

Mechanisms of Apoptosis in Skeletal Muscle

Peter J. Adhihetty⁽²⁾ and David A. Hood^(1, 2)

(1) Departments of Kinesiology and Health Science and (2) Department of Biology, York University, Toronto, Ontario

Abstract

Apoptosis is an important regulatory process that occurs during normal development and in the progression of specific diseases. Apoptosis can be induced by two alternative signaling routes: 1) external factors binding to membrane death receptors outside the cell, and 2) internal cellular events leading to the release of specific cell death molecules from mitochondria. Regardless of the mode of apoptotic initiation, the morphological characteristics of apoptosis are consistent in most cell types. However, apoptosis in skeletal muscle has been relatively unexplored. Muscle is unique in that it is multi-nucleated, and evidence suggests that individual myonuclear decay is a more frequent occurrence than wholesale myofiber cell death. This, along with the high concentration of endogenous apoptosis inhibitor molecules, may account for the relatively low frequency of apoptosis in skeletal muscle. Nonetheless, apoptotic events occur in skeletal muscle as a result of muscle disuse, ischemia/reperfusion, ageing, and exercise. However, the signaling pathways involved remain largely unknown. Since mitochondrial content can differ among muscle fiber types, and can be altered by variations in muscle use or disuse, the role of the mitochondrial pathway in mediating apoptosis in muscle during health and disease warrants further investigation.

Key words: caspases, exercise, mitochondria, myonucleus, reactive oxygen species.

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Morphological Features of Apoptosis

Apoptosis is a form of cell death that is crucial for normal development and tissue homeostasis. The defining morphological features of apoptosis include plasma membrane blebbing, nuclear breakdown, chromosomal fragmentation, and the bundling of cellular contents into vesicles called apoptotic bodies that are marked for phagocytosis [28]. During apoptosis, the plasma membrane is meticulously dismantled while maintaining mitochondrial integrity and cellular energy provision. Apoptotic cells are clearly distinguishable by phosphatidylserine externalization, identifying themselves for disposal through phagocytosis [52]. Apoptosis is characterized by unique morphological and biochemical alterations which make it distinct from necrotic cell death. This review will initially describe the molecular mechanisms of apoptosis, followed by a discussion of the evidence of its occurrence within skeletal muscle.

Apoptotic Signal Transduction

Apoptosis can be triggered by external and internal stimuli. The external pathway occurs via ligand-mediated activation of specific plasma membrane death

receptors, while the internal pathway originates within the mitochondrion.

Ligand-induced activation of apoptosis

Apoptosis can be induced by the binding of ligands to one or more of the extracellular receptors of the tumour necrosis factor receptor (TNFR) superfamily [7]. The most important ligands are the cytokine TNF- α and Fas ligand (FasL), which are present on cytotoxic T lymphocytes and other immune cells. The binding of these ligands to their receptors ultimately leads to the activation of a specific set of proteases crucial for the execution of apoptosis. These proteases are collectively referred to as caspases, and they are activated by proteolytic cleavage. Caspases have the ability to cleave and activate other caspases in a "cascade-like" fashion. This serves as an efficient and potent mechanism for amplifying the cell death signal [32]. Ultimately, caspase activation is responsible for the biochemical breakdown of cytosolic and nuclear targets leading to the distinct morphological features of apoptosis [43].

The response of cells to the binding of FasL to the Fas receptor is well characterized. This binding sequesters procaspase-8 to the cell membrane by an adaptor protein, Fas Associated Death Domain (FADD), which is

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bound to the Fas receptor [7]. Recruitment of procaspase-8 to the plasma membrane results in a feed-forward recruitment of more procaspase-8 proteins

[50; Fig. 1].

