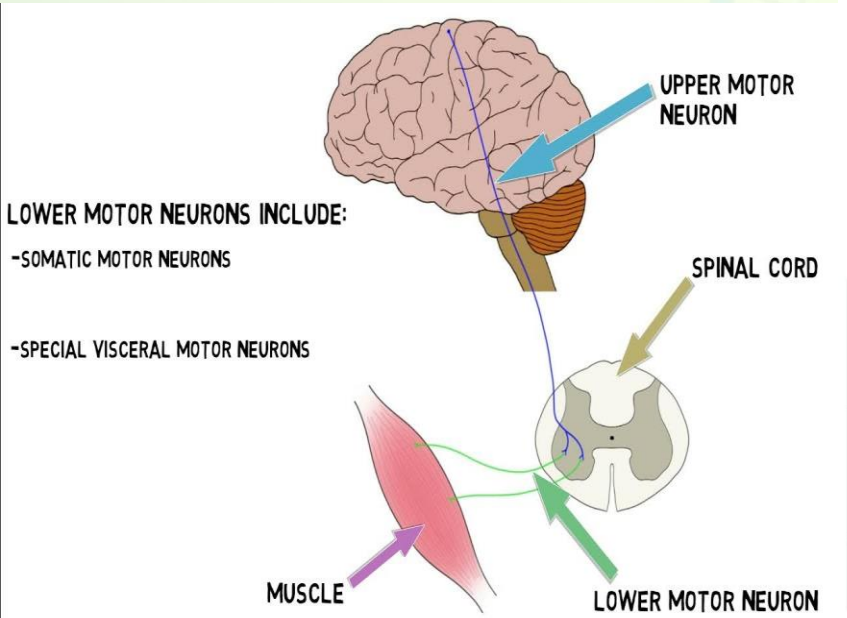
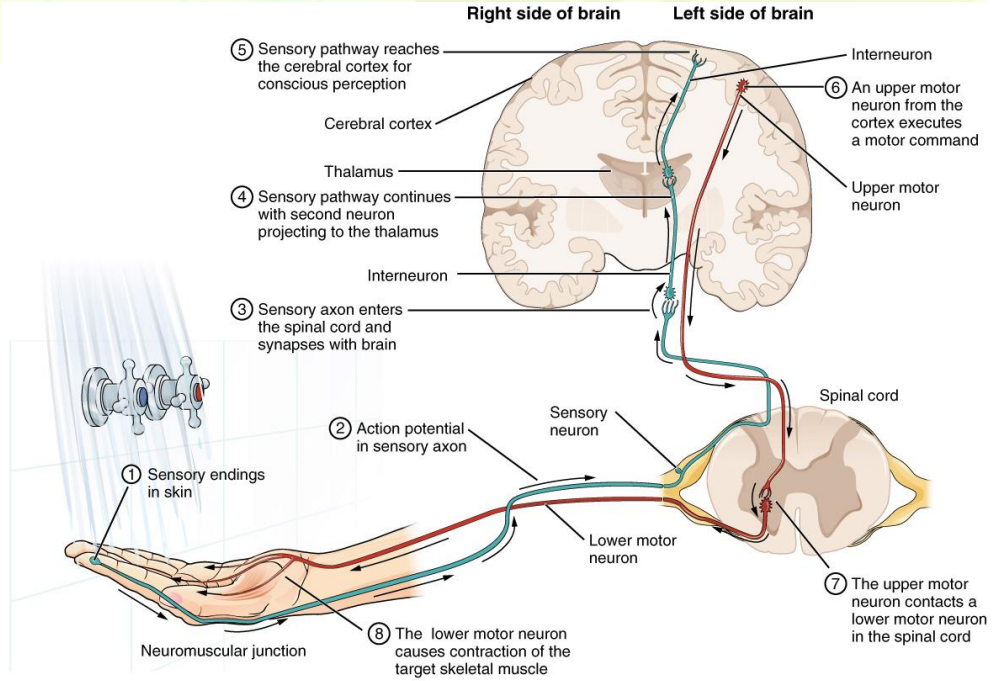
Role of Muscular Atrophy in ALS

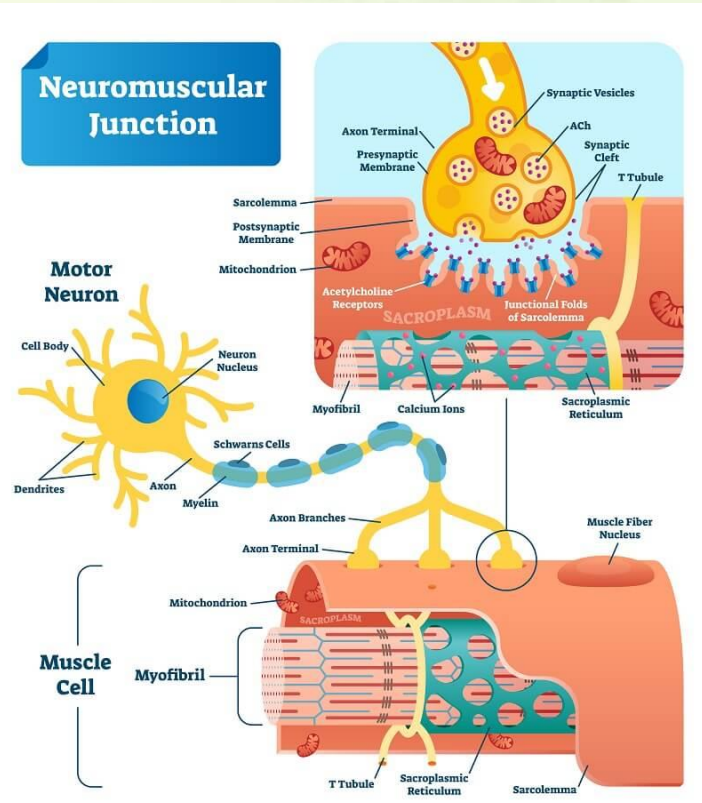
Studying ALS from Muscular Function's Perspective

Conventional Understanding of Motor Function

- Upper motor neurons (UMN) activate lower motor neurons (LMN).
- LMN then activates neuromuscular junction (NMJ).
- NMJ then activate motor function by releasing acetylcholine into the muscle mass.
- This is how every human body part moves (that includes the heart, lungs, tongue, throat, intestines, hands, and feet).
- But a lay person can only observe the loss of motor function on the limbs.







