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Muscle fatigue and exercise-related biomarkers in amyotrophic lateral sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder affecting motor neurons. The complex etiopathogenetic mechanism of ALS can lead to extensive alterations, including cortical changes, neuroinflammation, and changes in muscular structure. These ALS-derived alterations may contribute to fatigue, a symptom severely impacting patients' quality of life that is commonly associated with muscular exercise. Intriguingly, muscular exercise can be at once a promoter of motor neuron degeneration in predisposed patients as well as an effective non-pharmacological treatment of ALS. To fully disclose its therapeutic potential, muscular exercise must be tailored to patients' phenotypes, balancing potential benefits and risks that are unique to each ALS case. Biomarkers of muscular fatigue, with their potential for insight into inflammation and oxidation, can be used to ensure that the intensity of physical activity remains below the threshold level beyond which exercise might become harmful. In this review, the authors explore the concept of fatigue in ALS patients, focusing on fatigue generation, definition, detection, quantification, and treatment. The study discusses the most important fatigue biomarkers, putting them in relation to the mechanism of fatigue generation and with monitoring of muscular exercise as a possible treatment of fatigue.

Keywords

Amyotrophic lateral sclerosis (ALS), fatigue, biomarkers, muscular exercise

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disorder involving upper motor neurons (UMNs) and lower motor neurons (LMNs), with a median lifespan that ranges between two and five years. Typically, death occurs due to terminal deterioration of respiratory and swallowing muscles [1]. ALS is characterized by a wide spectrum of clinical manifestations, as caused by unbalanced deterioration of UMN and LMN, different spatial distribution of motor neurons' involvement, and specific disease courses [2].

The exact etiopathogenic mechanisms of ALS are still unclear. During the development of the disease, many cellular processes are affected by a catastrophic prion-like involvement of a large amount of motor neurons [3]. The accumulation of toxic aggregates of erroneously folded proteins and mRNA, alterations in nucleus transport mechanisms and axonal mobility, and mitochondrial malfunctions are all recognized amongst key alterations of cellular functionality in ALS [3]. The latter include alterations in fission and fusion processes, as well as alterations in mitochondrial cristae, as caused by genetic mutations in superoxide dismutase 1 (*SOD1*), vesicle-associated membrane protein-associated protein B and C (*VAPB)*, transactive response (TAR) DNA-binding protein (*TARDBP*), fused in sarcoma (*FUS)*, and coiled-coil-helix-coiled-coil-helix domain containing 10 (*CHCHD10*) [4, 5]. Among the etiopathogenic mechanisms of ALS, muscular exercise is reported to have putative detrimental effects on neurodegeneration by increasing skeletal muscle hyperactivity on vulnerable motor neurons. Muscle structure and function are intimately connected. The complexity of this relationship contributes to explaining why in ALS it is so challenging to find a clear-cut limit beyond which physical exercise may become detrimental.

In recent years, expanding knowledge of genetics has been instrumental in better understanding the several pathogenic mechanisms involved in motor neuron degeneration, at times unveiling a common link among different neurodegenerative disorders [4, 6]. Even in the case of patients lacking known pathogenic mutations, i.e., sporadic types of ALS, genetics may still play a pivotal role in predisposing to diseases development, likely increasing individual susceptibility to environmental factors, even though to which degree it happens is still a matter of debate [6, 7].

Regardless of its etiopathogenic mechanisms, ALS has a devastating impact on the life quality of both patients and caregivers. This calls for coordinated efforts in cures at the nexus of supplements, diet, and exercise [8]. In addition to progressive loss of mobility, respiratory troubles, and swallowing difficulties, the disease often causes limb pain, mood and sleep disorders, and social withdrawal [9]. One of the earliest and most commonly referred symptoms of ALS is fatigue, a complex symptom that accompanies several neurological diseases [10]. Patients suffering from neurodegenerative diseases, muscular disorders, and autoimmune diseases often refer to fatigue as a pivotal and multifactorial symptom that negatively impacts on daily life [11]. Yet, regardless of its centrality and importance, fatigue is often not appropriately managed, reinforcing the importance of studying the unmet needs that still come with this peculiar symptom.

