Molecular Mechanisms of Muscle Fatigue

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Muscle fatigue (MF) declines the capacity of muscles to complete a task over time at a constant load. MF is usually short-lasting, reversible, and is experienced as a feeling of tiredness or lack of energy. The leading causes of short-lasting fatigue are related to overtraining, undertraining/deconditioning, or physical injury. Conversely, MF can be persistent and more serious when associated with pathological states or following chronic exposure to certain medication or toxic composites. In conjunction with chronic fatigue, the muscle feels floppy, and the force generated by muscles is always low, causing the individual to feel frail constantly. The leading cause underpinning the development of chronic fatigue is related to muscle wasting mediated by aging, immobilization, insulin resistance (through high-fat dietary intake or pharmacologically mediated Peroxisome Proliferator-Activated Receptor (PPAR) agonism), diseases associated with systemic inflammation (arthritis, sepsis, infections, trauma, cardiovascular and respiratory disorders (heart failure, chronic obstructive pulmonary disease (COPD))), chronic kidney failure, muscle dystrophies, muscle myopathies, multiple sclerosis, and, more recently, coronavirus disease 2019 (COVID-19). The primary outcome of displaying chronic muscle fatigue is a poor quality of life.

neuroinflammation skeletal muscle atrophy muscle function fatigue

1. Muscle Architecture

The musculoskeletal system is one of the central organ systems in the body. It consists of muscles, tendons, cartilage, ligaments, connective tissues, and nerves.

There are three main types of muscle tissue: skeletal, cardiac, and smooth [1]. Skeletal muscles are fibrous tissues found in humans or animals mainly attached by tendons to the skeleton's bones. They can contract/shorten upon neuro-mediated calcium stimulation, thereby moving the whole body while maintaining the position of parts of the body.

A skeletal muscle is made up of multiple fascicles, and each one includes numerous muscle fibers (**Figure 1**, [2]). The muscle fibers are, in turn, composed of myofibrils. The myofibrils are composed of overlapping, protein-made, thick (myosin) and thin (actin) myofilaments highly organized as sarcomere units, which are de facto the contractile units of the muscle. The sheaths made of connective tissue that encapsulate the bundle of myofibrils, muscle fibers, and the outer side of the muscle are named endomysium, perimysium, and epimysium, respectively.