Problem with Conventional Understanding of ALS

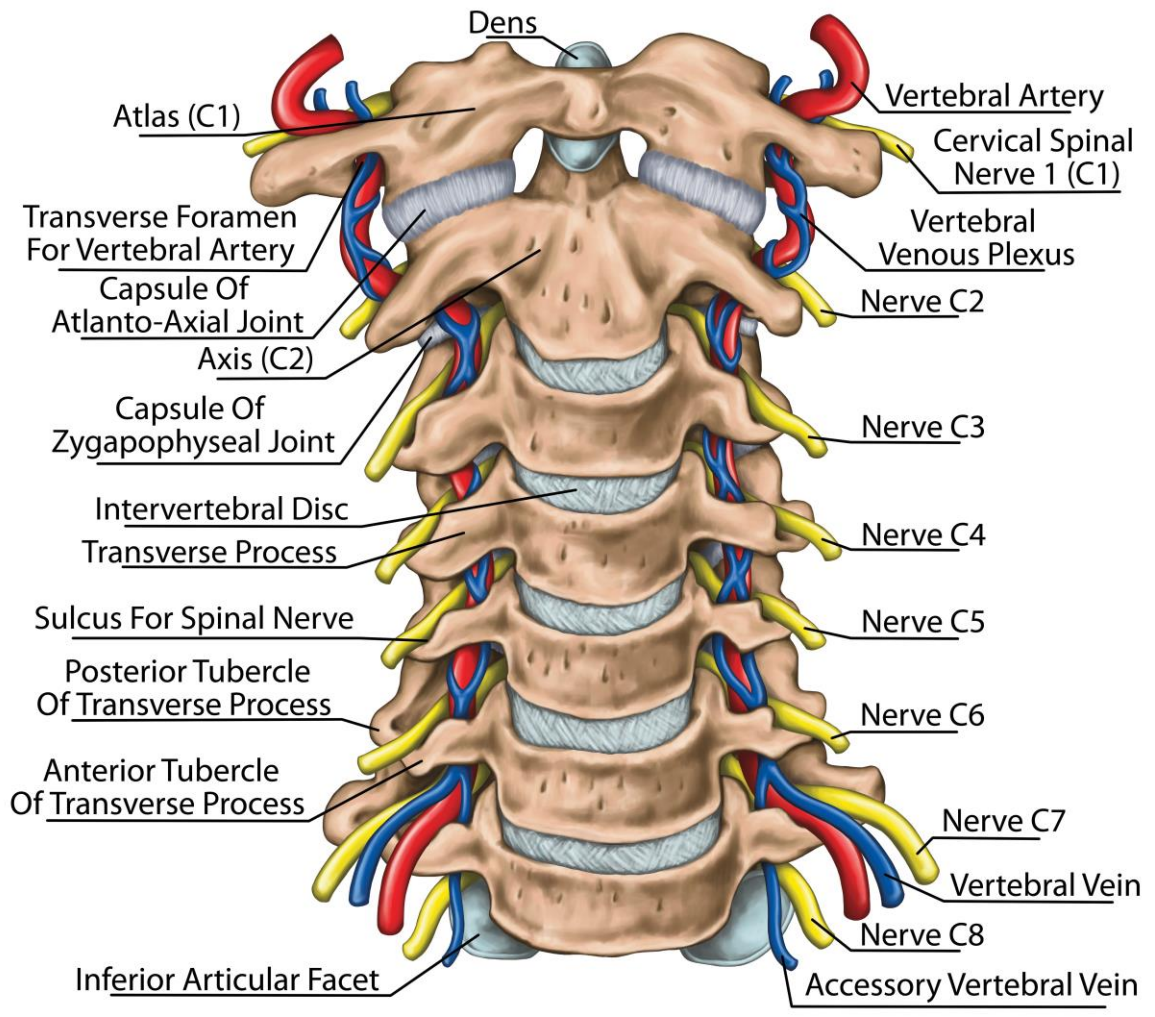
- Neurologist believe loss of UMN & LMN are what contribute to ALS.
- **Sensory nerves are not affected among ALS patients.**
- Motor neurons can lose its function just from glutamate excitotoxicity, much the same way a hair dryer loses power due to overloaded circuit.
- We also have a chick and egg problem: "**Does the loss of motor function contribute to muscle atrophy**" or "**does muscle atrophy contribute to the loss of motor function**"?
- In other words, "**Can ALS primarily result from muscle atrophy instead of the degeneration of UMN & LMN**"?

Example 1 – Astronauts live in the orbit for more than 2 weeks

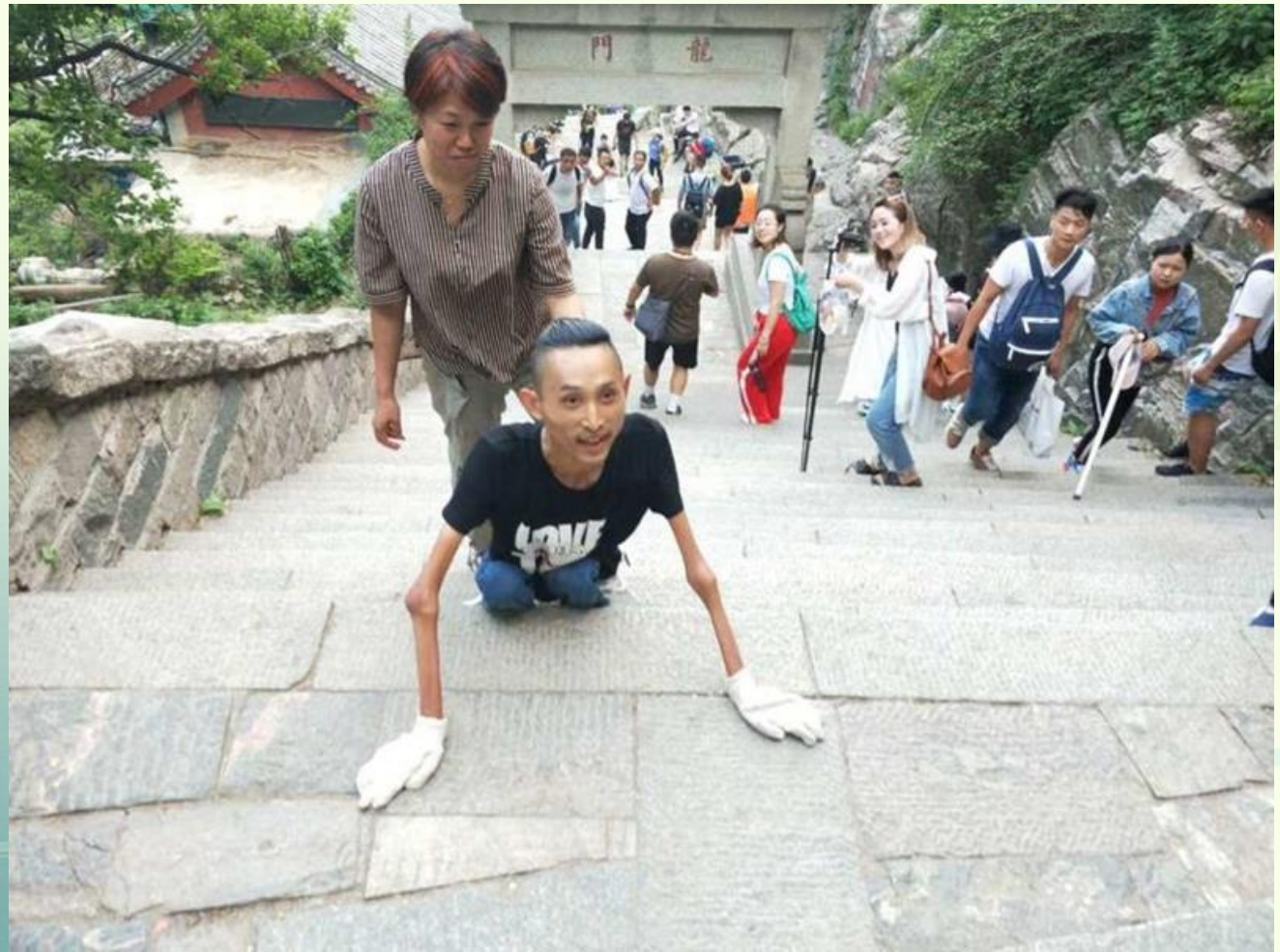
- We all know due to the loss of gravity in the orbit, astronaut must exercise 4 hours per day in the orbit to prevent muscle atrophy.
- Otherwise, they would not be able to stand on their own when they return to Earth
- This is the first example that the loss of muscle mass (muscular atrophy) contribute to the loss of motor function, even though UMN, LMN and their corresponding motor functions are perfectly normal.
- They can regain muscle mass with training whereas ALS patients cannot be due to glutamate excitotoxicity problem.

