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Intramuscular mechanisms of overtraining

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A B S T R A C T

Strenuous exercise is a potent stimulus to induce beneficial skeletal muscle adaptations, ranging from increased endurance due to mitochondrial biogenesis and angiogenesis, to increased strength from hypertrophy. While exercise is necessary to trigger and stimulate muscle adaptations, the post-exercise recovery period is equally critical in providing sufficient time for metabolic and structural adaptations to occur within skeletal muscle. These cyclical periods between exhausting exercise and recovery form the basis of any effective exercise training prescription to improve muscle endurance and strength. However, imbalance between the fatigue induced from intense training/competitions, and inadequate post-exercise/competition recovery periods can lead to a decline in physical performance. In fact, prolonged periods of this imbalance may eventually lead to extended periods of performance impairment, referred to as the state of overreaching that may progress into overtraining syndrome (OTS). OTS may have devastating implications on an athlete's career and the purpose of this review is to discuss potential underlying mechanisms that may contribute to exercise-induced OTS in skeletal muscle. First, we discuss the conditions that lead to OTS, and their potential contributions to impaired skeletal muscle function. Then we assess the evidence to support or refute the major proposed mechanisms underlying skeletal muscle weakness in OTS: 1) glycogen depletion hypothesis, 2) muscle damage hypothesis, 3) inflammation hypothesis, and 4) the oxidative stress hypothesis. Current data implicates reactive oxygen and nitrogen species (ROS) and inflammatory pathways as the most likely mechanisms contributing to OTS in skeletal muscle. Finally, we allude to potential interventions that can mitigate OTS in skeletal muscle.

1. Introduction

Exercise is arguably the most potent stimulus that triggers skeletal muscle adaptations during chronic endurance and resistance training. These adaptations range from increased endurance due to mitochondrial biogenesis and angiogenesis, to increased strength from hypertrophy in skeletal muscle. It is also well-known that the adaptation is dependent on exercise intensity, i.e. when exercise is performed to exhaustion, resulting in fatigue, it creates a metabolic drive that initiates a more powerful downstream activation of genes responsible for skeletal muscle remodeling than moderate exercise [1,2]. While exercise is necessary to trigger and stimulate muscle adaptations, the post-exercise recovery period is equally critical in providing sufficient time for metabolic and structural adaptations to occur within skeletal muscle [3–5]. These cyclical periods between fatigue and recovery form the basis of any effective exercise training prescription to improve muscle endurance and strength. However, we are currently lacking scientific knowledge of how long the recovery periods should be to receive optimal adaptation in skeletal muscle. Moreover, elite athletes and high-performance individuals might struggle to allow time for recovery between their exercise sessions and competitions where they, as required at the top level, are supposed to perform at their utmost capacity. Imbalance between the fatigue induced from intense training/competitions, and inadequate post-exercise/competition recovery periods can lead to a decline in physical performance. In fact, prolonged periods of this imbalance between fatigue and recovery may eventually lead to extended periods of performance impairment, referred to as the state of overreaching that may progress into overtraining syndrome (OTS). The prevalence of overreaching and OTS is difficult to establish as specific diagnostics are absent, but studies report that ~30% of both young athletes (< 18 years) and elite athletes (> 18 years old) have experienced overreaching/OTS at least once [6–9]. However a prevalence of as high as ~60% in male and female elite runners have been described [10].

Performance decrements accompanying overreaching will require days to weeks for recovery, but appropriate rest will ultimately lead to performance increases. However, if the overreaching is extreme and/or combined with insufficient downtime (i.e. rest, recovery) it will advance into OTS [8,11]. OTS is defined by persistent underperformance despite > 2 months of recovery, joined with changes in mood and absence of symptoms/diagnosis of other possible causes of underperformance [8,9,11,12]. OTS has been attributed to both central (psychological, neurological) and peripheral (intramuscular) mechanisms [8,9,11,12]. In this review, we will focus on intramuscular

