

Apoptosis and exercise

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ABSTRACT

PHANEUF, S., and C. LEEUWENBURGH. Apoptosis and exercise. *Med. Sci. Sports Exerc.*, Vol. 33, No. 3, 2001, pp. 393–396. This brief review will discuss an exciting new area in exercise science, namely the role of apoptosis or programmed cell death in exercise. Apoptotic cell death differs morphologically and biochemically from necrotic cell death, although both appear to occur after exercise. Accelerated apoptosis has been documented to occur in a variety of disease states, such as AIDS and Alzheimer's disease, as well as in the aging heart. In striking contrast, failure to activate this genetically regulated cell death may result in cancer and certain viral infections. We will discuss factors that may activate apoptosis during and after exercise and the importance of cell turnover after exercise. We will also discuss differences in apoptosis between lymphocyte and skeletal muscle cells. We speculate that exercise-induced apoptosis is a normal regulatory process that serves to remove certain damaged cells without a pronounced inflammatory response, thus ensuring optimal body function.

Apoptosis is a highly regulated form of cell death that is characterized by specific morphological, biochemical, and molecular events (29). It is essential for the normal development of multicellular organisms (11) and is involved in cell turnover in healthy adult tissues (8). Apoptosis also plays a critical role in removing unwanted and potentially dangerous cells, such as tumor cells (32) and cells infected by viruses (31). Disorders such as cancer, AIDS, Alzheimer's disease, and rheumatoid arthritis are thought to be, at least partially, a result of aberrant regulation of apoptosis (11). Recently, apoptosis has gained the interest of many exercise scientists because, in addition to necrotic cell death, evidence indicates that apoptotic cell death also occurs with exercise.

There are distinctive differences between necrotic and apoptotic cell death that can be observed and measured. Necrosis occurs when a cell suffers lethal injury and is characterized by swelling, rupturing of the cell, and inflammation. This type of injury is typically seen with eccentric exercise and is characterized by delayed-onset muscle soreness (8). Apoptosis, however, is a normal, genetically controlled event characterized by cell shrinkage, membrane blebbing, and chromatin condensation. Additionally, activation of endogenous endonucleases and caspases (ICE-like proteases named after interleukin-1 β converting enzyme) results in irreversible DNA fragmentation along with fragmentation of the cell into membrane-bound apoptotic bod-

ies. These apoptotic bodies are subsequently phagocytosed by surrounding cells or macrophages (15,30). An often-used marker to determine apoptosis is DNA fragmentation into oligonucleosome-sized fragments of approximately 180 base pairs, which form a DNA "ladder" upon gel electrophoresis. Understanding the differences between apoptosis and necrosis could lead to possible therapeutic interventions to reduce excessive apoptotic cell death.

A variety of internal and external signals regulate the expression of genes that control the initiation of apoptosis (11,12,19,23). Internally, genes will express proteins that initiate apoptosis (i.e., Bax, Fas, p53, etc.) and proteins that inhibit apoptosis (Bcl-2, Bcl-XL), and the outcome for the cell (death vs survival) depends on the ratio of the genes expressed. For example, high levels of Bcl-2 relative to Bax promote survival, whereas the reverse ratio promotes death (21). These genes express their specific proteins in the nucleus (p53), mitochondria (Bcl-2, Bcl-XL, and Bax), and other subcellular organelles.

Strenuous exercise modulates several factors, which may alter apoptosis in a variety of tissues. Currently, there is evidence for exercise-induced apoptosis occurring in lymphocytes and skeletal muscle. For example, glucocorticoids, growth-factor withdrawal, reactive oxygen species (ROS), a rise in intracellular Ca²⁺ levels, and tumor necrosis factor (TNF) are some of the signals that can induce apoptosis (11,12,19). Some of these factors originate from the extracellular milieu (glucocorticoids, TNF) and will interact with intracellular or extracellular proteins that may trigger cell death. Increases in glucocorticoid secretion, intracellular calcium concentrations, and reactive oxygen species production have been shown to occur during strenuous exercise and have the potential to induce apoptosis (6,8,10,16).