Example 2 – severe degeneration of C5-C7 contribute to lost of hand and finger's motor function yet its motor neuron & motor function are normal

- Cervical nerve 5 (C5) provides sensation to the upper part of your upper arm down to your elbow.
- Cervical nerve 6 (C6) controls the extensor muscles of your wrist and is involved in the control of your biceps. C6 also provides sensation to the thumb side of your forearm and hand.
- Cervical nerve 7 (C7) controls your triceps and wrist extensor muscles.
- Extreme dehydration, such as drinking tea entire life instead of water, can leads to degenerate of some or all 34 spinal vertebral discs.
- If the vertebral discs between C4, C5, C6 and C7 are badly degenerated. That alone will shut down the neuron signal from cervical nerves C5 to C7, patient can lose the hand and finger's motor function without any physical lose of UMN & LMN.
- This is further complicated by the muscle atrophy from prolong lack of stimulation for C5, C6 and C7 cervical nerves.
- Patient can easier tell the different, if their hand can't move yet can feel the touch, that is ALS, if the hand can't move and feel numb, or cannot feel the touch or pain when the muscle of hand is pinched, that is not ALS.



Example 3 – ALS patient move around with hand



- This picture from China Daily shows an ALS patient, Zhang Wei, scaling Huashan Mountain in Shaanxi province with his hands.
- Unless the motor function on the shoulder and hand are intact, there is no way he can scale the Huashan mountain.
- This is an example that muscle atrophy may precede the loss of motor functions among ALS patients.
- If that is true, can it be possible that ALS is not primarily a motor neuron disease, but a muscular atrophy disease?

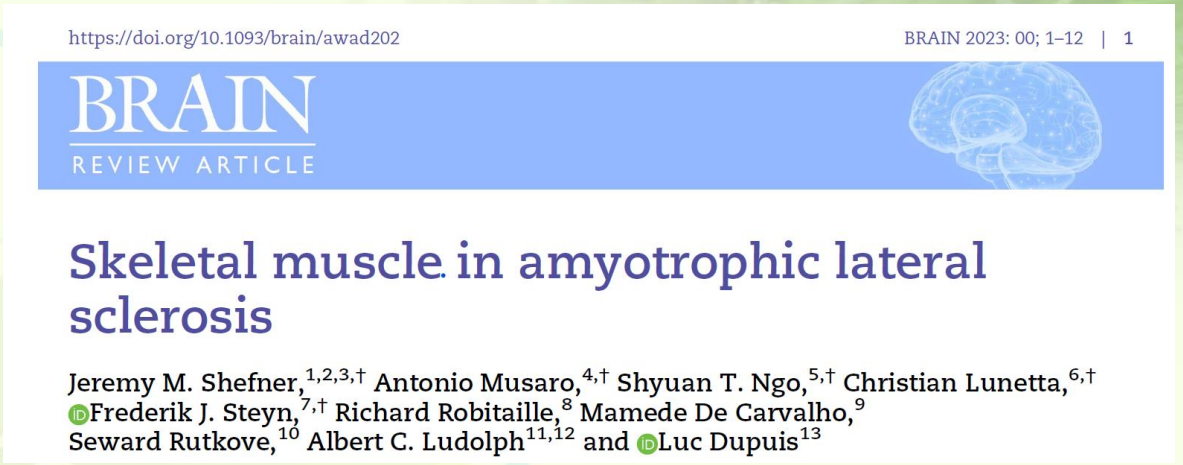
The Dying-Back Hypothesis

- Definition: The 'dying back' hypothesis suggests that ALS is a distal axonopathy that progresses in a retrograde fashion from peripheral tissue (including skeletal muscle).[1, 2, 5]
- Alterations in skeletal muscle have been observed in the early stages of the disease.[1]
- In ALS, NMJ degradation has been found to occur before motor neuron loss.[1, 3, 4]
- According to Scaricamazza et al., "understanding the molecular mechanisms involved in skeletal muscle degeneration may help develop therapeutic strategies that preserve muscle function, slow down the disease progression, and improve ALS patients' quality of life."[1]

1. Scaricamazza S, Salvatori I, Ferri A, Valle C. Skeletal Muscle in ALS: An Unappreciated Therapeutic Opportunity? Cells. 2021 Mar 2;10(3):525.
2. Dadon-Nachum, M.; Melamed, E.; Offen, D. The "dying-back" phenomenon of motor neurons in ALS. J. Mol. Neurosci. **2011**, 43, 470–477
3. Fischer, L.R.; Culver, D.G.; Tennant, P.; Davis, A.A.; Wang, M.; Castellano-sanchez, A.; Khan, J.; Polak, M.A.; Glass, J.D. Amyotrophic lateral sclerosis is a distal axonopathy: Evidence in mice and man. Exp. Neurol. **2004**, 185, 232–240.
4. Loeffler, J.-P.; Picchiarelli, G.; Dupuis, L.; Gonzalez De Aguilar, J.-L. The Role of Skeletal Muscle in Amyotrophic Lateral Sclerosis. Brain Pathol. **2016**, 26, 227–236.
5. Tsitkanou, S., Lindsay, A. and Della Gatta, P. (2019), The role of skeletal muscle in amyotrophic lateral sclerosis: a 'dying-back' or 'dying-forward' phenomenon?. J Physiol, 597: 5527-5528.

Quotes from Review Paper by Shefner et al.

- "In ALS, multiple studies indicate that skeletal muscle dysfunction might contribute to progressive muscle weakness, as well as to the final demise of neuromuscular junctions and motor neurons. Furthermore, **skeletal muscle has been shown to participate in disease pathogenesis** of several monogenic diseases closely related to ALS"
- "In ALS, the degeneration of neuromuscular synapses is a central and early feature of the disease,… which is observed before signs of motor units loss/reinnervation. **In this context, the contribution of skeletal muscle to neuromuscular junction (NMJ) dismantlement has been largely overlooked**."



- "Using *in vitro* systems, Picchiarelli *et al*., Badu-Mensah *et al*., and Ding *et al*. demonstrated that intrinsic morpho-functional deficits of the ALS skeletal muscle affect human NMJ integrity and function."