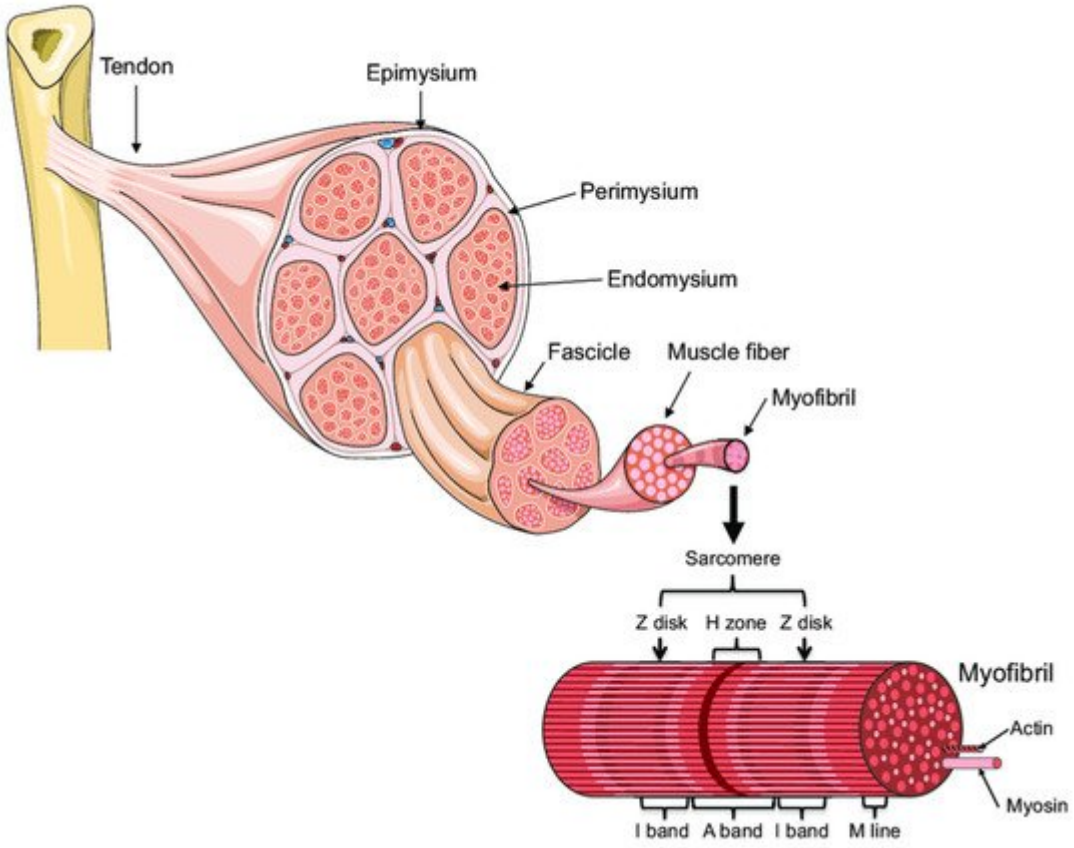

Figure 1. Muscle organization (A. Bonetta and LF Bonewald, originally adapted from Servier Medical Art—https://smart.servier.com/smart_image/tendon-anatomy/ accessed on 1 October 2021) [2].

Skeletal muscles contract via electric stimuli originating from the central nervous system (CNS). The impulses travel along nerves called motor neurons that insert into the muscle cells and branch, along with the blood vessels, into the epimysium and perimysium. The axons of the neurons then fork through the perimysium and enter the endomysium to innervate individual nerve fibers. The transfer of electrical signal from the motor neuron to the muscle fiber, which makes the latter contract, is facilitated by neuromuscular junctions. They are chemical synapses between a motor neuron and several muscle fibers, like those between regular neurons. Following stimulation by a nerve impulse, the terminal nerve releases the chemical neurotransmitter acetylcholine from synaptic vesicles. Acetylcholine then binds to nicotinic receptors located on an area of muscle fiber called motor endplate that entails folded sarcolemma. This binding opens the nicotinic receptor channels, and sodium ions flow into the fiber, depolarizing the muscle fiber membrane. The action potential generated spreads along the entire membrane to initiate excitation–contraction coupling. Propagation of the action potential is coupled to the release from the sarcoplasmic reticulum of calcium ions needed for contraction.

It is important to recall that not all skeletal muscle fibers are the same. There are broadly two types of skeletal muscle fibers, slow-twitch or type I and fast-twitch or type II muscle fibers [1]. Slow-twitch muscle fibers are resilient and mobilized for sustained, prolonged submaximal (aerobic) exercise and postural control. They contain numerous mitochondria and myoglobin and are highly aerobic compared to fast-twitch fibers. They are abundantly supplied with blood since more capillaries surround them. Fast-twitch muscle fibers generate more tension and

more powerful forces, but for shorter durations, and fatigue rapidly. They are more anaerobic with less blood supply.

2. Muscle Fatigue

Muscle fatigue can be classified as:

(1) *temporary* due to strenuous physical activities and is caused by accumulation in the intracellular space of working muscles with intermediary energy metabolism waste (e.g., lactate) or depletion of their energy-rich compounds (e.g., muscle glycogen store).