This review aims at characterizing in detail the role and importance of fatigue in the larger spectrum of ALS-related phenotypes. Here, the study explores the concept of fatigue and its role as the main indicator of exercise-related failure in ALS patients according to the most recent scientific literature. The authors start by defining fatigue, and then move to describe how fatigue can be quantified, and eventually treated in relation to the motor activity itself.

Fatigue in ALS

Fatigue is a multifaceted symptom known to have a strong impact on the quality of life in ALS patients [12]. It is defined by Gibbons et al. [13] as "reversible motor weakness and whole-body tiredness [...] predominantly brought on by muscular exertion and [...] partially relieved by rest". Differently from sleepiness, neurological fatigue usually does not improve after sleeping, although patients often describe it as a sensation of physical and/or mental exhaustion [13]. Fatigue can also be defined as an objective decline in the ability of a muscle to contract to maximum force [14] or as the decreased ability to generate appropriate amounts of muscle force during on-going contractile activity [15]. Fatigue is widely reported in several studies with the self-reported registry of patients' symptoms, more than 50% on average, up to 80% at times [10]. McElhiney et al. [16] conducted a large study including over 200 patients, finding that fatigue was related to a lower mean physical disability score, a lower pulmonary forced vital capacity, and a faster rate of progression.

As in the case of other neuromuscular diseases, fatigue in ALS has a dual nervous system origin, both central and peripheral [10, 12]. The death of large motor units leads to a reduction of peripheral neural drives, at which point re-innervation occurs by surviving smaller motor neurons, hence shifting the balance from type II fibers towards type I fibers. Being the latter slow-twitch, mitochondrial-rich, and less prone to fatigue (as they rely more on mitochondrial oxidative phosphorylation), this shift causes an altered and often incomplete balance towards less performing and more fatigable muscle fibers, thus contributing to generating fatigue [12, 17]. Moreover, mitochondrial dysfunctions that occur in ALS may lead to energetic depletion, which in turn may contribute to further fatigue [18]. Impaired ATP production due to mitochondrial abnormalities causes a switch towards anaerobic metabolism during fatiguing stimulation [17, 18]. Additionally, UMN dysfunction causes a reduced voluntary drive, leading to central fatigue [12]. Several studies have been aimed at better characterizing the exact amount of central and peripheral contributions to fatigue in ALS. Among them, Kent-Braun and Miller [19] measured the compound muscle action potentials (CMAP) of seven ALS patients and six healthy controls after a tetanic stimulation, during an intermittent voluntary submaximal isometric exercise (ankle dorsiflexion). Muscle properties were inferred by measuring the maximum rate of tetanic force development and the half-time tetanic force relaxation. At the end of the exercise, the ALS group had a 1/3 increase in added force in response to a stimulus train imposed during maximal voluntary contraction, while the healthy group did not show any changes, possibly indicating a central activation failure, thus supporting the role of a central origin in ALS-related fatigue [20]. In another study, Schillings et al. [21] developed a model to simultaneously calculate the central and peripheral components of fatigue during sustained maximal voluntary contraction. They evaluated the force obtained from maximal voluntary contraction and compared it to the force generated by superimposing twitches with electrical stimulation, representative to the force of the muscle activated as a whole [10, 12, 21].

Transcranial magnetic stimulation (TMS) can be used to track motor thresholds after a voluntary contraction. In these regards, several papers [22, 23] reported a significant reduction in the short-interval intracortical inhibition following the voluntary contraction in healthy controls, while non-significant changes were detected in ALS patients. These findings may be put in relation to the degeneration of inhibitory GABAergic intracortical circuits that might significantly correlate with the development of fatigue in ALS [22]. By examining the silent period with TMS, a decreased intracortical inhibition is detected in the later stages of the disease, thus confirming the hypothesis of dysfunction of inhibitory corticospinal influences in ALS [21, 22]. Using a train of supramaximal stimuli at 50 Hz, Sharma et al. [24] found a decline in ALS patients both in maximum voluntary force and tetanic force, suggesting a greater fatigability in ALS. Being the decline similar among voluntary force and tetanic force, one may assume that fatigue had a peripheral origin as well. In these experiments energy metabolites changed during the exercise, with proton signal intensity tending to be lower in ALS patients compared with controls, suggesting that fatigue might be related to impaired muscular activation and to muscular membrane alterations [12, 17].