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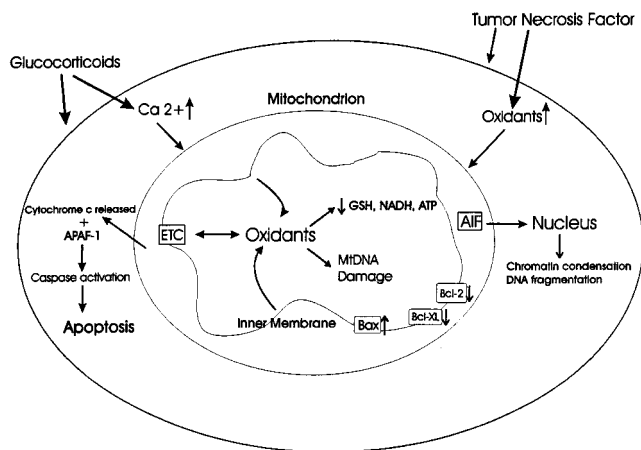


FIGURE 1—Apoptosis and exercise: simplified scheme on possible signals affecting apoptosis after exercise. Exercise causes increases in oxidant production by the electron transport chain (ETC) or oxidant production induced by increases in Ca^{2+} concentration. Oxidants or other yet unknown factors could have a direct impact on the levels of glutathione (GSH), ATP, NADH, and on oxidative mitochondrial DNA damage. Other factors, such as tumor necrosis factor and glucocorticoids, may have similar actions or operate by different mechanisms inducing cell death. All these factors may effect mitochondrial proteins, such as Bcl-2, Bcl-X_L, and Bax, (“check point proteins” for cell death) and lead to the release of cytochrome c from the mitochondria. Cytochrome c release or other yet unknown signaling pathways may lead to “the point of no return” and activate caspases, resulting in apoptosis (programmed cell death). Other proteins released from the mitochondria, such as apoptosis inducing factor (AIF) located in the mitochondrial intermembrane space may be caspase-independent and translocate to the nucleus, causing large-scale DNA fragmentation. Signals and proteins responsible for apoptosis may vary remarkably between cell types and may also originate from the extra cellular milieu and the nucleus.

Although the exact mechanism by which apoptosis occurs is unclear and may differ depending on cell type or stimulus, recent evidence suggests that mitochondria play a key role in regulating apoptosis in vertebrates (see Fig. 1). Increased oxidant production in mitochondria of muscle during acute exercise has been shown to damage DNA and proteins (2,16). Significant amounts of DNA damage could alter the expression of anti- and pro- apoptotic proteins and initiate the apoptotic process. In addition, other factors, such as increased oxidant production and decreased glutathione levels, can trigger mitochondria to release caspase-activating proteins, such as cytochrome c and apoptosis-inducing factor (AIF). The release of cytochrome c from mitochondria into the cytosol is an early apoptotic event. Cytosolic cytochrome c will bind to apoptosis protease-activating factor (Apaf-1) and ATP. This complex is capable of activating caspase-9, which is responsible for initiating the proteolytic cascade of events resulting in apoptosis (12). We will review the cell types that seem to be susceptible to apoptosis during exercise and the potential apoptotic mechanisms involved. We will also examine whether chronic exercise training can prevent cell loss. Additionally, we will provide directions for future investigation in this relatively new area in exercise physiology, which could have tremendous implications on health and disease. For example, discovering the mechanisms of how apoptotic cell loss due to aging may be ameliorated by exercise training could have implications

on a variety of disease processes characterized by heart or skeletal muscle atrophy.