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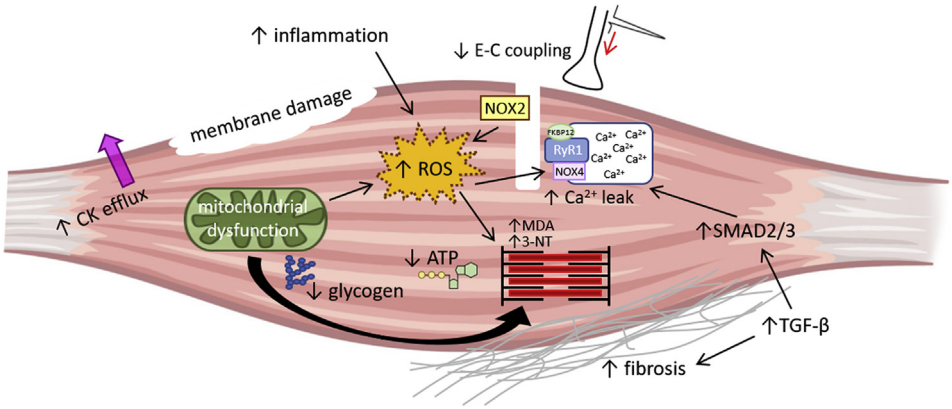

Fig. 1. Illustration picturing potential intramuscular mechanisms of OTS, including glycogen depletion, membrane damage, creatine kinase efflux, reduced excitation-contraction (E–C) coupling, inflammation and cytokine signaling with e.g. enhanced TGF-β1 signaling, mitochondrial dysfunction and increased ROS signaling. Current data implicates ROS and inflammatory pathways as the most likely mechanisms contributing to OTS in skeletal muscle.

mechanisms that results in impaired skeletal muscle contractile function following exhaustive exercise and elucidate how these can lead to OTS (Fig. 1).

2. Prolonged low-frequency force depression, a potent contributor to OTS

Physiological assessments of OTS by coaches and athletes has been limited due to difficulties in employing practical tests to assess skeletal muscle performance in the field [13]. However, in studies where muscle function was investigated, muscle weakness was a defining symptom of overtraining in elite athletes [7,14–16]. The muscle force produced during an exercise session, which allows us to e.g. run, jump and breathe, is primarily muscle contractions carried out at the submaximal level. Prolonged low-frequency force depression (PLFFD) is defined as a persistent exercise-induced reduction in submaximal force that can last for several days or weeks during the post-exercise recovery period [17–21]. This means that PLFFD underlies the long-lasting sensation of muscle weakness during the post-exercise recovery period. Thus, one consequence of PLFFD is that depressed submaximal force will require greater perceived effort to perform any given exercise task, which implies that repeated periods of PLFFD without recovery could potentiate or even exacerbate OTS in skeletal muscle. This greater voluntary effort required to compensate for the depressed submaximal force associated with PLFFD may also accelerate muscle fatigability by requiring increased recruitment of muscle fibers and higher motor unit discharge rates to maintain a given force. Indeed, fatigue is a defining symptom of OTS and muscle weakness associated with PLFFD may underlie the impaired exercise capacity [8,22]. PLFFD was first described in a human exercise study by Edwards and colleagues (1977), but since then, accumulating evidence has shown that PLFFD following fatigue can be replicated in single muscle fibres [23–26]. Thus, the primary cause of PLFFD appears to lie within muscle itself. Intriguingly, the prevailing hypotheses of underlying intramuscular causes of OTS [8] (Fig. 1) are also proposed causes of PLFFD [19,23,24,26–28], i.e. glycogen depletion, ultrastructural damage, inflammation, and oxidative stress, which will be discussed in more detail below.