Inflammation as a source of fatigue in ALS

Among central and peripheral fatigue generators, an important role is held by inflammation, which usually occurs in neurodegenerative disorders. Both the immune system and inflammation may have a role in the development of neurotoxicity, as supported by the detection of the infiltration of T cells in the central nervous system (CNS) [25], and by the associated observations of glial activation, peripheral immune cell infiltration, and presence of reactive astrocytes in the same anatomical sites of injured motor neurons [26]. The intricacy and mutual reciprocity between the several factors of these complex mechanisms urged researchers to coin the term inflammasome, contributing to both disease development and disease progression [27].

Studies [26, 27] reported that with the onset of ALS symptoms, microglia diminish their expression of anti-inflammatory and growth factors, and increase production of proinflammatory factors, contributing to neurodegenerative disorders. Thanks to the growing body of knowledge about pathogenic mechanisms in ALS, it is increasingly clear that a link exists between motor neuron degeneration and inflammation [27].

For instance, aggregates of the TAR DNA-binding protein 43 kDa (TDP-43, a hallmark of several forms of ALS) were reported to interact with the nuclear factor kappa light chain enhancer of activated B cells (NF-κB), a transcription factor regulating the production of proinflammatory factors [28]. The *FUS* gene, a gene mutated in about 5% of the familial ALS, is able to co-activate NF-kB as well, being able to induce a tumor necrosis factor (TNF)-mediated death [29].

In chronic inflammation at disease onset, aberrant astrocytes express glial fibrillary acid protein (GFAP), S100β and connexin 43, and markers of astrocytic damage that surround the dying motoneurons in hSOD1 mutant mice model [26]. Other markers of monocytic and astrocytic damage, e.g., the soluble monocyte differentiation antigen CD14 and the astrocytic calcium-binding protein S100β, can be detected in the cerebral spinal fluid of patients with ALS. An increased ratio of CD14 to S100β has been suggested as a prognostic indicator in ALS patients [30]. In mutated *SOD1* mice models, microglia activation is able to activate the pro-apoptotic caspase-1 and IL1β [26]. In ALS patients, the serum concentration of IL-18 was observed to be increased, and caspase-1 expression was shown to be augmented in brain tissues [31]. However, all those mechanisms that lead to faulty aggregation of proteins are powerful triggers of inflammation, and they trigger autophagolysosomal systems towards inducing cellular-death pathways [26]. Mitochondrial damage, endoplasmic reticulum stress response, and axonal damage also promote inflammation, contributing to both motor neuron degeneration and disease progression, together with polymorphisms in cytokine and chemokine receptors [32].

By increasing muscular damage, inflammation may exacerbate peripheral fatigue. On the other hand, several studies have highlighted that proinflammatory cytokine can reduce the production of tetrahydrobiopterin, a cofactor in the metabolism of tyrosine and tryptophan, respectively the precursors of dopamine, noradrenaline, epinephrine, and serotonin [17, 33]. The degradation of tryptophan due to inflammation may also explain the role of inflammation in central fatigue [17]. Increased inflammation is seen in the periphery in both depression and fatigue [33]. The increased permeability of the blood-brain barrier due to inflammation eases the entrance of inflammatory molecules or immune cells into the CNS, leading to both structural and functional changes, including those in the hippocampus [17, 33]. Therefore, all the products of microglial activation in response to acute injury may be used as biomarkers of inflammation and as indirect biomarkers of fatigue.

