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REVIEW

Mechanisms of eccentric contraction-induced muscle damage and nutritional supplementations for mitigating it

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Abstract

Eccentric contraction (ECC) often results in large and long-lasting force deficits accompanied by muscle soreness, primarily due to muscle damage. In this sense, exercises that involve ECC are less desirable. Paradoxically, exercise training that includes a substantial eccentric phase leads to a more powerful activation of the genes responsible for skeletal muscle remodeling (e.g., hypertrophy) than other types of training that emphasize a concentric or isometric phase. Therefore, effective strategies that lessen ECC-induced muscle damage will be of interest and importance to many individuals. The purpose of this brief review is to highlight the published literature on the effects of ECC and/or nutritional supplementations on proteins, lipids, metabolic and ionic changes, and enzyme activities in skeletal muscles subjected to an acute bout of ECC. First, we discuss the potential mechanisms by which ECC causes muscle damage. Previous findings implicate a Ca^{2+} overload-oxidative modification pathway as one possible mechanism contributing to muscle damage. Thereafter, the efficacy of two nutritional supplementations, i.e., L-arginine and antioxidant, is discussed because L-arginine and antioxidant would be expected to ameliorate the adverse effects of Ca^{2+} overload and oxidative modification, respectively. Of these, L-arginine ingestion before ECC seems likely to be the effective strategy for mitigating ECC-related proteolysis. More studies are needed to establish the effectiveness of antioxidant ingestion. The application of effective strategies against muscle damage may contribute to improvements in health and fitness, muscle function, and sports performance.

Keywords Mechanical stress · Ca^{2+} overload · Proteolysis · Oxidative stress · L-arginine

Abbreviations

ARG L-Arginine.
$[Ca^{2+}]_m$ Calcium concentration in myoplasm.
CK Creatine kinase.
DHPR Dihydropyridine receptor.
DOMS Delayed-onset muscle soreness.
ECC Eccentric contraction.
GSH Reduced glutathione.
JP Junctophilin.
NAC *N*-acetylcysteine.
NO Nitric oxide.
NOS NO synthase.
ROS Reactive oxygen species.
RYR Ryanodine receptor.
SR Sarcoplasmic reticulum.

Introduction

Skeletal muscles undergoing repetitive or sustained contractions show a progressive loss of the ability to produce a desired force. Eccentric contraction (ECC) is a contraction in which skeletal muscles are stretched while contracting. When the contraction includes a substantial eccentric phase, the decline of muscle performance tends to be greater and require a longer period for recovery than with concentric and isometric contractions (Kanzaki et al. 2010). This phenomenon is mainly due to muscle damage, which is

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characterized by swelling, delayed-onset muscle soreness (DOMS), increased serum creatine kinase (CK) (Sewright et al. 2008), inflammation (Liao et al. 2010), triad deformation (Takekura et al. 2001), sarcomere inhomogeneity (Allen 2001), increased membrane permeability (Lavender and Nosaka 2006), proteolysis (Kanzaki et al. 2018), or a combination of these.

ECC is a part of normal activities such as a transition from standing to sitting on a chair, walking downstairs, lowering a heavy weight, or joint movements controlled by agonist/antagonist muscle pairs (Allen 2001). It is well recognized that proper physical activity can attenuate the risk of developing chronic diseases, including cardiovascular diseases, hypertension, diabetes, and obesity (Kenney et al. 2015). Force depressions accompanying ECC-related muscle damage may contribute to the sensation of muscle weakness (Cheng et al. 2020), which, in turn, reduces the motivation for engaging in exercise. Moreover, the majority of competitive athletes employ resistance training as an important component of their overall training. Muscle damage is much more pronounced after resistance training than after other types of training such as endurance and sprint training. In particular in less-trained athletes, muscle damage resulting from training tends to be greater compared to well-trained athletes. As previously shown, disruption of myofibers and high force induced by ECC stimulate subcellular pathways involved in protein synthesis (Hedayatpour and Falla 2015). In this sense, muscle damage is a necessary stimulus for adaptation. However, long-lasting muscle damage may decrease the training efficiency, owing to the positive relationship between the extent of muscle damage and the time required for recovery (Paulsen et al. 2010). Therefore, effective strategies that attenuate ECC-induced muscle damage and facilitate muscle damage recovery will be of interest and importance to many individuals.

The extensive researches published to date have indicated that muscle damage can involve mechanical, metabolic and/or ionic factors (Allen 2001; Zhang et al. 2008; He et al. 2018). Of these, studies performed over the last years have implicated a Ca^{2+} overload-oxidative modification pathway as a major cause of muscle damage (Zhang et al. 2008; Tidball 2011; Kanzaki et al. 2017). On the basis of these findings, it is reasonable to assume that a blunting of the adverse effects of Ca^{2+} overload-oxidative modification may mitigate ECC-related muscle damage. The way of lessening the modification should be practical, in order to apply it in daily activities. In the first part of this brief review, we will discuss the potential mechanisms by which ECC causes muscle damage. In the later part, we will discuss nutritional supplementations that mitigate muscle damage, with a focus on L-arginine and antioxidants. The work reviewed here leads us to suggest the nutritional supplementation that inhibits an activation of calpains, Ca^{2+}-activated neural proteases, as the effective strategy against muscle damage. However, with the consideration that part of muscle damage causes specific muscle adaptations (e.g., greater gains in strength and muscle mass), it is possible that treatments to greatly ameliorate muscle damage suppress the desirable effects of ECC.