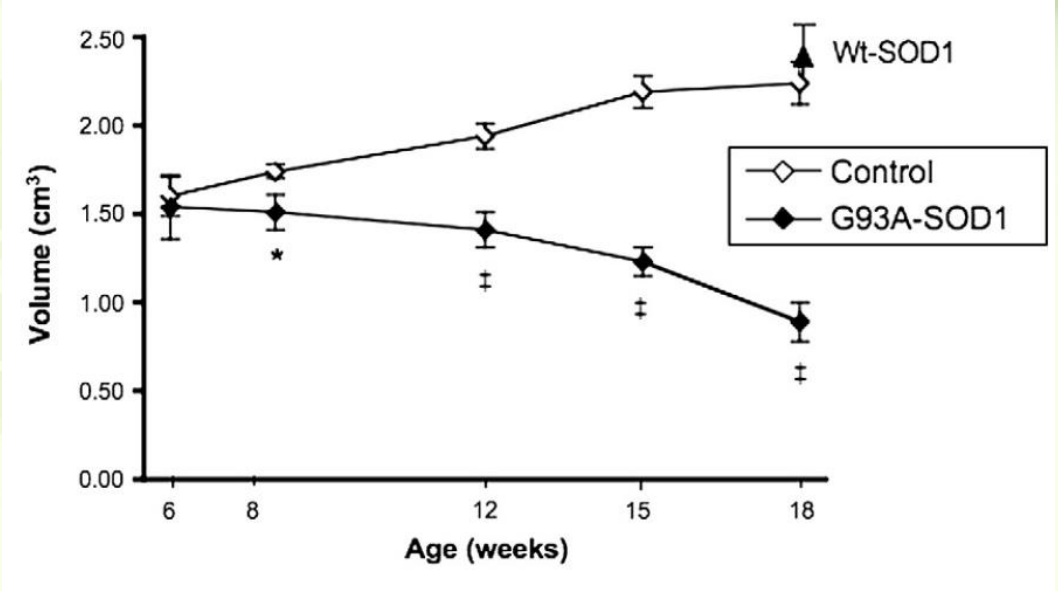
Shefner JM, Musaro A, Ngo ST, Lunetta C, Steyn FJ, Robitaille R, De Carvalho M, Rutkove S, Ludolph AC, Dupuis L. Skeletal muscle in amyotrophic lateral sclerosis. Brain. 2023 Jun 16:awad202.

A longitudinal ALS mice study using MRI shows muscle atrophy **precedes** neuronal degeneration (Marcuzzo, 2011)

- Muscle weakness occurred 4 weeks prior to clinical onset and 2-10 weeks prior to brain neurodegeneration in an MRI examination of ALS mice

Observation	Time onset (weeks)
Muscle weakness	8
Brain neurodegeneration	10-18
Onset of ALS clinical symptoms	12

- Confirmed by histological analysis



Hind limb muscle volume in SOD1 ALS and control mice from weeks 6 (n=5 per group) to 18 (n=7 per group from week 8)

Marcuzzo S, Zucca I, Mastropietro A, de Rosbo NK, Cavalcante P, Tartari S, Bonanno S, Preite L, Mantegazza R, Bernasconi P. Hind limb muscle atrophy precedes cerebral neuronal degeneration in G93A-SOD1 mouse model of amyotrophic lateral sclerosis: a longitudinal MRI study. Exp Neurol. 2011 Sep;231(1):30-7.

New Biotic LLC
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ALS is due to metabolic disorder with food-based Glutamate

The 1987 study by Plaitakis and Caroscio suggests ALS patient less effectively metabolize food-based glutamate into harmless glutamine.

Abnormal Glutamate Metabolism in Amyotrophic Lateral Sclerosis

Andreas Plaitakis, MD, and James T. Caroscio, MD

Glutamate levels were determined in the fasting plasma of 22 patients with early-stage primary amyotrophic lateral sclerosis (ALS) and compared to those of healthy and diseased controls. There was a significant increase (by approximately 100%, $p < 0.0005$) in the plasma glutamate of the ALS patients as compared with the controls. Oral glutamate loading (60 mg of monosodium glutamate per kilogram of body weight, taken orally after overnight fasting) resulted in significantly greater elevations in the plasma glutamate and aspartate levels in the ALS patients than in the controls. Glutamate, a potentially neuroexcitotoxic compound, is thought to be the transmitter of the corticospinal tracts and certain spinal cord interneurons. A systemic defect in the metabolism of this amino acid may underlie primary ALS.

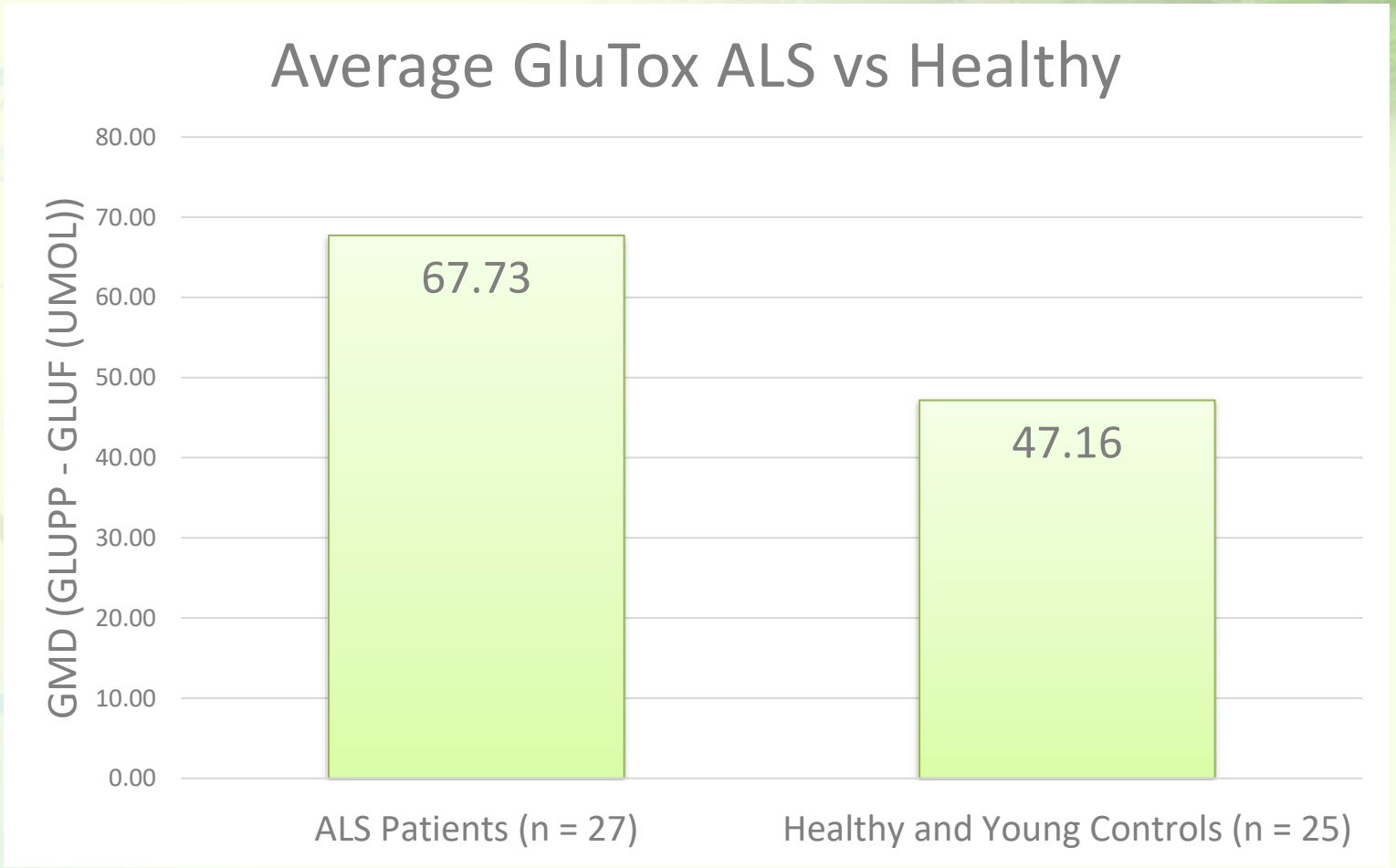
Plaitakis A, Caroscio JT: Abnormal glutamate metabolism in amyotrophic lateral sclerosis. Ann Neurol 22:575–579, 1987

