Intramuscular responses with muscle damaging exercise and the interplay between multiple intracellular networks: A human perspective

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1 Background

The past decade has seen an explosion of research examining the impact of exercise on various levels of cellular function. As this research continues, it has become more evident that multiple areas of regulation and interaction converge to yield the expressed outcome. While exercise has been investigated in all kinds of shapes and sizes, research examining the impact of muscle-damaging exercise has garnered particular interest from researchers. This type of exercise provides an opportunity to examine an in vivo response to further characterize involved mechanisms while also offering translatable outcomes that can impact recommendations related to clinical populations as well as physically active populations such as athletes, both recreational and competitive, tactical officers and legions of military personnel. An overwhelming amount of literature exists in both animal and human models and of the research performed in humans; the lion’s share of this research has primarily examined the sampling of blood as the primary tissue of involvement. Nicely, this research has masterfully characterized both peak and time-course responses to an array of variables that are impacted by muscle-damaging exercise. While this research has been expertly reviewed and presented elsewhere (Lee et al., 2002; Michailidis et al., 2007; Nikolaidis et al., 2008; Paschalis et al., 2007), the focus of the present paper will be centered on discussing intramuscular human work that has examined a number of mechanistic pathways which are impacted by muscle-damaging exercise. Specifically, scientific findings related to mechanisms related to inflammation, oxidative stress, apoptosis and proteolysis will all be discussed. (see Fig. 1)

2 Eccentric exercise and damage

Muscle contraction is primarily broken up into two distinct phases: concentric and eccentric contractions. Dynamic muscle contractions are characterized by concentric and eccentric phases; the concentric phase involves shortening and the eccentric phase involves lengthening against an external load. Another perspective commonly shared when defining or characterizing an eccentric contraction are those contractions which occur against an external force that is greater than the force produced which results in lengthening of the involved muscle (aka. a forced-lengthened contraction) while contracted and generating tension. Within any given movement, the agonist muscle will often (but not always)...
go through both a concentric and eccentric phase of contraction and for this reason eccentric contractions are considered an essential part of any movement, particularly those conducted as part of exercise (Dickinson et al., 2000; Lindstedt et al., 2001; Nikolaidis et al., 2008). Interestingly, direct comparisons between concentric and eccentric contractions will reveal that at a given power output, concentric contractions require more oxygen cost (Beltman et al., 2004; Dudley et al., 1991a) and more neural involvement (Enoka, 1996; Hortobagyi et al., 1996). For example, (Moritani et al., 1987) found that, for a given force, eccentric contractions in the biceps brachii of men were associated with much less EMG activity than concentric contractions, evident by less motor unit recruitment and rate modulation, thereby suggesting possible de-recruitment of motor units during the eccentric phase of a dynamic muscle contraction. This depression of voluntary activation occurring during maximal eccentric contractions may be explained from data demonstrating that the augmented feedback from peripheral sensory receptors during eccentric contractions seems to be suppressed by centrally- and peripherally-mediated presynaptic inhibition of Ia afferents (Duchateau and Enoka, 2008). Therefore, when damage to the muscle tissue is considered, eccentric contractions are responsible for an overwhelming majority of the resulting damage (Kendall and Eston, 2002; Paschalis et al., 2005) since this loading profile (high force with low motor unit recruitment) places a high mechanical stress on the muscle fibers.

A number of symptoms exist related to muscle damage and for this reason a number of different approaches have been employed by scientists to evaluate and report the extent to which muscle damage has occurred. Typical symptoms include an increase in muscle soreness, muscle swelling, structural damage and a leakage of proteins typically located within the myofibril unit (e.g., creatine kinase, myoglobin, troponin, etc.) while maximal strength or force production typically experiences reductions (Kerkisick et al., 2008, 2010; Morton et al., 2009; Willoughby et al., 2003). These ‘classic’ symptoms have been studied to a great degree (Kerkisick et al., 2008, 2010; Morton et al., 2009; Willoughby et al., 2003), while other features of eccentric contractions are accepted in the literature, but require more investigation and include activation of various cellular pathways (thus, the purpose of this review) (Kerkisick et al., 2008, 2010), alterations in glycogen metabolism (Costill et al., 1990) and insulin regulation (Kirwan and del Aguila, 2003).