Biomarkers of fatigue

Fatigue plays a pivotal role in influencing the quality of life in patients with neuromuscular disorders and calls for prompt detection and take-over. Aiming to this, several efforts have been put into applying appropriate scales to better categorize the spectrum of fatigue. Fatigue is quantified by scales that cover the physical and mental aspects of fatigue, whereas fatigability can be quantified by measuring the decline in performance on a given task [11]. Several scales are currently available to measure fatigue: among them are the 2- and 6-minute walking distance test (2MWDT and 6MWDT), four specific items in the 36-Item Short Form Survey (SF-36) (namely: items 23, 27, 29, and 31), the functional assessment of chronic illness therapy-fatigue (FACIT-F), and the fatigue severity scale (FSS). These scales are routinely used to assess fatigue in ALS [10, 12, 17].

In addition to scales, several biomarkers have been proposed to track fatigue, with the underlying idea that changes in the expression or state of a protein can be correlated with the risk or progression of a disease, or with the susceptibility of the disease to a given treatment [15]. Different categories of biomarkers might ease the detection of fatigue or of defective metabolic pathways associated with ALS.

Neurophysiological biomarkers of fatigue

Neurophysiological techniques are theoretically suitable as indirect biomarkers of fatigue. Since LMN degeneration causes muscular hypotrophy and muscular consumption, the derived condition of progressive sarcopenia is considered one of the leading causes of fatigue in neuromuscular diseases [34]. Moreover,

LMN degeneration forces survival motor neurons to innervate larger motor units, thus reducing the threshold of fatigue occurrence and perception.

In this view, motor unit number estimation (MUNE) may give an indirect insight on fatigue, as it estimates the number of intact motor units that innervate a single muscle. In accordance with the general formula of dividing the maximum response amplitude by the mean of all motor unit amplitudes, this technique records the maximum response generated by activation of all motor units in the muscle and compares it to an estimate of individual motor unit size [30].

MUNE studies in ALS typically show reduced motor unit numbers but increased motor unit amplitude when compared to healthy controls. In the studies on MUNE performed at the early stages of the disease, this compensation in amplitude led to potential amplitudes of compound motor action at normal ranges until MUNE values dropped below 10% of normal. At the same threshold, clinical atrophy appears as well. MUNE may be used in clinical practice to estimate disease progression, often being correlated to other parameters, including the clinical scale of muscular force and disease-related disability [30]. Similar to MUNE, the neurophysiological index (NI) combines the measures of CMAP, distal motor latency, and F-wave frequency. Although it does not precisely correlate to the number of surviving motor units, some studies have shown a reduction in NI values during the disease course as compared to baseline [30], suggesting that it may be used as a biomarker of disease progression.

Biochemical markers of peripheral muscle fatigue

Since general biomarkers for neurological fatigue are lacking, muscular biomarkers may be used to infer information about general fatigue. In ALS, biomarkers of muscle fatigue are easily obtained and are useful to achieve information about muscular detriment. However, effective biomarkers of muscle fatigue may not be easily individuated, since they can overlap with biomarkers of muscle damage.

In fatigue, models of muscular fatigue biomarkers are mainly obtained during or after exercise. Hence, a possible categorization of muscle fatigue and related biomarkers could be based on putting them in relation to the type of exercise or workload carried out, or on evaluating the timing in which they are detectable in relation to the timing of the start and end of physical exercise. Depending on the duration of muscular exercise, different biomarkers may be used, and their return to normal values depends on the rate of muscular recovery. This is related to the different energy demands that the different timing of exercise requires, e.g., anaerobic exercise lasting only few seconds, mixed aerobic-anaerobic exercise lasting up to 1 min, and aerobic exercise lasting more than 1 min [15].

