Nitric oxide: Is it the cause of muscle soreness?

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\textbf{A B S T R A C T}

Skeletal muscle hosts all of the isoforms of nitric oxide synthase (NOS). It is well documented that nitric oxide (NO) regulates force generation and satellite cell activation, and therefore, damage repair of skeletal muscle. NO can also activate nociceptors of C-fibers, thereby causing the sensation of pain. Although delayed-onset of muscle soreness (DOMS) is associated with decreased maximal force generation, pain sensation and sarcomere damage, there is a paucity of research linking NO and DOMS. The present mini-review attempts to elucidate the possible relationship between NO and DOMS, based upon current literature.

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\textbf{Introduction}

Although, delayed-onset of muscle soreness (DOMS) has been extensively studied, the phenomenon is still not completely understood. DOMS peaks about 1–3 days after unaccustomed exercise bouts, the result of lengthening contractions \cite{1,2}. The etiology of DOMS is still vague \cite{3}, even after a number of different hypotheses have described the most important symptoms, which are, pain, decreased maximal force generation, and altered permeability of sarcolemma. It was suggested long ago, that DOMS is associated with mechanical damage. In 1977 Abraham \cite{4} measured the ratio of hydroxyproline/creatine in the urine samples of subjects with and without muscle soreness, and the increased ratio indicated significant damage to connective tissue. From biopsy samples, Friden and co-workers \cite{5,6} reported that DOMS-associated muscle damage included intermyofibrillar sarcoma disturbances, and Z-band streaming, especially in Type 2 fibers \cite{7–10}. This observation was confirmed later using magnetic resonance techniques \cite{10,11}. Edema formation has also been observed in subjects suffering from DOMS \cite{12}. A large increase in creatine kinase (CK) and myoglobin concentrations in the circulation are two of the most often used associated markers of DOMS and indicate increased permeability of the sarcolemma \cite{13–18}. However, it has been reported that the magnitude of DOMS is not always associated with the magnitude of other markers of muscle damage \cite{19}.

In normal conditions, tissue damage is associated with inflammation, which, besides the well known beneficial effects, could also result in some of the discomforts of DOMS \cite{20}. Based upon...
this hypothesis, naproxen sodium, which is a potent anti-inflammatory drug, has been used to attenuate the consequences of DOMS. However, significant effects have not been observed on the extent of pain, CK release, or force generation [21]. Among the indirect markers of DOMS, the altered ratio of oxidized to reduced glutathione indicates that, as a result of inflammation or calcium efflux, reactive oxygen species (ROS) are generated [3,22]. The generation of ROS is often observed during aerobic exercise, in which the mitochondrial electron transport chain is one of the main sources of ROS [23–25]. However, the fact that eccentric muscle action with high tension and relatively low oxygen demand, such as downhill running, more readily causes DOMS than running with the same intensity on a flat surface [22], suggests that the main source of the generated free radicals is not the mitochondrial electron transport chain, but rather is due to secondary sources such as inflammatory agents [26].

The possible involvement of NO in DOMS was originally suggested by Radak et al. [27]. In that study the subjects developed DOMS after eccentric exercise. NO was measured from skeletal muscle biopsy samples of control subjects and subjects suffering from DOMS. The NO content was evaluated using electron spin resonance methodology, and an approximate 30% increase in NO levels was detected in skeletal muscle of subjects suffering from DOMS. This finding was associated with a significant decrease in maximal force generation. Since that time a number of important studies have been published on the role of NO in skeletal muscle function [28,29], and the findings include down regulation of force production [30,31], sensation of pain, and/or damage repair [32–36]. Although these studies were not always conducted during DOMS, the findings could be readily applicable to DOMS. Therefore, the present review attempts to highlight the role of NO in DOMS, especially in conditions that are associated with factors of DOMS, such as decreased maximal force generation, sensation of pain, and damage repair.

**Muscle soreness-induced suppression of maximal force generation and the potential role of nitric oxide**

Tiidus and Ianuzzo [37] reported that, in subjects with a high degree of muscle soreness, individuals were unable to lift weights corresponding to 90% max, for even one repetition, 48 h post-exercise. There is general agreement that DOMS results in decreased force generation 1–4 days after DOMS-induce exercise bouts [38,39], although Nosaka et al. [19] reported dissociation between the magnitude of DOMS and loss of muscle strength.

Our group was the first to describe a possible relationship between a DOMS-associated decrease in maximal force generation and NO concentration [27]. We have suggested that the DOMS-induced increase in NO formation that could suppress force generation was, at least in some part, a protective mechanism to prevent further damage induced by maximal contraction. However, at that time it was unclear whether NO was capable of down-regulating skeletal muscle contraction.