What causes ECC-induced muscle damage?

Mechanical stress

Mechanical stress is a measure of internal resistance exhibited by a material when an external force is applied to it. Due to a stretch with ECC and a contraction of the myofibril, some if not all of organelles would be exposed to mechanical stress in muscles undergoing ECC (hereafter, referred to as "ECC muscles"). Mechanical stress has been associated with ECC-induced muscle damage. Forty years ago, Friden et al. (1981) showed that running down many flights of stairs resulted in morphologic changes in the Z lines, including broadening and streaming of the Z-line material in humans (Fig. 1). Their findings are not necessarily direct proof for mechanical Z-line disruption, as they only examined biopsy samples obtained several days after ECC. The structural disturbance might be secondary resulting from protease activation (see below). However, subsequent studies on animals demonstrated overstretched half-sarcomeres (Talbot and Morgan 1996) and disrupted triad structure (Takekura et al. 2001) immediately after ECC.

Sarcomere inhomogeneity (i.e., a difference in strength among sarcomeres) has been implicated as the cause of sarcomere overstretching. Owing to sarcomere inhomogeneity, weaker sarcomeres elongate with repeated ECCs. In such sarcomeres, the thick and thin filaments would fail to correctly reinterdigitate (Allen 2001). However, overstretching occurs in only a few sarcomeres (Talbot and Morgan 1996), and loss of force production from some sarcomeres does not necessarily affect the force developed by the myofibrils (Allen 2001). Moreover, the force production of myofibrils isolated from human muscles is only marginally reduced after ECC, despite broadening of the Z lines (Kamandulis et al. 2017). Overall, accumulated data make it unlikely that the long-lasting force depressions in ECC muscles are due to direct ECC-induced mechanical stress (Newham et al. 1983; Pasquet et al. 2000; Yu et al. 2004; Kamandulis et al. 2017).

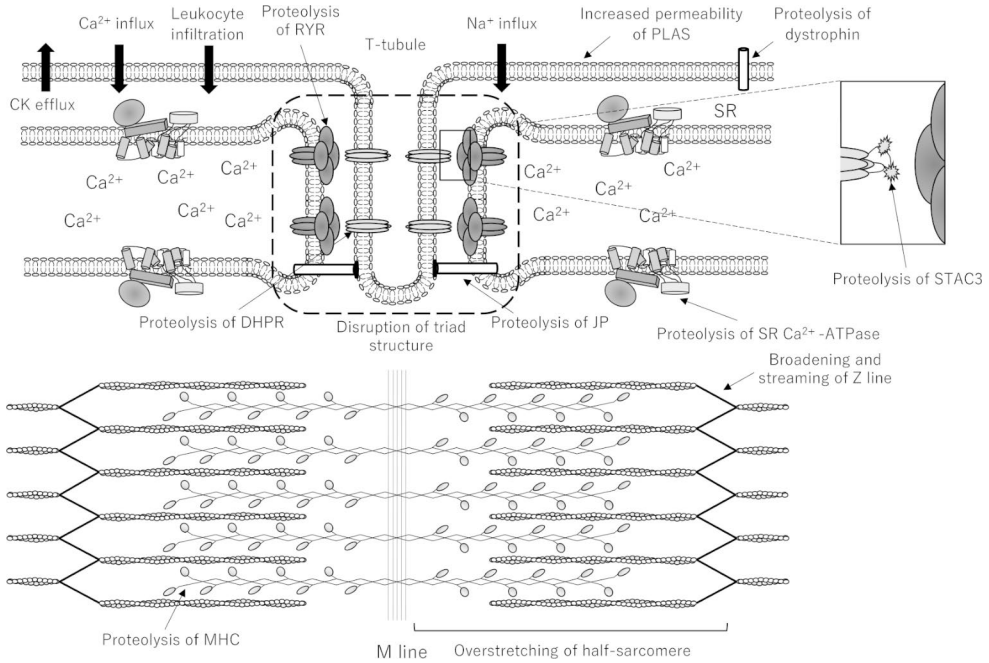

Fig. 1 Illustration picturing eccentric contraction-induced muscle damage
In skeletal muscle undergoing eccentric contraction, a decline of force production is greater and require a longer period for recovery compared to concentric and isometric contractions. This is because of muscle damage, which is characterized by cleavage of various proteins, increased membrane permeability, efflux and influx of ions and proteins, and ultrastructural changes. CK, creatine kinase; DHPR, dihydropyridine receptor; JP, junctophilin; MHC, myosin heavy chain; RYR, ryanodine receptor; SR, sarcoplasmic reticulum; T, transverse

