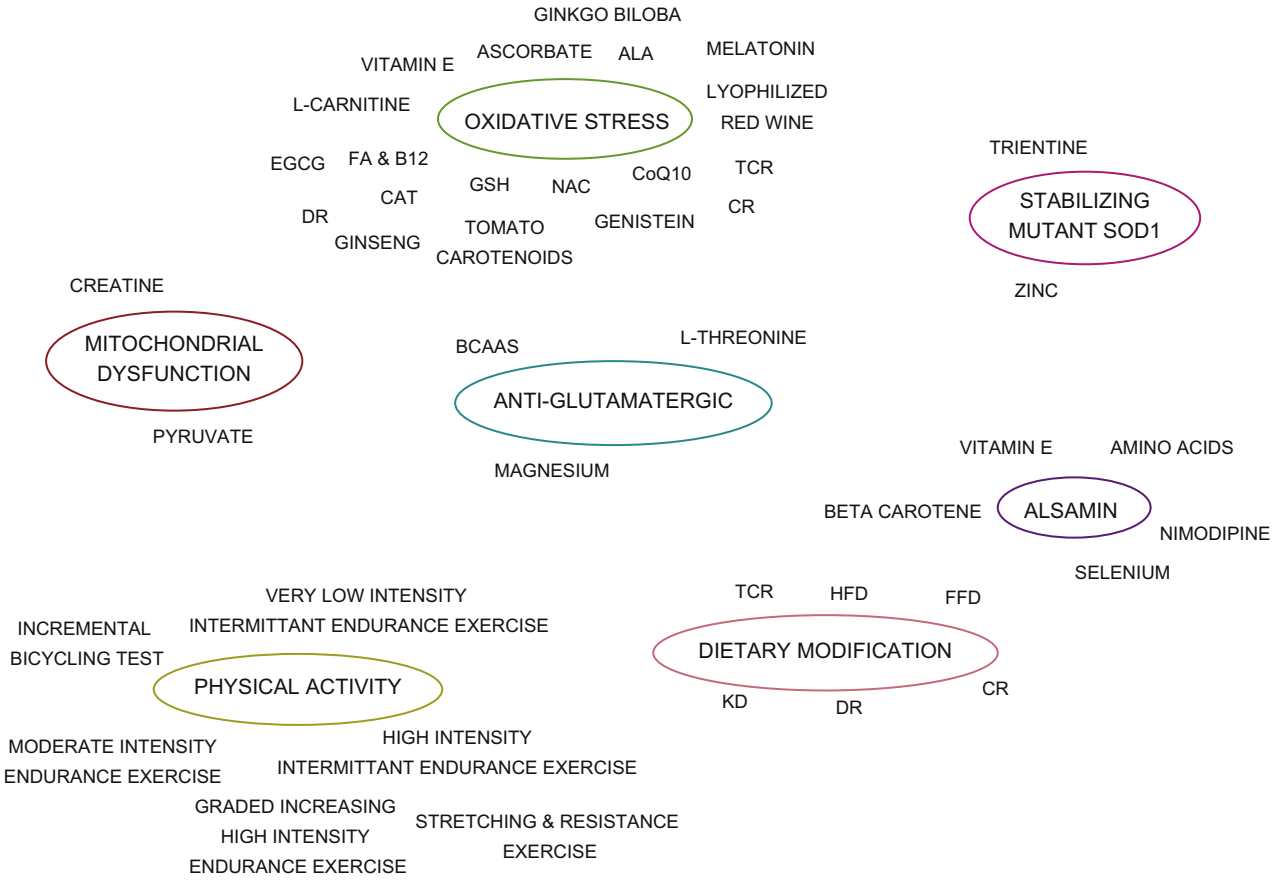

Fig. 2. Nutritional and exercise-based interventions in the pathogenesis of ALS and the specific mechanisms they target. A summary of all of the interventions discussed including all antioxidants, antiglutamatergic agents, dietary modifications, cocktail treatments, physical activity, as well as treatments which stabilize mutant SOD1 and treat mitochondrial dysfunction.

pathologically similar,[2] it is possible that they may differ biologically in some respects.[223] The mutant model may only offer a limited scope of what treatments might be effective in humans with the sporadic form of the disease.

Another possible explanation of why some interventions have been singularly successful in animals involves the time frame when the intervention is administered. Most interventions in animal models are initiated prior to clinical onset and continue until endpoint, with the hope of delaying disease onset and progression and extending lifespan. However, administering an intervention prior to the onset of disease in humans with SALS is improbable. On the other hand, initiating an intervention before disease onset in animal models may prove beneficial if replicated in individuals with FALS during a clinical trial. Early administration may help delay disease onset, slow disease progression and prolong lifespan in this population. However, it is impractical to submit a subset of the population free of any symptoms to interventions in the hopes of delaying disease onset, prolonging survival and modifying prognosis. The time commitment, cost, labour and ethical concerns necessary to conduct such a study are prohibitive. Hence, interventions in humans are limited to studies initiating treatment after clinical diagnosis. Alternatively, studies could be initiated prior to diagnosis in patients with a family history of ALS.

Additional problems leading to the failure of the applicability of mouse models to clinical trials deal with a bias in the relevant published work, and failures in experimental methodology. In a review by Benatar , they revealed that the majority of researchers only publish studies showing a beneficial effect, as opposed to studies showing lack of treatment effect.[223] The problem that arises with this bias is two-fold: firstly, it contributes to unnecessary redundancy in experimental interventions, and secondly, it may lead to potentially harmful interventions done in clinical trials at the expense of patients. In regards to methodology, many animal studies exhibit serious flaws, including a lack of randomization and blinding of experimenters.[224]

Furthermore, a weakness in some of the published data is that researchers fail to make a distinction between statistical and clinical significance. As explained by Benatar et al., an increase in survival of 1 d may be statistically significant given a large enough sample size but is not appreciable or significant in a clinical sense.[224] Other potential weaknesses encountered in clinical trials concern issues regarding statistical power, including a high drop-out rate and lack of patient compliance, which ultimately reduces the efficacy of the intervention. In reference to dietary interventions in humans, it is difficult to ascertain the most effective dosage of a particular nutritional aid. It is possible that the intervention may enhance a biochemical or molecular pathway in the absence of a functional effect. Thus, a focus on evaluating these biochemical/molecular variables through blood, urine, muscle and CNS analysis is useful to consider alongside the assessment of functional outcomes such as time to survival and progression of disease.

Despite the pros and cons observed in the interventions mentioned above, there is still strong evidence that non-pharmaceutical interventions offer some hope in delaying this debilitating disease. Riluzole, which specifically targets glutamate excitotoxicity, is the most prescribed therapy for ALS patients. In spite of the

obvious benefits of using Riluzole, the resulting marginal effect on survival is not a cure or an endpoint in the treatment of ALS. The annual cost of Riluzole treatment in the US amounts to $10 000/patient, and £2865/patient in the UK, and this exorbitant cost proposes some speculation on whether the modest therapeutic effect of the drug is warranted.[225] The limited success of Riluzole is most likely due to the multiple pathways proposed to be involved. It is likely that successful and effective therapy will involve a combination of nutritional and physical activity interventions, in order to operate on a number of different mechanisms involved in the pathogenesis of ALS. A comprehensive approach to treating this multifaceted disease would not only be ideal in slowing the progression of ALS, but also enhancing the quality of life during the duration of the disease. Establishing successful treatment for ALS is a critical aspect of research in this area, and the more promising nutritional and exercise interventions mentioned in this review should be thoroughly considered to ensure that these patients are being treated in the most effective manner possible.

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