Table 1

Table 1: Nutritional therapies targeting oxidative stress

Study	Animal/ Human	Intervention	Principal Findings	Reference
Vitamin E	G93A mice *High copy* - Males & females	- Starting at age 30 d, mice were supplemented with: 1) Vitamin E group: 200 IU vitamin E + 8 mg selenium/kg of food, or 2) Control diet group: 75 IU vitamin E + 0.15 mg selenium/kg of food (treatment, n = 23; control, n = 23)	- 18-fold increase in stored liver content of vitamin E at age 70 d - Supplemented mice had a 60% improvement in average levels of wheel activity after clinical onset vs. control mice (ran 45 km farther, P = 0.002) - Vitamin E supplemented mice showed delay in clinical onset by 12 - 15 d vs. G93A mice on control diet (14%, P < 0.005)	(Gurney et al., 1996)
Vitamin E	Human - Men & women	- Double-blind, placebo RCT - Participants were randomized into: 1) 500 mg of vitamin E twice/d + 50 mg of Riluzole, or 2) placebo + 50 mg of Riluzole - Duration: 12 mo - Mean duration of disease prior to study was 1.2 years in the vitamin E group & 1.1 years in the placebo group. (treatment, n = 144; control, n = 144)	-No effect on the rate of motor function deterioration (assessed by the modified Norris limb scale) or survival -According to the ALS Health State scale, patients supplemented with vitamin E were more likely to remain in the milder disease state A vs. non-supplemented control (32% progressed from state A to state B in treatment group vs. 44.5% in control group, P = 0.045) -After 3 mo of treatment, plasma measurements revealed an increase in GPx activity (11%, P = 0.039) & a decrease in markers of lipid peroxidation (13%, P = 0.005) vs. control group	(Desnuelle et al., 2001)
Vitamin E	Human - Men & women	- Double-blind, placebo RCT Participants were randomized into: 1) 1 g of vitamin E 5 X/d (total of 5 g/d), or 2) placebo - Duration: 18 mo - Mean duration of disease prior to study was 20 mo in the vitamin E group & 25 mo in the placebo group. (treatment, n = 83; control, n = 77)	- No difference in the Norris limb score between the treatment & control groups (P = 0.32) - The vitamin E group showed a trend toward a lower vital capacity vs. the placebo group (P = 0.07) - There were less patients in need of intermittent assisted ventilation in the vitamin E group (3%; 1 in 33 after 18 mo) vs. the placebo group (33%; 8 in 24 after 18 mo)	(Graf et al., 2005)
Vitamin E	Human - Men & women	- Observational study Participants received either: 1) 600 mg of vitamin E & 100 mg of Riluzole/d, or 2) 100 mg of Riluzole/d - Duration: 8 mo - Mean duration from disease onset prior to study was 19.6 mo in both groups (treatment, n = 8; control, n = 25)	- Trend toward a decline in FRS score in both groups at 4 & 8 mo (P value not reported) - No difference in the HRQoL sections of the SF-36 or in SEIQoL score at baseline, & after 4 & 8 mo between the groups	(Galbussera et al., 2006)

Study	Animal/ Human	Intervention	Principal Findings	Reference
Folic acid (FA) & Vitamin B12	G93A mice *High copy* - Males & females	- Starting at age 42 d, G93A mice were randomized into 1 of 4 groups: 1) oral administration of 0.9% saline 2) oral administration of 4 mg FA/kg body weight/d 3) oral administration of 0.2 mg B12/kg body weight/d, or 4) oral administration of 4 mg FA & 0.2 mg B12/kg body weight/d (treatment groups, n = 12/group; control, n = 12)	- Groups 2 & 4 showed a delay in clinical onset by 6 (6.5 d) & 8% (8.4 d), respectively ($P < 0.05$) and an increase in survival by 7 (9 d) & 10% (13 d), respectively ($P < 0.05$) vs. group 1 - B12 alone showed no difference in delaying clinical onset or increasing lifespan vs. group 1 - Groups 2 & 4 had lower plasma levels of Hcy (61 & 69%, respectively, $P < 0.01$) vs. group 1, whereas group 3 did not alter Hcy levels vs. group 1 - Groups 2 & 4 decreased iNOS (~38 & 69%, respectively), TNF-α (~56 & 78%, respectively), cleaved caspase (~50 & 66%, respectively) & PARP (~50 & 67%, respectively), and upregulated Bcl-2 protein content (~2 & 2.6-fold, respectively) vs. group 1 ($P < 0.01$ for group 2 & $P < 0.001$ for group 4)	(Zhang et al., 2008)
Catalase (CAT)	G93A mice *High & low copy* -Males & females	- *High copy* G93A mice & WT mice: starting at age 30 – 46 d, mice were supplemented through an osmotic pump with: 1) 720 – 900 U/d CAT 2) PUT-CAT, or 3) PBS (each treatment, n = 10; control, n = 10) - *Low copy* G93A mice, mice which overexpress normal SOD1 & WT mice: starting at age 30 - 45 d, mice were injected i.p. 2 X/week with: 1) CAT (60,000 U/kg) 2) PUT-CAT (60,000 U/kg), or 3) PBS (10 mL/kg) (treatment, n = 8; normal SOD1, n = 10; WT, n = 10)	- Subcutaneous administration of PUT-CAT in *high copy* mice delayed clinical onset by 26% (21 d, $P < 0.001$) vs. PBS-supplemented high copy mice, and by 12% vs. CAT-supplemented high copy mice (10 d, $P < 0.01$) - Injection of PUT-CAT in *low copy* mice delayed clinical onset by 15% (30 d, $P < 0.001$) vs. PBS-supplemented low copy mice, and by 7% vs. CAT-supplemented low copy mice (15 d, $P < 0.01$) - No difference in lifespan was observed in *high copy* G93A mice on any treatment - In *low copy* mice, there was a trend towards increased survival by 10 d (4%) with PUT-CAT	(Reinholz et al., 1999)
CAT	G93A mice *Low copy* -Males & females	- Starting at age 35 – 42 d, mice were supplemented through an osmotic pump with: 1) 450 U/d of PUT-CAT, or 2) PBS (treatment, n = 24; control, n = 7)	- PUT-CAT delayed clinical onset by 5% (11 d, $P < 0.001$), and increased survival by 12% (29 d, $P < 0.001$) vs. PBS-supplemented mice	(Poduslo et al., 2000).

Study	Animal/ Human	Intervention	Principal Findings	Reference
Glutathione (GSH)	Human - Gender not mentioned	- Open, randomized, crossover trial - 1st 12 weeks: Group A: GSH (600 mg/d) (n = 16) Group B: received only symptomatic drugs Wash-out period-1 week - Next 12 weeks: Group B: GSH (600 mg/d) Group A: symptomatic drugs - Duration: 6 mo - Mean duration of disease prior to study was 10.8 mo for Group A & 11.4 mo for Group B (group A, n = 16; group B, n = 16)	- No differences found between supplemented & unsupplemented patients in muscle strength, Norris scale & bulbar scale - There was a trend for FVC percent. In the 1st period, group A (–0.52) vs. group B (-0.70) (P = 0.08), as compared with the 2nd period, group A (-0.65) vs. group B (-0.52) (P = 0.07) No effect in modifying progression of the disease	(Chio et al., 1998)
N-acetyl cysteine (NAC)	G93A mice *Low copy* -Males & females	- NAC treatment started at age 120 d - 1st experiment: NAC solution (50 mg/mL) was injected subcutaneously at a dose of 0.5 mg/g of body weight daily (treatment, n = 14; control, n = 29) - 2nd experiment: NAC (10 mg/mL) was supplemented in drinking water resulting in plasma concentrations of 0.5 – 2 μg/mL (treatment, n = 7; control, n = 8) - Control group was unsupplemented G93A mice	- Neither experiment resulted in differences in clinical onset & survival between supplemented & unsupplemented G93A mice	(Jaarsma et al., 1998)
NAC	G93A mice *High copy* -Males & females	- Starting at age 4 – 5 weeks (28 – 35 d), G93A mice were given 2 mg/kg/d NAC in drinking water (4.5 mL/mouse) - Control group was unsupplemented G93A mice (treatment, n = 24; control, n = 18)	- Mice in treatment group showed preserved rotorod performance (15 weeks for control vs. 16 weeks for treatment group, P = 0.046) and an increase in survival by 7% vs. unsupplemented G93A control mice (~9 d, P < 0.05)	(Andreassen et al., 2000)