Oxidative stress

A substantial amount of evidence suggests that exercise-induced force loss is ascribable, at least in part, to modification by reactive oxygen species (ROS), the production of which increases with intense and prolonged muscle contractions (Powers et al. 2011; Cheng et al. 2016). The dominant ROS in skeletal muscles are superoxide and its downstream derivatives (i.e., hydrogen peroxide and hydroxyl radicals). The major sites of superoxide production in skeletal muscles are complexes I and III of mitochondria, NADPH oxidase, phospholipase A_2, and xanthine oxidase (Allen et al. 2008; Cheng et al. 2016). In muscles undergoing vigorous isometric contractions, superoxide, but not hydrogen peroxide and hydroxyl radicals, accounts for decreased sarcoplasmic reticulum (SR) Ca^{2+} release, which, in turn, leads to reduced force production (Bruton et al. 2008; Cheng et al. 2015; Watanabe and Wada 2016; Watanabe et al. 2019). This functional impairment of the SR is immediately manifested after the end of a fatiguing contraction and takes only hours to recover (Watanabe and Wada 2016; Watanabe et al. 2018). Considering that impaired SR function persists for several days after ECC (Kanzaki et al. 2017), factors other than superoxide generated during muscle contractions seem likely to be associated with ECC-induced SR impairment.

Each muscle fiber is surrounded by a plasma membrane, called the sarcolemma. It is mainly composed of lipid molecules arranged perpendicular to the surface of the fiber, forming two layers. One of the ECC-specific alterations is an increase in the permeability of the sarcolemma to ions and proteins (i.e., membrane damage) (Figs. 1 and 2). ROS are capable of modifying unsaturated fatty acid, which is a component of all cell membranes. This modification leads to lipid peroxidation, thus affecting cellular membrane integrity (Gissel 2005). Phospholipase A_2 inhibitors and ROS scavengers provide protective effects against altered membrane permeability (Duncan 1987; Howl and Publicover 1990). It is plausible, therefore, that ECC-induced increases in membrane permeability are ascribable, at least in part, to ROS-related modification of the sarcolemma (Fig. 2).

Why does membrane damage mainly occur in ECC muscles, even though ROS levels are elevated in not only ECC muscles but also muscles undergoing isometric contraction? An increase in the free Ca^{2+} concentration in the myoplasm ($[Ca^{2+}]_m$), which occurs in ECC muscles (see below), is likely to be involved in this regard. Elevated $[Ca^{2+}]_m$ facilitates superoxide synthesis in mitochondria, and by phospholipase A_2 and xanthine oxidase (Fig. 2). Membrane damage can stimulate muscle invasion by leukocytes, which have the potential to increase tissue damage (Figs. 1 and 2) (Paulsen et al. 2012). Neutrophils promote the formation of superoxide and hypochlorous acid via NADPH oxidase and myeloperoxidase, respectively (Gissel 2005; Tidball 2011). Additionally, macrophages produce nitric oxide (NO) that