3. Underlying intramuscular causes of OTS

3.1. The glycogen hypothesis

Excitation-contraction (E-C) coupling [29] and the force producing machinery (cross-bridge cycling) [30] are two energy-demanding processes in skeletal muscle, which are further potentiated by physical exercise. For instance, Ca^{2+} pumping by the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) is purportedly responsible for ~50–80% of the total energy cost in skeletal muscle [31,32]. Furthermore, one ATP molecule is required for each myosin head (myosin/myofibrillar ATPase) that will interact with actin to generate force in the cross-bridge cycling [30]. Glycogen is a multi-branched polymer of glucose molecules that serves as an energy storage form. Glycogen is found in a variety of tissues, but quantitatively high in skeletal muscles (and the liver). The large quantity of glycogen in skeletal muscle reflects its important role of rapidly providing muscle cells with ATP, which display a high and rapidly shifting energy turnover. Moreover, glycogen is located in close proximity to energy-consuming sites in skeletal muscle, e.g. SERCA and myofibrillar ATPase [33]. Thus, intramuscular glycogen depletion can be a significant contributor to fatigue and impaired post-exercise recovery [27,34–36]. The rate of muscle glycogen re-syntheses is slow and takes hours to several days to fully restore [37–41]. Nevertheless, intramuscular glycogen levels restore faster than the months-long duration of OTS.

The glycogen hypothesis of OTS states that exercise-induced muscle glycogen depletion is linked to decreased performance [42]. Based on this hypothesis, long-term carbohydrate supplementation was recently tested as an intervention to prevent or mitigate OTS in rodents [43]. Although a trend towards attenuated OTS-induced performance decrements in running and muscle atrophy with carbohydrate supplementation was observed, it did not reach statistical significance. Furthermore, glycogen supplementation was not able to protect against muscle damage in rodents [43], assessed by oxidative stress markers and creatine kinase (CK) levels [43]. On the other hand, a noticeable glycogen dependence has been observed in the post-exercise recovery of PLFFD. For instance, we have shown that submaximal force recovery is absent in muscles not provided with glucose, i.e. in muscles not able to resynthesize glycogen [27]. Thus, low intramuscular glycogen appears to contribute to PLFFD and OTS, but muscle glycogen content alone cannot explain the mechanism underlying PLFFD and/or OTS.

3.2. Exercise-induced muscle damage and OTS

Exercise-induced muscle damage is a condition characterized by e.g. loss of muscle strength, swelling, delayed onset muscle soreness (DOMS), ultrastructural myofibrillar disruption, systemic efflux of myocellular enzymes and proteins (e.g. CK), or a combination of these [44–46]. It is well recognized that muscle damage is pronounced after repeated eccentric contractions (i.e., lengthening) [47–49]. For instance, running downhill and limb deceleration (drop jumps) are two common movements of repeated eccentric muscle contractions that will induce muscle damage [19,21,49], which lead to both maximal as well as submaximal force depression (i.e. PLFFD) [19]. Moreover, there appears to be a temporal association between the extent of loss of muscle strength after exercise, and the time required to restore muscle strength back to normal, i.e. the more the muscle strength decreases immediately after exercise the longer it takes to recover [49–51] but if the next exercise bout takes place before full recovery, it could contribute to the negative performance spiral that can lead to overreaching

and OTS. Overall, the multitude of symptoms initiated with muscle damage can last from weeks to over a month, including prolonged depression in muscle strength [21,44,46,48], hence the muscle damage-induced loss of muscle strength matches the duration of the performance decrements in OTS. However, what are the underlying cellular and molecular explanations for the loss of strength introduced by exercise-induced muscle damage and the recovery/regeneration that follows injury, and what is the evidence, if any, that these mechanisms are potential causes of OTS?

3.2.1. Mechanical damage not directly responsible for exercise-induced loss of force

Mechanical damage of the muscle fiber ultrastructure has been proposed to explain exercise-induced muscle damage and loss of muscle strength [19,52,53]. For instance, loss of z-disc integrity (i.e. z-disc streaming) and hence loss of the sarcomeric boundaries and the anchoring site for the contractile protein actin in skeletal muscle [19,52]. However, despite a wider z-disc, the force production of isolated myofibrils from human muscle biopsies were only marginally reduced following 100 repeated drop jumps [19]. Nonetheless, impaired shortening velocity following eccentric exercise could indicate dysfunction in crossbridge kinetics that could contribute to decreased muscle power generation [54].