As highlighted throughout, most of the damage is due primarily to an interaction between a novel exercise stimulus and one that is primarily eccentric in nature. While distinct and clear characterization of the mechanisms behind muscle damage remains elusive, a number of excellent reviews have been prepared and the interested reader is highly encouraged to consult these works (Armstrong, 1984; Armstrong et al., 1991; Clarkson and Sayers, 1999). In accordance with this work (Howatson and van Someren, 2008), a predominant and attractive theory of muscle damage breaks the process upon into three phases. The first phase has contributions from both metabolic and mechanical stress, which the latter is thought to be the primary contributor. Mechanical stresses occur due to the greater production of force generated during eccentric contractions and the known physiological concept of non-uniform lengthening of sarcomeres, which upon lengthening has resulted in visible muscle damage under magnification and is commonly referred to a Z-band streaming (Friden et al., 1983). This seems to be due to the mechanism in which force generation during an eccentric contraction differs because the cross-bridges are detached mechanically and with greater force than undergoing the detachment involving ATP splitting that occurs during concentric contractions (Enoka, 1996). Disruption of the myofilibrillar structure leads to an imbalance of calcium homeostasis and is considered to be the secondary phase of muscle damage. Increases in intramuscular calcium exacerbate damage and facilitates a cascade of events that alter several cellular structures. A loss of membrane integrity leads to the leaking of intramuscular proteins into the circulation while the known infiltration of neutrophils and then macrophages leads to contributions from the inflammatory cascade. In conclusion, a number of factors such as speed of contraction, fiber-type, intensity of contraction, novelty of the exercise bout and length of the involved muscle all converge to impact the resulting damage (Howatson and van Someren, 2008). Fortunately, researchers have nicely characterized a number of outcomes related to soreness, force production, swelling or circumferences, and intramuscular proteins with peak values typically being achieved within 24–48 h after the damaging session with most variables returning to normal after 72–96 h (Kerkisick et al., 2008, 2010; Nikolaidis et al., 2007; Paschalis et al., 2005; Stupka et al., 2001; Willoughby et al., 2003).

3. Intramuscular inflammatory changes

As will be discussed throughout this review and like the other pathways involved in eccentric damage, only a small number of studies have sampled human muscle tissue surrounding multiple prescriptions of eccentric exercise to examine how inflammatory components are activated and interact with other aspects of skeletal muscle physiology. Initial human work completed by Stupka and investigators required untrained men (n = 8) and women (n = 8) to complete a single exercise bout comprised of eccentric muscle contractions (36 repetitions of unilateral leg press and 108 repetitions of unilateral leg extensions at 120% of their concentric 1RM) that was intended to invoke muscle damage. Muscle biopsies were collected from both the exercised and control legs of all participants 48 h after the damaging exercise. Intramuscular granulocyte and leukocyte counts increased for both men and women while men tended to have more infiltrated leukocytes (p = 0.052) than women (Stupka et al., 2000). To build upon this work and in response to repeated bouts of damaging, eccentric exercise of the knee extensors (36 unilateral leg press repetitions and 100 unilateral leg extension repetitions at 120% concentric 1RM) was completed with each bout being separated by 5.5 weeks, Stupka reported increased macrophage levels in both men and women after eccentric exercise while levels of infiltrated neutrophils were only found to significantly increase in women after the 2nd eccentric bout (men experienced pronounced mean changes in this variable, but they were not statistically significant, p > 0.05) (Stupka et al., 2001). Also using a repeated bout design, Willoughby reported immediate increases in serum levels of cortisol after two repeated bouts of eccentric exercise (7 × 10 repetitions at 150% concentric 1RM) separated by 3 weeks that persisted through a 24 h window as well as increases in the mRNA and protein content of cortisol’s receptor, the glucocorticoid receptor (Willoughby et al., 2003). Collectively, these studies consistently highlighted the findings that eccentric exercise in both genders leads to an infiltration of early-response cells to the damage area. An excellent study with intriguing outcomes by Malm and colleagues had subjects perform either uphill (4 degrees) or downhill (4 or 8 degrees) running for 45 min. Uphill runners performed at 75% VO2Max (45.2 ml/kg/min), the 4 degree downhill runners ran at 50% VO2Max (23.9 ml/kg/min) and the 8 degree downhill runners, due to concern for safety, ran at a speed they were comfortable (3% VO2Max not reported; 32.2 ml/kg/min). Muscle samples were collected 48 h after completion of the exercise bout. Interestingly, the authors reported that no changes in circulating levels of inflammatory cells were found in exercising subjects versus non-exercised controls. Furthermore, eccentric exercise (downhill running) was not found to increase skeletal muscle inflammation 48 h after the exercise bout, despite increased levels of soreness and creatine kinase (Malm et al., 2004).
A few years removed, other investigations by the Willoughby group utilized real-time polymerase chain reaction (RT-PCR) in conjunction with repeated muscle biopsies to report a fairly comprehensive look at a number of cell components closely linked to the inflammatory cascade (several of which had never been reported upon by the literature). While both studies did not incorporate exercise models that exclusively required eccentric contractions (one used traditional resistance training and the other used downhill running), the utilized biochemical technology and the sampling of muscle tissue before and through 24 h warrants their inclusion due to the relative lack of available studies that exclusively utilized eccentric exercise (Buford et al., 2009a,b). After warming up, 24 active women completed a lower body resistance exercise that consisted of 90 repetitions at 80% 1RM equally dis-bursed among the machine squat, leg press and leg extension exercises. Muscle samples were collected both before and 3 h after completion of the exercise bout and a significant up-regulation of multiple inflammatory markers was found. Specifically increases in TNFα, IL1β, IL6, SOCS2, COX2, SAA1, SAA2, IKKB, cfos and jun B were found while no changes were found for IL2, IL5, IL10 and IL12. The lack of changes for these latter proteins was not surprising as no other reported studies have documented changes, making it more possible that these interleukins are not mediated by acute exercise stress (or at least under conditions in which they are often measured). Additionally, the authors indicated that many previous studies have reported similar changes in a number of the genes found to be significantly up-regulated in their study (Buford et al., 2009b; Louis et al., 2007). Another study by the same research group had 29 males complete a 45-min downhill running protocol at 60% VO2 Max (Buford et al., 2009a). Muscle biopsies were collected before, 3 and 24 h after completion of the exercise bout for determination of mRNA expression of a number of targets related to inflammation. As before a number of markers were increased in response to the stressful exercise bout that corresponded with increases in soreness and serum levels of creatine kinase (indirect markers of muscle damage). For example, mRNA expression of IL-6 (a key pro-inflammatory cytokine produced in skeletal muscle) was increased at both 3 and 24 h while IL-8 and COX2 were increased 3 h after exercise. To link the changes in muscle damage with inflammatory involvement, soreness levels were found to be correlated with IL-8 at 24 h while serum creatine kinase levels were related to NKKβ at baseline. Regardless, the authors concluded that an acute bout of downhill running increased the expression of the mRNA associated with IL-6, IL-8 and COX2. Finally, one of the only studies to examine intramuscular changes associated with inflammation in humans after damaging, eccentric-only muscle contractions was completed by Hubal et al. (2008). In this study, seven male subjects performed one of two exercise bouts separated by 4 weeks. Both bouts required a total of 300 muscle contractions, but one session utilized only eccentric contractions while the other bout required only concentric contractions. Skeletal muscle biopsies were collected 6 h after the exercise bout and were analyzed to determine the mRNA content of several genes related to inflammation. When damaging, eccentric contractions were utilized, three of the probed genes were found to increase from the first to the second exercise bout and these included ZFP36, CEBPD and MCP1. Conversely, none of the genes were found to alter their expression after the concentric only protocol (Hubal et al., 2008). The changes associated with MCP1 were of the greatest interest as co-localization of this transcript was identified via immunohistochemistry with macrophage and satellite cell populations in the muscle, which suggested that alterations in cytokine signaling may mediate muscle adaptation to exercise.