ALS patient lose their ability to digest food-based glutamate

- All food, whether it plant-based or animal-based, as long as it contains protein, it will have glutamate in it.
- NBI developed a serum glutamate challenge test in 2017 to confirm the findings of Plaitakis and Caroscio.
- On the average ALS patients lose 43.62% of their ability to convert food-based glutamate into glutamine compared to healthy controls.

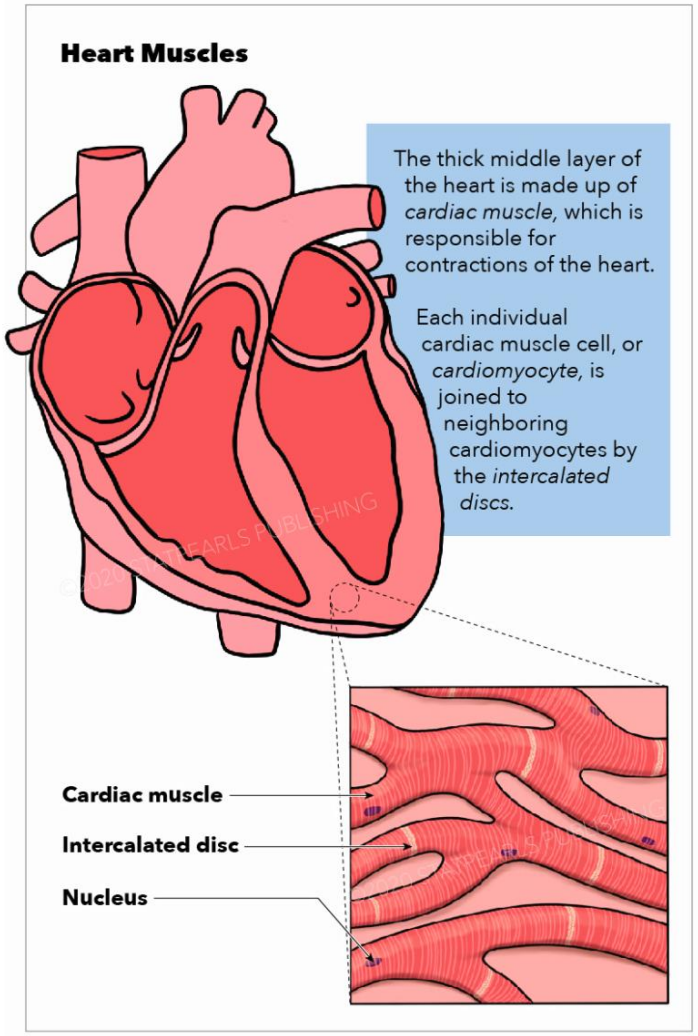
Glutamate Conversion in ALS Patients

Glutamate metabolism deficiency (GMD) of ALS patients is **43.62%** greater than healthy and young controls

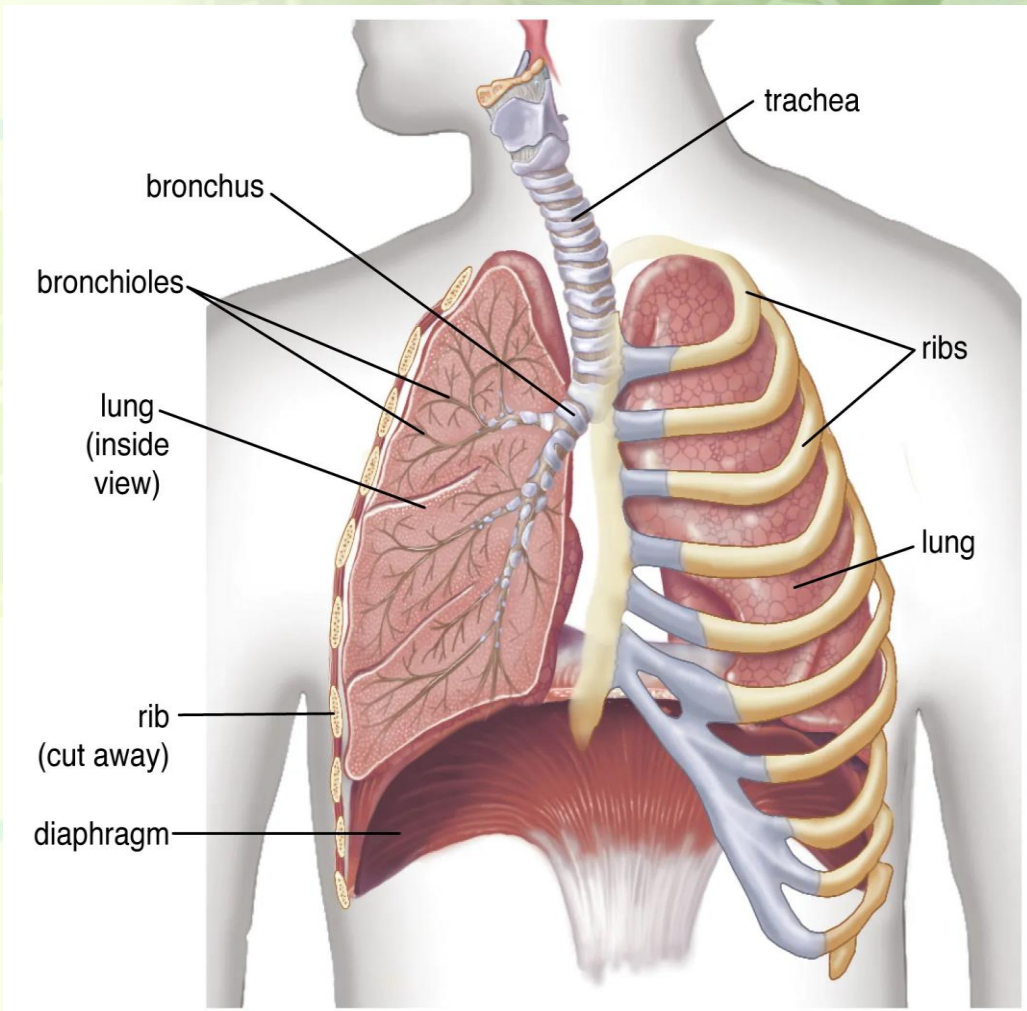


The consequent of muscle atrophy on heart and lung

- Human's muscles are made up of 50-60% glutamine.
- When muscle lose 43.62% of glutamine, gradual atrophy of the muscle mass is unavoidable.
- Muscular atrophy is not limited to limbs.
- Cardiac muscle is needed for the heart to pump blood. Atrophy of heart muscle will mean reduced blood circulation. That alone could lead to atrophy of the limbs.
- The diaphragm muscle is needed for the lung to breathe. Atrophy of the diaphragm will mean patients cannot breathe.