Moreover, despite evidence of increased sarcolemmal membrane tearing and permeability as shown by elevated creatine kinase levels into blood plasma [47,55], measurements of M-wave properties (amplitude, duration, area) from surface electromyography shows that sarcolemmal excitability is unchanged under conditions that cause marked force depression and PLFFD [54,56]. The absence of a significant change in the M-wave suggests that failure in neuromuscular transmission and sarcolemmal excitation are not closely related to the force decrease, but instead imply that peripheral intramuscular mechanisms are responsible for the loss of force. Overall, accumulated data [19,44,54,56–58] suggests that the ultrastructural changes are signs of damage and/or remodeling, but are not directly responsible for the force decrements. Instead, intramuscular modifications targeting the E-C coupling and/or cross-bridges appear accountable for the weakness. Specifically, exercise-induced inflammation and oxidative stress targeting proteins involved in muscle contraction and force production are major candidates potentially responsible for the force and performance decrements in OTS [23,44,50,57,59–66].

3.3. Exercise-induced inflammation and cytokine production

The local classical signs of inflammation include pain, heat, redness, swelling and loss of function, i.e. it has many commonalities with symptoms of exercise-induced muscle damage. In fact, inflammation is an acknowledged key process in muscular repair and regeneration [67,68] and under non-pathophysiological conditions (e.g. after exercise-induced muscle damage) intramuscular inflammation is a tightly coordinated and dynamic process that eventually leads to adaptive remodeling, e.g. skeletal muscle hypertrophy [50,57,62,67,69]. Among the myeloid lineage cell types (including monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, and megakaryocytes to platelets) [70] that enter muscle following damage, macrophages are most clearly demonstrated as positive regulators of regeneration [44,50,63,71,72]. Macrophages demonstrate a wide continuum of phenotypic diversity, on one hand, macrophages can be activated to the M1 ($F4/80^{+}/CD68^{high}/CD206^{-}$) phenotype by e.g. proinflammatory cytokines or other myeloid cells [73]. Interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) are well-characterized proinflammatory cytokines that activate macrophages to the M1 phenotype. At the other hand, macrophages can be activated to the M2 ($F4/80^{+}/CD68^{low}/CD206^{+}$) phenotype by anti-inflammatory cytokines, including interleukin 4 (IL-4), IL-10 and IL-13[73]. Moreover, M1 and M2 macrophages appear functionally coupled to distinct stages of myogenesis in muscle regeneration. For instance, it has been shown that depletion of macrophages at the time of M1 to M2 transition reduced muscle growth, repair and regeneration, and perturbed the expression of the muscle-specific transcription factor MyoD in skeletal muscle from mice that had undergone hindlimb unloading and reloading as a model of muscle damage [74]. However, the spatial and temporal coordination of macrophage-mediated signaling of inflammation and muscle regeneration is not fully understood, but several cytokines, including TNFα, IFNγ, IL-6, and IL-10, appears to play key roles in muscle regeneration [44,50,63,64]. In accordance, non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to negatively impact satellite cell activity, translational signaling and protein synthesis in human biopsies after acute exercise and chronic resistance training (both concentric and eccentric contractions) [57,59–61]. Moreover, it was recently shown that in healthy young men and women which performed 8 weeks of supervised resistance training, the NSAID ibuprofen (1200 mg/day) compromised resistance exercise-induced muscle strength and muscle hypertrophic adaptations, which was accompanied by ibuprofen-induced downregulation of IL-6 expression [62].

Although there are beneficial effects of inflammation in the short term, a chronic inflammatory response will be deleterious ultimately resulting in decreased muscle function with reduced mitochondrial respiration and muscle weakness [75–81]. For instance, overexpression of IL-6 causing chronically elevated IL-6 levels in skeletal muscle, results in lowered force production, reduced expression of proteins in the mitochondrial electron transport chain, and diminished respiratory capacity [81]. Moreover, exercise-induced muscle damage can persist for weeks and trigger macrophage activation where several cytokines (incl. TNFα, IFNγ, IL-6, and IL-10), appear to be involved [44,50,63,64]. Thus, repeated strenuous exercise can induce a persistent intramuscular molecular cytokine signature, which shares commonalities with disease states of chronic inflammation (e.g. rheumatoid arthritis [80]) which is accompanied by muscle weakness [76–78,80]. As a result, repeated strenuous physical activity with too short recovery periods that induces soluble factors which prolongs the duration of inflammation will most certainly lead to decreased muscle function and may well be a key component in OTS.