Lactate, ammonia, hypoxanthine, and xanthine

Intense exercise may cause acidosis leading to ATP depletion. At the beginning of the exercise, ADP is converted to ATP. As the exercise increases in intensity and duration, the ATP:ADP ratio decreases due to ATP consumption, and the total adenine nucleotide pool decreases due to adenosine monophosphate (AMP) deamination. To maintain the ATP:ADP ratio, energy-rich phosphate groups are transferred from one ADP to another, each time resulting in one ATP and one AMP. Prolonged or intense exercise can activate AMP-kinase, a key sensor of cellular energy stress involved in long-term metabolic adaptations, such as the increase in the number of mitochondria. Because AMP degradation is enhanced during exercise, serum ammonia and intracellular inosine monophosphate (IMP) levels concomitantly increase. The progressive accumulation of the deaminated products IMP and ammonia cause fatigue. This explains, for example, the lower fatigue tolerance in patients with AMP deaminase deficiency [35]. Monitoring acidosis may thus give an idea of muscle fatigue since the decreased ATP activity affects the ryanodine receptor function and impairs the functioning of ATPases pumps in several myocytes [15, 35].

Acidosis leads to a shift in ATP production from aerobic to anaerobic processes, thus producing lactate. Hypocalcemia and hypomagnesaemia may follow this condition and are easily detected in blood tests. Since ammonia closely follows the lactate response during exercise, it lends itself to monitoring muscle fatigue. In clinical practice, the ischemic test (a forearm exercise under ischemic conditions in a fasted subject) can

easily reflect acidosis condition, enabling to easily obtain the dosage of serum lactate and ammonium, prior to, during, and after the test. Likewise, purine derivatives hypoxanthine and xanthine are increased in serum and urine after prolonged exercise. Since they are directly correlated with the amount of ATP consumed inside the cell, they are considered good biomarkers of muscle fatigue [15].

Reactive-oxygen species, antioxidants, and biomarkers of inflammation

Neuro-inflammation is an important factor that causes and follows motor neuron damage, muscle degeneration, and the occurrence of neurological fatigue. High levels of oxidative stress biomarkers and a reduction of antioxidant factors are observed in ALS patients [26, 27]. For instance, the nuclear factor erythroid 2-related factor 2 (Nrf2) protects against oxidative stress and cell death induced by the SOD1-mutant protein and increases the resistance of glial cells to oxidative stress by increasing glutathione (GSH) levels in SOD1-mutant protein mice [36]. Studies *in vitro* found that motor neuronal cell lines expressing TDP-43 mutants exhibited shortened neuritis and higher oxidative stress, an effect partially reversed using the Nrf2 enhancer. Thus, all markers of motor neuron injuries may give an indirect measure of muscle fatigue. For instance, markers like reactive oxygen species (ROS) and reactive nitrogen species (RNS), cytokines like TNF, interleukin 1b (IL-1b), IL-10, IL-4, IL-6, chemokine, and brain-derived neurotrophic factor (BDNF) are easily measured in blood, giving a picture of inflammatory state [26, 36].

Under physiological conditions, ROS are required to control biochemical processes, including cell differentiation, neurogenesis, antioxidant gene expression, and immune system regulation [37, 38]. However, conditions of muscular stress, including muscular inflammation related to some types of muscular exercise, may increase oxidative stress, thus leading to an oxidation of proteins, lipids, or nucleic acids at the basis of signaling pathways inducing necrosis and apoptosis [38]. In healthy subjects, muscle fibers can prevent oxidative damage, so that during training reproducing a muscular stress condition, the total antioxidant capacity (TAC) shows an increase at the beginning of the exercise and after high-volume training. This suggests that the antioxidant defense system is activated during exercise. Muscle cells are able to create a protective environment against oxidative stress, recruiting anti-inflammatory molecules like IL-6, IL-8, IL-15, BDNF, fibroblast growth factor 21, and follistatin-like-1 [15, 38].

ROS can activate transcription factors known to regulate IL-6, a cytokine acting both as pro-inflammatory (in monocytes and macrophages), and as anti-inflammatory (in myocytes), which is easily detectable in blood in conditions of increased inflammation [38]. However, in conditions of neurodegenerative disorders, the increased oxidative stress can exceed the muscular antioxidant capacity [15, 38], making ROS and TAC potential biomarkers. The dosage of ROS and TAC are easily measured in a blood test, and their dosage coupled with biomarkers of oxidative damage [such as lipid peroxidation biomarkers like thiobarbituric acid-reactive substances (TBARS), and isoprostane, protein oxidation biomarkers–advanced oxidation protein products (AOPPs) or biomarkers of antioxidant capacity], may give a more accurate picture of muscle fatigue [15]. While ROS immediately peaks after exercise, TBARS significantly decreases post-exercise, likely in relation to the time required to activate the biological pathways underlying the cellular redox state after an aerobic exercise [39].