4. Intramuscular oxidative stress changes

A close examination of the available literature reveals an abundance of published studies which have examined changes in oxidative stress in the blood after muscle-damaging exercise. One of the initial studies to report on these changes was published in 1989 (Maughan et al., 1989) and since that time period, the amount of available research continues to grow with a number of reviews...
available (Fisher-Wellman and Bloomer, 2009; Nikolaidis et al., 2008). Blood carries a bevy of markers associated with all aspects of oxidative stress and considering the relative ease of sampling blood at distinct time points relative to other tissues, the abundance of research utilizing this tissue should come as no surprise. Moreover, blood plays a near-ubiquitous role in maintaining the redox state of the cell and works to both deliver and remove antioxidants and oxidants alike from the involved tissue beds. An excellent review prepared by Nikolaidis and colleagues highlighted the critical importance of sampling time (Michailidis et al., 2007) relative to oxidative stress assessment in the blood; a consideration which arguably holds more importance when discussing the more invasive sampling of muscle tissue. Highlights from this work and a number of other papers reveal that in response to exercise and in particular damaging exercise markers of oxidative stress and levels of antioxidants change. Sacheck et al. required younger and older men to complete a downhill running bout on a treadmill for 45 min at 75% VO2Max both before and after 12 weeks of supplementation with vitamin E (1000 IU/day) or a placebo. Following the exercise bout, both young and older men experienced increased levels of creatine kinase (a myofibrillar protein used as a marker of muscle damage), isoprostanes and malondialdehyde (both are markers of lipid peroxidation) while levels of 8-hydroxy-2′-deoxyguanosine (a marker of DNA oxidation) were unaffected (Sacheck et al., 2003). Childs et al. 2001 had human subjects complete an eccentric exercise bout that involved the upper arm musculature. Immediately following the damaging exercise bout, supplementation with either a placebo or a combination of vitamin C + N-acetyl-cysteine (NAC) occurred for 7 days. Blood-based markers of muscle damage (e.g., lactate dehydrogenase, creatine kinase and myoglobin) were all elevated 2, 3 and 4 days after the injurious exercise bout. Moreover, markers of oxidative, specifically lipid peroxidation (8-isoprostane and lipid hydroperoxides) and antioxidant enzyme levels were all increased after injury (Childs et al., 2001). Paschalis and colleagues had ten healthy but untrained females complete a single bout of muscle-damaging muscle contractions on the knee extensors. Assessments of muscle damage (isometric torque, soreness, and creatine kinase levels) and oxidative stress (reduced glutathione [GSH], oxidized glutathione [GSSG], thiobarbituric-acid reactive substances [TBARS], protein carbonyls, catalase, uric acid, bilirubin and total antioxidant capacity [TAC]) were all assessed before, 24, 48 and 72 h after the exercise bout. All markers of oxidative stress changed in a manner to indicate increased oxidative stress which peaked around 48 h (except for TBARS) after the exercise bout (Paschalis et al., 2007). Using a repeated-bout design, Nikolaidis and investigators had twelve females complete two bouts of 75 eccentric muscle contractions of the knee extensors separated by 3 weeks. A comprehensive mix of muscle damage and oxidative stress markers were assessed in the blood up to 7 days after each exercise bout. All muscle damage markers changed significantly after exercise to indicate damage while all oxidative stress markers changed in a manner to indicate increased oxidative stress with the majority of markers peaking around 3 days after the exercise bout. Similar changes occurred after the second damage bout (3 weeks later), but almost universally the associated markers experienced attenuated or blunted responses when compared to the same time point found in the first exercise bout (Nikolaidis et al., 2007). Finally, a 2010 paper by Fatouros had trained soccer players either play simulated games or serve as controls. Blood samples were taken and analyzed for markers of muscle damage and oxidative stress and significant increases in oxidative stress were consistent across the measured markers throughout the 3 days recovery period (Fatouros et al., 2010). As can be clearly seen, consistent increases in numerous markers of both muscle damage and oxidative stress can be seen in human blood for 2–4 days after completing a damaging exercise bout.