There are data from rodents that supports the link between cytokines and OTS, however, further experiments in humans are necessary to elucidate the relationship between inflammation and excessive exercise [82]. For example, in an experimental setting, overtraining was induced in rats by 11 weeks of motorized treadmill running, which resulted in decreased physical performance accompanied by increased cytokine levels of e.g. TNF-α, IL-4, IL-6, IL-10 as compared with a sedentary control group and a moderate trained control group [83]. Remarkably, the levels of TNF-α, IL-6, and IL-10 remained elevated even after a two week recovery period after the last exercise session [83]. Nevertheless, how can cytokines lead to decreased performance in skeletal muscle, i.e. decreased force production? Cytokines are known to increase the production of reactive oxygen species (ROS) and in turn, ROS can promote release of pro-inflammatory cytokines [67,80,84–86]. In the next chapter we will discuss how ROS can cause an imbalance in the redox state of the muscle, resulting in impaired exercise performance as evident in athletes with OTS [12,87].

3.4. Oxidative stress and decreased muscle function in OTS

Athletes with OTS exhibit exercise-induced oxidative stress [12,87], which is thought to be caused by an imbalance in the intramuscular redox state that triggers inflammatory signaling, resulting in impaired force production and exercise performance. In line with the data from human studies showing that the degree of exercise-induced muscle inflammation depends on the type of exercise and extent of loss of force [57,66,88], the amount and impact of the oxidative stress on muscle performance appears dependent on type of exercise and intensity

[19,66]. The time course of exercise-induced oxidative stress in skeletal muscle is unclear, but transcriptional analyses of skeletal muscle biopsies after endurance exercise indicated transcriptional activity of oxidative-stress related genes (e.g. transforming growth factor β (TGF-β1), phospholipase A2) 96h post-exercise [66]. However, exercise-induced increases in ROS can also have direct effects on the force production in skeletal muscle [23,65].

Exercise-induced increases in ROS has been associated with muscle fatigue and impaired post-exercise recovery [89]. For instance, Reid et al. showed already 25 years ago that the general antioxidant *N*-acetylcysteine (NAC) alleviated the fatigue-related force decline when human subjects performed repeated submaximal contractions [90]. This finding established the mechanism that ROS production increases in skeletal muscle during physical exercise and ameliorating the resulting oxidative stress with antioxidants lessens the force decrease. Another study that investigated the direct effect of ROS on muscle force generation in unfatigued muscle, showed via exposure of isolated muscle fibers to oxidizing (i.e. H_2O_2) and reducing agents (i.e. DTT) that shifting the redox state of the muscle also had implications on the muscle force generation [91]. Specifically, these findings revealed that an unfatigued muscle is mostly in a reduced state and upon exposure to mild oxidation, ROS increases contractile force to a state considered "optimal redox balance" [91]. However, continued exposure of the muscle fiber to ROS caused force depression due to excessive oxidation, which may represent the state of severe fatigue and OTS [91] (Fig. 2). Since these landmark studies though, the role of ROS and oxidative stress in fatigue and force depression has been a subject of intense debate. For instance, it is known that ROS are produced to the greatest extent during metabolically demanding high-intensity exercise [92]. However, it was recently shown that treating muscle with potent antioxidants that inhibited ROS production from the major cellular sites (e.g. mitochondria and NADPH oxidase 2 (NOX2)) during one session of high-intensity stimulation did not mitigate the fatigue-induced decline in contractile force [23]. One plausible explanation why no effect was seen with antioxidants in direct conjunction with one session of fatigue-induced decline in contractile force [23] is that the ROS produced during the contractions was transient which shifted it to an "optimal redox balance" that was beneficial for the force generation [24,25,91,93], and direct application of antioxidants could not reduce the redox state of the fiber and thus no altered muscle performance was observed (Fig. 2). However, several human and animal studies show that chronic treatment with antioxidants to remove ROS can hamper the beneficial effects of endurance training [94–96], probably because continuous antioxidants intake/application neutralizes the oxidative stress and also the beneficial effects of ROS on e.g. force generation and signaling leading to mitochondrial biogenesis. In comparison to acute and brief increases in ROS with exercise in healthy skeletal muscle, several chronic diseases showing symptoms of skeletal muscle dysfunction and muscle weakness, including rheumatoid arthritis (RA) [76–78], Duchenne muscle dystrophy [97], malignant hyperthermia [98], and even in normal ageing [99] show chronic intramuscular increases in ROS and oxidative stress (Fig. 2). Similar to chronic disease, OTS may represent a state of chronic oxidative stress. For instance, blood markers of oxidative stress (e.g. depletion of reduced glutathione (GSH)) can persist for longer than a month following an ultra-endurance running event [100]. Athletes categorized as chronically suffering from OTS (> 6 months) show increased levels of the oxidative stress marker malondialdehyde (MDA) adducts at baseline and reduced blood plasma antioxidant capacity (i.e., oxygen radical absorbance capacity) [101]. In chronic conditions with oxidative stress and muscle weakness, antioxidant treatment has been shown to be beneficial in restoring muscle force production [75,77,102]. Thus, increases in ROS can have both good and bad effects on skeletal muscle contractile function and fitness, and the outcome probably depends on a combination of factors, e.g. the type of ROS, the size of ROS increase, the duration of ROS/RNS elevation (e.g. milliseconds *vs* hours and months), as well as the site of ROS production and accumulation [103–105].

