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REVIEWS: CURRENT TOPICS

Tumor necrosis factor α signaling in skeletal muscle: effects of age and caloric restriction

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Abstract

Past the age of 50 years, aging individuals lose muscle mass at an approximate rate of 1–2% per year. This age-related muscle atrophy, termed *sarcopenia*, can have significant effects on individual health and quality of life and can also impact the socioeconomic status. Sarcopenia is due to both a decrease in the number of fibers and the atrophy of the remaining fibers. The mechanisms causing loss of fibers have not been clearly defined, but may likely involve apoptosis. Elevated levels of circulating tumor necrosis factor α (TNF-α) and adaptations in TNF-α signaling in aged skeletal muscle may be contributing factors for the activation of apoptosis. These adaptations may be fiber-type specific, which could explain the selective loss of type II fibers, vs. type I fibers, in the aging process. Caloric restriction, a proven antiaging intervention, is known to attenuate the loss of muscle mass and function with age. Furthermore, caloric restriction has been shown to attenuate the age-associated adaptations in TNF-α signaling in skeletal muscle, which may be a possible mechanism by which CR prevents apoptosis and the loss of muscle fibers with age. The potential role of TNF-α in the progression of sarcopenia will be discussed, as well as the effects of life-long caloric restriction on TNF-α signaling.

Keywords: TNF-α; NF-κB; Skeletal muscle; Caloric restriction; Apoptosis; Aging

1. Introduction

Past the age of 50 years, aging individuals lose muscle mass at an approximate rate of 1–2% per year [1,2]. This age-related muscle atrophy, termed *sarcopenia*, can have significant effects on individual health and quality of life and can also impact the socioeconomic environment. Sarcopenia affects a growing population, occurring in more than 40% of the elderly over the age of 80 years and 10–25% under the age of 70 years [1,2]. The loss of muscle mass and strength that comes with age can lead to increased incidence of falls and, therefore, injuries such as hip fractures [1]. General weakness can reach levels that result in the loss of independent living, thereby affecting quality of life. Muscular strength is highly predictive of disability and all-cause mortality [3,4]. Rantanen et al. [3] found that men ranging in age between 45 and 68 years with the lowest handgrip strength had the highest risk of disability and experienced functional limitations 25 years later. Annual medical costs imposed by physical frailty, including the

Abbreviations: AIF, apoptosis-inducing factor; AP-1, activating protein-1; Apaf-1, apoptosis protease activating factor-1; ARC, apoptosis repressor with caspase-associated recruitment domain; Bad, Bcl-2 antagonist of apoptosis; Bak, Bcl-2 homologous antagonist/killer; Bax, Bcl-2-associated protein X; Bcl-2, b-cell lymphoma-2; Bcl-X_L, B cell lymphoma X long; Bid, BH3 interacting domain death agonist; cIAP-1,2, cellular inhibitor of apoptosis protein-1,2; cFlip, FADD-like interleukin-1 beta converting enzyme inhibitory protein; dATP, deoxyadenosine triphosphate; FADD, fas-associated death domain; IκB-α, inhibitor of κB; IKKγ, IκB kinase; IL-1, 6, 15, interleukin-1, 6, 15; NF-κB, nuclear factor-kappa B; Omi/HtrA2, high temperature requirement A2; RIP1, receptor interacting protein-1; Smac/Diablo, second mitochondrial activator of caspases/direct IAP binding protein with low pI; TNF-α, tumor necrosis factor α; TNFR1, 2, TNF receptor 1,2; TRADD, TNF receptor-associated death domain; TRAF2, TNF receptor-associated factor-2; TUNEL, terminal dUTP nick end labeling; XIAP, X-linked inhibitor of apoptosis protein.

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effects of sarcopenia, are estimated to reach ~$130 billion by the year 2030. In the year 2000, 1.5% ($18.5 billion) of the total direct healthcare costs in the United States were attributable to sarcopenia [5]. The amount spent due to sarcopenia equates to more direct costs spent due to osteoporosis; yet, little effort is made to increase public awareness to prevent sarcopenia [5]. It is estimated that by reducing the prevalence of sarcopenia by 10%, this would save $1.1 billion per year in healthcare costs in the United States [5]. Hence, sarcopenia and physical frailty are significant public health problems.

Sarcopenia is due to both a decrease in the number of fibers and the atrophy of the remaining fibers. By the age of 80 years, we lose ~30–40% of the number of muscle fibers in some types of muscles, mostly those containing type II muscle fibers [6]. Hypoplasia and atrophy in aging muscle occur largely through mechanisms not clearly defined. Multiple components such as altered central and peripheral nervous system innervation, altered hormonal status and altered caloric and protein intake are among the sources believed to contribute [7–9]. A more recent proposal has speculated toward the involvement of inflammatory mediators as potential etiological factors [7,9,10]. Others have reported elevation of inflammatory cytokines in concert with aging [11–13]; however, limited information exists that documents the role of cytokine signaling in sarcopenic muscle. Tumor necrosis factor α (TNF-α), a central mediator orchestrating cellular inflammatory and apoptotic signaling pathways, has been postulated as playing a contributory role in aging processes [7,10,14]. Elevated TNF-α levels have been reported with age in heart, liver, kidney and brain tissues [13,15], and compromising skeletal muscle in elderly individuals [16].