In addition to TAC, GSH and glutathione peroxidase (GPX) are easily tested in serum and saliva. They are scavengers of ROS and hydrogen peroxide (H_2O_2), respectively, and the former increases with high-volume training, while the latter is able to scavenge at low-volume training. At higher exercise workloads, the production of H_2O_2 exceeds GSH and GPX capabilities, leading to a loss of muscle contractility, and to an increase in muscle fatigue [15, 40].

Concerning the redox balance and its relationship with exercise in ALS patients, Pasquinelli et al. [41] measured oxidative parameters in ALS patients during an exercise protocol using a myometer at a forearm in which the contractile force increased incrementally. Plasmatic levels of AOPPs, Ferric reducing antioxidant power (FRAP), and total thiol (t-SH) groups remained stable during the short-lasting exercise protocol, suggesting a lack or delayed exercise-related kinetic curve for these redox biomarkers in ALS patients. On the contrary, levels of lactate during each step of the exercise protocol increased similarly in ALS patients compared to healthy controls. Interestingly, they found different results in patients carrying

different polymorphisms in the *PGC-1α* gene, which is activated by muscle contraction and brings fast-to-slow muscle fiber conversion. Patients harboring the Ser428Ser genotype showed higher levels of AOPPs [at 50% of maximal voluntary contraction (MVC) force and at recovery] and lactate (at 30% and 50%) during exercise compared to Gly482Gly patients [41].

IL-6 can function as a myokine, increasing in response to muscle contractions [42]. It increases with exercise duration, reaching the maximum levels immediately post-exercise, and returning to resting levels a few hours after the exercise ends [15]. During exercise, IL-6 may act in a hormone-like manner to mobilize extracellular substrates or augment substrate delivery. It expresses its anti-inflammatory role by inhibiting TNF-α and IL-1 and activating IL-1RA and IL-10. TNF-α is a pro-inflammatory cytokine able to induce apoptosis and inflammation [26, 42].

Finally, since the neutrophil-to-lymphocyte ratio is reported to be correlated to the degree of neuroinflammation in ALS patients [43], blood cell count may also be considered to add information on inflammation.

Treatment of fatigue and the role of exercise in ALS patients

Once fatigue is detected and quantified, there is the need to reduce its symptoms by therapeutic means. Although no specific treatments for fatigue in ALS exist, several therapeutic options are available.

According to a recent Cochrane review [10], modafinil showed significant results in clinical trials testing clinical outcomes in regard to FSS and to measures of depression and sleepiness. Modafinil acts by stimulating the release of norepinephrine and dopamine from synaptic terminals, thus elevating hypothalamic levels of histamine. Creatine, which increases the maximum availability of energy output in anaerobic activities, may also have a positive effect on muscle strength and fatigue. Preliminary studies showed that natural molecules such as curcumin, a natural antioxidant compound, might reduce oxidative stress, and be combined with disease-modifying therapies [44]. Among non-pharmacological approaches, resistance exercise like treadmill ambulation and muscular exercise showed a reduction in fatigue at FSS. Repetitive TMS (rTMS) is a well-established therapeutic strategy that, by increasing cortical plasticity, may be used to boost motor rehabilitation and treat mood disorders [43, 45]. In a clinical trial, the patients group performing rTMS at 5Hz on the motor cortex for 2 weeks showed a reduction in FSS *versus* the control group which performed a sham intervention [46]. Future applications of nanomaterials bear promise towards the treatment of ALS fatigue. The increasing feasibility of tissue-specific delivery of oxygen using nanobubbles makes it a promising approach to compensate for hypoxia, a potential complement to current therapeutic approaches [47]. Although still in a preliminary testing phase, antioxidant materials coupled with carbon nanotubules have been shown to be able to support the regeneration of neuronal stem tissues [48] and the reduction of oxidative damage [49]. Similarly, selenium-based nanoparticles have recently shown neuroprotective effects on other neurological disorders as well [50].