(2) *chronic* due to either:

(*i*) muscle atrophies (muscles waste away) due to immobilization, also called disuse atrophy, the presence of chronic inflammation in cardiovascular and respiratory disorders (e.g., heart failure, chronic obstructive pulmonary disease (COPD)), trauma, critical illness, medication (PPAR agonism),

(*ii*) muscle atrophy with aging (sarcopenia), or

(*iii*) neurogenic muscle atrophy due to obstructions or interference with different stages of nerve signal propagation from CNS to motor neuron plate due to disease or spinal injury. Depending upon location, it can be divided into central and peripheral [3]. Central fatigue is initiated at the central nervous system (CNS), such as in multiple sclerosis, thereby decreasing the neural drive to the muscle [4][5]. In contrast, peripheral fatigue is generated by changes at or distal to the neuromuscular junction such as in (a) autoimmune diseases caused by abnormal autoimmune reactions targeting neuromuscular synaptic proteins, as in Graves disease, Guillain-Barré syndrome, and myasthenia gravis, or (b) muscular dystrophies (MDs) due to genetic defects. MDs are progressive and debilitating diseases characterized by muscle wasting and progressive weakness.

3. Reduction of Muscle Mass and Function

The main underlying factor behind the loss of muscle mass, malnutrition, and negative nitrogen balance is an increase in skeletal muscle protein degradation. This occurs on a background of inflammatory responses to trauma or infection, increased circulating cytokine, glucagon, epinephrine, and glucocorticoid treatment, hyperglycemia-mediated secondary infections, and induction of muscle insulin resistance. The immobilization is also an important factor triggering the preferential myosin loss, atrophy, and loss of specific force in fast- and slow-twitch muscle fibers with the loss of strength, especially in the quadriceps and extensors [6]. The critical implications of muscle protein loss extend to poor clinical outcomes such as wound healing, decreased ambulation, and increased risk of thromboembolic complications. There is also evidence that trauma and sepsis can lead to pulmonary complications due to a catabolic response in the respiratory muscles [7], extending to peripheral skeletal muscles [8].

Although the amount of protein that is degraded in healthy subjects of a given age equals typically the amount of protein synthesized, the whole-body protein turnover (protein synthesis + protein degradation) decreases gradually with ageing after peaking through puberty.

4. Major Molecular Mechanisms of Underlying Muscle Wasting

Skeletal muscle proteolysis is regulated by at least four metabolic pathways: (1) ubiquitin ATP-dependent proteasome (UPP), (2) autophagy lysosomal, (3) calcium-dependent, and (4) myostatin mediation. Animal models have previously shown that most, but not all, cellular proteins are degraded by the proteasome pathway [9]. Ubiquitinated proteins are then recognized, unfolded, and degraded by the multicatalytic 26S protease (proteasome) complex (**Figure 2**). The action of several enzymes achieves ubiquitination: ubiquitin-activating (E1) enzyme, ubiquitin-conjugating (E2) enzymes, and ubiquitin ligases (E3). Two muscle-specific ubiquitin ligases, muscle atrophy F-box (MAFbx) and muscle RING finger 1 (MuRF1), have emerged as critical regulators of skeletal muscle proteolysis under catabolic conditions [10]. In line with the above, the finding of increased concentrations of ubiquitinated proteins in muscle homogenates from myopathic septic patients [11] and a several-fold increase in the levels of 20S proteasome (the catalytic core of the 26S proteasome complex) mRNA and protein expression in seriously ill patients that need immediate life support [12][13] would highlight the magnitude of protein breakdown in critical illness. The overall elevated proteolysis rate can also be clinically identified from increases in urinary 3-methyl histidine excretion, an index of myofibril protein breakdown [14].