Graphics by Emma Gregory; Source: Anthony Saxton; Muhammad Ali Tariq; Bruno Bordoni

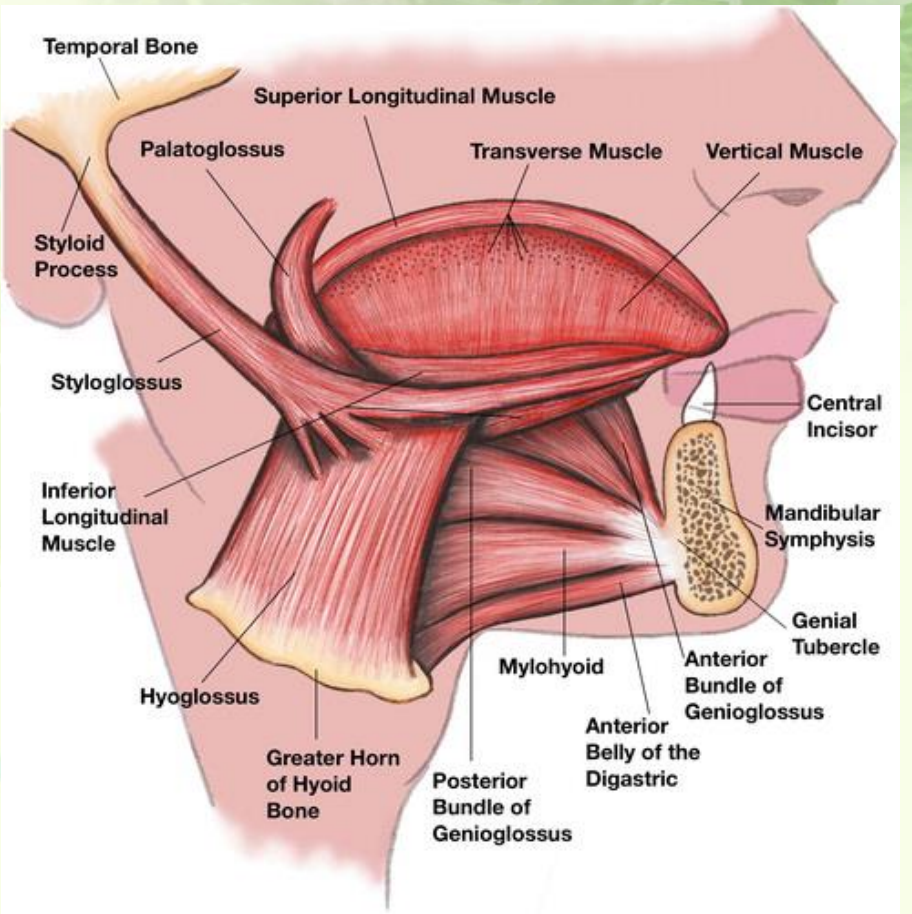


The consequent of muscle atrophy on the tongue

The tongue is a muscular structure that consists of two types of muscles:

1. **Intrinsic Muscles - Innervated by Hypoglossal nerve CN XII**
   - superior longitudinal muscle
   - inferior longitudinal muscle
   - transverse muscle
   - vertical muscles

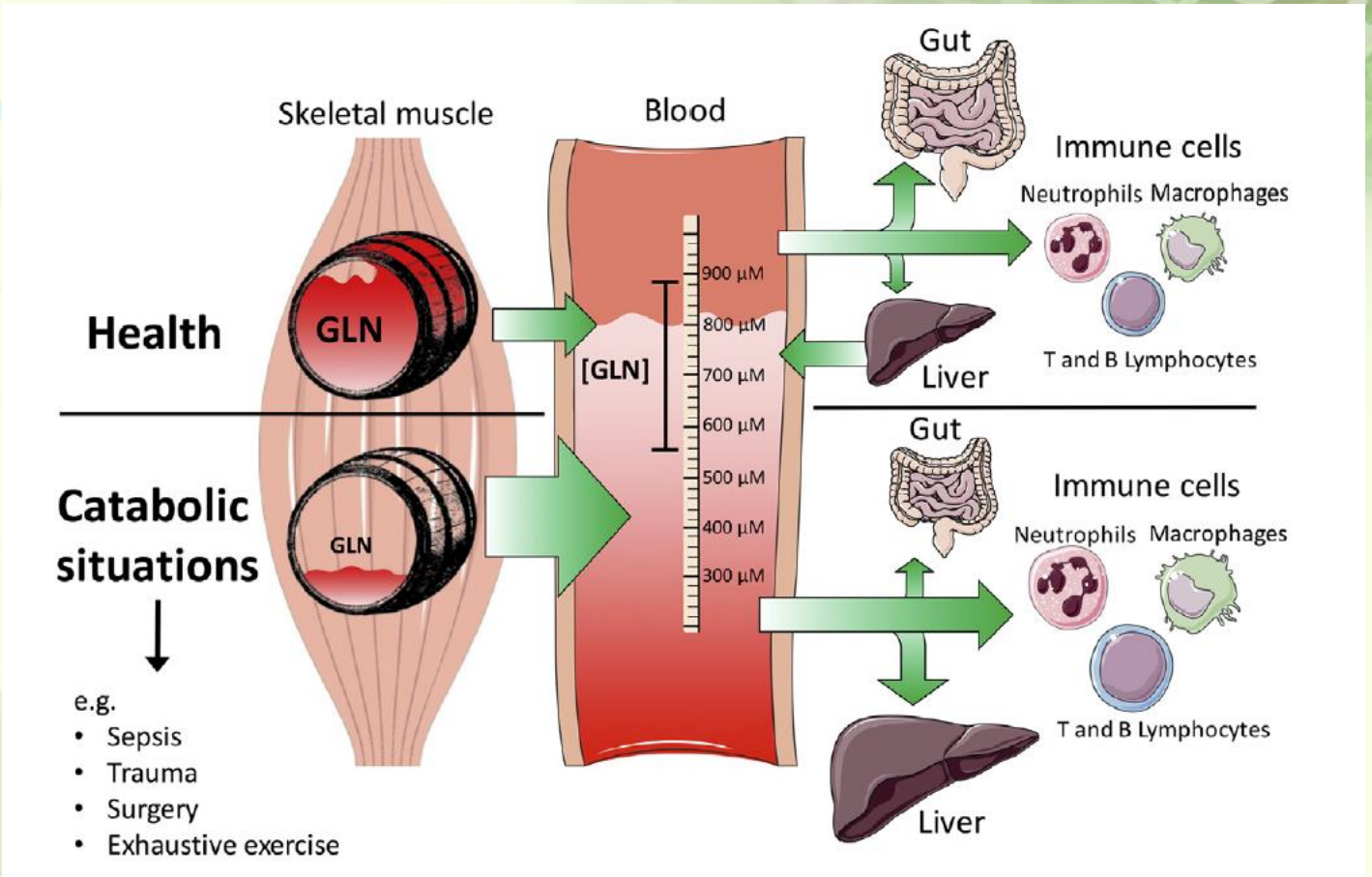
   These 4 muscles control tongue rolling and facilitating speech, eating and swallowing.
2. **Extrinsic Muscles – Innervated by Hypoglossal & Vagus nerve**
   - **Genioglossus muscle** controls protrusion ('sticking the tongue out') and depression of the tongue.
   - **Hyoglossus muscle** controls depression and retraction of the tongue.
   - **Styloglossus muscle** controls retraction and elevation of the tongue.
   - **Palatoglossus muscle** controls elevation of the posterior tongue