Besides pharmaceutical and technologic approaches to therapy, the role of physical exercise has been debated for a long time. In the past, the main idea was to avoid physical activity since it may increase muscular consumption. In the last years, however, more and more studies pointed out that short-lasting muscular training that does not reach a threshold of muscular fatigue is considered to be beneficial for ALS patients [45]. However, other reasons for uncertainty in promoting sports in ALS derive from previous studies that associated strenuous exercise with higher ALS incidence, as observed in athletes and professional football and American football players [6, 51]. According to this interpretation, an intense and prolonged exercise may induce a massive increase in ROS and calcium concentration, with consequent motor neuron degeneration [38].

In predisposed patients, very intense physical activity may simultaneously act on changing cortical plasticity in the motor cortex and in stressing muscles. The role of physical exercise in ALS was recently pointed out in a work by Julian et al. [52] that demonstrated that ALS shares polygenic risk genetic factors with several conditions, among which moderate to high levels of exercise. Transcriptomic analysis

corroborated this result, revealing that genes whose expression resulted altered in acute exercise were enriched with known ALS risk genes (including *C9ORF72*), and with ALS-associated variants of unknown significance [6].

Since intense physical exercise can lead to an increase in oxidative stress, ALS patients seeking exercise as a treatment for fatigue must be followed by a physiotherapist with an experience in neuromuscular diseases, able to establish the correct fatigue threshold and the correct amount of muscular exercise [51]. In this view, biomarkers may help in defining this threshold, and in defining a physical program tailored to the patients' clinical picture, since the spectrum of motor neuron diseases is widely heterogeneous and the muscular involvement is different. Although intense and excessive physical exercise may promote oxidative stress, moderate and routine exercise is associated with numerous benefits on cardiovascular, endocrine, and even neuromuscular factors. Adequate exercise training can enhance endogenous antioxidant defense systems, while intense and strenuous exercise may lead to ROS overproduction [38].

It can be assumed that physical exercise can have positive or negative effects on oxidative stress depending on the load, specificity, and basal level of training [15]. In previous studies, Siciliano et al. [38] tried to understand if exercise might be considered as a therapeutic strategy in neuromuscular diseases, and Zhu et al. [53] conducted a meta-analysis to establish which exercise better improved respiratory function, fatigue, and quality of life in ALS patients [38, 53]. These studies showed that intense aerobic exercise increases oxygen consumption with a consequent rise in ROS production, while for low exercise intensity [< 50% of maximal oxygen uptake], better antioxidant activity is present. After exercise, positive changes in redox status appear several hours after the exercise ends, likely following the time required for antioxidant gene transcription activation, mRNA maturation, and mRNA translation into protein. Thus, aerobic exercise should be prolonged in time and performed routinely [38].

Referring to anaerobic exercise, data are more controversial. The muscular exercise that creates a condition of hypoxia in muscle cells, like in the case of some type of anaerobic exercises, produces an increase in xanthine oxidase (XO), an enzyme that generates ROS during ischemia-reperfusion. Elevated lactate levels, an alteration of the oxidative status, an increase in the lipid peroxide concentration, and a decreased SOD activity were all found to occur in anaerobic exercise. However, the picture is arguably more complex, and other studies found an increase in antioxidant enzymes. Also in the case of anaerobic training, moderate-intensity training rather than maximal exercise may be able to improve adaptive responses to oxidative stress [38, 54]. In ALS-mice models, aerobic training led to an extension in the lifespan of SOD1-mutated mice, which showed higher levels of antioxidant activity. Mitochondria in trained ALS-mice models showed a reduction in lipid peroxidation compared to those in ALS-mice devoid of training. In the same study, ALS mice subjected to swimming or to running on a treadmill showed delayed spinal motor neuron death and preserved larger motor neurons. In contrast, mice forced to high-intensity training showed motor neuron loss and an increased proportion of motor neurons with small soma areas. Authors of these studies argued that running was a high-impact exercise, which recruited only small motor neurons and generated more oxidative stress than swimming, which was a low-impact exercise that recruited both small and large motor neurons [55]. On this same line, endurance exercises may protect skeletal muscle against the excessive activation of autophagy and ubiquitin-proteasome system, upregulating mitochondrial metabolism and preventing fiber-type transformation [56].