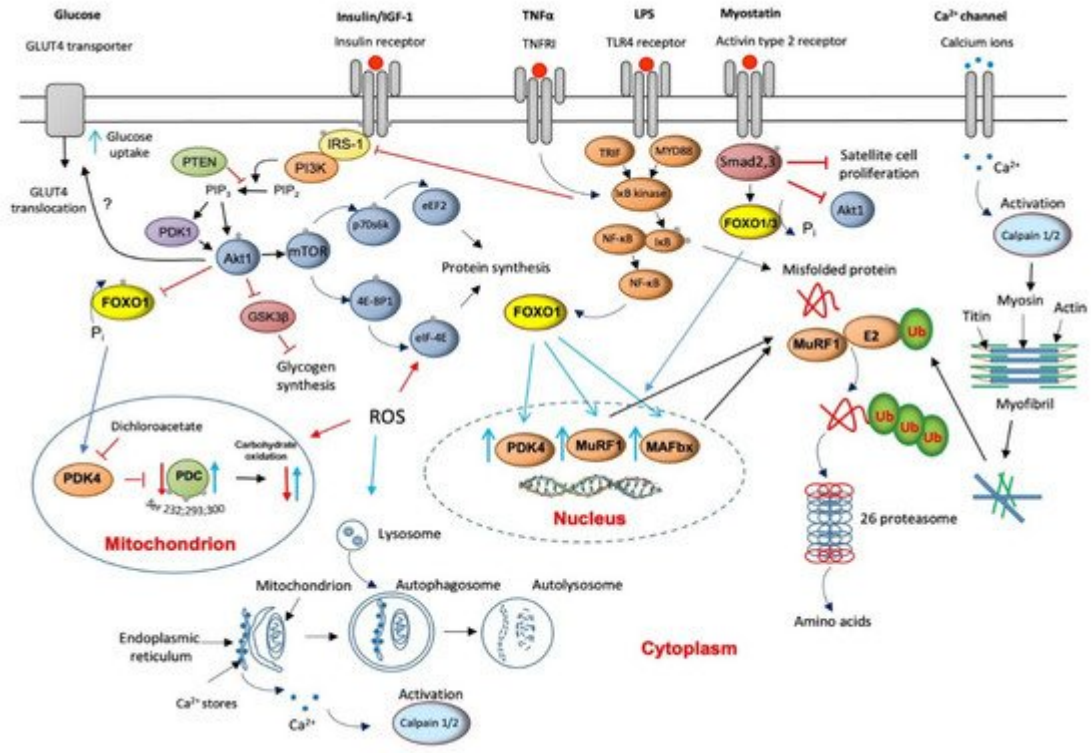

Figure 2. A schematic illustration of the main molecular mechanisms underlying muscle wasting and thereby to muscle fatigue (red lines/arrows indicate downregulation, blue lines/arrows indicate upregulation).

Concurrent with the increased expression of the 20S, the muscle-specific ubiquitin ligases, MAFbx and MuRF1, are also up-regulated in critical illness [12][15], suggesting that an upstream control of the components of the ubiquitin-proteasome pathway exists (**Figure 2**). Several cytokines that increase oxidative stress have received attention as potential underlying triggers to up-regulation of the UPP [16]. Although they are not exclusive, the most cited cytokines are tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6).

5. The Role of Lysosomal Autophagy in Muscle Protein Breakdown

Autophagy is a highly conserved catabolic process in which lysosomes engulf cytoplasmic macromolecules and organelles for degradation. Although basal levels of autophagy support healthy maintenance of metabolic homeostasis through sustaining a balance between protein synthesis and degradation and organelle biogenesis and degradation, amplified autophagy due to metabolic and inflammatory diseases also contributes to the disruption of the protein synthesis–degradation cycle. Previous animal-based research suggested that in cachexia, lysosomal-mediated protein degradation can account for almost one-third of muscle protein loss [17]. These membrane-enclosed organelles contain several acidic pH 5-optimal proteases, including cathepsins B, H, and D, and many other hydrolases, for example, aspartate proteases and Zn^{2+} metalloproteases. Some cytosolic proteins and even cellular organelles are degraded in lysosomes after being engulfed in autophagic vacuoles that fuse with lysosomes (**Figure 2**). In line with animal studies, investigations in humans have confirmed that muscle cathepsin-D mRNA expression and enzyme activity increase in, for example, cachexia and trauma [18].