Furthermore, oxidative stress contributes to PLFFD in skeletal muscle and both could act as potent promoters of OTS. Within skeletal muscle, ROS appears to promote PLFFD by either reducing Ca^{2+} release from the sarcoplasmic reticulum (SR) mediated by the ryanodine receptor (RyR1) or myofibrillar Ca^{2+} sensitivity, and this appears to depend on the major origin of the ROS-producing cellular site (i.e., mitochondria, cytosol), and on the ROS species interacting with various intracellular proteins (i.e., superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), nitric oxide (NO), peroxynitrite ($ONOO^{\bullet-}$)) [23,24,26,28]. In a state potentially representing chronically elevated oxidative stress in OTS, H_2O_2 treatment of rat skinned muscle fibers decreased myofibrillar Ca^{2+} sensitivity and cross-bridge force [106]. A mechanism was revealed whereby prolonged and elevated [H_2O_2] interacts with the Fe^{2+} on myoglobin, to generate hydroxyl radicals, which then oxidizes the major cytosolic antioxidant enzyme, glutathione, to decrease myofibrillar Ca^{2+} sensitivity via irreversible oxidation on the contractile apparatus [106].

Another intramuscular mechanism by which oxidative stress interferes with cross-bridge cycling and force production is by oxidative post-translational modifications (PTMs) on actin [76] (Fig. 1). Using mass spectrometry we recently identified a specific set of oxidative MDA and 3-nitrotyrosine (3-NT) PTMs on skeletal muscle actin from mice and humans with chronic inflammation (i.e. RA), which caused impaired actin polymerization, reduced myofibrillar force production and muscle weakness [76].

Animal models might have been criticized for not completely mimicking OTS in humans, however, the multifunctional cytokine TGF-β1 has been found in transcriptional analyses of skeletal muscle from both animal models and humans with OTS [66,107]. Thus, animal models might be a promising tool for future mechanistic studies in order to further understand cellular and molecular aspects of intramuscular OTS. TGF-β1 belongs to the transforming growth factor superfamily together with myostatin and activin A, and known to have catabolic