Although ALS-mice models employed in therapeutic trials may show encouraging results, findings may eventually not be confirmed in the following phases on patients due to the inherent complexity of the interrelation between muscular functions and structures. Still, mice models may disclose useful insights into the effects of pharmacological and non-pharmacological therapeutic approaches that are later confirmed in humans and may eventually lead to changes in therapeutic approaches and outcomes. Several studies on humans confirmed these findings and showed that physical activity led to an improvement in respiratory function [57], on ALS functional rating scale (ALSFRS) [58], and a reduced decline in leg strength [59]. Other studies did not confirm these beneficial effects [60, 61], though an improvement in mood and in quality of life was still often detected. The meta-analysis from Zhu et al. [53] revealed that

combined programs of aerobic exercise, resistance exercise, and standard rehabilitation were the best in improving quality of life and in reducing fatigue in ALS patients, while exercise programs of aerobic and resistance training showed the highest potential to improve ALS patients' physical function. Overall, literature findings mostly agree in supporting resistance exercise programs as a non-pharmacological adjuvant therapeutic approach in ALS [38, 53].

Conclusions

The involvement of UMN and LMN in ALS and the complex pathogenetic mechanism of the disease contribute to the multifaceted origin of fatigue, a symptom frequently reported and capable of severely affecting the quality of life of ALS patients. While treating fatigue related to ALS, one must consider other conditions that can increase fatigue perception, like depression, drugs against spasticity, sleep disorders, respiratory disturbances, malnutrition, and riluzole itself. These are all considerations that must be kept in mind when approaching fatigue in ALS, and highlight the need for a holistic approach to tackle this symptom. Also due to its intrinsic complexity and elusiveness, fatigue is not always treated to complete satisfaction of patients. Exercise may have a beneficial role in ALS patients, although remains a challenge to achieve the correct balance of intensity of workload and fatigue threshold, requiring experienced teams. In motor neuron diseases, characterizing the precise effect of exercise on redox balance is of the utmost importance, since an excess increased oxidative stress due to exercise may contribute to disease development. In this sense, the pleiotropic effects of exercise in inducing adaptive and constitutive modifications in the motor unit function and structure need to be gauged accurately in ALS. This would be important to answer the crucial question as to which type and amount of muscular exercise is capable, possibly when combined with disease-modifying pharmacological treatments, to provide positive effects and slow down the otherwise ineluctable course of neurodegeneration and muscle wasting in ALS. In ALS-related fatigue, it is important to carefully assess the several facets of patients' symptoms in order to address each aspect with tailored treatments. Treatments should therefore move towards a multidisciplinary dimension, and be open to technological innovation, in which different specialists contribute to improving patients' quality of life.

Abbreviations

ALS: amyotrophic lateral sclerosis

AMP: adenosine monophosphate

AOPPs: advanced oxidation protein products

FSS: fatigue severity scale

GSH: glutathione

LMNs: lower motor neurons

MUNE: motor unit number estimation

ROS: reactive oxygen species

SOD1: superoxide dismutase 1

TAC: total antioxidant capacity

TMS: transcranial magnetic stimulation

TNF: tumor necrosis factor

UMNs: upper motor neurons

Declarations

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Author contributions

FB: Conceptualization, Investigation, Writing—original draft, Writing—review & editing. LB, GR, LC, GS: Conceptualization, Investigation, Validation. GS: Supervision. All authors read and approved the submitted version.

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The authors declare that they have no conflicts of interest.

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