Atrophy of these tongue muscles will affect speech, eating and swallowing functions

What ALS patient should know about Glutamine?

- In **health**, glutamine is produced by skeletal muscle to support other bodily functions.[1]
- However, under **catabolic situations** where muscles are under excessive stress, like in ALS, then glutamine can be depleted in skeletal muscle.[1]
- Glutamine supplementation can restore glutamine in skeletal muscle.[1]



(Cruzat, 2015)

1. Tirapegui, J., Cruzat, V.F. (2015). Glutamine and Skeletal Muscle. In: Rajendram, R., Preedy, V., Patel, V. (eds) Glutamine in Clinical Nutrition. Nutrition and Health. Humana Press, New York, NY.

What ALS patient should know about Glutamine?

- The loss of glutamate-metabolizing *bacteria* leads to loss of the gut's ability to metabolize food-based glutamate into glutamine
- This may lead to the loss of about **43.62%** of glutamine.
- About 80% of the body's glutamine is found in skeletal muscle.[1]
- Glutamine makes up about 50-60% of the free amino acids found in skeletal muscle.[1]
- Researchers estimate we consume about 3 to 6 grams glutamine daily in our diet
- Prolong deficiency of glutamine can lead to atrophy of tongue, diaphragm, heart, hand and feet muscles.
- Muscular atrophy precedes the loss of motor function in ALS patients.[2, 3]
- This could shift our understanding of ALS from motor neuron dysfunction toward muscular atrophy

1. Cruzat V, Macedo Rogero M, Noel Keane K, Curi R, Newsholme P. Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. Nutrients. 2018 Oct 23;10(11):1564.
2. Scaricamazza S, Salvatori I, Ferri A, Valle C. Skeletal Muscle in ALS: An Unappreciated Therapeutic Opportunity? Cells. 2021 Mar 2;10(3):525.
3. Shefner JM, Musaro A, Ngo ST, Lunetta C, Steyn FJ, Robitaille R, De Carvalho M, Rutkove S, Ludolph AC, Dupuis L. Skeletal muscle in amyotrophic lateral sclerosis. Brain. 2023 Jun 16:awad202.

How is ALS Different than Starvation?

- Top-down approach
- Because of glutamate excitotoxicity in ALS, upper and lower motor neurons are persistently activated.
- This leads to the repetitive activation of muscle, which may be clinically presented as fasciculation.
- This persistent depolarization and repolarization of motor neurons during fasciculation may cause intracellular K^+ to become deficient.
- K^+ deficiency may delay or prevent repolarization, which would prevent termination of muscle activation.
- This can lead to cramps and muscle stiffness.
- The hypermetabolism of muscle and **prolonged deficiency in glutamine** would then lead to glutamine depletion in muscle tissue.[1]

1. Tirapegui, J., Cruzat, V.F. (2015). Glutamine and Skeletal Muscle. In: Rajendram, R., Preedy, V., Patel, V. (eds) Glutamine in Clinical Nutrition. Nutrition and Health. Humana Press, New York, NY.

Role of Acetylcholine in Muscular Atrophy

- Fasciculation is a form of repetitive muscle activation through the excessive release of acetylcholine (ACh) **from the axon terminals** of **lower** motor neurons.[1, 2]
- Chronic fasciculation leads to excessive release of acetylcholine in the neuromuscular junction. This may lead to the over expression, then under expression, and lastly dysfunction of acetylcholine receptors (AChRs) in the neuromuscular junction.
- In a study by Cappello, it was reported that AChRs of ALS patients have significantly less affinity than those of healthy subjects.[3]
- Furthermore, sodium/potassium ion pump dysfunction at the neuromuscular junction has been implicated in ALS.[4] This may prevent normal repolarization. This is similar to the mechanism seen in Isaac's Syndrome, where the lack of repolarization causes permanent muscle cramps.
- We postulate that overexposure to acetylcholine and the excessive release of potassium in the extracellular space leads to cramps, the dysfunction of AChRs, and the eventual depletion of ACh. This may result in ineffective muscle activation that leads to muscle weakness **and loss of muscle function**.