Study	Animal/ Human	Intervention	Principal Findings	Reference
NAC	Human -Men	- Double-blind, RCT Participants were randomized into: 1) Subcutaneous injection of 50 mg/kg NAC/d, or 2) placebo - Duration: 12 mo - Mean duration from disease onset prior to study was 40 mo in the NAC group & 36 mo in the placebo group, and from diagnosis to inclusion in the study was 27 mo in the NAC group & 23 mo in the placebo group (treatment, n = 54; control, n = 56)	- There was a trend between treatment & control groups in the linear rate of decline in for disability (P = 0.06) - There was an increase in deterioration of bulbar function in the treatment group (P < 0.01) & an increased deterioration in MRC score (P < 0.01), myometry (P = 0.01) & bulbar function (P < .01) in patients with *bulbar onset* receiving treatment.- After 12 mo, 35 patients (65%) supplemented with NAC & 30 (54%) given placebo were still alive (P = 0. 31) - In a subgroup of 81 patients with *limb onset*, 28 patients (74%) receiving NAC & 22 (51%) receiving placebo were alive at the end of the study (P = 0.06). - In a subgroup of 29 patients with *bulbar onset*, 7 patients (44%) receiving NAC & 8 (62%) receiving placebo were alive at the end of the study (P = 0.36)	(Louwerse et al., 1995)
Alpha-lipoic acid (ALA)	G93A mice *High copy* -Males & females	- Starting at age 28 d, 100 mg of ALA/kg of body weight /d (based on a food intake of 5 g/d) - Control group was unsupplemented G93A mice (treatment, n = 8 -14; control, n = 21 - 29)	- ALA supplemented G93A mice had a 22% improvement in rotorod performance (P < 0.05) & a 13% increase in weight (P < 0.01) compared to unsupplemented G93A mice - ALA increased survival by 7 d (6% increase) compared to unsupplemented G93A mice (P = 0.02)	(Andreassen et al., 2001a)
Lyophilized red wine	G93A mice *High copy* -Males & females	- Treatment onset varied between age 43-66 d - Treatment administered to G93A mice in drinking water. - The concentration of polyphenolic compounds expressed as GAE was 4,824 mg/L. - Daily intake of GAE was approximately 20 mg/mouse. - Control group was unsupplemented G93A mice (treatment, n = 7; control, n = 8)	- Significant extension in lifespan observed in mice supplemented with lyophilized red wine (152 ± 2 d) compared to unsupplemented G93A mice (144 ± 3 d), equivalent to a 6% increase (P = 0.017)	(Esposito et al., 2000)
Lyophilized red wine	G93A mice *High copy* -Males & females	- Starting at age 30-40 d, mice were supplemented with 5 mg/mL of lyophilized red wine in water - Control group was unsupplemented G93A mice (treatment, n = 8; female controls, n = 7)	- Lyophilized red wine increased mean survival time by 15% (21 d, P =0.017)	(Amodio et al., 2006)

Study	Animal/ Human	Intervention	Principal Findings	Reference
Co-enzyme Q10 (CoQ10)	G93A mice *High copy* -Males & females	- Starting at age 50 d, mice were supplemented with 200 mg of CoQ10/kg of body weight - Control group was unsupplemented G93A mice (treatment, n=16; control, n = 13)	-CoQ10-supplemented mice showed a 4% extension in lifespan (control, 135 d compared to treatment, 141d; $P < 0.05$)	(Matthews et al., 1998)
Epigallocatechin gallate (EGCG)	G93A mice *High copy* -Males & females	- Starting at age 60 d, mice received either an intraoral injection of: 1) 1.5 μg EGCG/g body weight/d 2) 2.9 μg EGCG/g body weight/d 3) 5.8 μg EGCG/g body weight/d dissolved in 0.5 mL of 0.9% sterile NaCl, or 4) 0.5 mL of 0.9% sterile NaCl/d (control group) (treatment, n = 11/group; control, n = 11)	- A dosage of 2.9 & 5.8 μg/g delayed clinical onset by about 13.9 & 13.2 d (~11 - 12% vs. control), rotorod failure by about 19.2 & 20.1 d (~16-17% vs. control) & disease endpoint by about 19.3 & 20.4 d (~ 15 - 16% vs. control), respectively ($P < 0.01$ for all) - Duration between clinical onset and endpoint (i.e. disease progression) was also prolonged by 5.4 & 7.3 d (~63 - 85% vs. control), respectively ($P < 0.05$ for all) - Immunoblotting of spinal cord homogenates showed inhibition of caspase 3 (76%, $P < 0.01$), PARP (69%, $P < 0.01$)& cytochrome *c* release (82%, $P < 0.01$) vs. WT mice - Immunoreactivity of PI3-K (2.8-fold, $P < 0.01$), pAkt (1.9-fold, $P < 0.01$), pGSK-3B (2.3-fold, $P < 0.01$) proteins were enhanced by EGCG vs. WT mice	(Koh et al., 2006)
EGCG	G93A mice *High copy* - Males & females	- Starting at age 42 d, mice received either an intraoral injection of: 1) 10 mg EGCG/kg body weight/d, or 2) an equivalent volume of injection vehicle (treated G93A, n = 11; treated WT, n = 6; control G93A, n = 11)	- Rotorod performance was improved in supplemented G93A mice (results not shown, $P < 0.05$) - EGCG treatment in G93A mice delayed clinical onset by 9% (1.4 weeks, $P < 0.05$) and extended lifespan by 10% (1.8 weeks, $P < 0.05$) vs. control G93A mice - Motor neurons were preserved in the anterior horns of the spinal cords from supplemented G93A mice vs. control G93A mice at age 120 d (~71%, $P < 0.01$) - Microglia activation was reduced in supplemented vs. control G93A mice at age 120 d (~30%, $P < 0.01$) - There was a reduction in the protein content of iNOS in supplemented vs. control G93A mice (~56%, $P < 0.01$) - Immunoreactivity and protein content of activated NF-kB is reduced in the spinal cord of supplemented vs. control G93A mice (~63% & 56%, respectively, $P < 0.01$) - Immunoreactivity of activated caspase 3 is reduced in spinal cord of supplemented vs. control G93A mice (~69%, $P < 0.01$)	(Xu et al., 2006)

Study	Animal/ Human	Intervention	Principal Findings	Reference
Ginseng	G93A mice *High copy* -Males & females	- Starting at age 30 d, G93A mice supplemented with: 1) 0 μg of ginseng/mL of drinking water (control group) 2) 100μg of ginseng/mL of drinking water, or 3) 200 μg of ginseng/mL of drinking water. - Daily intake per animal averaged 6 – 7 mL/d, thus average intake was 0, 40 or 80 mg/kg ginseng, respectively - Control group was unsupplemented G93A mice (treatment groups, n = 6/group; control, n = 13)	-Treatment groups receiving 100 & 200 ug/mL demonstrated prolonged clinical onset of disease by ~23% (116 d vs 94 d, P < 0.001) & extended survival by ~5% (139 d vs. 132 d, P < 0.05) compared to unsupplemented G93A controls	(Jiang et al., 2000)
Ginkgo biloba	G93A mice *High copy* -Males & females	- Starting at age 21 d, treatment groups received either 0.022% (200 mg/kg/d) (n = 40) or 0.045% (400 mg/kg/d) of EGb761 extract - Control group was unsupplemented G93A mice (treatment, n = 40; control, n = 40)	- EGb761 prevented age-associated loss in body weight for both sexes compared to controls (53% difference at age 120 d for males, 10% difference at age 120 d in females), as well as improved motor performance in males only (> 2-fold at age 110 d, P < 0.05) - Male G93A mice had an extension in lifespan with both 0.022% (137.9 ± 2.3 d) & 0.045% (138.2 ± 1.9 d) EGb761, as compared to unsupplemented G93A controls (126.0 ± 2.0 d) (~ 9% increase, P < 0.001) - Neither dose of EGb761 was able to increase survival in females - 200 mg/kg EGb761 protected against loss of spinal cord anterior motor horn neurons in male mice (62% increase vs. unsupplemented G93A controls, P < 0.001), but not in females	(Ferrante et al., 2001)
Tomato carotenoids	G93A mice *High copy* -Males & females	- Starting at age 29 d, treatment group received tomato food pellets (10% lyophilized powder), while control mice were fed a modified Altromin MTdiet (milk proteins & serum) (treatment, n = 10; control, n = 8)	The tomato-enriched diet did not affect either the disease onset, survival or the decline in motor performance as assessed with the rotorod test	(Esposito et al., 2007)