There is increasing evidence that the myofiber loss in aging muscle is due to activation of apoptosis, a cell suicide executed via specific signaling pathways [17–19]. The stimulus activating apoptosis and which signaling pathways are responsible for the myofiber loss have not been elucidated. Because TNF-α is known to induce apoptosis in various cell types, it may be likely that TNF-α-induced apoptosis may contribute to myofiber loss in sarcopenia. Furthermore, the proven antiaging regimen of caloric restriction has shown to alter circulating levels of TNF-α and TNF-α signaling in skeletal muscle. The potential role of TNF-α in the pathogenesis of sarcopenia will be discussed, as well as the effects of life-long caloric restriction on TNF-α signaling.

2. Apoptotic and antiapoptotic signaling involving TNF-α

Tumor necrosis factor α is known to have pleiotropic effects on cells acting as an inflammatory cytokine inducing antiapoptotic signals or can induce cell death by activating apoptotic signals. The cellular signaling response to TNF-α is complex and seemingly depends on the cell type and physiological conditions. Discussed below are the general understanding of apoptosis signaling and the role of TNF-α in modulating the apoptotic response.

Apoptosis is executed by specific cellular signaling pathways and is therefore characterized by specific biochemical and morphological events. Some of these identifying features of apoptosis include chromatin condensation and DNA fragmentation into mono- and oligonucleosomes, cellular shrinkage, maintenance of organelle membrane integrity and membrane blebbing forming apoptotic bodies that are engulfed by macrophages or neighboring cells. Death via apoptosis ensures death of a single cell without causing an inflammatory response and therefore is not disruptive to surrounding tissues.

Apoptosis is mediated by activation of a variety of cysteine proteases, known as caspases. Caspases normally exist in an inactivated state called procaspases but can be activated by proteolytic cleavage and subsequent heterodimerization. Initiation of apoptosis involves activation of a caspase cascade in which "initiator" caspases (i.e., caspase-8, caspase-9, caspase-12) first become activated and then cleave and activate "effector" caspases (i.e., caspase-3, caspase-6, caspase-7). The effector caspases carry out the proteolytic events that result in cellular breakdown and demise. There are 14 known mammalian caspases (i.e., caspase-1 through caspase-14) that participate in the apoptotic process depending on the stimulus and respective signaling pathway activated and/or cell type undergoing apoptosis. The two major pathways extensively described in the literature include the mitochondrion-mediated and receptor-mediated apoptotic signaling. These signaling pathways can independently induce apoptosis in various cell types; however, apoptosis induced by TNF-α and its corresponding receptor-mediated signaling often involves cross talk with the mitochondrial-mediated signaling. For this reason, both apoptotic signaling pathways are extensively described below.

2a. Mitochondrion-mediated apoptotic signaling

Mitochondria play a central role in the induction of apoptosis (Fig. 1). Upon stimulation, mitochondria can release cytochrome *c* into the cytosol, which forms a complex, known as the apoptosome, with procaspase-9, apoptosis protease activating factor-1 (Apaf-1) and deoxyadenosine triphosphate (dATP). Once the apoptosome is formed, procaspase-9 can cleave and activate itself. The active enzyme caspase-9 can cleave and activate effector caspases such as procaspase-3, which leads to the typical morphological features of apoptosis. This process is highly regulated at a number of levels. First, cytochrome *c* released from the mitochondria is regulated. The b-cell lymphoma-2 (Bcl-2) family of proteins was the first described to affect the release of cytochrome *c*. This family consists of a number of proteins, which are antiapoptotic or proapoptotic. For example, Bcl-2 and B cell lymphoma X long protect against cytochrome *c* release and are therefore