6. Muscle Calpains' Expression Is Increased in Muscle Wasting

Calpains are cytosolic Ca^{2+}-activated, non-lysosomal, cysteine proteases, of which μ- and m-isoforms (also known as calpain-1 and calpain-2, respectively) are the most ubiquitously expressed, including in skeletal muscle. Calpain-1 and calpain-2 are believed to degrade cellular 'cement' proteins, such as titin, vinculin, talin, desmin, and troponin within the myofibrils' architecture (**Figure 2**). The action of these proteases is a prerequisite for the UPP and lysosomal pathways to degrade the released actin and myosin and other ubiquitin-tagged cytoskeletal proteins. Since cytosolic Ca^{2+} concentrations are consistently elevated in sepsis, chronic inflammation, and ischemia [19], it would not be unreasonable to assume that calpain-1 and calpain-2 activation will also be a feature of critical illness. Indeed, increased expression and activity of calpain-1 and calpain-2 is well documented in human muscle wasting models [20].

7. Muscle Myostatin Expression Is Increased in Muscle Wasting

Myostatin or growth differentiation factor 8 (GDF8) is a member of the transforming growth factor (TGF)-α superfamily, which functions as a negative regulator of satellite cell proliferation and differentiation, muscle growth, and development (**Figure 2**). This is thought to occur through the myostatin-mediated inhibition of the myogenic regulators' myoblast determination protein 1 (MyoD), muscle-specific basic helix-loop-helix (bHLH) transcription factor (myogenin), and myogenic factor 5 (Myf5) [21]. Myostatin is also important in the regulation of human muscle mass because it is involved in the control of both muscle protein synthesis by inhibiting anabolic signaling, which is translation initiation, through inhibiting the Akt/mTOR)/p70S6k signaling pathways [22] and muscle protein breakdown [23]. Consistent with this role, increased myostatin mRNA and protein expression appears to be a significant feature of human skeletal muscle wasting in multiple noncommunicable diseases [12].

8. Apoptosis Is Increased in Muscle Wasting

It also appears that activation of apoptotic signal transduction (programmed muscle cell death) during muscle denervation is another player involved in regulating denervation-induced muscle atrophy along the four major earlier-presented protein degradation pathways [24]. Although myonuclei's apoptosis may contribute to the loss of muscle mass, the mechanisms underlying this process are still largely unknown. The study by Siu et al. [24] indicated that the apoptotic Bax and Bcl-2 proteins, both members of the bcl-2 family, and the mitochondria-associated apoptotic factors, including cytochrome c, the second mitochondria-derived activator of caspase (Smac/DIABLO), and the apoptosis-inducing factor (AIF), were all increased in denervated muscles. Moreover, denervation augmented the protein content of heat shock protein 70 kDa (HSP70). In contrast, the mitochondrial isoform of superoxide dismutase (MnSOD) protein content was reduced, which indicated that denervation might have induced cellular and/or oxidative stress.

Conversely, some argue that the skeletal muscle does not undergo apoptosis during either atrophy or programmed cell death, aka apoptosis, thereby supporting the theory that the nucleus persists once a muscle fiber has acquired it [25].

9. Critical Illness Is Associated with Muscle Insulin Resistance

Patients with critical illness develop insulin resistance, which refers to a normal physiological concentration of insulin producing a less than expected biological response. Present evidence suggests that the simultaneous up-regulation of the central muscle UPP-protein degradation (via muscle-specific ligases MuRF1and MAFbx) [26] and muscle insulin resistance [27] share, via Akt1, PPARδ, and the FOXO family of transcription factors, a common signaling pathway. It appears, therefore, that FOXO1 is a gatekeeper of a central crossroad between the most important proteolytic pathway (UPP) and insulin resistance. The involvement of the FOXO1 transcription factor in the etiology of muscle insulin resistance in critical illness is mediated via increased pyruvate dehydrogenase kinase 4 (PDK4) transcription [12]. Since PDK4 specifically phosphorylates (inactivates) pyruvate dehydrogenase complex (PDC), it can be therefore accepted as a rate-limiting factor in carbohydrate (CHO) oxidation [28]. Insulin, along with

muscle contraction, is the most important physiological activator of PDC [28]. Although insulin is an effective treatment in increasing whole-body glucose disposal rate, its use confers an increased risk of hypoglycemia, which inadvertently increases mortality [29].