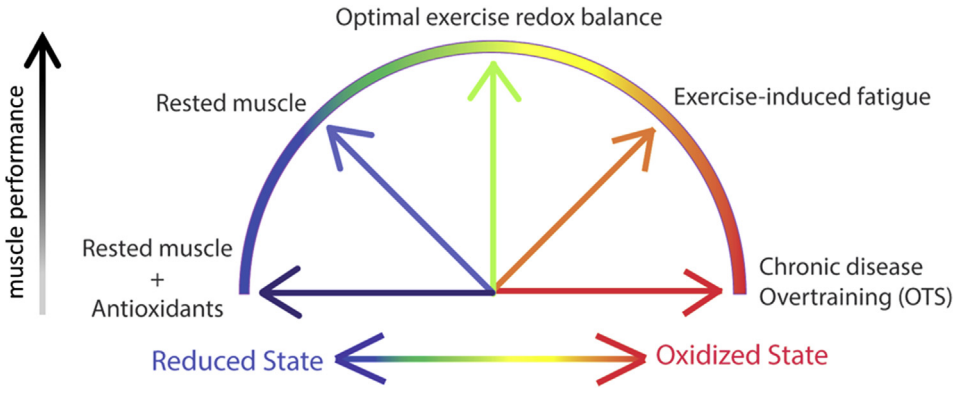

Fig. 2. Cartoon illustrating the proposed bell-shaped relationship between redox state and performance in skeletal muscle. In the rested state, muscle fibers appear in a semi-reduced redox state and can become oxidized during exercise to an "optimal exercise redox balance" at which the muscle can reach peak performance. Muscle fibers can become overly oxidized during fatiguing exercise and even further in OTS and chronic disease which leads to a reduced muscle performance. On the opposite end, an exceedingly reduced fiber will also result in lower muscle performance.

effects on skeletal muscle [108,109]. TGF-β1 can be secreted by macrophages and is known to induce pathological fibrosis [110,111], but other cells e.g. microvascular smooth muscle cells have also been reported to release TGF-β1 and contribute to fibrosis [108,112,113]. For instance, TGF-β1 can activate myofibroblasts to deposit extracellular matrix, of which a major component is collagen and fibrosis formation [109–111]. Furthermore, patients with peripheral artery disease (PAD) exhibit decreased muscle function that is associated with oxidative stress and mitochondrial defects [114], i.e. skeletal muscle biopsies (gastrocnemius) from PAD patients that exhibit increased levels of oxidative stress markers (e.g. carbonylation (DNP), and lipid hydroperoxides) [114] also have higher TGF-β1 expression which correlates with increasing collagen deposition [108]. Moreover, TGF-β1 has been shown to provoke skeletal muscle weakness by phosphorylation and activation of the SMAD2/3 transcription factor, leading to NOX4 transcription which in turn induces oxidative PTMs on the Ca^{2+} release channel RyR1 [102]. Oxidative PTMs (e.g. DNP [102,115]) of RyR1 can lead to reduced binding of the 12-kDa FK506-binding protein (FKBP12) to the channel which contributes to SR Ca^{2+} leak that is considered an underlying mechanism of muscle weakness in several pathological conditions, including diaphragm weakness during mechanical ventilation and in bone metastases [102,115]. Moreover, increased SMAD2/3 signaling and decreased running performance and grip strength have been observed in muscle from mice where overtraining was induced by running [116].

Oxidative PTMs on proteins involved in excitation-contraction coupling (e.g. RyR1) or cross-bridge cycling (e.g. actin, myosin) has, to our knowledge, not been investigated in muscle samples from OTS subjects. However, exhaustive endurance exercise ranging from days to weeks in mice (swimming, running) and human (cycling) have shown a progressive increase in PTM on RyR1 (nitrosylation and phosphorylation) which correlated inversely with FKBP12 binding to RyR1 and increased open probability of RyR1, indicative of enhanced Ca^{2+} leak [117]. These results suggest that during exercise, remodeling of the RyR1 macromolecular complex with FKBP12 dissociation results in leaky channels that play a role in limiting exercise capacity. The same physiological mechanisms that impair exercise capacity during chronic exercise are likely beneficial during acute exercise, but with repeated exhaustive exercise without recovery period, this may contribute to OTS (Fig. 1). Thus, given that OTS mimics a state of chronic inflammation and oxidative stress, oxidative PTMs modifications of contractile proteins [65,76,106,117,118] are a potential intramuscular mechanism of the decreased force production in OTS.