Studies which have incorporated collection of human skeletal muscle to determine changes in oxidative stress after damaging exercise are limited, a point highlighted by Nikolaidis et al. (2008). In this review, the authors highlighted human studies which examined changes in lipid (Child et al., 1999; Meydani et al., 1993; Saxton et al., 1994), protein (Saxton et al., 1994) and DNA oxidation (Radak et al., 1999). Changes in malondialdehyde (MDA) and conjugated diene levels, both markers of lipid peroxidation, inside the vastus lateralis of people who had just completed damaging exercise experienced no changes. As nicely highlighted throughout (Nikolaidis et al., 2008), two studies (Meydani et al., 1993; Saxton et al., 1994) revealed pronounced percentage changes (39–68% increase in these markers from 2 to 5 days after exercise) of MDA and conjugated dienes, but due to the low statistical power, no statistically significant differences were revealed (p > 0.05). The investigation by Saxton et al. (1994) had 14 males perform a single exercise bout consisting of either eccentric or concentric muscle contractions, which were separated by 4 weeks. Interestingly, intramuscular determination of protein carbonyl concentrations was found to be higher immediately after concentric vs. eccentric contractions (Saxton et al., 1994), a finding that may be viewed as somewhat counterintuitive to anticipated response to differing styles of muscle contraction. Furthermore, Radak et al. concluded that intramuscular levels of DNA oxidation (8-OHdG) in six females were increased 24 h after an eccentric exercise when compared to levels that were measured at baseline (prior to the damage bout); these findings overlapped with a 11% decrease in force production and an increase in self-reported soreness (Radak et al., 1999). Since that review article, Kerksick and colleagues examined the impact of a 14-day prophylactic bout of antioxidant supplementation (n-acetyl-cysteine [NAC] and epigallocatechin gallate [EGCG]) on blood and muscle based changes in young, healthy men before completing a damaging bout of 100 eccentric muscle contractions of the knee extensors (Kerksick et al., 2010). Skeletal muscle samples were collected while fasted before, 6, 24 and 48 h after completion of the damaging exercise bout. Independent of the antioxidant supplementation, all subjects displayed evidence of muscle damage (decrease in force, increase in soreness, and an increase in creatine kinase). Serum levels of 8-isoprostane did not change while serum level of superoxide dismutase tended to increase in all groups. One of the more involved studies which utilized human skeletal muscle and eccentric exercise was completed by Theodorou et al. (2011). Study participants were required to supplement in a double-blind fashion with either vitamin C and vitamin E or a placebo for 11 weeks. After 4 weeks of supplementation, all subjects completed the first of two eccentric exercise bouts that consisted of five sets of 15 eccentric muscle contractions of the knee extensors (Kerksick et al., 2010). Study participants were required to supplement in a double-blind fashion with either vitamin C and vitamin E or a placebo for 11 weeks. After 4 weeks of supplementation, all subjects completed the first of two eccentric exercise bouts that consisted of five sets of 15 eccentric muscle contractions of the knee extensors. While continuing the supplementation protocol, subjects then completed 4 weeks of eccentric training which consisted of two sessions per week identical to the testing sessions. After completing the 4 weeks eccentric training period, a second eccentric damage testing bout was completed. Skeletal muscle samples were collected at four time points from each subject: immediately before and 3 days after completing both eccentric damage testing bouts. Blood samples and indicators of muscle were also collected before and 1, 2, 3, 4 and 5 days after each eccentric damage testing bout. As expected, widespread indicators of muscle damage occurred in addition to changes in redox status indicative of oxidative stress. This study is valuable because it was the first and the only investigation that has examined blood and intramuscular changes and weeks of eccentric training. Results failed to support any utility of antioxidant supplementation in...
either the blood or muscle. Due to the low sample size of participants in each group (n = 4) who completed muscle collection, the authors of this study did not use traditional statistics due to their knowledge of low statistical power from their design. While their results should be interpreted cautiously, it is important to highlight that consistent indicators were evident from this study to indicate the several markers of oxidative stress were increased above pre-damage levels 3 days after this exercise bout was completed (Theodorou et al., 2011). Additionally, when baseline levels of oxidative stress markers were compared before and after the exposure of eccentric training, most values indicated an adaptation suggesting improved protection from oxidative damage.