This caspase-8 accumulation forms a death-inducing signaling complex (DISC) at the plasma membrane, re-

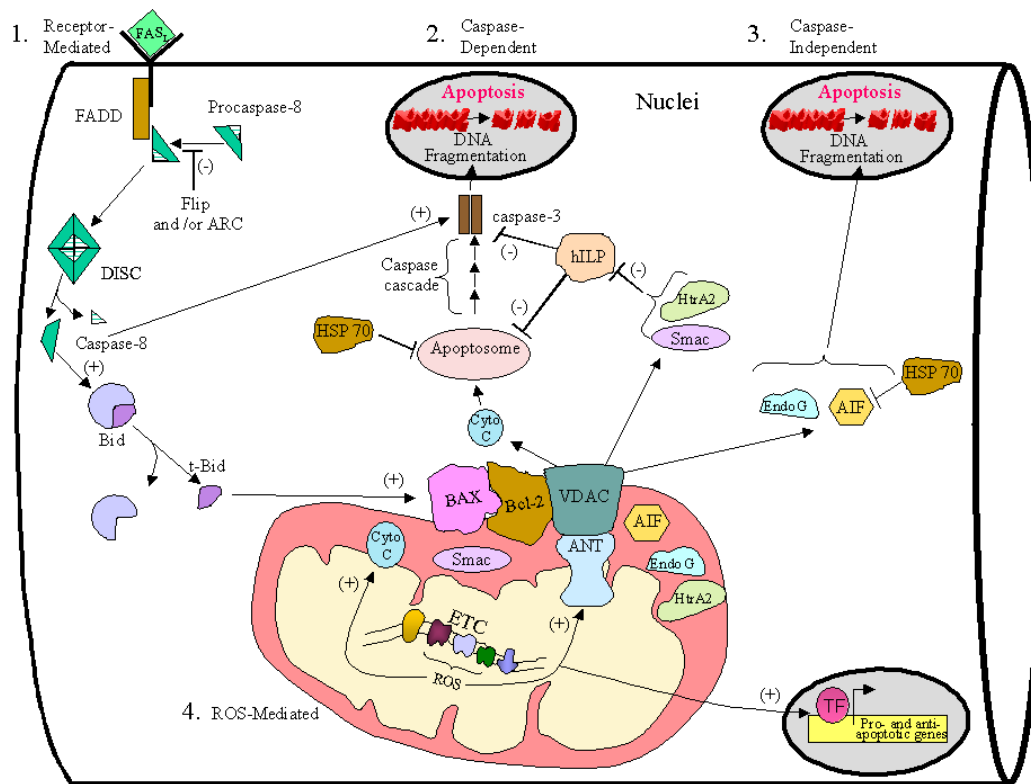


Figure 1. Overview of the extrinsic and intrinsic pathways leading to either caspase-dependent or -independent apoptosis.

1) Receptor-mediated apoptosis. Binding of FasL to its receptor evokes procaspase-8 recruitment to the adaptor protein FADD. This binding recruits additional procaspase-8 molecules to form a death-inducing complex (DISC) which activates caspase-8. FLIP and ARC prevent procaspase-8 from binding to the FADD adaptor molecule to impede the apoptotic pathway. Upon activation, caspase-8 can mediate the cleavage of caspase-3 and/or the pro-apoptotic Bid to form truncated Bid (tBid). Direct activation of caspase-3 by caspase-8 leads to DNA fragmentation and apoptosis. tBid translocates to the mitochondria where it interacts with Bax to facilitate the release of pro-apoptotic molecules from the intermembrane space of the mitochondrion. The Bid-induced release of these molecules can induce apoptosis either through a caspase-dependent or -independent pathway. This release is dependent on the formation of the mitochondrial permeability transition pore (mtPTP), composed of the inner and outer membrane proteins ANT and VDAC, respectively. Additionally, the mtPTP is regulated by the anti- and pro-apoptotic proteins, Bcl-2 and Bax, respectively. **2) Caspase-dependent apoptotic pathway.** Cytochrome c release, provoked by either tBid or mitochondrial ROS formation, promotes the assembly of the apoptosome. This complex activates caspase-9, leading to a series of proteolytic cleavages known as the caspase cascade. The terminal cleavage involves the activation of caspase-3, known as the executioner caspase. Caspase-3 translocates to the nucleus to induce genomic DNA fragmentation by the activation of DNases and deactivation of DNA repair enzymes. Inhibitor of apoptosis proteins such as hILP, can prevent the progression of the apoptotic signaling cascade by binding to caspase-9 or caspase-3. However, mitochondrial release of Smac/Diablo and HtrA2 can relieve this inhibition, thus promoting the unimpeded progression of the caspase cascade, leading to apoptosis. Additionally, HSP70 can directly bind to APAF-1, an apoptosome component, to inhibit the caspase-mediated apoptotic pathway. **3) Caspase-Independent Pathway.** Upon release, AIF and Endo G induce apoptosis by directly translocating to the nucleus without involvement of caspases. HSP70 can bind to AIF to prevent its translocation to the nucleus. **4) ROS-Mediated Pathway.** The mitochondrial electron transport chain (ETC) is the primary source of reactive oxygen species (ROS) within the cell. ROS are involved in apoptosis by a) altering the conformation of mtPTP components to facilitate the release of pro-apoptotic proteins, b) provoking cytochrome c release from the inner membrane, and c) activating transcription factors responsible for both pro- and anti-apoptotic gene expression.