Study	Animal/ Human	Intervention	Principal Findings	Reference
L-Carnitine	G93A mice *Low-copy* -Males & females	- Starting at age 16 weeks (~112 d), G93A male mice were supplemented with 1 mg/mL (~50 mg of L-carnitine/kg body weight/d) in drinking water - Control group was unsupplemented G93A mice (treatment, n = 20; control, n = 20) - <u>After</u> disease onset, mice were injected subcutaneously with 100 µl of saline (control group) or 100 µl of L-carnitine-containing saline (1 mg/mL, 5 mg/kg body weight)	- L-carnitine in the drinking water delayed disease onset (~17 d, 10%) vs. unsupplemented G93A controls ($P < 0.001$) - L-carnitine in the drinking water prevented apoptosis in muscle & extended lifespan (71%, $P < 0.01$) - Even after disease onset, subcutaneous L-carnitine injections increased lifespan (13 d, 46% for males; 14 d, 60% for females) vs. saline-injected G93A controls ($P < 0.001$) - Treatment suppressed hind limb muscle & spinal cord injuries, while decreasing lipid peroxidation (51% decrease in muscle, $P < 0.01$; 56% decrease in spinal cord, $P < 0.01$) & protein oxidation (79% decrease in muscle, $P < 0.01$; 46% decrease in spinal cord, $P < 0.01$)	(Kira et al., 2006)
Genistein	G93A mice *Low copy* - Males & females	- Starting at age 75 d, G93A mice received either: 1) genistein (16 mg/kg twice everyday except weekends), or 2) vehicle (control group, i.p 6 d/week (treatment, n = 26; control, n = 28)	- Disease onset & endpoint was delayed in vehicle-treated female vs. vehicle-treated male mice ($P < 0.001$ & $P < 0.01$, respectively) - Genistein treatment delayed disease onset & endpoint in male mice ($P < 0.02$ & $P < 0.02$, respectively) - Treated females did not exhibit a delay in disease onset in comparison to untreated females	(Trieu and Uckun, 1999)
Melatonin	a)G93A mice *High copy* - females b) Humans - Men & women	- Treatment started at age 28 d or at clinical onset Mice received either: 1) 0.5 mg of melatonin (dissolved in 1% ethanol)/mL of drinking water. 2) controls received 1% ethanol in drinking water (treated, n = 29; control, n = 25) ALS patients enrolled in a safety trial of melatonin received: 1) 300 mg of melatonin/d mixed with equal amounts of hard fat were designed into suppositories (n = 31) - Duration: 24 mo - Mean duration of disease prior to study was ~ 22 mo	- In the mice, between age 56 d & 105 d, mean daily melatonin uptake was 88.3 mg/kg body weight, decreasing to 56.9 mg/kg body weight between age 112 d & 140 d - Disease progression (time between clinical onset & endpoint) was delayed in the treated vs. untreated mice by 10.2 d (25%, $P = 0.007$, n = 25 - 29/group), and after mice failed the rotorod test by 7.3 d (74%, $P = 0.033$, n = 15/group) - Lifespan was extended in the treated (n = 29) vs. untreated mice (n = 25) by 5.9 d (4%, $P = 0.009$), but there was no difference in clinical onset between groups - The age at which most mice died was delayed by 7 d (not significant) - Significance for increased lifespan & disease progression were lost when melatonin treatment was started after disease onset <u>*ALS patients*</u> - ALS FRS scores did not change over the course of 12 mo - Protein carbonyls decreased in ALS patients after > 4 mo of treatment compared to baseline levels (~13%, $P = 0.005$, n = 19)	(Weishaupt et al., 2006)

Abbreviations: ALA, alpha lipoic acid; ALS, amyotrophic lateral sclerosis; ALS FRS, ALS functional rating scale; CAT, catalase; CoQ10, coenzyme Q10; EGCG,

epigallocatechin gallate; FA, folic acid; FVC, force vital capacity; GAE, Gallic acid equivalent; GPx, glutathione peroxidase; GSH, glutathione; Hcy, homocysteine; HRQoL, Health-Related Quality of Life; iNOS, inducible nitric oxide synthase; NAC, N-acetylcysteine; PARP, poly (ADP-ribose) polymerase; PBS, phosphate buffered saline; PUT-CAT, putrescine catalase; RCT, randomized controlled trial; SALS, sporadic ALS; SEIQol, Schedule for the Evaluation of Individual Quality of Life-Direct Weighting; SF-36, Short-Form 36-Item Health Survey; SOD1, superoxide dismutase 1; TNF-α, tumour necrosis factor-α; WT; wild-type.

Table 2

Table 2: Dietary modifications

Study	Animal/ Human	Intervention	Principal Findings	Reference
Caloric restriction (CR) *Long term*	G93A mice *High copy* -Males & females	- Starting at age 40 d, CR mice received 60% of the average intake of the AL diet (CR, n = 8; AL, n = 6)	- CR mice weighed 31% less than AL mice (P < 0.001) - Body condition was 36% lower in the CR group vs. the AL group (P = 0.034) & it decreased over time (P < 0.001). - Ability to move was 27% lower in the CR vs. AL group (P = 0.075) & it decreased over time (P < 0.001). - Fall time was 65% higher in the CR vs. AL mice (P = 0.039), & fall speed was 33% higher in CR vs. AL mice (P = 0.009), using the rotorod test - Time & speed rapidly decreased after disease onset - CR mice reached clinical onset 10% sooner (P < 0.001) & endpoint 6% sooner (P = 0.09) vs. AL mice	(Hamadeh et al., 2005)
Transient caloric restriction (TCR) *Short term*	G93A mice *High copy* -Males & females	- Starting at age 40 d, TCR mice received 60% of the average intake of the AL diet - When TCR mice lost 30% of their body weight, they were placed on an AL diet until endpoint, otherwise all TCR mice were placed on an AL diet from age 55 d until endpoint - Period of TCR was 13 – 15 d (TCR, n = 15; AL, n = 17)	- Maximum weight loss in TCR mice was ~25% (P < 0.001) - Body condition was 13% lower in TCR vs. AL mice (P = 0.025), with TCR males having lower body condition vs. AL males (17%, P = 0.003), AL females (25%, P < 0.001) and TCR females (25%, P = 0.005) - TCR had no effect on Paw Grip Endurance - TCR males reached clinical onset 5 d sooner than TCR females (5%, 0.036) - TCR mice had faster disease progression vs. AL mice, with males developing disease 6 d faster than females (23%, P = 0.076) - TCR males had a faster rate of reaching endpoint vs. TCR females (9%, P = 0.034), AL males (9%, P = 0.022) & AL females (11%, P = 0.044)	(Hamadeh and Tarnopolsky, 2006)
Dietary restriction (DR) *Intermittent*	G93A mice *Low copy* -Males & females	- Starting at age 42 d, DR mice were fed 60 -70% of AL group's diet on alternate days (intermittent feeding regimen) (DR, n = 6; AL, n = 6)	- The difference in disease onset in the DR vs. AL group was not significant (236 d vs. 269 d, respectively) - Disease progression was lower in the DR (5 d) vs. the AL group (11 d) by 55% (P < 0.01) - Histochemical analyses in the lumbar spinal cord indicate extensive motor neuron degeneration in both groups compared to WT mice used as controls for this experiment	(Pedersen and Mattson, 1999)

Study	Animal/ Human	Intervention	Principal Findings	Reference
Ketogenic diet (KD)	G93A mice *High copy* -Males	- Starting at age 50 d, male mice were placed on either: 1) a KD (caloric composition, fat 60%, carbohydrate 20%, protein 20%), or 2) a standard control diet (fat 10%, carbohydrate 70%, protein 20%) - both diets contained equal percentages of cholesterol per gram (treatment, n = 6; control, n = 4; WT, n = 5)	- KD-fed mice had more than 3.5 fold higher blood ketones compared to G93A mice on standard control diet (P = 0.012) - KD-fed mice weighed 23% more than G93A mice on standard control diet at study endpoint (P = 0.070) - KD-fed mice lost 50% of baseline motor performance 25 d later than G93A mice on standard control diet (P = 0.027) - There was no difference in lifespan between KD fed vs. G93A mice on standard control diet - KD-fed mice had ~38% more motor neurons in the ventral horn compared to controls at endpoint (9.4 ± 1.1 vs 6.8 ± 0.6, p = 0.03); WT: 13.6 ± 1.3 motor neurons/spinal cord section	(Zhao et al., 2006)
High-fat diet (HFD)	G86R mice -Males & females	- Starting at age 4 weeks (~28 d), mice were fed a high-fat diet (HFD) - Starting at age 6 weeks (~42 d), mice were fed either: 1) regular chow supplemented with 21% (w/w) butter fat & 0.15% (w/w) cholesterol, or 2) regular chow (control group) (treatment, n = 13; control, n = 12)	- From age 60 - 105 d, body weights of HFD mice were higher than normally fed mice (13 – 40%, P < 0.05) - HFD extended mean survival by 20% vs. normal diet (20 d, P < 0.05) - HFD also improved motor neuronal function in G86R mice by increasing large cells (> 600 μm^2) in the ventral horns of the lumbar spinal cord (2.3-fold, P < 0.05)	(Dupuis et al., 2004)
Fast food diet (FFD)	G93A mice *Low copy* -Males	- Starting at age 6 weeks (~42 d), male mice were fed: 1) a fast food diet (FFD; 38% carbohydrates, 47% fats, & 15% protein), or 2) a control diet (64% carbohydrates, 17% fats, & 19% protein) (treatment, n = 12; control, n = 12)	-Mice on the FFD continuously gained body weight until they became symptomatic in comparison to control mice who either lost or maintained weight - FFD delayed clinical onset vs. control diet - Mice on the FFD survived 22% longer than mice on the control diet	(Mattson et al., 2007)