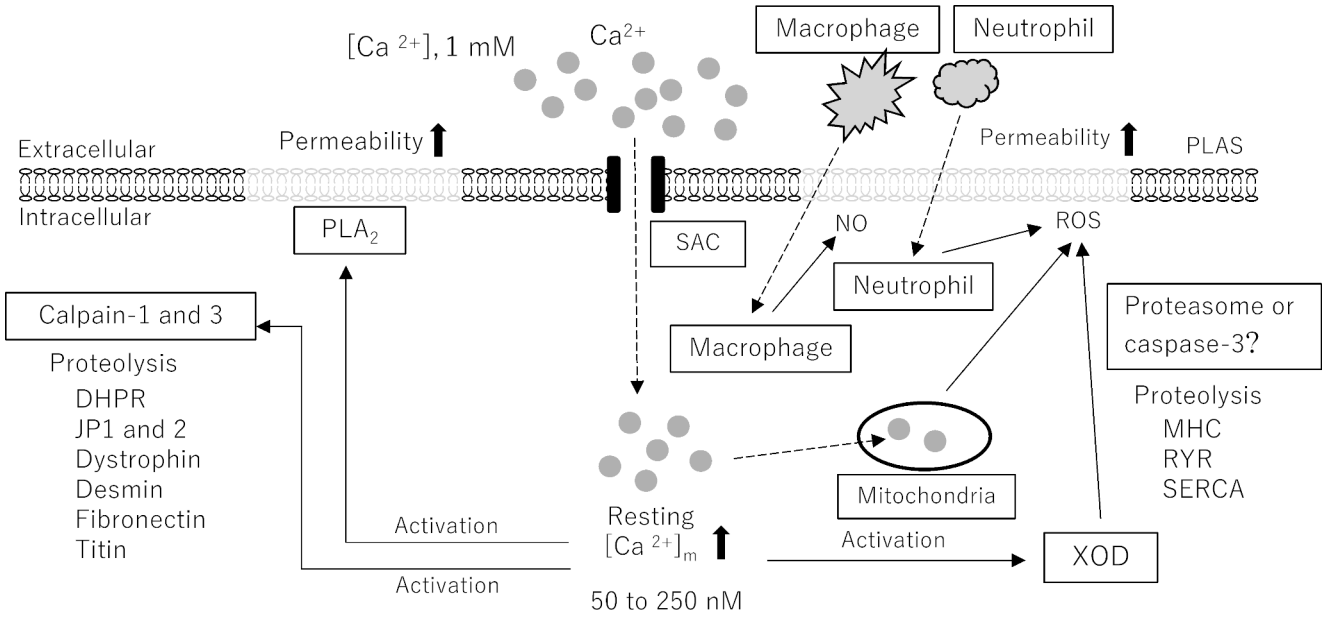

Fig. 2 Pathways involved in eccentric contraction-induced muscle damage
Muscle damage is initiated by influx of Ca^{2+} via stretch-activated channel (SAC). The Ca^{2+} influx results in an increase in the concentration of free Ca^{2+} in the myoplasm ($[Ca^{2+}]_m$), which, in turn, leads to elevated production of reactive oxygen species (ROS) and activated proteases. $[Ca^{2+}]$, Ca^{2+} concentration; DHPR, dihydropyridine receptor; JP, junctophilin; MHC, myosin heavy chain; NO, nitric oxide; PLA_2, phospholipase A_2; RYR, ryanodine receptor; SR, sarcoplasmic reticulum; XOD, xanthine oxidase

lyses the cell membrane (Tidball 2011), thus exacerbating membrane damage. It is important to note that macrophages play a role in both muscle repair/remodeling and membrane damage (Tidball 2011; Hody et al. 2019).

The precise time course of oxidative stress during and after ECC remain uncertain, presumably because of technical limitations for measurements of ROS and their short lifetimes (Lamb and Westerblad 2011; Cheng et al. 2016). However, based on previous findings, it is envisaged that oxidative stress with ECC has the following time course: *(1)* As mentioned above, ROS produced during isometric contractions elicit impaired muscle function (Bruton et al. 2008; Watanabe et al. 2019). However, given the rapid recovery of the impairment (Watanabe and Wada 2016), it is conceivable that the amount of ROS generated during muscle contractions including ECC is not high enough to cause irreversible modifications of proteins and lipid. *(2)* The elevated $[Ca^{2+}]_m$ at rest (see below), which promotes ROS generation, begins immediately after ECCs and lasts for days (Lynch et al. 1997; Zhang et al. 2008). It appears most likely, therefore, that the effect of $[Ca^{2+}]_m$-mediated ROS also persists for the same period. *(3)* Leukocyte infiltration does not occur immediately after ECC, but occurs hours to days after ECC (Tidball 2017). With leukocyte infiltration, the deleterious effect of ROS would be exaggerated.

Ca^{2+} overload and calpains

$[Ca^{2+}]_m$ is maintained at approximately 50 nM in quiescent muscle fibers, whereas the extracellular $[Ca^{2+}]$ is ~1 mM (Allen et al. 2008). Although the permeability of the sarcolemma for Ca^{2+} is very low, passive diffusion of Ca^{2+} into the cells continuously occurs, owing to the presence of a great electrochemical gradient across the membrane. Muscles are equipped with systems that precisely regulate the $[Ca^{2+}]_m$, such as SR Ca^{2+}-ATPase, Na^+/Ca^{2+} exchanger, and mitochondria (Gunter and Pfeiffer 1990; Deval et al. 2002; Tupling 2004). In normal conditions, the resting $[Ca^{2+}]_m$ remains almost constant owing to the large Ca^{2+} storage ability of the SR.

ECCs are widely recognized to evoke an increase in the resting $[Ca^{2+}]_m$ (Lynch et al. 1997; Zhang et al. 2008; Kanzaki et al. 2014). For example, in rat skeletal muscles, an acute bout of downhill running causes an increase in the $[Ca^{2+}]_m$ to ~250 nM that lasts for days (Lynch et al. 1997). $[Ca^{2+}]_m$-related degradation of myofibrillar proteins (e.g., desmin and titin) can be mitigated by treatment with streptomycin, a blocker of stretch-activated channels in the sarcolemma (Zhang et al. 2012), suggesting that Ca^{2+} influx is mediated through a stretch-activated channel (Fig. 2) (Guharay and Sachs 1984). Action potential-induced Ca^{2+} release via the ryanodine receptor (RYR), a Ca^{2+} channel of the SR, from the SR lumen increases the $[Ca^{2+}]_m$ to approximately 0.3–1.2 μM (Olsson et al. 2020). However,

the increased $[Ca^{2+}]_m$ rapidly decreases to the resting levels, since upon cessation of SR Ca^{2+} release, myoplasmic Ca^{2+} is transported by SR Ca^{2+}-ATPase back into the SR lumen. This short-period elevation ($<\sim 0.5$ s) in the $[Ca^{2+}]_m$ does not induce alterations that cause muscle damage. However, a long-lasting increase in the resting $[Ca^{2+}]_m$ seems to cause problems associated with muscle damage.