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sulting in caspase-8 activation [50]. Activated caspase-8 can then directly cleave and activate caspase-3. Caspase-3 exerts its apoptotic effect by cleaving key structural proteins of the plasma membrane and nuclear envelope leading to the structural breakdown of the cell [43]. However, this enzyme mediates its primary effects within the nucleus of the cell. Activated caspase-3 translocates from the cytosol to the nucleus where it cleaves and deactivates an inhibitor of caspase-activated DNase [ICAD; 33]. This releases caspase-activated DNase (CAD), an endonuclease, enabling the cleavage of genomic DNA. This DNA fragmentation is one of the hallmark morphological features of apoptosis. Concurrently, caspase-3 inactivates poly (ADP-ribose) polymerase (PARP) and DNA-dependent protein kinase which are two key DNA repair enzymes [43]. Thus, the combined effect of activating CAD and inhibiting DNA repair enzymes accounts for the unimpeded degradation of genomic DNA observed during apoptosis [33, 43].

Alternatively, caspase-8 can also mediate apoptotic cell death by cleaving a member of the Bcl-2 family, Bid, into a small 15kDa pro-apoptotic fragment called truncated Bid (tBid). tBid then translocates from the cytosol to the mitochondria where it binds with Bax, another pro-apoptotic Bcl-2 member to induce the release of cytochrome c [17; Fig. 1]. Thus, the ligand-mediated death pathway can produce apoptotic cell death by mitochondrial-dependent or -independent pathways.

Mitochondrial pathway

Mitochondria are intimately involved in apoptosis because of two main characteristics. First, the mitochondrial intermembrane space contains several pro-apoptotic proteins which can lead to cell death upon release into the cytosol. Second, mitochondria are the primary producers of reactive oxygen species (ROS) which can have both direct and indirect effects on apoptosis.

Release of pro-apoptotic factors from the mitochondria

Mitochondria are composed of outer and inner membranes separated by an intermembrane space. The inner membrane is impermeable to large molecules and forms a barrier to the inner mitochondrial matrix. The difference in permeability barriers, along with proton movement, creates a difference in charge ($\Delta\psi$) between the matrix and intermembrane space, providing the electrochemical gradient necessary for ATP production. The mitochondrial membranes contain numerous contact sites which are composed of the voltage dependent anion channel (VDAC) of the outer membrane, and the adenine nucleotide translocase (ANT) of the inner membrane [12; Fig. 1]. The association of ANT, VDAC, Bax and cyclophilin D, forms a pore in the mitochondrial membranes, termed the mitochondrial permeability transition pore (mtPTP; outlined below). Despite some controversy over the role of the mtPTP in apoptosis, the majority of data supports the formation of the mtPTP to facilitate the release of pro-

apoptotic factors during apoptosis. The release of molecules from the intermembrane space of the mitochondrion occurs concurrently with the dissipation of the membrane potential. Thus, a change in permeability of the mitochondrial membrane is typically used to indicate mtPTP formation.