Exercise-Induced Apoptosis in Lymphocytes

One area of great interest to the exercise scientist is the adverse effect exhaustive exercise may have on the programmed cell death of immune cells. It is well known that various types of stresses, such as heat, anxiety, and physical stress, can influence immune system function (9,18). For example, acute exercise stress is associated with a lower lymphocyte functional response. In Nieman’s “J-shaped model,” it is suggested that exercise can enhance or reduce immunity depending on the frequency, duration, and intensity of the exercise (20). In rats, thymic involution has been shown to occur under a variety of conditions including running to exhaustion, physical restraint, and exposure to cold (4,27). The thymus is the site of T-cell production (lymphocytes involved in cell-mediated immunity) and a decrease in the total number of T-cells will result in thymic involution. In 1993, Concordet and Ferry (9) found that thymocyte apoptosis, as evidenced by DNA fragmentation, was induced in rats that performed two treadmill runs to exhaustion (with runs separated by a 24-h rest period). Apoptosis was also evident immediately after the first run to exhaustion and continued to be detectable 24 h post exercise. Furthermore, the induction of apoptosis appeared to be mediated by glucocorticoid receptors because RU-486 (also known as mifepristone and used as an antiprogesterin), a potent glucocorticoid receptor antagonist, decreased DNA fragmentation in rats that experienced mild physical stress compared with controls. However, exercise may not always induce apoptosis in thymocytes. A recent study by Hoffman-Goetz et al. (13) found that mice subjected to a sub-maximal treadmill run of 90 min and sacrificed 2 h post exercise had a lower percentage of apoptotic thymocytes and more viable thymocytes than control mice, despite elevated levels of plasma corticosteroids (13).

Increased oxygen consumption during exercise—compared with periods of rest—and the corresponding increase in reactive oxygen species levels (6,10,16) may enhance antioxidant enzyme activity and capacity (14). This response to acute exercise may be different depending on the exercise protocol used in the two studies (9,13) and therefore may help to explain the discrepancy in the data. In addition, the apoptotic response of thymocytes during exercise may be species-dependent. Furthermore, the mobilization of antioxidants, such as vitamin C, in these two species may be different and may have led to fewer apoptotic thymocytes in mice. It also appears that oxidative stress may influence thymocyte-induced apoptosis. Azanbor and Hoffman-Goetz (5) found that exhaustive exercise results in increased lipid peroxides (indices of membrane oxidative stress) and decreased radical-protective enzymes, such as superoxide dismutase and catalase, in thymic and splenic tissues. Therefore, this may point toward a role of radical-induced apoptosis (5). However, this study did not document whether there was an increase in lymphocyte

apoptosis after exercise. Importantly, in humans, lymphocyte apoptosis has been documented to occur immediately after and 24 h after an exhaustive exercise bout (18).

In summary, because there are relatively few published results on the subject of exercise-induced apoptosis in thymocytes, definitive conclusions cannot be drawn. Additionally, the lack of control for factors that can affect the results complicates the matter. These factors include the following: different exercise protocols used, time of sampling, and methods used to assess apoptosis. This area is in desperate need of further investigation because a functional loss of specific immune cells may explain the higher incidence of respiratory tract infections seen in highly-trained athletes.

Exercise-Induced Apoptosis in Skeletal Muscles

Prolonged physical activity can cause skeletal muscle damage, with eccentric activity (lengthening contractions) being more damaging than isometric activity (8). Previously it was believed that the damage was largely due to inflammatory and necrotic processes, but recent evidence indicates an important role for apoptosis in adult muscle fibers during and after eccentric exercise (22,23,25,26).

Using gel electrophoresis, DNA fragmentation has been shown to occur in the muscles of dystrophin-deficient (*mdx*) mice 48 h after a night of spontaneous wheel running (25,26). Additionally, myonuclear ubiquitination increased in both normal and *mdx* runner mice compared with normal, sedentary mice. Ubiquitin is a highly conserved stress protein found in all eukaryotic cells. It can attach to various cellular proteins which signifies to the cell that these proteins should be degraded. Sandri et al. (25) also found decreased Bcl-2 levels in *mdx* mice after exercise. A low level of this antiapoptotic mitochondrial protein may partly explain the greater incidence of apoptosis after acute exercise.

Apoptosis also occurs in the muscles of normal mice after exercise as evidenced using electron microscopy to detect morphological characteristics of apoptotic nuclei, TUNEL staining, and gel electrophoresis to detect DNA fragmentation (22,23). Western blotting of pro- and anti-apoptotic proteins showed decreased levels of Bcl-2 relative to Bax immediately after exercise, whereas the ratio was reversed 96 h post exercise. Essentially, this would promote cell death immediately after exercise while cell survival would be promoted 4 d later.