Collectively, results from studies incorporating eccentric damage consistently demonstrate that this type of exercise increases an array of markers indicative of oxidative stress in the blood. When human muscle is considered, the number of available studies goes down immensely, but consistently findings from study designs that vary to a great extent also indicate the oxidative damage to this tissue is occurring. However, only one available study has been performed with eccentric training in humans and overall more questions exist than what are answered at this point. For this point, investigators are highly encouraged to consider identifying if the time course of intramuscular changes differs greatly from those characterized in the blood. This will aid future investigations with developing efficient study designs that maximize the risk-to-benefit ratio of muscle collection and hopefully will allow for more fruitful outcomes. An additional point which has yet to be explored fully in human muscle, but has been characterized in animal models (Ji et al., 1992; Leeuwenburgh et al., 1997; Powers et al., 1994) is the notion that muscle fiber type may impact the extent to which changes in oxidative stress markers occur. In this respect, Quindry and colleagues correlated fiber type with changes in blood markers of oxidative stress and more specifically revealed that type II fiber content was corrected with post-exercise serum levels of protein carbonyls (Quindry et al., 2011).

5. Intramuscular apoptotic changes

An area that has garnered more interest relative to cellular involvement with damaging exercise is that related to apoptotic mechanisms. For years, scientists operated under the notion that muscle tissue damaged from eccentric contractions was primarily mediated through inflammatory pathways which ultimately resulted in necrosis of the affected tissue (Abu-Shakra et al., 1997), however, the work of Carroro and Sandri introduced another possibility where they documented the presence of apoptotic nuclei in damaged muscle tissue (Carroro and Franceschi, 1997). Apoptosis is known to be a highly conserved type of cell death that plays a critical role in tissue homeostasis as well as disease-associated processes (Quadrilatero et al., 2011). Stupka et al. (2000) completed in what is believed to be the first human investigation to examine the impact of eccentric muscle contractions on apoptotic activity in healthy human skeletal muscle. In this study, groups of eight men and eight women provided muscle samples before and 48 h after the eccentric damage bout (12 sets of 12 eccentric contractions using muscles of the lower body at 120% 1RM) along with a series of blood samples also surrounding the damage bout. Significant indications of muscle damage (e.g., 20% disk streaming, increased serum creatine kinase) were found and significantly greater numbers of cells that stained positive for the apoptotic protein, bcl-2, were found in men when compared to women. Results from this study provided some of the first evidence that apoptotic cells were found in the skeletal muscle of previously healthy individuals (Stupka et al., 2000). A later study by Willoughby had nine males complete two bouts of eccentric exercise (7 sets of 10 repetitions at 150% 1RM) on the knee extensors separated by 3 weeks. Blood, strength and soreness was determined before, 6, 24, 48 and 72 h after each exercise bout while muscle samples were collected before, 6 and 24 h after each exercise bout (Willoughby et al., 2003). Intramuscular determinations of caspase-3 content and activity were made whereby caspase-3 is known to be key regulatory component of apoptotic activity (Du et al., 2004). In addition, intramuscular DNA content was determined as a corollary to apoptotic involvement and results from this study revealed that caspase-3 activity was significantly elevated both 6 and 24 h after both eccentric exercise bouts with greater levels occurring after the initial damage bout while total DNA content exhibited decreases of a similar pattern (Willoughby et al., 2003). To follow up on this research, two studies completed by Kerksick and colleagues examined the impact of gender and antioxidant supplementation on blood and muscle based changes in young, healthy men and women (Kerksick et al., 2008, 2010). The first of these investigations compared a group of eight men and eight women in response to completing a single bout of 70 eccentric muscle contractions of the dominant knee extensors at 150% 1RM. Before as well as 6, 24, 48 and 72 h after the exercise bout, strength, soreness and blood samples were taken while muscle samples were collected after 6 and 24 h. Men reported higher levels of soreness when compared to women while strength changes appeared to be independent of gender. Interestingly, females appeared to have both higher