The Bcl-2 family of proteins regulates the status of the mtPTP. There are pro-apoptotic (i.e. Bax, Bak, Bok) and anti-apoptotic family members (i.e. Bcl-2, Bcl-X_L, Bcl-w; cf. [1] for review). These proteins can effectively neutralize or titrate the function of one another by forming heterodimers. Thus, the relative proportion of pro- and anti-apoptotic proteins appears to be an important contributing factor in determining cellular fate when faced with a pro-apoptotic stimulus. Bcl-2 is an integral anti-apoptotic protein located in the outer membrane. Overexpression of Bcl-2 has been shown to prevent mtPTP formation and inhibit cytochrome c release to impede the progression of the apoptotic pathway [29]. In contrast, overexpression of Bax facilitates mtPTP formation and causes the release of cytochrome c. Studies have revealed that both Bax and Bak undergo translocation from the cytosol to associate with VDAC, the primary outer member component of the mtPTP, to induce cytochrome c release [41; Fig. 1]. Additionally, Bcl-2 and Bax have also been shown to interact with ANT, the inner member component of the mtPTP, to regulate cytochrome c release [13; Fig. 1]. Thus, the association of Bcl-2 family members with either the inner and/or outer portions of the mtPTP appears to regulate the formation of the channel.

Apoptosis induction by cytochrome c release

Upon release from the mitochondrion, cytochrome c binds to apoptotic activating factor-1 (APAF-1) in the presence of dATP or ATP [36]. This causes APAF-1 to undergo a conformational change to expose its caspase recruitment domain (CARD), resulting in the recruitment of procaspase-9. This complex is termed the apoptosome, and it triggers a caspase cascade (Fig. 1). The final proteolytic cleavage involves the activation of caspase-3, ultimately causing the fragmentation of genomic DNA and cell death, as described above. However, the release of cytochrome c does not necessarily mean that apoptosis is imminent. This is because the cell is equipped with endogenous caspase inhibitors that are capable of terminating the cell death signaling pathway, and these can be considered "death checkpoints" for the cell. Interestingly, these inhibitors are highly expressed in skeletal and cardiac muscle suggesting that these tissues may have a unique resistance to apoptosis [18, 26, 31]. For example, FLICE/caspase-8 inhibitory protein (FLIP) is an enzyme homologous to caspase-8 which competes with endogenous caspase-8 for binding to FADD (Fig. 1), thereby inhibiting DISC formation [26]. Another caspase inhibitor is called the apoptosis repressor with a caspase recruitment domain (ARC), and it

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selectively interacts with initiator or upstream caspases making them inoperative, thereby suppressing apoptosis [42]. ARC inhibits cell death induced by the Fas pathway by binding to caspase-8 and rendering it dysfunctional [31]. Thus, ARC and FLIP can be considered upstream caspase repressors which appear to be primarily responsible for inhibiting receptor-mediated apoptosis. In contrast to these, another group of proteins termed inhibitors of apoptosis proteins (IAPs), have both upstream (caspase-9) and downstream (caspase-3) inhibitory targets to prevent apoptosis [18]. A human IAP-like protein (hILP) appears to inhibit apoptosis via direct binding to both caspases-9 and -3 to effectively suppress their activation [18]. hILP appears to be primarily expressed within the sarcolemmal fraction of skeletal muscle, which implies that it may predominantly inhibit the receptor-mediated apoptotic pathway in this tissue.