NO is produced by nitric oxide synthase (NOS) from L-arginine following an oxygen dependent process. Skeletal muscle hosts the three types of NO isoforms: inducible NO (iNO), endothelial NO (eNO), and neuronal NOS (nNOS) [40]. The main NO generating enzyme in skeletal muscle is nNOS, which is encoded to dystrophin [29,41]. It is quite clear that NO can regulate muscular function, force generation, and even affect the structure of skeletal muscle [42,43]. This regulatory process includes the NO mediated reversible inhibition of cytochrome oxidase, which affects oxygen uptake [44]. NO can control the IGF-I/p70 S6 kinase signaling pathway during muscle growth [45]. NO has also been implicated in protein S-nitrosylation of skeletal muscle and therefore, could modulate ATP-ase activity [46], as well as an exaggerated exercise-induced fatigue response [47]. It is clear that sarcolemmal nNO is an important regulator of blood flow to active skeletal muscle [29]. It appears that during eccentric exercise the induction of nuclear factor kappa-B, which is one of the main mediators of the inflammatory process [48], modulates the transcription of all of the isoforms of NO [49]. This observation could illustrate an important link between DOMS-inflammation and NO induction. In addition, the exercise induced NO generation appears to be important for the induction of interleukin-6, interleukin-8, hem oxygenase and HSP78 [50]. Indeed, NO, with a relatively long half-life, is an important signaling molecule [44,51,52]. However, because of the gaseous nature of NO, a transmitter would be advantageous to work specifically on targeted sites. Indeed, cyclic guanosine monophosphate (cGMP) is generated by NO, transmits the signal, and readily turns on downstream targets [53].

It is known that both inhibitors and donors of NO can result in decreased force generation [54], which demonstrates that the dose response of NO follows a bell-shaped hormetic curve [55–57]. The hormesis curve is a dose–response phenomenon [58,59] which can be described by low dose caused activation and high dose caused inhibition, a phenomenon which is generally true for other free radicals [58,60,61]. Although, nNOS are readily up-regulated by exercise training [53] an increased level of NO or cGMP, can interact via a variety of pathways, to down regulate force production. One of the first studies to report a causative link between NO generation and decreased force generation showed that iNO inhibitors maintained force levels in the diaphragm [62]. Andrade and co-workers [63] have studied the effects of NO donors and cGMP inhibitors on Ca++ sensitivity and force generation of single fibers, and concluded that NO can impair Ca++ sensitivity, especially on actin filaments. Similarly to these earlier findings, Galler et al. [64] reported that, in skinned fibers, increased levels of NO resulted in decreased force generation through direct interaction of NO with force-generating fibers. It was also revealed that NO, through oxidizing contractile thiol proteins and by the depression of actomyosin ATP-ase, could mediate force development [64]. Moreover, increased levels of NO have been shown, in rabbit skeletal muscle, to result in a decreased number of cross-bridges and hence decreased force generation [65]. NO levels also negatively affect the force generation of cardiomyocytes [66], suggesting that NO and cGMP could have similar affects on contractile function in myocardium as in skeletal muscle. However, there are few studies which report on the effects of NO mediated changes after eccentric exercise and only one in which the investigators studied the possible link between NO and force generation [27]. In another study, in which the effects of eccentric exercise were studied on NO content, it has been shown that eccentric exercise increases nitrate concentration [67], and iNOS activity [68–70] but the link between eccentric exercise induced NO and force production requires further study. Nonetheless, DOMS can be induced by eccentric exercise or by an unaccustomed exercise load [21,71,72]. The results of these studies would seem to suggest that enhanced NO production could impair force production in skeletal muscle [57] and the muscle soreness-associated increase could be a protective mechanism for skeletal muscle to prevent the possibility of maximal force generation and extensive damage [27].

**Is nitric oxide involved in muscle soreness?**

In a critical review, Armstrong [1] suggested that the pain, during DOMS, is due to the activity of macrophages and possibly the by-products of damage-induced exercise that accumulate in the interstitium, activating the endings of group-IV sensory neurons. The activation of macrophages could be a result of inflammation,
which would lead to the involvement of prostaglandins and the related pain. However, the supplementation of prostaglandin suppressing agent flurbiprofen did not significantly affect the sensation of pain, which suggests the existence of other factors [15]. Intramuscular fluid pressure, measured after concentric and eccentric bouts of muscle contraction, and two days after the exercise, increased with eccentric contraction, and was associated with the resultant pain [5]. Thus, the investigators suggested that eccentric exercise-induced intramuscular fluid mediated pressure is a causative factor of pain sensation. In 1994 Miles and Clarkson [73] stated that the exact cause of DOMS-related pain remained a mystery. The theory of prostaglandin mediated pain gained support later on, when it was shown that indomethacin treatment decreased this eccentric exercise mediated pain. The pain was probably sensed by polymodal-type nociceptors [74]. However, it must be noted that aspirin supplementation, (a potent inhibitor of prostaglandin synthesis) has been tested a number of times and found not to be effective to attenuate DOMS [75]. Glutamate could be one mediator of DOMS-associated pain, since DOMS increases glutamate concentration significantly in skeletal muscle (when measured by microdialysis), and supplementation of hypertonic saline causes a significant increase in dialysate glutamate concentration and decreased pain [76]. The presence of glutamate at the site of inflammation in the skeletal muscle is not surprising, since it is an important fuel for inflammatory cells [77].