Although it remains unclear as to how and why apoptosis is induced in adult skeletal muscle after exercise, there are many plausible hypotheses that warrant further investigation. One of the leading hypotheses is that during exercise, muscle metabolism is increased, which leads to an increased production of reactive oxygen species. Significant amounts of oxidants can produce DNA damage and thereby directly induce apoptosis. Type I muscle fibers, which are predominantly oxidative, may be overwhelmed by the amount of reactive oxygen species produced and may not be able to effectively scavenge them. Additionally, the stress of the exercise increases catecholamine levels, which promote the

induction of apoptosis. The increase in reactive oxygen species and glucocorticoids, as a result of exercise, could then signal the cell to undergo apoptosis. Further evidence in favor of this hypothesis is that exercise training, which has been shown to upregulate certain antioxidant enzymes (17,24,28), partially, attenuates the loss of skeletal muscle myonuclei due to apoptosis after hindlimb suspension (3). This important study by Allen et al. (3) also documented that growth hormone and insulin-like growth factor I (GH/IGF-1) given alone or in combination with exercise training prevented the loss of myonuclei after hindlimb suspension. Because muscle cells are multinucleated, they may differ in their apoptotic response as compared with mononucleated cells. This study showed that some, but not all, nuclei stained positive for the TUNEL method and it is unclear how many of the muscle cells were entirely lost to apoptosis.

Furthermore, skeletal muscle inactivity due to chronic heart failure could potentially influence apoptosis. Indeed, recent research showed that apoptosis occurs in skeletal muscle myocytes in about 50% of patients with chronic heart failure, possibly due to inactivity (1). Importantly, patients with apoptosis-positive skeletal muscle myocytes exhibited a significantly lower $\dot{V}O_{2max}$ and lower Bcl-2 expression as compared with biopsies of healthy patients. The mechanisms by which apoptosis is induced in skeletal muscle with exercise and/or inactivity is uncertain, and it is important to note that they may very well differ.

The possibility that mitochondrial-produced oxidants during exercise have a direct effect on apoptosis has not yet been directly investigated. It seems very plausible that disturbances in mitochondrial homeostasis, i.e., DNA damage, inner mitochondrial membrane damage, and increases in calcium, could eventually result in the release of pro-apoptotic factors, such as cytochrome c from the intermembrane space. However, a recent study found that human skeletal muscle cytosol lacked the ability to activate type-II caspases by a cytochrome c-mediated pathway (7). Apoptosis protease-activating factor (Apaf-1) was also not present in human skeletal muscle. These data suggest that apoptosis in human skeletal muscle may not operate via a mitochondrial mechanism. However, skeletal muscle biopsies were taken from humans at rest rather than from acutely exercised individuals. Exercise could have activated a variety of proteins needed for the complete activation of caspases. In summary, apoptosis has been documented in skeletal muscle after exercise, the mechanism is unknown, and it is unclear if these cells are indeed entirely lost by an apoptotic process or just temporarily "marked" as undergoing apoptosis.

Perspective

Apoptotic cell death induced by exercise in tissues exposed to specific stresses (calcium, glucocorticoids, radicals) may be a normal process used to remove partially damaged cells. Excessive and/or eccentric exercise may cause significant mechanical damage, followed by an inflammatory response, leading to necrosis and apoptosis.

Apoptosis is a partially reversible process and therefore, therapeutic interventions could be tested to attenuate this process. Research is needed to investigate alterations in pro- and anti-apoptotic proteins during long-term exercise training. Alterations in these death/survival proteins may possibly explain why exercise training partly prevents the loss in muscle cells and muscle mass with age. Furthermore, from most studies it is unclear 1) exactly which cells and how many cells are lost due to apoptosis and necrosis and 2) exactly when cells are irreversibly lost by an apoptotic process. Future research is needed to determine the molec-

ular signals enhancing lymphocyte and skeletal muscle apoptosis in order to develop a therapeutic rationale for muscle fiber protection and restoration of contractile force and interventions to protect immune function.

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