Apoptosis induction by factors suppressing apoptosis inhibitor proteins

Inhibitor of apoptosis proteins (IAPs-noted above) prevent apoptosis by directly binding to the enzymatic site within caspases, resulting in functional inactivation. Relief of the inherent IAP suppression of caspases is achieved by the mitochondrial proteins termed the second mitochondrial activator of caspases /direct IAP binding protein with low pI (Smac/Diablo) and high temperature requirement protein [HtrA2/ also called Omi; 22, 56]. These proteins are released from the mitochondrial intermembrane space upon an apoptotic stimulus and bind to IAPs. This eliminates IAP inhibitory activity, promotes caspase activation, and leads to apoptosis.

Apoptosis induction by AIF and Endonuclease G release

Apoptosis induction can also be mediated independently of apoptosome formation and caspase activation [16]. Specifically, the mitochondrial intermembrane proteins, apoptosis inducing factor (AIF) and endonuclease G (Endo G), are released from the mitochondrial intermembrane space and translocate to the nucleus to induce apoptosis in the absence of caspase activation [35, 57]. This provides an alternative and more direct cell death pathway. AIF is found in many human tissues including both heart and skeletal muscle [16]. Evidence has shown that AIF is translocated from the mitochondrial intermembrane space to the nucleus during apoptosis, and it is responsible for changes in nuclear morphology such as chromosome condensation, rippling of the nuclear contour, and DNA fragmentation ([57]; Fig. 1). Additionally, AIF has been shown to be crucial in inducing apoptosis during embryonic development, and it is vital for normal mouse morphogenesis [27].

Endo G is a mitochondrial nuclease which functions similar to caspase-activated DNase [CAD; 37]. It acts in partnership with the nuclear-bound proteins, exonuclease and DNase I, to facilitate DNA fragmentation

[60]. To date, the expression profile and physiological roles of Endo G within heart and skeletal muscle have not been investigated.

Apoptosis can occur with the release of a variety of mitochondrial factors through a caspase-dependent (cytochrome c, Smac/Diablo, HtrA2/Omi) or caspase-independent (AIF, Endo G) cell death pathway. Thus, apoptotic pathways appear to contain redundant molecular mechanisms. However, the physiological situations dictating the utilization of these differential pathways have yet to be determined. As a whole, the evidence clearly implicates mitochondria as a crucial bridge in the transduction of apoptotic stimuli. The recent observation of caspases located not only in the cytosol, but also within mitochondria [48], adds an additional, yet unresolved, complexity to the involvement of mitochondria in apoptosis. Since the release of Smac/Diablo and AIF appear to be dependent on caspase activation following an apoptotic insult, this suggests that cytochrome c release must occur prior to the release of these proteins [2, 5]. Thus, cytochrome c release may act as an initial trigger resulting in a caspase-dependent feedback loop to facilitate the release of other pro-apoptotic mitochondrial proteins.

Apoptosis and Reactive Oxygen Species

Mitochondrial respiration involves the sequential transfer of electrons through a succession of oxidation/reduction reactions involving inner mitochondrial membrane proteins. However, inefficient transfer of electrons can produce a variety of unstable and potentially damaging reactive oxygen species (ROS; Fig. 1). Mitochondria are the primary site of ROS production within the cell, and the mitochondrial matrix has 5-10 fold higher concentrations of ROS than that of the cytosol [15].

ROS are proposed to initiate early triggering events in the apoptotic pathway. ROS can directly induce the dissociation of cytochrome c from the inner mitochondrial membrane and cause its subsequent release from the organelle [44]. However, the accumulation of ROS within the matrix is limited by mitochondrial antioxidant enzymes, including phospholipid hydroperoxide glutathione peroxidase (PHGPx), glutathione peroxidase (GPx) and Mn-superoxide dismutase [Mn-SOD; 19, 44].

ROS can also indirectly influence the apoptotic pathway by activating mitogen activated protein kinases (MAPKs) and various redox-sensitive transcription factors involved in the expression of both anti- and pro-apoptotic gene expression [10, 24]. ROS appear to target proteins containing a thiol group which is sensitive to oxidation. This alters the conformation of the protein and changes its activity.