Besides nociceptors, opioid receptors could also be activated, since the administration of morphine-6-glucuronide has been shown to decrease DOMS-associated pain [78]. Both nociceptors and most of the opioid receptors are sensitive to NO. A recent study reports that DOMS-associated pain is due to the mechanical stress related activation of nociceptors by bradykinin (which can produce NO) and neuro growth factor prolongs this pain [79].

The available information suggests that DOMS-associated pain, sensed by opioid receptors, mechano and nociceptors, can be activated by increased fluid pressure, prostaglandin, bradykinin, glutamate, ATP, or NO, and the pain is sustained by neuro growth factor (Fig. 1). However, it is not clear whether the joint activation of opioid, mechano and nociceptors are obligatory to DOMS. The unaccustomed exercise associated sensation of pain appears to be very complex and, despite significant findings on the nature of substrates that are able to cause pain, it remains unknown when and how the receptors are activated and the possibility of cross-talking between substances is still a mystery.

Muscle damage and repair

As mentioned above, unaccustomed exercise-induced muscle damage was observed decades ago, but, interestingly, mechanical studies on the repair process are lacking. Many observations have been made about the level of fitness, stretching, or physiotherapeutic treatments which can decrease DOMS and facilitate repair (please see the reviews [80–85]).

We hypothesize that NO, which can mediate decreased force production and elevate pain sensation during DOMS, plays a very important role in the repair of muscle damage. One of the first investigations on the role of NO in skeletal muscle damage was reported by Anderson [35] who mechanically crushed muscle of wild, NO knock-out or NO inhibited mice and observed very different repair processes to reveal the importance of NO in damage repair. It followed that NO facilitates the activation of satellite cells, which are located in the basal lamina of skeletal muscle, and this is one of the first steps in the repair process. Moreover, it appears that the beneficial function of NO in damage repair is not just restricted to satellite cell proliferation and differentiation but also to fusion. Hence, NO and the signaling agent of NO, cGMP, the antagonist of myostatin, activate follistatin [86], which is a negative regulator of myogenesis. Despite the well described role of NO in the repair of muscle injury, it is possible that the mechanism controlling repair after unaccustomed exercise-induced damage might be different from those after crush injury. Interestingly, treadmill running related overuse of tendons results in increased NO production, which suggests a role in the repair process [87]. The induction of mechanical damage to gastrocnemius muscle has been shown to result in increased NO formation, which is believed to initiate a signaling process for damage repair [88]. In addition, the importance of NO to muscle function, has been demonstrated as NO inhibition resulted in severe reduction in walking speed of rats [89].

Therefore, one can suggest that NO, which is generated during muscle contraction as a result of nNOS [29], eNOS [90–92] and iNOS [93,94] activation, could cause decreased force generation, pain as a protective mechanism but also obligatory to the repair process.
by satellite cell activation, proliferation, and fusion by the activating follistatin (Fig. 2). This suggestion is in accordance with the observation that mdx mice (lack of dystrophin anchored nNOS, thus genetically mimicking Duchenne muscular dystrophy), suffer from impaired skeletal muscle repair [95]. The fact that nNOS splice variants such as nNOSα and nNOSβ (located in the golgi system) deficient mice are extremely myopathic [29], further supports the role of NO in muscle damage repair and muscle physiology. Moreover, nNOS is involved in eccentric exercise and caused damage because it has been observed that nNOS blocker supplementation increases the eccentric exercise induced damage of desmin and dystrophin [96].

Conclusion

DOMS is a very complex mechanism, with well characterized symptoms, including decreased maximal force generation 1–3 days post-exercise, pain sensation and mechanical damage to skeletal muscle and connective tissue. Although, the etiology of DOMS cannot be described by a single agent, the present review emphasizes the possible role of NO in DOMS, which indeed could be important in decreased maximal force generation, pain, and damage repair. Despite the well established role of NO in muscle physiology, more research is necessary to further clarify its effects in DOMS.

Perspectives

It is well established that unaccomodated exercise induces muscle damage and pain. NO could be one of the causes of pain sensation, hence NO-blockers or NO binding agents could have the potential to attenuate pain. However, it must be mentioned that pre-treatment with NO inhibitors may result in reduced blood flow in skeletal muscle, resulting in reduced performance. Therefore, in order to increase endurance capacity one may have to pay the price of a little pain.

On the other hand, NO appears to be important for the activation of satellite cells that are required for damage repair. Hence, NO donors could promote damage repair. Therefore, it can be hypothesized that pre-treatment of NO inhibitor and post-treatment of NO activator could have beneficial effects on DOMS. There is a wide array of commercially available products that aim to increase blood flow by activating NO production in skeletal muscle, but one has to note that these products can cause hypotension and/or redistribution of blood away from the skeletal muscle. Therefore, the manipulation of NO production in skeletal muscle is not without risk, and muscle specific NO stimulators could have the potential to increase blood flow during exercise and by the activation of satellite cells and follistatin stimulate damage repair and even muscle hypertrophy.

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