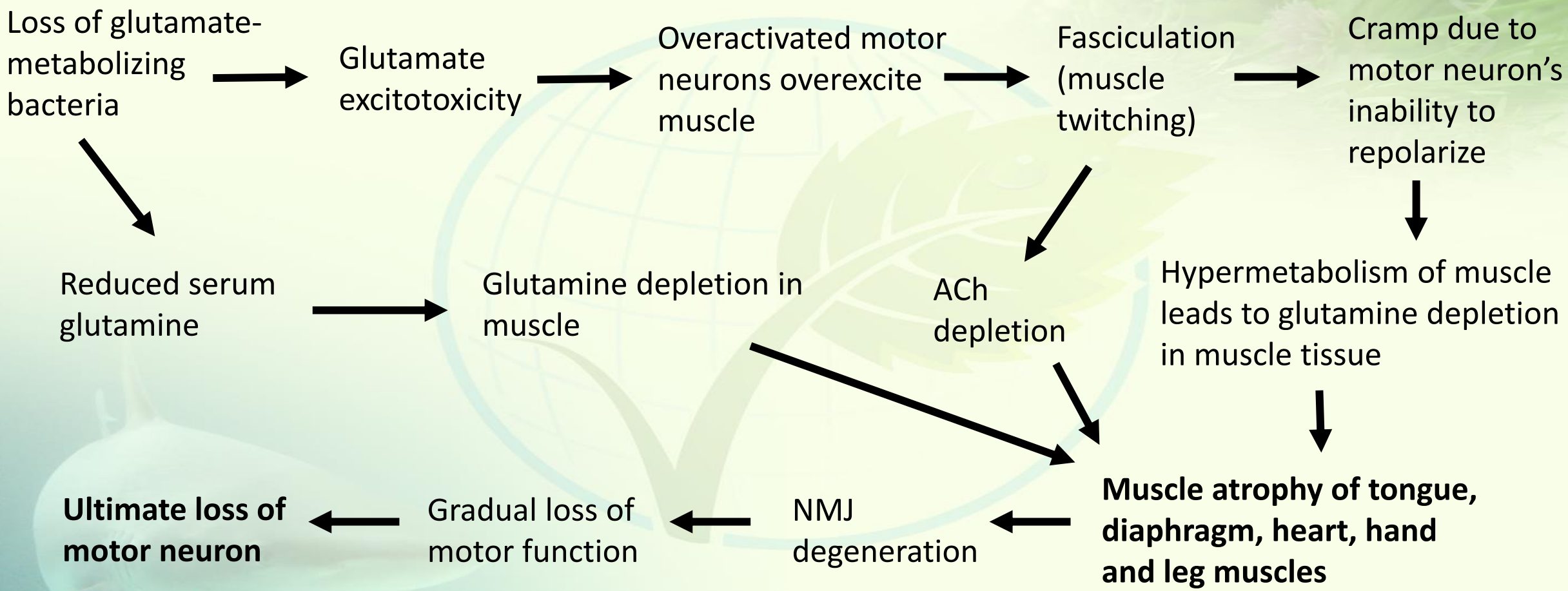
1. Leite MA, Orsini M, de Freitas MR, Pereira JS, Gobbi FH, Bastos VH, de Castro Machado D, Machado S, Arrias-Carrion O, de Souza JA, Oliveira AB. Another Perspective on Fasciculations: When is it not Caused by the Classic form of Amyotrophic Lateral Sclerosis or Progressive Spinal Atrophy? Neurol Int. 2014 Aug 8;6(3):5208.Scaricamazza S, Salvatori I, Ferri A, Valle C. Skeletal Muscle in ALS: An Unappreciated Therapeutic Opportunity? Cells. 2021 Mar 2;10(3):525.
2. Adeyinka A, Kondamudi NP. Cholinergic Crisis. [Updated 2023 Aug 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-.
3. Cappello, V., & Francolini, M. (2017). Neuromuscular Junction Dismantling in Amyotrophic Lateral Sclerosis. *International Journal of Molecular Sciences , 18*, 2092.
4. Kiernan, M. C., Vucic, S., Cheah, B. C., Turner, M. R., Eisen, A., Hardiman, O., et al. (2011). Amyotrophic lateral sclerosis. *The Lancet , 377* (9769), 942-955.

Role of Acetylcholine in Muscular Atrophy

- In addition to dysfunctional AChRs at the NMJ and loss of intracellular potassium, the over release of acetylcholine in the synapse may eventually deplete the cholinergic synapse of acetylcholine and its related enzymes
- ACh recycling and packaging enzymes, acetylcholineesterase (AChE) and vesicular ACh transporter (VAChT), have both been found to be reduced in ALS patients.[1]
- Furthermore, microassay analysis of choline acetyltransferase (ChAT), an enzyme used for the biosynthesis of acetylcholine and the most specific indicator for cholinergic synaptic function, showed less ChAT activity in ALS patients than in control patients.[2]
- ChATs were significantly reduced in motor neuron somas and cholinergic synaptic terminals early on, even before motor neuron loss and neuromuscular junction detachment.[3]
- The loss of ACh recycling and packaging mechanisms may lead to ACh depletion from the axon terminal as unrecycled ACh will dissipate from the synapse into the extracellular space.
- ACh depletion may lead to reduced muscle activation, leading to muscle weakness and **loss of muscle function**.[4]

1. Campanari ML, García-Ayllón MS, Ciura S, Sáez-Valero J, Kabashi E. Neuromuscular Junction Impairment in Amyotrophic Lateral Sclerosis: Reassessing the Role of Acetylcholinesterase. Front Mol Neurosci. 2016 Dec 27;9:160.
2. Kato, T. (1989). Choline acetyltransferase activities in single spinal motor neurons from patients with amyotrophic lateral sclerosis. *J Neurochem , 52* (2), 636-640.
3. Casas, C., Herrando-Grabulosa, M., Manzano, R., Mancuso, R., Osta, R., & Navarro, X. (2013). Early presymptomatic cholinergic dysfunction in a murine model of amyotrophic lateral sclerosis. *Brain Behavior , 3* (2), 145-58.
4. https://www.rupahealth.com/post/poor-short-term-memory-muscles-weakness-and-constipation-are-symptoms-of-low-levels-of-this-neurotransmitter#:~:text=Low%20levels%20of%20acetylcholine%20can,Sclerosis%2C%20dementia%2C%20and%20Alzheimer's

Simple flow chart for alternate pathway of ALS pathology



How much Glutamine do ALS patients need?

- The amount and frequency of glutamine ALS patients should take depends on how bad the condition of their muscular atrophy is. In general, clinical trials use doses of 5 to 45 grams per day for up to six weeks with no negative side effects.
- Mayo Clinic recommends adults to take 30 grams per day in divided doses (5 g taken 6 times a day) for up to 16 weeks for patients who suffer short bowel syndrome.[1]
- Typically, a fixed daily dose of 20-35 g/24 hours or variable dose of < 1.0 (usually 0.2 to 0.3) g/kg of body weight is recommended.[2]

1. Mayo Clinic. Drugs and Supplements: Glutamine (Oral Route). https://www.mayoclinic.org/drugs-supplements/glutamine-oral-route/proper-use/drg-20064099
2. Tirapegui, J., Cruzat, V.F. (2015). Glutamine and Skeletal Muscle. In: Rajendram, R., Preedy, V., Patel, V. (eds) Glutamine in Clinical Nutrition. Nutrition and Health. Humana Press, New York, NY.

What is the best way to take Glutamine

- Glutamine is an amino acid. As such, it can be directly absorbed by the microvilli of the small intestines without going through the digestion process.
- Regeneration of human body, including regrowing muscle mass, happens only in the sleep.
- **Therefore, the best way to take Glutamine powder is to mix 5 to 10 gram of L-Glutamine in a cup of warm water. Let it sit for 5 to 10 minutes to allow L-Glutamine to dissolve completely in the warm water. Then take it on an empty stomach (2 hours after dinner or before sleep).**

What is the implication?

- Many papers suggest muscular atrophy precedes the loss of motor function in ALS.[1]
- Other papers suggest loss of motor function precedes the loss of motor neurons in a dying-back way for ALS[2, 3]
- Could this finding suggest ALS is a progressive muscular atrophic disease similar to SMA4?
  - Loss of glutamate-metabolizing bacteria contributes to the deficiency of glutamate to glutamine conversion. Glutamine is needed to maintain muscle function and prevent muscular atrophy.
  - While recolonizing glutamate-metabolizing bacteria is a long and difficult process, supplementing glutamine on an empty stomach may have potential to prevent muscular atrophy, stopping the progression of ALS completely from the day of formal diagnosis.

If validated in the near future, the progression of ALS may be prevented by taking proper amount of glutamine.

1. Shefner JM, Musaro A, Ngo ST, Lunetta C, Steyn FJ, Robitaille R, De Carvalho M, Rutkove S, Ludolph AC, Dupuis L. Skeletal muscle in amyotrophic lateral sclerosis. Brain. 2023 Jun 16:awad202.
2. Scaricamazza S, Salvatori I, Ferri A, Valle C. Skeletal Muscle in ALS: An Unappreciated Therapeutic Opportunity? Cells. 2021 Mar 2;10(3):525.
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