Abbreviations: AL, ad libitum; CR, caloric restriction; DR, dietary restriction; FFD, fast-food diet; HFD, high-fat diet; KD, ketogenic diet; TCR, transient caloric restriction; WT, wild-type

Table 3

Table 3: Nutritional therapies which stabilize mutant SOD1

Study	Animal/ Human	Intervention	Principal Findings	Reference
Trientine & ascorbate	G93A mice *Low copy*	- Starting at age 45 d, mice were administered trientine (0.2% w/v in distilled water) & ascorbate (0.8% w/w mixed into standard animal food) - Control group was unsupplemented G93A mice (treatment, n = 8; control, n = 7)	- Treatment increased mean body weight - Treatment delayed the onset of disease by 8.4% (16 d, P = 0.030) compared to unsupplemented G93A controls - Treatment delayed disease endpoint by 10.4% (25 d, P = 0.045) compared to unsupplemented G93A controls	(Nagano et al., 1999)
Trientine & ascorbate	G93A mice *High copy* -Males	Experiment 1 was initiated at age 50 d: - High dose trientine (0.5% w/v) - Ascorbate (0.8% w/w) - Low dose trientine (0.2% w/v) - Combination of trientine (0.5% w/v), ascorbate (0.8% w/w) - Control group was unsupplemented G93A mice (all treatments, n = 8; control, n = 7) Experiment 2 was initiated after disease onset: - High dose trientine (1.2% w/v) - Ascorbate (0.8% w/w) - Low copy trientine (0.2% w/v) - Combination of trientine (1.2% w/v), ascorbate (0.8% w/w) (all treatments, n = 8; control, n = 7)	- The combined treatment group had less motor function deterioration (18%, P = 0.043) compared to unsupplemented G93A controls - In Experiment 1, there was no difference in clinical onset between any treatment group & unsupplemented G93A controls - High trientine, ascorbate & combination delayed endpoint by 5 – 8% (7 – 10 d) vs. unsupplemented G93A controls (P < 0.05) - In Experiment 2, there was no difference between the high doses of trientine, ascorbate or combination on delaying endpoint.	(Nagano et al., 2003)
Trientine	G93A mice *High copy* -Males & females	- Starting at age 28 d, trientine was added to drinking water at concentrations of 0.2% & 0.4% - Amount was based on an average intake of 5 mL/d which resulted in doses of 400 & 800 mg/kg/d - Control group was unsupplemented G93A mice (treatment, n = 8 -14; control, n = 21 - 29)	- Both doses delayed weight loss by 15% from age 126 d onwards (P < 0.05) compared to unsupplemented G93A controls - Both the 0.2 & 0.4% trientine improved motor performance in G93A mice from age 119 d onward vs. unsupplemented G93A controls (P < 0.05) - 0.2% trientine extended survival by 6.2% (~8 d, P < 0.01), whereas the 0.4% concentration showed an 8% extension in lifespan (~10 d, P < 0.001)	(Andreassen et al., 2001a)

Study	Animal/ Human	Intervention	Principal Findings	Reference
Zinc	G93A mice *High copy* -Males & females	- Starting at age 50 d, G93A mice were placed on: 1) a Zn-deficient diet (control group, < 2 ppm), or 2) supplemented with 30 ppm of Zn^{2+} (males, n = 20; females, n = 9) - Starting at 30 or 50 d, male G93A mice were supplemented with either: 1) a Zn-deficient diet 2) 30 ppm = ~6 mg of Zn^{2+}/kg/d 3) 60 ppm = ~12 mg of Zn^{2+}/kg/d, or 4) 90 ppm = ~18 mg of Zn^{2+}/kg/d in drinking water (all groups, n = 9)	- G93A mice on Zn-deficient diet survived 7 d less (5% decrease) than G93A mice supplemented with 30 ppm of Zn^{+2} (~6 mg of Zn^{2+}/kg/d, $P < 0.001$) & developed hind limb weakness 6 d earlier (6% increase, $P < 0.02$) - Supplementation with 60 ppm of Zn^{2+} (~12 mg of Zn^{2+}/kg/d) delayed death by 11 d compared to G93A mice on a Zn-deficient diet (9%) - 90 ppm of Zn^{2+} (~18 mg of Zn^{2+}/kg/d) caused death in 70% of mice	(Ermilova et al., 2005)
Zinc	G93A mice *High copy* -Males & females	- Starting at age 50 d, G93A mice were placed into 3 groups: 1) Low Zn^{2+} group: (75 mg/kg body weight) 2) High Zn^{2+} group: (375 mg/kg body weight) 3) Control group (normal diet) (all groups, n = 11)	- Survival was lower (11 d, 8%) in mice supplemented with high dose (375 mg/kg body weight) of Zn^{2+} vs. unsupplemented mice ($P < 0.05$), with no difference between low dose Zn^{2+} and unsupplemented mice or between high and low doses - Zn^{2+} treatment did not alter clinical onset or disease progression	(Groeneveld et al., 2003a)

Table 4

Table 4: Nutritional therapies targeting mitochondrial dysfunction

Study	Animal/ Human	Intervention	Principal Findings	Reference
Creatine	G93A mice *High copy* -Males & females	- Starting at age 70 d, G93A mice were supplemented with either: 1) 1% creatine, or 2) 2% creatine in the diet. - Control group was unsupplemented G93A mice (1%, n = 7; 2%, n = 7; control, n = 6)	- 2% creatine increased levels of creatine by 37% vs. unsupplemented G93A mice ($P < 0.01$) - Creatine enhanced motor performance from 116 to 136 d of age vs. unsupplemented controls - Oral administration of 1% & 2% creatine resulted in a dose-dependent increase in survival by 9% ($P < 0.05$) & 17% ($P < 0.001$), respectively vs. unsupplemented control mice - Survival was extended by 13 d with 1% creatine ($P < 0.05$) & by 26 d with 2% creatine ($P < 0.001$), which is superior to Riluzole, which extends survival by 13 d in this model - 1% creatine offered complete protection against neuronal loss in ventral horns vs. unsupplemented controls at age 120 d ($P < 0.01$) - Mice fed 1% creatine showed no increase in 3-nitrotyrosine in the spinal cord vs. unsupplemented controls at age 120 d	(Klivenyi et al., 1999)
Creatine	G93A mice *High copy* -Males & females	- Starting at age 4 weeks (~28 d), G93A mice received either 2% creatine (w/w) in food or a standard diet (treatment, n = 12; control, n = 14) - Starting at age 4 weeks (~32 d), another group of G93A mice were supplemented with 1, 2 & 3% creatine (n = 12 - 14 mice/group)	- 2% creatine attenuated weight loss, with unsupplemented mice having lower body weights (10 – 11%, $P < 0.05$) - 2% creatine delayed loss of motor control by 15 d ($P < 0.05$) & improved motor performance (rotarod) from age 114 d until 149 d (1 – 5-fold, $P < 0.02$) vs. unsupplemented controls - All concentrations of creatine resulted in an extension in lifespan (6%, $P < 0.05$), but the 2% treatment showed the greatest results (15%, $P < 0.001$)	(Andreassen et al., 2001b)
Creatine	G93A mice *High copy* - Males & females	-Starting at age 60 d, G93A & WT mice mice received either: 1) creatine (2% w/w in food), or 2) unsupplemented diet (treatment, G93A = 15, WT = 10; control, G93A = 15, WT = 10)	- Body weight, rotorod performance grip strength, muscle ATP content & glycogen content did not differ between the G93A groups - Tetanic contractions did not differ between the G93A groups, but there was a strong trend for increased relaxation speed in the EDL of creatine-treated G93A mice (66%, $P = 0.06$) - There was a trend for increased total creatine in the soleus of supplemented vs. unsupplemented G93A mice (39%, $P = 0.09$) - Relative EDL weight (mg/g) was higher in supplemented vs. unsupplemented G93A mice (25%, $P < 0.05$)	(Derave et al., 2003)