10. ROS Involvement in Muscle Wasting

Lengthy periods of inactivity, bed immobilization, and disease (e.g., cachexia) show decreases in both muscle contractile function and muscle fiber size. It is recognized that the inactivity-mediated changes in muscle fibers, although not limited to this, result from a concurrent increase in muscle protein degradation and a decrease in protein synthesis [30][31][32]. While many facts about the mechanism underlying protein degradation are known, the signaling pathways controlling muscle protein balance remain undetermined. A trigger factor often named is the excess production of reactive oxygen species (ROS). The main site of ROS production is in the cytoplasm. One of the most well-known sources of ROS is the nitric oxide (NOX) family NADPH oxidase enzymes, which are proteins that transfer electrons across cellular membranes [33]. At large, the electron acceptor is oxygen, and the product of the electron transfer reaction is superoxide. The biological function of NOX enzymes is, therefore, to generate reactive oxygen species. In addition to NOX-dependent ROS production, the nitric oxide synthases (endothelial [eNOS], neuronal [nNOS], and inducible [iNOS]) are also sources of ROS [34]. The observation that prolonged periods of contractile inactivity also led to increased production of reactive oxygen species (ROS) in muscle fibers suggests that ROS could be an important signaling molecule contributing to muscle atrophy. Theoretically, increased ROS can accelerate proteolysis and autophagy [35] and depress protein synthesis via (1) limiting the ability of the mechanistic target of rapamycin (mTOR) to phosphorylate the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), which is an inhibitor of the eukaryotic translation initiation factor 4F (eIF-4F) complex [31] or (2) the 5’ AMP-activated protein kinase (AMPK) pathway [34]. Yet, it is still controversial whether oxidants are a major contributor to disuse muscle atrophy. Therefore, it is prudent to say that, although ROS production in skeletal muscle is associated with muscle wasting, it remains much of a debate as to whether oxidative stress is a cause or consequence of muscle atrophy [36].

11. Muscle Wasting with Ageing Sarcopenia

Sarcopenia is a progressive loss of muscle mass and function in the absence of any noticeable disease. It is frequently used to describe a collection of cellular signaling pathways’ responses, which, over time, contribute to the accumulation of damaged cells and a collection of outcomes such as decreased muscle strength, reduced mobility and function, increased fatigue, increased risk of metabolic disorders, and increased risk of falls and skeletal fractures [37]. Most importantly, sarcopenia can occur at any age because of disuse or malnutrition. In younger individuals, the loss of muscle mass is reversible, whereas, in older subjects, the muscle loss appears irrecoverable. With age, there is an increased susceptibility to contraction-induced injury and a decreased ability to recover from injury leading to muscle atrophy and weakness [38]. As the abundance and recruitment of satellite cells or muscle stem cells are low in the ageing myofibrils, skeletal muscle regeneration, growth, and maintenance are severely impaired [39]. Of note, the reduction in the number of satellite cells in type II fibers of atrophic muscle

in elderly individuals is notably remarkable, and such a fiber type-specific reduction in satellite content could represent an important factor in the etiology of sarcopenia [40]. The age-related motor unit remodeling (muscle fibers are progressively denervated or reinnervated by compensating neurons) leads to muscle force and power loss, thereby adding further weight to enduring muscle fatigue [41]. Although with age, there is a general marked reduction (35%) in the cross-sectional area of muscle compared with younger individuals [42] along with a lower strength in all types of muscle fibers, the power in the muscles of men remains greater than that in women mainly because of the greater muscle mass that men possess, and, therefore, is less fatigable [43]. An increase in fat infiltration–lipotoxicity of muscle may be an additional factor contributing to progressive muscle fatigue in the elderly [44].