baseline levels as well as higher serum levels of superoxide dismutase (a predominant endogenous antioxidant enzyme) in response to the exercise bout along with lower serum levels of 8-isoprostane (a marker of lipid peroxidation) when compared to men. Muscle samples were analyzed for changes in bax, bcl-2, cytochrome c, bax:bcl-2 ratio and cell death. Independent of gender, eccentric exercise increased intramuscular levels of bax (a pro-apoptosis protein), while levels of bcl-2 (an anti-apoptosis protein) were higher only in women. No changes were found to occur with cytochrome c for either gender in response to eccentric exercise. When the bax:bcl-2 ratio was computed (an overall index of apoptosis with higher levels indicating an increased expectation of apoptotic activity), lower levels were revealed in women after 6 h and returned to similar baseline levels after 24 h. To further substantiate these findings, a non-specific measure of cell death also revealed men to have greater levels of cell death at all time points when compared to women along with decreased levels of total DNA up to 24 h after the eccentric damage. A later study by Kerksick had young college-aged men complete a single bout of isokinetic eccentric muscle contractions after following a 2-week supplementation regimen with n-acetyl-cysteine (NAC) and epigallocatechin gallate (EGCG), a predominant catechin found in green tea, while having blood and muscle samples collected at various time point surrounding the exercise damage bout (Kerksick et al., 2010). Muscle samples were collected before as well as 6, 24 and 48 h after the damaging exercise bout and again increases from baseline were found to occur for both the bax and bcl-2 proteins. Changes in the concentrations of these proteins have been implicated in the literature to promote (bax) or limit/prevent (bcl-2) apoptosis (Quadrilatero et al., 2011). No changes were found in cytochrome c, caspase-3 content or activity as well total DNA content. However, contrasts made for caspase-3 enzyme activity at 48 h were found to be significantly greater in all groups after eccentric exercise when compared to value at baseline and 6 h after the damage bout (Kerksick et al., 2010). While no impact was found for the chosen supplementation regimen, the authors again determined that acute responses to eccentric damage does involve activation and increased expression of key components of the apoptotic pathways. Similar findings were made by Park et al. when they determined increased concentrations of the mitochondrial apoptotic proteins (e.g., bax, bcl-2, bax:bcl-2 ratio) occur.
curred in the blood of human subjects in response to different bouts of downhill treadmill running (40 min at ~70% \( \text{VO}_2\text{Max} \)) (Park et al., 2011).

6. Intramuscular proteolytic changes

In the last 10–15 years the scientific literature has greatly expanded detailing mechanisms of proteolysis as well as key points of regulation and associated areas of “crosstalk” amongst related pathways. Collectively, proteolytic pathways include the cathepsins, calcium-mediated calpains, the ubiquitin proteolytic system and its components as well as the caspase family. Overwhelming, discussions of proteolytic involvement within human muscle surrounding muscle damage include the calpains and the ubiquitin system. Uniquely, the calpain system works to dismantle the architecture of the myofiber and as its name indicates it is particularly responsive to changes in cytosolic calcium (Belcastro et al., 1998). Secondary to the trauma and myofiber destruction which can occur from heavy bouts of eccentric bouts, increases in calcium commonly occur and are widely considered to be a primary signal to drive downstream events (Belcastro, 1993; Gissel and Clausen, 2001). The ubiquitin system works throughout all cell types, but has been shown in research to be primarily responsible for the breakdown of myofibrillar proteins (Glass, 2005). The ubiquitin proteolytic system is commonly outlined to have five distinct parts that all work together to achieve the breakdown of proteins. The first component, ubiquitin, is the pathways namesake and is a ubiquitinous protein that upon its activation by the next component, the E1 ubiquitin activating proteins, is attached several times (commonly referred to as polyubiquination) to damaged or improperly folded proteins. This act of tagging is often viewed as being a signal which commits the “flagged” protein for destruction. The third component is the E2 ubiquitin conjugating proteins, the fourth component are the E3 ligase proteins of which two key candidate genes, MaFbx (atrogen-1) and MuRF1, have been identified along with the final, the proteasome core that completes the act of breaking down those proteins identified by the system to be damage or in need of repair (Glass, 2005).