Study	Animal/ Human	Intervention	Principal Findings	Reference
Creatine	G93A Mice *Low copy* -Males & females	- Starting at age 40 d, G93A mice received either: 1) standard chow (control group) 2) Riluzole (100 μg/mL of drinking water) 3) creatine (2% w/w in food), or 4) a combination of Riluzole & creatine (Riluzole, n = 13; creatine, n = 14; combination, n = 15; control, n = 17)	- G93A mice supplemented with creatine displayed elevated total levels of creatine in cerebral hemispheres (5%) & spinal cord (8%), but not skeletal muscle ($P < 0.05$) - Clinical onset was delayed by ~12 d in all treatment groups vs. control (~7%, $P < 0.05$); no difference observed between treatments -Female mice supplemented with creatine exhibited an 8 d delay in onset of clinical symptoms (4%, $P < 0.05$) & a reduction in severity of symptoms (41%, $P < 0.05$) at the time mice were sacrificed vs. supplemented males - All animals were sacrificed at 199 d of age (survival not analyzed) - At 199 d, severity of clinical signs was less with all treatments vs. unsupplemented control ($P < 0.05$); no difference observed between treatments - Separate treatments with Riluzole & creatine were both effective in delaying disease onset & clinical disability up to 199 d; no additional benefit was conferred by the combined treatment - Treatments had no effect on the number of neurons in ventral horns of the lumbar region of the spinal cord	(Snow et al., 2003)
Creatine	Human -Men & women	- ALS patients were administered 20 g of creatine/d for 7 d (loading period), followed by 3 g of creatine/d for 3 & 6 mo (maintenance period) - There was no control group - Duration: 7 d loading period + 6 mo maintenance period - Mean duration of disease prior to study was 22.5 mo (n = 28)	- MVIC increased after 7 d of 20 g/d supplementation in 20 patients (70% in knee extensors, $P < 0.05$) & in 15 patients (53% in elbow flexors, $P < 0.04$) compared to pre-treatment values - Post-treatment MVIC of knee extensors was higher vs. pre-treatment ($P < 0.03$) in patients with *bulbar onset* - Fatigue test improved after 7 d of supplementation in 11 patients (39% in elbow flexors) & in 9 patients (32% in knee extensors) - During the 6-mo maintenance period of 3 g of creatine/d, all the examined parameters exhibited a linear progressive decline, especially MVIC in upper & lower limbs, except for a trend toward higher BMI	(Mazzini et al., 2001)

Study	Animal/ Human	Intervention	Principal Findings	Reference
Creatine	Human -Men & women	- Double-blind, placebo controlled, randomized sequential trial - ALS patients were given either 5 g of powdered creatine monohydrate in 250 mL of water twice daily or placebo - Duration: 16 mo - Mean duration of disease prior to study was 1.4 years for treatment group & 1.5 years for control group (treatment, n = 88; control, n = 87)	- Creatine supplementation did not affect disease progression (assessed by MVIC, FVC, functional status & quality of life) - There was no difference in survival between the groups, with 68% of patients in the treatment group surviving compared to 66% in the control group	(Groeneveld et al., 2003b)
Creatine	Human -Men & women	- Double-blind, placebo RCT - ALS patients were randomized to receive either: 1) 20 g of creatine monohydrate/d for 5 d (loading period), followed by 5 g/d (maintenance period), or 2) placebo - Duration: 5 d loading period + 6 mo maintenance period - Mean duration of disease prior to study was 1.7 years in treatment groups & 2.2 years in control group (treatment, n = 50; control, n = 54)	- Body weight & BMI declined for treatment & control groups, but rate was not different between control & treatment. - No difference in rate decline of MVIC, ALSFRS-R & MUNE between treatment & control group. - Similar rates of noncompliance with study parameters regarding creatine intake in treatment & control groups according to urinary creatine (6 of 31 control subjects had higher concentrations at the 3 mo time point; 6 of 37 treatment subjects had lower concentrations at the 3 mo time point)	(Shefner et al., 2004)
Creatine	Human -Men & women	-Double-blind, placebo RCT -ALS patients were ranodmized to receive either: 1) 10 g of creatine monohydrate/d for 5 d (loading period), followed by 5g/d (maintenance period), or 2) placebo -Duration: 5 d loading period + 9 mo maintenance period -Mean duration of disease prior to study was 1.5 years in the treatment group & 1.9 years in the control group (treatment, n = 53; control, n = 54)	-No difference in rate of decline of MVIC, in the slope of declining fatigue indices, maximal FVC, SF-12 quality of life index, ALSFRS-R or survival between treatment & control group.	(Rosenfeld et al., 2008)

Pyruvate	G93A mice *High copy* -Males & females	- Starting at age 70 d, G93A mice received either: 1) sodium pyruvate (i.p. injections of 1000 mg/kg/week), or 2) PBS (control group, i.p. injections of 1000 mg/kg/week) (treatment, n = 19; control, n = 19)	- Pyruvate improved motor performance (rotorod) vs. control mice starting at postnatal week 16 (~112 d) (75%, $P < 0.05$), & increased weight by 10% starting at week 16 ($P < 0.05$) - No difference in disease onset, but mean survival increased by 10.5% (~12 d) with pyruvate treatment ($P < 0.05$) - Pyruvate treatment reduced nitrotyrosine immunoreactivity, gliosis, but increased Bcl-2 staining in the spinal cord	(Park et al., 2007)
Pyruvate	G93A Mice *High copy* - Males & females	- Starting at age 70 d, G93A mice received either: 1) pyruvate (500 mg/kg/d), or 2) saline injections (control group, i.p 6 d/week (treatment, n = 8; control, n = 8)	- No effect on onset or motor decline with pyruvate treatment vs. control - No difference in survival between the treatment & control groups	(Esposito et al., 2007)

Abbreviations: ALSFRS, ALS functional rating scale; BMI, body mass index; EDL, Extensor Digitorum Longus; MUNE, motor unit number estimation; MVIC, maximum voluntary isometric contraction; RCT, randomized controlled trial

Table 5

Table 5: Anti-glutamatergic agents

Study	Animal/ Human	Intervention	Principal Findings	Reference
Branched chain amino acids (BCAAs)	Human - Men & women	- Double-blind, parallel-group RCT - ALS patients received: 1) 6.4 g of valine, 12 g of leucine & 8 g of isoleucine/d, or 2) placebo - Duration: 12 mo - Mean duration of disease prior to study was 1.8 years in treatment group & 1.9 years in the control group (treatment, n = 11; control, n = 11)	- Mean spinal scores were higher in the BCAA treated patients vs. controls (9%, $P < 0.1$ at 3 mo; 24%, $P < 0.1$ at 6 mo 32%, $P < 0.02$ at 9 mo) - The changes in bulbar scores did not differ between the groups.	(Plaitakis et al., 1988)
BCAAs	Human - Men & women	- Open, therapeutic trial - ALS patients received: 1) 1.6 g of valine, 3 g of leucine & 2 g of isoleucine 4 X/d, or 2) control patients on therapy other than BCAAs - Duration: 12 mo - Mean duration of disease prior to study was 18.4 mo in BCAA treated *bulbar* ALS patients, 17.8 mo in control *bulbar* ALS patients, 19.2 mo in BCAA treated *non-bulbar* ALS patients & 18.1 mo in control *non-bulbar* ALS patients (BCAA treated *bulbar*, n = 8; control *bulbar*, n = 8; BCAA treated *non-bulbar*, n = 8; control *non-bulbar*, n = 8)	- A decline in Norris was evident & progressive for all groups, but this was not significant - The number of drop-outs due to death or admission to a hospital was the same between the treated patients & the control group - Plasma glutamate levels in supplemented patients were comparable to control patients	(Testa et al., 1989)