Calpains are nonlysosomal, Ca^{2+}-activated neural proteases. Skeletal muscles express not only the ubiquitous calpains, calpain-1 and calpain-2, but also a muscle-specific calpain referred to as calpain-3 (Murphy 2010). Ca^{2+} triggers an autolytic process in calpain-1, which reduces the $[Ca^{2+}]_m$ required for the activation of this calpain from 3 to 50 to 0.5-2 μM. Further, autolysis of calpain-2 reduces the required $[Ca^{2+}]_m$ for its activation from 400 to 800 to 50–150 μM (Goll et al. 2003). Although it is unclear whether the autolysis of calpain-3 results in a decrease in the $[Ca^{2+}]_m$ ($<\sim 1$ μM) necessary for its activation, calpain-3 is proteolytically active only after it autolyzes itself in a Ca^{2+}-dependent process (Diaz et al. 2004). Direct evidence for the involvement of calpains in ECC-related proteolysis has been presented in experiments showing that treatments with a calpain inhibitor and streptomycin, or the use of a Ca^{2+}-free solution can attenuate the proteolysis of various proteins (Fig. 2) (Zhang et al. 2008, 2012; Kanzaki et al. 2017). In vitro studies have indicated that calpains can cleave numerous myofibrillar and cytoskeletal proteins, including e.g., titin, desmin, dystrophin, troponin, myosin light chain 1, STAC3 and RYR (Shevchenko et al. 1998; Goll et al. 2003; Murphy 2010; Ashida et al. 2021). However, this may not be necessarily the case in vivo. For instance, treatment with MDL-28,170, a calpain inhibitor, prevents cleavage of the dihydropyridine receptor (DHPR) and junctophilin (JP), whereas it has little effect on the RYR (Kanzaki et al. 2017).

The mechanism of the degradation of RYR in ECC muscles is uncertain. One likely possibility is the involvement of a ubiquitin-related proteasome that is activated a few days after ECC (Fig. 2) (Kanzaki et al. 2017). Although the proteasome pathways act "downstream" of calpain in myofibrillar proteins (Solomon and Goldberg 1996), they might play a pivotal role in the direct degradation of the RYR. In support of this idea, the proteasomes have been demonstrated to downregulate the inositol 1,4,5-trisphosphate receptor, which is structurally and functionally related to the RYR (Mackrill 1998). Although calpains and proteasomes do not directly degrade the myosin heavy chain, which primarily governs the force production by the contractile apparatus (Powers et al. 2007), a decrease in this protein occurs in ECC muscles (Ingalls et al. 1998; Kanzaki et al. 2010). A candidate for the cleavage of the myosin heavy chain is the protease caspase-3, which is activated by calpains, given that its activation promotes the degradation of the actomyosin complex (Du et al. 2004; Powers et al. 2007).

Not only the SR but also mitochondria can sequester myoplasmic Ca^{2+}. $[Ca^{2+}]_m$ homeostasis is better maintained in female than in male muscles (Watanabe et al. 2020), because estrogen, an ovarian hormone, functions to maintain the mitochondrial function (Ribas et al. 2016). These findings raise the possibility that male muscles might be more damaged by ECC than female muscles. This possibility is reinforced by human studies showing higher levels of serum CK after ECC in males than in females (Sewright et al. 2008). In contract, the extent of ECC-induced force loss and DOMS was shown to be similar for females and males (Sewright et al. 2008; Lee et al. 2017). Additionally, a recent meta-analysis indicated that apart from the CK response, there were no sex-differences in ECC-related muscle damage (Morawetz et al. 2020).

Nutritional supplementations for mitigating muscle damage

The aforementioned mechanisms for ECC-induced muscle damage suggest that inhibition of calpain activation and/or oxidative modification may be effective strategies that mitigate muscle damage. Nutritional consideration is one of important components for achieving this (Markus et al. 2021). The efficacy of two nutritional supplementations, i.e., L-arginine (ARG) and antioxidants, is discussed in this section because ARG and antioxidants would be expected to ameliorate the adverse effects of Ca^{2+} activation and oxidative modification, respectively.

L-Arginine

In ECC muscles, calpain-1 is considered to play a central role in the proteolysis of some proteins (e.g., DHPR and JP) involved in excitation-contraction coupling, based on previous findings that *(1)* calpain-1 becomes autolytically activated a few days after ECC, during which DHPR and JP are degraded (Kanzaki et al. 2014, 2017); *(2)* in contrast, autolysis of calpain-3 occurs within approximately 24 h after ECC (Murphy et al. 2007; Kanzaki et al. 2014); *(3)* the degree of Ca^{2+}-induced excitation-contraction uncoupling is similar between normal and calpain-3-deficient muscles (Verburg et al. 2009); and *(4)* high levels of Ca^{2+} are required for the activation of calpain-2 (Goll et al. 2003) (see above).