12. Muscle Wasting with Chronic Chemical Exposure

Further related to developing muscle fatigue following exposure to a chemical is Gulf War syndrome or Gulf War illness (GWI). This term is an umbrella term that covers chronic and multi-symptomatic disorders affecting returned military veterans of the 1990–1991 Persian Gulf War. Many acute and chronic symptoms have been linked, including musculoskeletal weakness, muscle pain, and fatigue. One of the suggested underlying mechanisms for GWI is a chemically induced impairment of liver function, with the spillage of stored vitamin A compounds (“retinoids”) into the circulation in toxic concentrations, resulting in a chronic endogenous form of hypervitaminosis A [45]. Using a rodent model, Ramirez-Sanchez et al. [46] provided evidence that animals exposed to chemicals like those envisaged to have been used in the battlefield showed marked muscle atrophy, decrease in myofiber area and limb strength, and reduced treadmill time/distance. Muscle wasting pathway proteins were upregulated, while those promoting growth, mitochondrial function, and muscle ATP levels decreased. Proteomic analysis of muscle also documented unique alterations in the mitochondrial and metabolic pathways. Thus, like the outcomes described following exposure to prescribed medication, chemicals related to GWI adversely impacted key metabolic pathways leading to muscle atrophy and loss of muscle function.

Similarly, heavy metals, such as lead and mercury, or poisoning through environmental contamination also show, among many symptoms, muscle wasting and muscle fatigue. Since the leading target of this type of poisoning is the nerve, it is not surprising that this type of poisoning outcome is clinically classified as peripheral motor neuropathy. Peripheral neuropathy from lead poisoning typically affects the distal upper limbs [47]. Lead neuropathy can be due to demyelination or impairment of axon function. Consequently, and like the injury of lower motor neurons, the impulse coming from the CNS is impaired or even severed, and flaccid paralysis and induction of muscle atrophy and reduction in muscle mass, fiber cross-sectional area, and strength are rapidly developed.

13. Muscle Fatigue Associated with Neurogenic Muscle Atrophy Induced by Viruses

A classic example of a disease associated with chronic muscle fatigue is multiple sclerosis. Here, the suppressor function of regulatory T cells (Tregs), which have a role in regulating or suppressing other cells of the immune

system, thereby controlling the immune response to self and foreign particles (antigens), is impaired for vague reasons. We recently provided evidence that supports a novel mechanism underlying diminished Treg function in multiple sclerosis. Thus, infections that activate the toll-like receptor 2 (TLR2) in vivo (specifically through TLR1/2 heterodimers) could shift the ratio between the Treg and the HIV inhibitor T helper 17 (Th17) cells' balance toward a pro-inflammatory state in multiple sclerosis, thereby promoting disease activity and progression [48].

COVID-19 is a recent viral infection that has spread worldwide and has been identified to affect multiple organ systems, including the nervous system. There is now a mounting body of evidence to suggest the existence of a long-term, post-Covid muscle fatigue syndrome even after mild cases of viral infections. There is, so far, no evident description of the underlying pathology. Muscle deconditioning, immune- or virus-mediated neuropathy, and exercise hyperventilation have been hypothesized to play an essential role in developing debilitating symptoms [49]. Nevertheless, due to the presence of robust and durable systemic inflammatory responses to the viral load (such as the formation of ROS and NO by immune cells during chronic inflammation) in conjunction with a lengthy bed immobilization and medication initially intended to dampen the immune responses, which otherwise also stimulates muscle atrophy (e.g., the steroid dexamethasone), it may not be, therefore, surprising that these factors could contribute to accelerating the energy-independent proteolytic activation of protein degradation via calcium (calpains) and TNF-α (E3-ligases) along with the dexamethasone-induced upregulation of myostatin [50]. Collectively, these outcomes might explain the initiation of muscle atrophy and muscle fatigue.

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