Apoptosis and skeletal muscle

Skeletal muscle represents a unique tissue with respect to apoptosis because muscle cells are 1) multinucleated,

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2) they contain a variable mitochondrial content which is dependent on fibre type and the extent of training, and 3) they contain two morphologically and biochemically distinct mitochondrial pools. These factors contribute to the complexity of apoptotic mechanisms within skeletal muscle. To date, limited research has focused on determining the physiological and pathophysiological conditions which lead to apoptotic cell death within skeletal muscle. Initial experiments utilized cultured myotubes and myoblasts to determine whether well established pro-apoptotic stimuli could induce cell death. These cells displayed the hallmark biochemical and morphological characteristics of apoptosis, including cell membrane blebbing, the externalization of phosphatidylserine, caspase-3 activation and DNA fragmentation [39]. Thus, these experiments showed that postmitotic multinucleated tissue was capable of undergoing apoptosis. In addition, evidence of apoptotic cell death can be found in numerous skeletal muscle myopathies [51]. We will now consider the evidence for apoptosis during alterations in functional demand brought about by muscle disuse, ageing and exercise.

Muscle atrophy

Hindlimb suspension, microgravity and immobilization of skeletal muscle are established animal models used to induce muscle atrophy [3]. During the typical decrease in muscle mass associated with muscle unloading and immobilization, coincident reductions in the myonuclear number per fiber, and an increased incidence of DNA fragmentation as assessed by TUNEL staining are evident [3, 54]. In addition, these fibers also contain a higher proportion of morphologically abnormal nuclei with no evidence of altered sarcolemmal morphology [3, 54]. Thus, the data suggest that apoptotic mechanisms within skeletal muscle result in more extensive programmed “nuclear” death, rather than wholesale cellular decay [3]. Treatment of GH/IGF-1 and exercise in animals under unloaded conditions was able to attenuate the increase in TUNEL-positive myonuclei [3]. This implicates roles for GH/IGF-1 and exercise in mediating cell survival within skeletal muscle. However, the molecular signals leading to increased apoptosis and a reduction in myonuclear number are relatively unknown. A recent study has shown that the expression of the inhibitor of differentiation-2 (Id2) protein, a negative regulator of the myogenic transcription factor family, is elevated following 7 or 14 days of unloading in quail skeletal muscle [4]. Increased Id2 expression occurred coincident with the highest levels of caspase activation and the greatest loss of muscle atrophy during the unloading phase. This study suggests that Id2 may be involved in mediating the apoptosis-related atrophy of skeletal muscle associated with skeletal muscle unloading [4]. In addition, two weeks of microgravity significantly increased the protein levels of p53, a transcription factor which increases the expression of the pro-apoptotic Bcl-2 family member Bax [45]. Thus, these

studies represent the initial stages of attempting to unravel the signaling pathways and mechanisms responsible for the increased incidence of apoptosis following muscle unloading. In contrast, it is interesting to note that muscle overload resulting in hypertrophy is associated with the fusion of satellite cells to the adult fiber, thereby increasing the myonuclear number per fiber. Thus, the loading or unloading of muscle evokes a remodelling process that appears to maintain the nuclear-to-cytoplasm ratio [i.e. the myonuclear domain; 3].

Denervation of skeletal muscle results in progressive atrophy similar to that observed with muscle unloading paradigms. Early work revealed that denervated, atrophying muscle is associated with an increased incidence of DNA fragmentation [40]. One study illustrated that denervated muscle contained a greater Bax to Bcl-2 ratio compared to its innervated counterpart, indicative of a greater susceptibility to apoptosis [59]. However, some inconsistencies exist in the morphological and biochemical indices of apoptosis in denervated muscle. For example, denervated muscle fibers do not show increased caspase activity as assessed by immunohistochemical staining [40]. Another study showed that denervated rat muscle appears to possess a larger proportion of fibers with distinct apoptotic-like morphologies, but which stained weakly for TUNEL-positive myonuclei, suggesting little DNA fragmentation [11]. In contrast, denervation by spinal cord transection resulted in an increased number of apoptotic myonuclei in the soleus muscle [21]. This study supports the concept that myonuclear loss contributes to muscle atrophy following muscle disuse. Interestingly, it was shown that muscle mass can be maintained by exercise following denervation, and that this occurred coincident with decreased DNA fragmentation [21]. However, more work is required to clarify the spectrum of biochemical and morphological events which occurs in response to denervation.