In skeletal muscles, NO, which is synthesized from ARG primarily by neuronal NO synthase (NOS), is moderately generated in the resting state and produced in markedly increased levels with contractile activity (Dyakova et al. 2015). Previous in vitro studies have demonstrated that

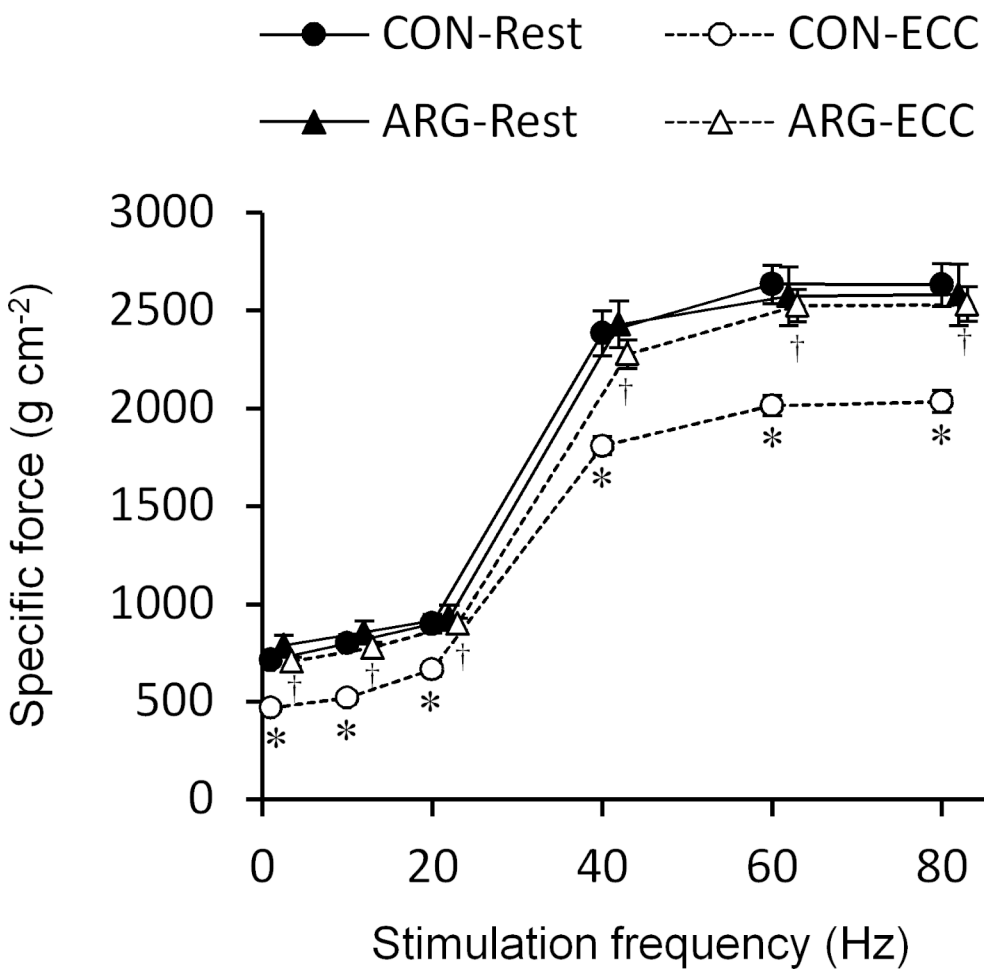

Fig. 3 Effects of L-arginine (ARG) ingestion and eccentric contraction (ECC) on force production. (Adapted from Kanzaki et al. 2018) AGR rats were provided water that contained ARG for 7 days starting from 3 days before the ECC protocol. ECCs were repeated in the anterior muscles for 200 cycles. Three days after ECC, isometric force was measured in situ. Values are means ± SEM. *$P < 0.05$, versus rested muscles within rats; $^{\dagger}P < 0.05$, versus ECC muscles from control rats. CON, control; mNm, milli-Newton-meters

treatment with NO donors can inhibit calpain-1 activation, which is mediated via *S*-nitrosylation of the enzyme (Liu et al. 2016). Lomonosova et al.(2014) reported that in rats, ingestion of ARG before downhill running evoked increases in NO production and reductions in exercise-induced calpain-1 mRNA up-regulation and in exercise-induced desmin degradation in soleus muscles. Their observations, together with previous findings from in vitro studies suggest that ARG may elicit decreased activation of calpain-1 via the down-regulation calpain-1 and/or *S*-nitrosylation, resulting in reductions in ECC-related muscle damage. To elucidate this, we recently examined the effect of ARG ingestion (Kanzaki et al. 2018). In that study, rats were given ARG for 7 days starting from 3 days before the ECC protocol. Three days after ECC, we observed that ARG attenuated the ECC-related force deficit (Fig. 3), autolysis of calpain-1, and proteolysis of DHPR and JP and increased the amounts of *S*-nitrosylated calpain-1. Given no changes in the calpain-1 content in ECC muscles (Kanzaki et al. 2014), our results indicate that the benefit of ARG is primarily due to calpain-1 inactivation via *S*-nitrosylation.