Keeping the focus of this review in mind, a number of other studies have reported using techniques ranging from stable isotope tracers and mRNA quantification of candidate intramuscular genes to indicate that rates of muscle breakdown increase in response to exercise while using bouts of traditional resistance exercise (those which involve both concentric and eccentric muscle contractions) (Dalbo et al., 2011; Phillips et al., 1999; Yang et al., 2006). For example, Yang and colleagues examined the change in mRNA expression of a number of genes linked to inflammation, calpain-mediated proteolysis, ubiquitin proteolysis and apoptosis in a group of young and older men in response to a modest bout of concentric-only resistance exercise (3 sets of 10 repetitions at 65% 1RM of bilateral knee extensions). Expression of a number of these genes indicated that proteolytic pathways are indeed activated in a time-dependent fashion ranging from 4 to 24 h after a modest bout of concentric-only resistance exercise (Yang et al., 2006). Using a stepping protocol (which involves both concentric and eccentric contractions) in a repeated fashion over an 8 weeks period, Vissing et al. identified and reported that soreness, creatine kinase, myoglobin and strength all changed to indicate muscle damage occurred. Muscle samples collected before, as well as 3, 24 and 168 h (7 days) after this exercise bout resulted in increases in calpain 2 and calpastatin mRNA levels at 24 h after both exercise bouts. No changes were found in calpain 1 and 3 mRNA levels, which is consistent with previous unpublished work from our lab (Kerksick, unpublished observations) (Vissing et al., 2008).

A deeper intramuscular investigation by Stupka et al. (2000) incorporated two (separated by 5.5 weeks) eccentric only exercise bouts (36 repetitions of unilateral leg press and 100 repetitions of unilateral leg extension, both at 120% concentric 1RM) in untrained men \((n = 8)\) and women \((n = 8)\). Muscle biopsies were collected from each leg 24 h after the exercise bout and were analyzed for markers related to inflammation, calpain activity, ubiquitin proteolysis and apoptosis. The authors reported that the muscle protein content of the regulatory calpain subunits were unchanged whereas the total content of ubiquitin-conjugated proteins were increased 24 h after both bouts of eccentric exercise. This led the authors to conclude that at their measured sampling points, activation of the ubiquitin-proteolytic system may be a contributing factor (Stupka et al., 2001). In what remains as the most comprehensive look into proteolytic regulation involving eccentric exercise in humans, Willoughby and colleagues had nine men complete two bouts of eccentric only exercise 3 weeks apart (Willoughby et al., 2003). Each bout consisted of 7 sets of 10 repetitions of the dominant knee extensors with an intensity that represented 150% of their concentric 1RM. Before as well as 6 and 24 h after both exercise bouts, muscle samples were harvested and both the mRNA expression and protein content of multiple markers of the ubiquitin proteolytic system (ubiquitin, E2, the 20S proteasome) was determined. In addition, mRNA expression and protein content of the glucocorticoid receptor was determined due to the known relationship between glucocorticoid physiology and proteolysis (Wallace and Cidlowski, 2001). Markers of muscle damage (soreness and serum levels of troponin-I) increased to suggest damage after both eccentric bouts along with greater values prior to the initial bout. Furthermore, across the board increases were seen in both the mRNA and protein content for the ubiquitin, E2 conjugating enzyme, the 20S proteasome as well as the glucocorticoid receptor as early as 6 h which continued throughout the 24 h sampling period. In accordance with previous repeated bout effect outcomes, levels for all of these intramuscular proteolytic components were greater after the first bout when compared to the second bout (Willoughby et al., 2003). Finally two studies, one by Willoughby et al. (2003) and another by Kerksick et al. (2010) reported time course changes in the overall level of myofibrillar protein levels before, 6 and 24 h eccentric exercise. In both studies, significant decreases in myofibrillar protein content were found to provide further support of increased proteolytic activity. Moreover, the Willoughby study demonstrated these changes after not one but two bouts of eccentric exercise that were separated by 3 weeks.