Study	Animal/ Human	Intervention	Principal Findings	Reference
BCAAs	Human - Men & women	- Double-blind, placebo RCT - ALS patients received: 1) 6 g of valine, 12 g of leucine & 6 g of isoleucine/d, or 2) placebo - Duration: 12 mo - Mean duration of disease prior to study was 11.6 mo in the treatment group & 13.4 mo in the control group (treatment, n = 61; control, n = 65)	- Out of the 126 patients recruited, 24 patients in the treatment group (39%) vs. 13 in the control group (20%) died - Patients treated with BCAA had a 92% chance of survival by 3 mo, 81% chance by 6 mo, 74% chance by 9 mo & 59% chance by 12 mo vs. 100%, 90%, 85% & 74% for the placebo group, respectively - The relative risk for death in treated patients was 2.3 times higher than for non-treated patients (P = 0.01) - Men had a 3.6 risk of death vs. women - Mortality was greater in treated vs. untreated patients who were 56 years of age or older (relative risk was 2.9 – 3.4) or who had an FVC < 60% (relative risk was 4.3) - Patients who died had greater changes in their disability scores, but these changes were not significant	(The Italian ALS Study Group, 1993)
BCAAs	Human -Men & women	- 24 ALS patients from The Italian ALS Study Group RCT (see above) receiving either treatment or placebo were assessed for plasma concentrations of large neutral amino acids & glutamic acid & the large neutral amino acid brain influx (treatment, n = 13; control, n = 11) - Plasma from 15 untreated ALS patients & 15 healthy volunteers was also analyzed	- Plasma BCAA concentrations increased 3 - 6-fold in the treated group compared to the patients receiving placebo or no treatment & to the healthy controls (P < 0.01) - Plasma glutamic acid concentration was higher in all ALS patients vs. healthy controls (84%, P < 0.01) - Plasma phenylalanine & tyrosine were lower in all ALS patients vs. healthy controls (~25 & 29%, respectively, P < 0.01), whereas tryptophan levels were not significantly different - BCAA brain influx of the treated group was 110% - 140% of that measured in the patients receiving placebo & in the healthy controls (P < 0.01) - The aromatic amino acid brain influx was lower in the treated group vs. the placebo group or healthy controls (16 -42%, P < 0.01)	(Bastone et al., 1995)

Study	Animal/ Human	Intervention	Principal Findings	Reference
BCAAs	Human -Men & women	- ALS patients and healthy controls received an oral load of sodium L-glutamate (60 mg/kg dissolved in 200 mL water) after an overnight fast - ALS patients were then supplemented for at least 2 weeks with: 1) 4 daily doses (30 min prior to food intake) of 3 g L-leucine, 2 g L-isoleucine and 1.6 g L-valine, or 2) a placebo (control group) - Glutamate loading was repeated - Duration: 2 weeks - Mean duration of disease prior to study was 14 mo (ALS patients, n = 6; healthy controls, n = 6)	- ALS patients had similar basal plasma glutamate and aspartate levels as controls - There was a difference in plasma glutamate levels during the three hours after loading ($P < 0.001$), with increased plasma glutamate levels at 30 and 45 min after loading ($P < 0.01$) in ALS patients vs. controls - ALS patients exhibited an increased plasma glutamate AUC compared to controls (2.6-fold, $P = 0.026$) - Peak concentration of glutamate and the ratio of plasma glutamate peak level:basal level were not different between the two groups	(Gredal and Moller, 1995)
BCAAs	Human -Men & women	- Double-blind, parallel group RCT - ALS patients received: 1) 12 g of L-leucine, 8 g of L-isoleucine & 6.4 g of L-valine powder daily (BCAA group) 2) 4 g of L-threonine powder & 160 mg of P-5-P (40 mg tablet) daily (L-threonine group), or 3) placebo - Duration: 6 mo - Mean duration from disease onset prior to study was 18.5 mo & from diagnosis to inclusion in the study was 7.3 mo (BCAA, n = 31; L-threonine, n = 32; control, n = 32)	- BCAA group gained more weight (0.2 kg) compared to the other 2 groups (L-threonine & placebo groups lost 1.1 & 3.2 kg, respectively; $P = 0.04$) - BCAA and L-threonine groups did not show significant differences in MRC scores, quantitative myometry, Norris score, bulbar score, activities of daily living, timed task or electrophysiological assessment - FVC was ~2.5 times greater in the BCAA and L-threonine groups vs. placebo ($P = 0.03$)	(Tandan et al., 1996)

Study	Animal/ Human	Intervention	Principal Findings	Reference
L-threonine	Human - Men & women	- Open, therapeutical trial Participants were randomized into: 1) 1 g of powdered L-threonine 4X/d (total of 4 g/d), or 2) received therapy cycles of vitamin B and carnitine (control group) - Duration: 12 mo - Mean duration of disease since onset prior to study was ~18 mo (treatment, n = 9; control, n = 4)	- No difference in the decline of the clinical assessment score (Norris score assessed every 3 mo until the duration of the study at 12 mo) was observed between groups - More control vs. treatment patients (9 vs. 4) complained of respiratory failure, despite higher bulbar involvement in the treatment group	(Testa et al., 1992)
L-threonine	Human - Men & women	- Double-blind, placebo RCT - Participants were randomized into: 1) 2 g of powdered L-threonine, or 2) placebo - Duration: 12 mo - Mean duration of disease prior to study was 1.8 years in the vitamin E group & 1.9 years in the placebo group. (treatment, n = 7; control, n = 8)	- No significant difference in mean scores of muscular testing, grip strength, cramps, fasciculations or therapeutic effectiveness - No significant difference in the decline of the clinical assessment score (Norris score assessed every 3 mo until the duration of the study at 12 mo) was observed between groups	(Blin et al., 1992)
Magnesium	G93A mice *High copy* -Males & females	- Starting at age 6 weeks (~42 d), low and high dose G93A mice were fed with cubes containing 200 mg/kg of Mg - Treatment groups were also provided with drinking solution containing Mg pidolate dissolved in water at 21.5 g/L (low dose) or 43 g/L (high dose). - Control group was unsupplemented G93A mice (high dose G93A, n = 15; low dose G93A, n = 14; unsupplemented G93A, n = 15)	- G93A SOD1 on high & low doses showed no difference in their onset of grip weakness at week 15, or in the progression of weakness after week 15 - G93A mice on high & low doses had tendency to be faster on the balance beam (data not shown) vs. unsupplemented G93A mice near endpoint (between weeks 15.5 and 16.5), but this was not statistically significant. - No statistical difference in onset of weakness or lifespan between treatment and control groups.	(Pamphlett et al., 2003)

Abbreviations: ALS, Amyotrophic lateral sclerosis; AUC, area under the curve; BCAAs, branched chain amino acids; FVC, force vital capacity; GS, grip strength; MRC, Medical Research Council; P-5-P, pyridoxyl-5-phosphate; RCT, randomized controlled trial

Table 6

Table 6: Antioxidant, amino acid & mineral cocktail

Study	Animal/ Human	Intervention	Principal Findings	Reference
Alsamin	Human -Men & women	- Double-blind, placebo controlled study SALS patients were assigned either: 1) Alsamin (cocktail of 135 IU vitamin E, 30 μg selenium, 4500 IU beta-carotene , and amino acids: , 0.15 g L-arginine, 2.0 g L-methionine, 4.0 g L-leucine, 3.0 g L-isoleucine & 2.0 g L-valine) & Ca^{2+} channel blocker nimodipine (20 mg) 2) a combination of selenium, vitamin E & beta-carotene 3) a combination of amino acids 4) nimodipine, or 5) a placebo taken 3 times/d - Duration: 63 d (each group, n = 7)	- There was progression in Norris score in all subgroups except the Alsamin group - Prior to treatment, GPx & SOD1 activities were significantly decreased in blood samples of SALS patients vs. controls - GPx activity and the amount of vitamin E was increased only through the combination of antioxidants, selenium, amino acids & nimodipine in SALS patients after 9 weeks	(Apostolski et al., 1998)