Muscle weakness and atrophy can also be produced by burn injury [63]. Muscle biopsies taken directly from an experimentally-induced burn site revealed DNA fragmentation assessed using TUNEL staining [63]. In addition, muscle biopsies sampled from regions distal from the initial site of thermal insult also showed DNA fragmentation [63]. This suggests that a blood-borne factor may be responsible for inducing apoptosis by a receptor-mediated pathway in burn injury. In this instance, the pharmacological inhibition of apoptosis following burn may prove clinically useful in attenuating muscle wasting [63].

Ischemia/reperfusion

Muscle damage and dysfunction can also be produced by ischemia followed by reperfusion. Although somewhat controversial, the likely mechanism involves the production of ROS during the reperfusion phase. In cardiac muscle, evidence of apoptotic myonuclei following ischemia/reperfusion is plentiful. However, in skeletal muscle the findings are both sparse and divided.

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One study has shown that an increased incidence of DNA fragmentation occurred coincident with a decline in tetanic muscle force following an ischemia/reperfusion event [58]. Treatment with a ROS scavenger during ischemia-reperfusion attenuated both the decreased force production, and the extent of DNA fragmentation [58]. These data imply that the increase in apoptotic myonuclei, as well as the muscle dysfunction, were due to ROS production during ischemia-reperfusion. However, a more recent study failed to find evidence of TUNEL-positive myonuclei during ischemia and reperfusion [30]. Thus, further research on apoptosis during ischemia/reperfusion in skeletal muscle is required.

Ageing

Ageing is an inevitable biological process characterized by the progressive deterioration of numerous physiological functions. Within skeletal muscle, ageing results in a significant loss in the number of fibres (i.e. sarcopenia), and demonstrable biochemical and morphological abnormalities [14]. The specific molecular mechanisms responsible for many age-related alterations remain elusive. However, it has been proposed that the accumulation of ROS-induced damage to macromolecules over the lifetime of an individual may play a role in the ageing process, possibly via apoptosis [55]. The fact that a progressive increase in ROS production occurs with increased age in a variety of tissues supports this idea [55]. A recent study has shown an increased incidence of apoptosis within aged rat skeletal muscle [20]. However, it remains to be determined whether this is directly attributable to a greater production of ROS.

The susceptibility of different skeletal muscle fiber types to apoptosis has received limited attention. This issue is intriguing since there are 3-4 fold differences in the mitochondrial content between type I (slow-twitch red), IIa (fast-twitch red), and IIb (fast-twitch white) fibers. A comparison of muscle fiber types would allow for the opportunity to examine the role of mitochondrial content on the extent of organelle-induced apoptosis within a given tissue type. Studies have shown that fast-twitch muscle fibers have a higher incidence of sarcopenia when compared to slow-twitch fibers. Whether these fibre type differences in sarcopenia are attributable to parallel variations in the age-related progression and regulation of apoptosis remains to be determined. However, there is some corroborating evidence which suggests that fast-twitch fibres are more susceptible to apoptosis than slow-twitch fibres [34, 61].

Exercise

The extent to which apoptotic mechanisms are evoked in normal, healthy muscle tissue following exercise is largely unknown. A significant increase in DNA fragmentation has been demonstrated in the skeletal muscle of mice and rats following extensive wheel running [6,

47]. The sarcolemmal membrane and contractile material were not damaged, indicating that the apoptotic process was restricted to nuclear domains [47]. This supports the idea that apoptosis within muscle appears to be a process which largely affects myonuclei, at least initially, thereby altering the nuclear-to-cytoplasm ratio while maintaining the structural integrity of the fiber.