7. Hypertrophy: does damage play a role?

As has been presented, a number of studies continue to investigate the impact of damaging exercise on mechanisms that are often characterized as bad (whether it is accurate or not) such as inflammation, oxidative stress, apoptosis and proteolysis. However, a complete discussion of potential mechanistic implications from an intramuscular perspective cannot occur without some discussion related to its potential mechanistic impact related to muscle hypertrophy. While the focus of this paper has intended to center on mechanistic involvement related to inflammation, oxidative stress, apoptosis and proteolysis, the work which has been completed regarding the role of damaging exercise in hypertrophy warrants at least a brief discussion. In this respect, several original studies (Adams et al., 2004; Dudley et al., 1991b; Hather et al., 1991; Higbie et al., 1996; Hortobagyi et al., 1996) and one recent review (Schoenfeld, 2012) has examined this relationship. Collectively, the original articles indicate that when compared to concentric (shortening) contractions, an eccentric (lengthening)
contraction appears to be responsible for more hypertrophic gains (Adam et al., 2004; Dudley et al., 1991b; Hather et al., 1991; Higbie et al., 1996; Hortobagyi et al., 1996). In support of this original work, Schoenfeld summarized an extensive body of literature while highlighting a few key areas that at the cellular level could offer some links to a hypertrophic response in the muscle. These areas included intracellular signaling via inflammatory cell activation which have been mechanistically linked to pathways associated with hypertrophy (e.g., ERK1/2, JNK, p38 MAPK, IGF1), satellite cell activity, and the IGF-1 signaling cascade which includes its well-characterized downstream components (i.e., the IGF1/Pi3K/Akt/mTOR/p70S6K and 4eBPI) and their association with improvements in translation initiation and efficiency (Schoenfeld, 2012). Currently, direct human research that has attempted to link damaging eccentric muscle contractions to modulations in skeletal muscle hypertrophy are extremely limited and remain an area of future inquiry.

8. Future directions and conclusions

Much progress has been made regarding our understanding of those intramuscular mechanisms involving inflammation, oxidative stress, apoptosis and proteolysis. Even with recent work providing more evidence than ever before, many more questions remain. One aspect that has changed is the overall feeling towards the contributions made by pathways involving inflammation and oxidative stress. Certainly, prolonged increases in both inflammation and oxidative stress are widely accepted to be negative as are situations in which the overall balance between pro-inflammatory and anti-inflammation as well as pro-oxidants or anti-oxidants strongly favors the former. However, more and more evidence continues to mount to indicate that small perturbations in inflammation and oxidative stress should be viewed as a good thing (dare we say, helpful) to the cell. For this reason, research in this area needs to be completed to ascertain what are the key factors, conditions or triggers that turn on pathways linked to inflammation and oxidative stress and eventually to identify which factors or situations leads to an unfavorable shift in this balance. When examining a transition from a course perspective both in the blood as well as the muscle, immediate increases seem to be more likely with markers associated with inflammation and oxidative stress while changes in apoptosis and proteolysis appear to be somewhat more prolonged (although not all evidence points to this). One thing that is becoming clearer is the inter-relatedness of the pathways. While fascinating, it is maddening from a research perspective as it becomes particularly challenging (nearly impossible) to only study one component, particularly as more evidence continues to indicate a level of inter-pathway dependence may be evident. Additionally, the inverse regulation of key targets associated with inflammation, oxidative stress and the like creates the need for more integrated investigations, ideally in the form of training studies, to identify what components make key contributions to the overall process of muscle protein regeneration and may in fact lead to an overall improvement in muscle hypertrophy, a point addressed in our review, but one in need of much more work. Across the board and as can be seen from our review of the literature, more human intramuscular work is needed. Initial work has offered a multitude of outcomes that begin to demonstrate and characterize the time course of changes which occur, but in many instances this work was focused on one particular area over the other. Future work should continue identifying key targets, but also work to extend their investigations across other cellular pathways to continue to unravel the web of interaction. Another area of need revolves around a better understanding of the relationship that exists between changes in various compartments throughout the physiological system. A number of studies have measured various components in the blood in response to stressful or damaging exercise, but only a few have taken a rather comprehensive look (Nikolaidis et al., 2008). From a time-course perspective, it certainly does seem that time-related changes bear resemblance across blood and muscle, but more work is needed in all areas. From this perspective, several questions remain namely; can blood changes be used as surrogate indicators of deeper tissue-level changes? Do magnitude and time-course changes seen in the blood reflect or relate to changes which occur at the tissue level? In many situations it does appear that changes in the blood resemble changes which occur in muscle tissue and it is our hope that as biochemical techniques get better characterized that more in-depth (multi-tissue) examinations will become the norm. Implications from these findings will certainly impact findings related to exercise and performance, but more importantly will provide additional information to aid in the treatment and prevention of any number of clinical conditions plagued with inflammation, proteolysis and eventual decrements in function. Moreover, it is our hope that future research will also aid in the treatment towards trauma victims and populations where muscle regeneration and optimal muscle function is needed to facilitate an ideal recovery.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References