Abbreviations: GPx, glutathione peroxidase; SOD1, superoxide disumutase 1

Table 7: Physical activity

Study	Animal/ Human	Intervention	Principal Findings	Reference
Incremental bicycling test	Humans - Men & women	- Eleven ALS patients underwent an incremental workload exercise on a cycloergometer (3 min exercise bouts, at a pedalling rate of 60 – 70 rpm, with 2 min rest intervals, at increasing workloads; exercise started at 10% of predicted normal maximal power output for each individual) - Control group consisted of 9 healthy untrained volunteers & 8 patients afflicted with chronic denervating process (CD) - Mean duration of disease prior to study was 13.8 mo	- In ALS & CD patients, mean basal levels of lactate were higher than in healthy controls (~88%, $P < 0.05$) - Exercised ALS patients had higher mean normalized peak lactate (34%) & its mean absolute value (48%) vs. exercised CD group ($P < 0.05$) - Lactate threshold in ALS patients was achieved at 40% – 50% of the predicted normal maximal power output, compared to 60% - 70% of the predicted normal maximal power output in healthy and CD controls	(Siciliano et al., 2001)
Incremental bicycling test	Humans - Men & women	- Ten patients underwent an incremental workload exercise on a cycloergometer (3 min exercise bouts, at a pedalling rate of 60 – 70 rpm, with 2 min rest intervals, at increasing workloads exercise; started at 10% of predicted normal maximal power output for each individual) - Control group consisted of patients afflicted with CD - Mean duration of disease prior to study was 12.9 mo	- In ALS patients, mean basal levels of lactate & lipoperoxides were higher than CD controls (~6% & 12%, respectively, $P < 0.05$) - Exercised ALS patients had higher lactate (50%) & lipoperoxide levels (13%) vs. exercised CD group ($P < 0.05$) - In the ALS patients there was a significant correlation between exercise lactate & lipoperoxide levels ($r = 0.97$, $P < 0.01$)	(Siciliano et al., 2002)
Moderate intensity endurance exercise	G93A mice *High copy* - Males & females	- Starting at week 7 (~49 d), treatment group exercised on motorized treadmill for 30 min/d, 5 d/week at 13 m/min - After week 17 (~119 d), mice showing consistent difficulties maintaining speed at 13 m/min were subjected to slower pace regimen - Control group was sedentary. (treatment, n = 30; control, n = 32)	- Treatment group had an extension in lifespan (~2%, $P = 0.007$) - Sex differences: males in treatment group had an extension in lifespan ~8% (129 d to 139 d, $P = 0.02$), whereas females showed trend in extension of lifespan ~4% (139 d to 144 d, $P = 0.1$)	(Kirkinezos et al., 2003)

Study	Animal/ Human	Intervention	Principal Findings	Reference
Moderate intensity endurance exercise	G93A mice *Low copy & high copy* -Males & females	- Treatment group trained for 45 min/d, 5 d/week, 16 m/min at 80% VO_2max on motorized treadmill - Sedentary groups did not exercise (*High copy* G93A mice: male exercise, n = 13; female exercise, n = 9; male sedentary, n = 13; female sedentary, n = 11 *Low copy* G93A mice: male exercise, n = 13; female exercise, n = 11; male sedentary, n = 13; female sedentary, n = 10 WT: exercise & sedentary, n = 16/group)	- Female sedentary low copy G93A mice experienced delayed onset compared with male low copy sedentary G93A mice (25 d, P = 0.04) - Exercise delayed onset in female low copy G93A mice compared to female low copy sedentary G93A mice (48 d, P = 0.03) - Sex did not influence total survival time in both high & low copy, sedentary G93A mice. - In female exercising high-copy G93A mice, a delay (P = 0.04) of 4 d in total survival time was observed vs. sedentary female mice.	(Veldink et al., 2003)
High intensity and very low intensity intermittent endurance exercise	G93A mice *High copy* -Males & females	G93A mice were divided into: 1) Treatment group, exercised on motorized running wheels (40 X 10 min running periods interrupted by rest periods of 5 min following every 10 min period for 10 h/d) 2) sedentary (low-activity) group, had same exercise regimen except that the wheel speed was fixed to slow motion (0.1 m⁄min) 3) control group left undisturbed in cages. - Duration: Treatment & sedentary groups were introduced to wheel at 3 weeks of age (~21 d); motorized intervention were introduced 2 weeks later (~35 d) (treatment, n = 12; sedentary, n = 13; control, n = 12)	- No differences in clinical onset as assessed by GS (P = 0.29), SL (P = 0.68) or TRT performance (P = 0.92). - Active group showed non-significant (P = 0.22) improvement in survival compared to sedentary (5.1%) & control group (3.6%) (Active group = 133.7 ± 3.2 d; sedentary group = 127.2 ± 3.2 d; control group = 129.1 ± 2.5 d) - No sex differences in lifespan.	(Liebetanz et al., 2004)

Study	Animal/ Human	Intervention	Principal Findings	Reference
Graded increasing high intensity endurance exercise	G93A mice *High copy* -Males & females	- Starting at age 40 d, treatment group received high-intensity endurance exercise training on motorized treadmill for 20, 25, & 30 min/d, 3 times/week for the first 3 weeks, followed by 45 min/d, 5 times/week for remainder of study - Training protocol had progressive increase in duration from 9 to 22 m/min - Control group did not exercise (treatment, n = 14; control, n = 25)	- Male mice lost weight earlier than female mice (males, 116 d; females, 132 d; $P < 0.05$) - Males in treatment group lost weight earlier than control group (treatment, 114 d; control, 126 d; $P < 0.05$); no difference in females - Rotorod performance declined in males: treatment group at 112 d ($P < 0.05$) & sedentary group at 119 d ($P < 0.05$). - Rotorod performance declined in females: treatment group at 126 d ($P < 0.05$) & sedentary group at 129 d ($P < 0.05$). - Exercise did not affect onset ($P > 0.05$) across the sexes, but it hastened clinical onset in male mice (age at clinical onset was ~97 d, $P = 0.062$). - Exercised female mice lived 14% longer than males in exercise group ($P < 0.0001$) - Female G93A mice had a greater probability of survival than male G93A mice (10%, $P = 0.02$). - Male control group lived 7.6% longer than treatment group ($P = 0.03$), while female control group lived 2.1% shorter than treatment group (not significant). Males lived 120 d in treatment group vs. 130 d in control group; females lived 137 d in treatment group vs. 140 d in control group.	(Mahoney et al., 2004)
Moderate intensity endurance exercise	Human - Men & women	- Prospective, controlled single blind study - ALS patients with respiratory insufficiency performed a ramp treadmill exercise up to anaerobic threshold, with the assistance of noninvasive ventilation (Bipap STD), while the control group of patients did not exercise (treatment, n = 8; control, n = 12) - Duration: 12 mo - Mean duration of disease prior to study was not reported	- FIM scores were higher in treated vs. untreated patients (~20 – 30%, $P < 0.03$), with no difference in Barthel scores (both are quality of life scores) - Spinal Norris scores were higher in treated vs. untreated patients (~36%, $P < 0.02$) - There was a difference in the slope of the RFT in treated vs. untreated patients ($P < 0.008$)	(Pinto et al., 1999)

Study	Animal/ Human	Intervention	Principal Findings	Reference
Stretching and resistance exercise	Human -Men & women	- Treatment group received program consisting of daily stretching & resistance exercises 3 X/week, whereas control group performed only daily stretching exercises. - Duration: 6 mo - Mean duration of disease (since onset) prior to study was 20.4 mo in the treatment group & 15.4 mo in the control group (treatment, n = 13; control, n = 14)	- Exercise group had higher ALSFRS scores (P = 0.01), higher SF-36 physical function subscale scores (P = 0.02), and decreased fatigue severity scores - No adverse events related to intervention (MVIC & FVC indicated no negative effects). - There was less decline in leg strength (P = 0.03) with exercise, as measured by MVIC. From baseline to end of study, % change in MVIC data ranged from -0.8% to -37% in control group and -47% to +35% in treatment group.	(Bello-Haas et al., 2007)
Moderate intensity endurance exercise	Human -Men & women	-Treatment group received moderate exercise program (15 min/d, twice daily) to improve muscle endurance - Control group did not perform any physical activity beyond usual daily life requirements -Duration: 12 mo (Note: At 9 and 12 mo intervals, too few patients for statistical evaluation) - Mean duration of disease (since onset) prior to study was ~20 mo (treatment, n = 14; control, n = 11)	- At 3 mo, treatment group showed less deterioration on FRS (27%, P < 0.001) and spasticity rating (57%, P = 0.005), but not on other parameters - Exercise group showed no change in complaints of fatigue. - Trend towards a smaller decline in motor function (MMT, 14% difference) and functional rating (FRS, 23% difference) for treatment vs. control group at 6 mo interval	(Drory et al., 2001)

Abbreviations: ALS, Amyotrophic lateral sclerosis; CD, chronic denervating process; FIM, Functional Independent Mobility; FRS, functional rating scale; FVC, force vital capacity; MMT, Manual muscle testing; MVIC, maximum voluntary isometric contraction; RFT, Respiratory Function Test; SL, stride length; TRT, tight rope test; WT, wild type