Based on the conversion ratio of 1:0.16 (rats vs. humans) (Cynober et al. 2016), the supplemental dose (611 mg kg-body wt^{-1} day^{-1}) used in our study (Kanzaki et al. 2018) is equivalent to 98 mg kg-body wt^{-1} day^{-1} in humans. Such an ARG dose has been confirmed to be safe (Kanzaki et al. 2018). Our subsequent study revealed that ingestion of soy protein isolate, which contains more than two times ARG than animal-based proteins (e.g., beef, egg, and milk), also reduced ECC-induced muscle damage (Kanzaki et al. 2019). The findings on ARG raise the possibility that ingestion of green leafy vegetables, such as spinach and beetroot, also has similar effects to that of ARG, with the consideration that these vegetables are rich in inorganic nitrate and that nitrate can be converted into NO (Lundberg et al. 2008).

The exact reason for the occurrence of ECC-associated calpain-1 activation in rats that do not ingest ARG, despite the elevated NO content after ECC, is uncertain (Fig. 2). One

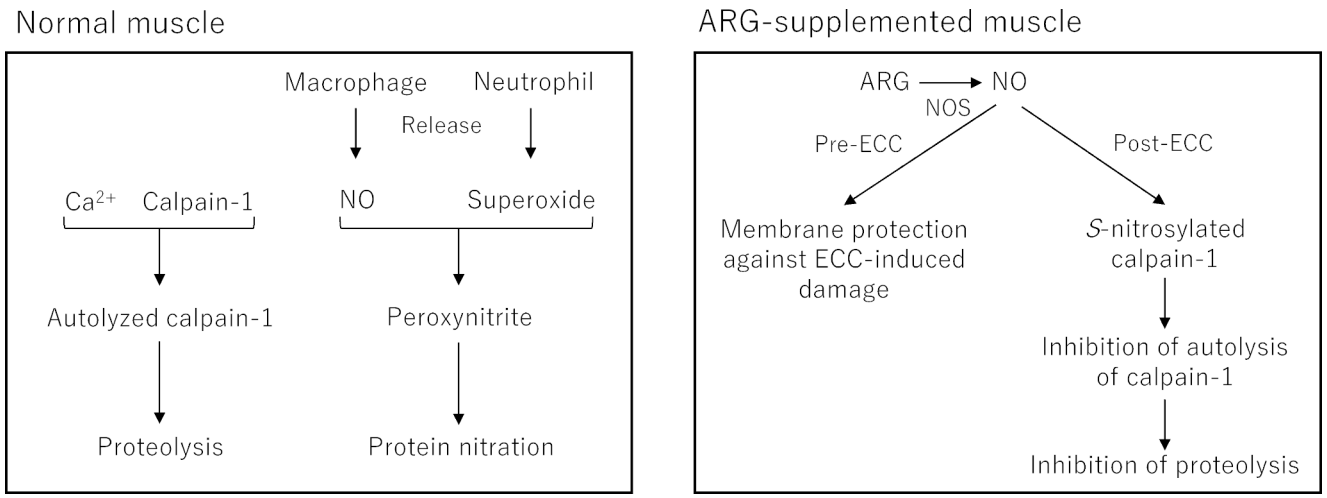

Fig. 4 Reaction of nitric oxide (NO) in normal and L-arginine (ARG)-supplemented muscles undergoing eccentric contraction (ECC) In normal muscles in which macrophages and neutrophils infiltrate into the cells, many NO molecules interact with superoxide molecules to form peroxynitrite. On the other hand, in ARG-supplemented muscles, elevated levels of NO lead to *S*-nitrosylation of calpain-1, which, in turn, inhibits autolysis of the enzyme. NOS, nitric oxide synthase

possible explanation is that the sources and reaction of ARG differs between normal and ARG-supplemented muscles. As mentioned above, damage of the sarcolemma results in infiltration of neutrophils and macrophages into the muscle cells, which, in turn, increases the concentrations of NO and superoxide. It might be expected that, under such conditions, many NO molecules would interact with superoxide to form peroxynitrite, resulting in decreased *S*-nitrosylation of calpain-1. Further, when NO levels are increased before ECC, the sarcolemma might be protected (Fig. 4). Support for these assumptions is provided by studies showing that *(1)* except for some pathologic conditions, peroxynitrate mainly causes nitration rather than *S*-nitrosylation (Begara-Morales et al. 2015); *(2)* protein nitration is increased a few days after ECC (Chiang et al. 2009); and *(3)* increased NOS ameliorates the sarcolemma damage that occurs with ischemia–reperfusion in rat hearts (Szelid et al. 2010).