3.5. Reduced mitochondrial capacity in skeletal muscle from subjects with OTS?

As mitochondria are the cellular powerhouse that generates ATP to fuel muscle force production, reduced oxidative phosphorylation will directly limit exercise performance and thus an obvious player that could contribute to OTS. Athletes with OTS exhibit performance decrements, reduced ability to perform high intensity exercise and persistent high fatigue ratings [9,22,119], which all can be linked to reduced mitochondrial capacity and a reduced maximum oxygen consumption (VO_{2max}). However, understandably, results from VO_{2max} tests are not a reliable physiological indicator of OTS, partly because one might not have a 'baseline' value to compare with, but more importantly the listed symptoms of OTS makes it impossible for the subject to perform at maximum capacity in a physiological test. Instead, *ex vivo* cellular respiration (using e.g. Seahorse or Oroboros instruments) [120,121] of muscle fibers would be a more direct, controlled and repeatable procedure to assess oxygen consumption and energy production rates. To our knowledge, no cellular respiration analyses of skeletal muscle are currently available, but mitochondrial respiration have been analyzed in skinned myofibers from rat myocardium in response to chronic exhaustive exercise [121]. The overtraining resulted in a reduced oxidative phosphorylation rate in myofibers from the overtrained group [121]. In skeletal muscle, lower levels of the mitochondrial oxidative enzyme citrate synthase have been reported in rats with OTS [122], indicative of reduced mitochondrial respiration that might contribute to the impaired performance of skeletal muscle in OTS.

4. Prevention and possible treatment options

OTS may have devastating effects on an athlete's career and thus prevention is of importance. Prevention includes carefully planned training programs that include regular monitoring by coaches and the athletes themselves to assess adaptation to training over both the short and long term. Measures suggested to prevent overtraining include minimizing abrupt increases in training loads, monitoring inadequate dietary intake and too frequent competition, individualizing and periodizing training plans, as well as allowing adequate post-exercise recovery and rest days into the training/competition program. Despite careful prevention, it is possible that athletes develop OTS anyways, and except providing adequate rest, no pharmacological treatment strategies are currently available. However, based on the intramuscular processes that appears to contribute to OTS, one obvious solution to mitigate the syndrome could be the use of antioxidants to alleviate the oxidative stress. We acknowledge that antioxidants (e.g., vitamin C and E) given to healthy individuals can have detrimental effects on endurance training adaptations [94–96]. However, here we imply that OTS more closely resembles a state of chronically elevated oxidative stress, such as in chronic disease, rather than exercise adaptation. Moreover, we have previously shown that the SOD/catalase mimetic EUK-134 is an antioxidant that can counteract muscle weakness induced by oxidative stress and thus could prove useful in improving muscle performance in athletes with OTS [75,77]. Using the same argument but instead that OTS mimics a state of chronic inflammation, an anti-inflammatory treatment option could be an alternative. However, in any case, more research is needed before giving any specific recommendations with doses and length of treatment.

5. Conclusion

OTS has severe implications on training/competition performance and hence may have devastating effects on an athlete's career. OTS is caused by chronic imbalances between exercise-induced fatigue and provision of sufficient post-exercise rest. Skeletal muscle accounts for approximately 40% of your body weight and is essential for our ability to move and breath. Here we showed that skeletal muscle is an important contributor to OTS with the long-lasting prolonged low-frequency force depression developed following exhaustive exercise as a potentially potent inducer of skeletal muscle weakness in OTS. Exercise causes an increase in pro-inflammatory cytokines, which in turn can increase muscle oxidative stress that results in a vicious cycle to further elevate inflammation. Although the effects of post-translational modifications of proteins involved in muscle force generation have not been examined in athletes with OTS, pathological conditions of chronic inflammation and increased oxidative stress have shown that oxidation of calcium-handling and contractile proteins can cause long-term skeletal muscle weakness and exacerbated fatigue. Other than providing adequate rest, there is no effective pharmacological treatment to counteract OTS and accelerate recovery. However, antioxidants and anti-inflammatory compounds may show promise in neutralizing the elevated oxidative stress and chronic inflammation in muscles of athletes with OTS, although further research is required to determine the effectiveness of these pharmacological strategies to treat OTS. Finally, novel animal models that mimic the progression from overreaching to OTS is needed to achieve further mechanistic understanding of this physical impairment, including deciphering the interaction between inflammation and oxidative stress in the development of the decreased muscle performance in OTS.

Author contributions

All authors contributed equally to the writing of this Review and all authors approved the final version of the manuscript.

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Declaration of competing interest

The authors declare no conflict of interest.

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