Figure 1

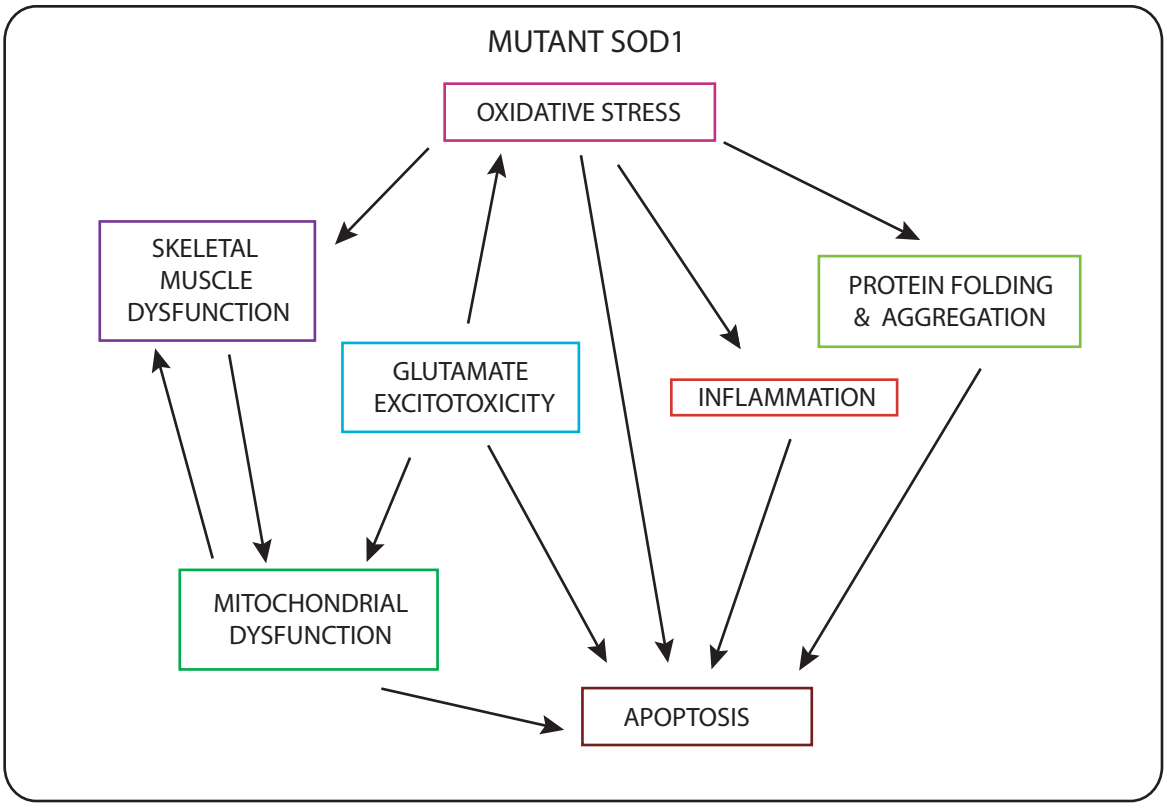
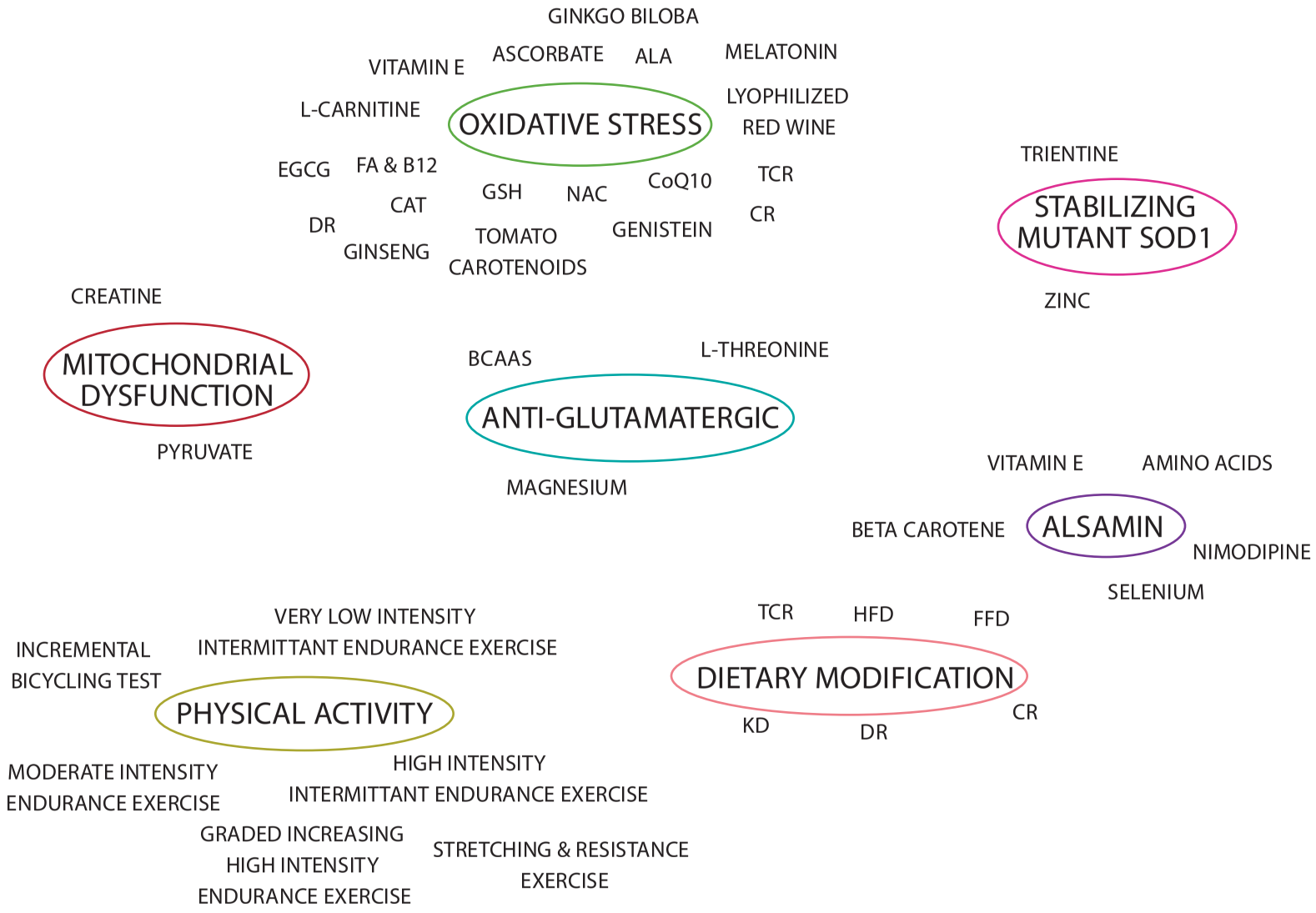

MUTANT SOD1
OXIDATIVE STRESS
SKELETAL MUSCLE DYSFUNCTION
GLUTAMATE EXCITOTOXICITY
PROTEIN FOLDING & AGGREGATION
INFLAMMATION
MITOCHONDRIAL DYSFUNCTION
APOPTOSIS

Figure 2


GINKGO BILOBA
VITAMIN E
ASCORBATE
ALA
MELATONIN
L-CARNITINE
OXIDATIVE STRESS
LYOPHILIZED
RED WINE
EGCG
FA & B12
GSH
NAC
CoQ10
TCR
CAT
CR
DR
TOMATO
GENISTEIN
GINSENG
CAROTENOIDS
TRIENTINE
STABILIZING
MUTANT SOD1
ZINC
CREATINE
MITOCHONDRIAL
DYSFUNCTION
PYRUVATE
BCAAS
L-THREONINE
ANTI-GLUTAMATERGIC
MAGNESIUM
VITAMIN E
AMINO ACIDS
BETA CAROTENE
ALSAMIN
NIMODIPINE
SELENIUM
VERY LOW INTENSITY
INTERMITTANT ENDURANCE EXERCISE
INCREMENTAL
BICYCLING TEST
PHYSICAL ACTIVITY
TCR
HFD
FFD
DIETARY MODIFICATION
CR
KD
DR
MODERATE INTENSITY
ENDURANCE EXERCISE
HIGH INTENSITY
INTERMITTANT ENDURANCE EXERCISE
GRADED INCREASING
HIGH INTENSITY
ENDURANCE EXERCISE
STRETCHING & RESISTANCE
EXERCISE

Author Agreement:

All authors significantly contributed to the work and approved the conception, drafting and final version of the manuscript.