Antioxidants

Antioxidants that are ingested to inhibit muscle damage include vitamins C and E, *N*-acetylcysteine (NAC), taurine, and honokiol (Childs et al. 2001; Chiang et al. 2009; Silva et al. 2011; He et al. 2018). Supplementation of both vitamins C and E before downhill runs has been shown to ameliorate decreases in isometric force, CK efflux, DOMS, and leukocyte apoptosis in humans (see below) (Shafat et al. 2004; He et al. 2015, 2018). Also, studies in rodents reveal that taurine or honokiol supplementation evokes decreases in superoxide production, serum CK levels, protein carbonylation, lipid peroxidation, and/or leukocyte infiltration (Silva et al. 2011).

As one of the most important nonenzymatic antioxidants, reduced glutathione (GSH) plays a critical role in protecting the muscle cells against oxidative stress (Sen 1998). NAC is a nonspecific antioxidant that functions as a sulohydryl group donor. It is also a cysteine precursor and its ingestion provides the body with cysteine. NAC ingestion leads to an increase in GSH concentrations since cysteine is the rate limiting amino acid in *de novo* synthesis of GSH (Pizzorno 2014; Gould and Pazdro 2019). It is possible, therefore, that NAC could directly and indirectly relieve ECC-induced muscle damage. However, there is no consensus with regard to the efficacy of NAC ingestion (Childs et al. 2001; Silva et al. 2008; Whitehead et al. 2008; Kerksick et al. 2010).

In contrast to the above-mentioned benefit of antioxidant, in human experiments, drop jumps leading to a long-lasting force decline induced only a minor elevation in ROS production (Kamandulis et al. 2017). Furthermore, although downhill runs resulted in increased ROS generation, the increase occurred only after the peak decline in muscle function and DOMS (Close et al. 2004). These findings suggest that ECC-related muscle damage may not be primarily influenced by the ROS produced during ECC, thus indicating that antioxidant ingestion may not be an effective strategy against muscle damage. In fact, ingestion of both vitamin C and NAC immediately after ECC was shown to exacerbate muscle damage in humans (Childs et al. 2001). Although the underlying reasons for the contradictory results remain unclear, differences in the exercise protocol, tissues examined, and analytical procedures all remain potentially important. Particularly, the contraction modes (i.e., drop jump, downhill run, ECC of knee extensor muscles, or electrical stimulation) and/or the number of ECCs loaded on muscles markedly differ across studies (Close et

al. 2004; Shafat et al. 2004; Kamandulis et al. 2017; He et al. 2018). These differences most likely result in variable degrees of muscle damage and/or distinct ROS levels in the cells, which would influence the effect of antioxidant ingestion. More elaborate studies with systematically controlled ECC protocols are needed to gain further insights into the effectiveness of antioxidant ingestion. Finally, it is worth noting that ingestion of antioxidants suppresses training-induced muscle adaptations by hampering the cellular signaling responsible for mitochondrial biogenesis (Strobel et al. 2011; Paulsen et al. 2014).

Concluding remarks

Pain and weakness are common symptoms of muscle damage. ECC, which induces muscle damage, is an unavoidable form of muscle contraction in daily life activities including sports. Although many individuals engage in sports activities to improve health and fitness, ECC-induced pain and weakness might discourage such efforts. Moreover, engaging in the next exercise bout before full recovery from the muscle damage caused by the previous bout might lead to prolonged periods of muscle performance impairment (Pereira et al. 2016; Cheng et al. 2020), which suggests that muscle contraction with a substantial eccentric component is a less desirable exercise.

Conversely, numerous studies have shown the importance of including the eccentric phase in muscle contractions. For instance, high-intensity eccentric exercise upregulates androgen receptor expression in humans, which facilitates muscle hypertrophy (Hedayatpour and Falla 2015). Moreover, the mechanical stretching of muscles is the primary stimulus for protein synthesis via the mechanistic target of rapamycin (Kenney et al. 2015). In addition, an exercise program that properly incorporates ECC would be expected to be beneficial in terms of increased muscle fatigue resistance, given that mitochondrial biogenesis is triggered by $[Ca^{2+}]_m$ elevation, which occurs in ECC muscles (Wright et al. 2007; Bruton et al. 2010; Tavi and Westerblad 2011). The active use of ECC, in which muscle damage is relieved in a physiologic manner, may contribute to improvements in health and fitness, muscle function, and sports performance. ARG ingestion before ECC seems likely to be an effective strategy for ameliorating ECC-related muscle weakness.

Author contributions KK and MW conceived of the review. KK, DW, and MW interpreted results of experiments. JS and MW prepared figures. KK, DW, and MW drafted manuscript. KK, DW, and MW edited and revised manuscript. KK, DW, JS, and MW approved final version of manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

Declarations

Disclosures No conflicts of interest, financial or otherwise, are declared by the authors.

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