Mechanical overload produced by supramaximal eccentric contractions is known to cause myofibril and cytoskeletal damage that leads to muscle injury [23]. To determine whether apoptosis is involved during eccentric contractions, rat muscle was electrically stimulated while in an extended position. The results indicated that some fibers contained TUNEL-positive nuclei, in conjunction with an increased expression of Bax and activated caspase-3 [9]. These data suggest that apoptosis may be involved during muscle remodelling and repair in response to certain types of functional demand [9].

Exercise can induce the expression of heat shock protein 70kDa (HSP70) within skeletal and cardiac tissue [38, 46]. HSP70 facilitates the folding and transport of nascent polypeptides into cellular organelles, and it is also capable of inhibiting the apoptotic pathway [8, 38, 49]. This protective molecular mechanism occurs downstream of cytochrome c release, and upstream of caspase 3 activation [35]. Using a cell-free system, it was demonstrated that HSP70 binds to APAF-1 to inhibit apoptosis by inhibiting apoptosome formation [8]. In addition, HSP70 has also been shown to inhibit the AIF-induced chromatin condensation of purified nuclei. Thus, HSP70 has emerged as an important protein capable of inhibiting both caspase-dependent (APAF-1 inhibition) and caspase-independent (AIF inhibition) apoptosis pathway (Fig. 1).

Moderate exercise has been shown to provide protection against ischemia/reperfusion injury in cardiac muscle [62]. However, the protective effect of exercise on the effects of ischemia/reperfusion in skeletal muscle is currently unknown. It is established that exercise can upregulate the antioxidant defence system within skeletal muscle tissue. This is beneficial since exercise itself also increases ROS production as a result of increased oxidative phosphorylation [53]. However, ROS are capable of activating numerous transcription factors and signal transduction pathways, and the magnitude of ROS production may dictate the direction of the adaptive response to exercise (Fig.1). It is possible that moderate endurance training provides a critical amount of oxidative stress needed to induce the upregulation of protective anti-apoptotic proteins, yet remain an insufficient stimulus to evoke an apoptotic response. Whether the contractile activity-induced upregulation of both the anti-oxidant defence system as well as the anti-apoptotic proteins is capable of effectively thwarting pro-apoptotic stimuli to avoid cell death induction, has yet to be determined.

Conclusions

The reviewed literature indicates that skeletal muscle apoptosis occurs under a variety of physiological conditions. However, many of the mechanisms and signaling pathways involved in activating apoptosis in muscle have yet to be elucidated. The multinucleated composition of skeletal muscle suggests that the probability of wholesale cell death is relatively low, and this is substantiated by the literature. Individual myonuclear decay appears to be a much more common event within muscle tissue, ultimately leading to cellular remodelling. Thus, it may be difficult to detect many of the typical indicators of apoptosis which focus on extensive cellular breakdown and DNA fragmentation. In addition, based on the relatively high basal expression of endogenous caspase inhibitors, skeletal muscle appears to possess an inherent resistance to apoptosis.

One of the unique aspects of skeletal muscle is its ability to undergo mitochondrial biogenesis in response to chronic contractile activity [25]. As discussed above, mitochondria play an instrumental role in provoking apoptosis in a number of cell types. Determining the role of exercise in conferring protection against DNA fragmentation and myonuclear decay requires that we know the contractile activity-induced expression of pro- and anti-apoptotic factors, and their interaction with mitochondria. In addition, it is well established that skeletal muscle contains two morphologically and biochemically distinct mitochondrial subfractions, subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria. Whether these two mitochondrial populations show differential expression of pro- and anti-apoptotic proteins, and/or respond differentially to pro-apoptotic stimuli remains to be established. Future investigation in these areas will help us unravel the role of exercise in ameliorating the cell death pathways in muscle atrophy, ischemia/reperfusion, ageing, and diseased muscle.

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Address correspondence to:

D.A. Hood, PhD., Department of Biology, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3, fax (416) 736-5698, tel. 736-2100 ext. 66640, Email dhood@yorku.ca.

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