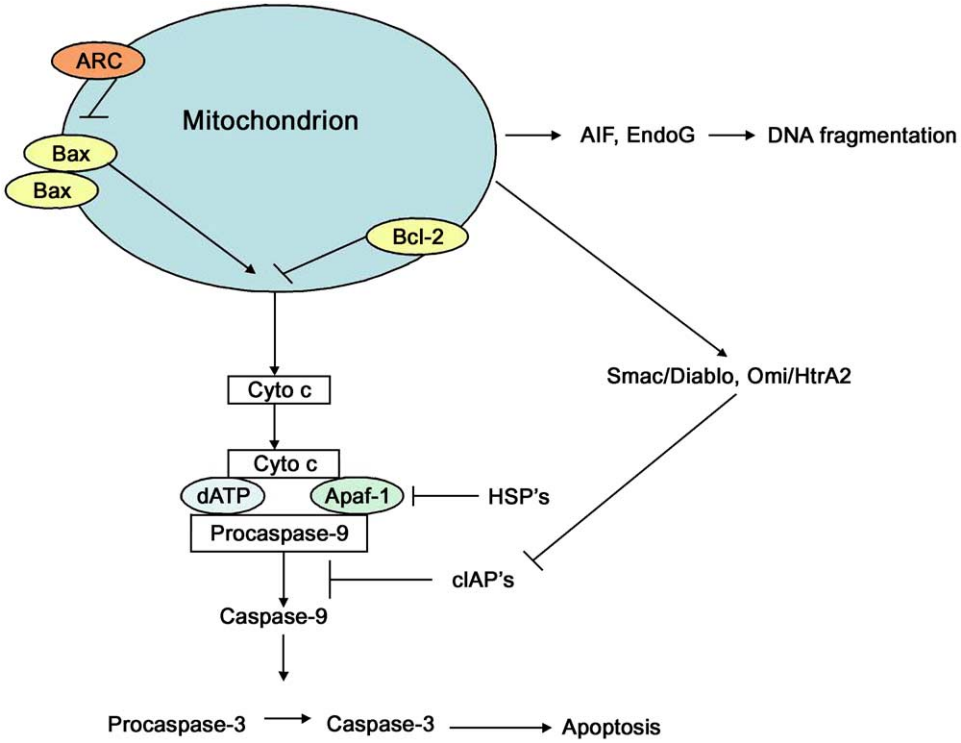

Fig. 1. Mitochondrion-mediated apoptotic signaling. Cytochrome *c* (cyto c) release from the mitochondrion leads to apoptosome (cyto c, dATP, Apaf-1 and procaspase-9) formation and activation of procaspase-9. Active caspase-9 cleaves and activates procaspase-3, which leads to apoptosis. Bcl-2-associated protein X favors cyto c release, whereas Bcl-2 and ARC inhibit cyto c release. Heat shock proteins inhibit apoptosis by inhibiting recruitment of Apaf-1 to the apoptosome. Cellular inhibitor of apoptosis proteins inhibit apoptosis by inhibiting activation of procaspase-9. Second mitochondrial activator of caspases/direct IAP binding protein with low pI and Omi/HtrA2 are released from the mitochondrion and inhibit cIAPs, relieving their inhibitory effect on apoptosis. Apoptosis-inducing factor and EndoG are also released from the mitochondrion and translocate to the nucleus where they induce DNA fragmentation in a caspase-independent manner.

antiapoptotic, whereas Bcl-2-associated protein X (Bax), Bcl-2 homologous antagonist/killer (Bak), Bcl-2 antagonist of apoptosis and BH3 interacting domain death agonist (Bid) favor cytochrome *c* release and are therefore proapoptotic. The ratio and interaction of the Bcl-2 family antiapoptotic and proapoptotic proteins determines the fate of cytochrome *c* release from the mitochondria. Often, the Bcl-2/Bax ratio is used as an indicator of apoptotic potential where a high ratio protects against apoptosis and a low ratio favors apoptosis. Apoptosis repressor with caspase-associated recruitment domain (ARC) is another protein that regulates cytochrome *c* release. Upon stimulation, ARC translocates from the cytosol to the mitochondrial membrane and prevents cytochrome *c* release [20]. Recent data show that ARC may prevent apoptosis by binding to Bax and interfering with its activation, which would ultimately protect against cytochrome *c* release [21]. A second level of regulation is the inhibition of apoptosome formation by various heat shock proteins. Heat shock protein 70 and 90 can associate with Apaf-1 to prevent the recruitment and activation of procaspase-9 to the apoptosome [22,23]. A third level of regulation involves the inhibition of caspases by the inhibitor of apoptosis proteins (IAPs). The IAPs [i.e., X-linked inhibitor of apoptosis protein (XIAP), cellular inhibitor of apoptosis protein-1 (cIAP-1), cIAP-2] can bind to cleaved and activated caspase-9 and caspase-3 to inhibit their enzyme activity and to prevent apoptosis. Lastly, the mitochondria can release additional proteins, along with cytochrome *c*, to relieve the inhibition exerted by the IAPs so indeed apoptosis can be executed. These proteins include second mitochondrial activator of caspases/direct IAP binding protein with low pI (Smac/Diablo) and high temperature requirement A2 (Omi/HtrA2) [24–26].

Mitochondria can also release proapoptotic proteins that are not involved in the activation of the caspase cascade. Mitochondria can release apoptosis-inducing factor (AIF) and endonuclease G (EndoG), which translocate to the nucleus to induce chromatin condensation and DNA fragmentation in a caspase-independent manner [27,28].

In summary, mitochondria play a central role in the execution of apoptosis. Mitochondria can release proteins that function to activate the caspase cascade or proteins that can directly induce chromatin condensation and DNA fragmentation in a caspase-independent manner. These processes are regulated at multiple levels in order to maintain precise control over cell death and survival when

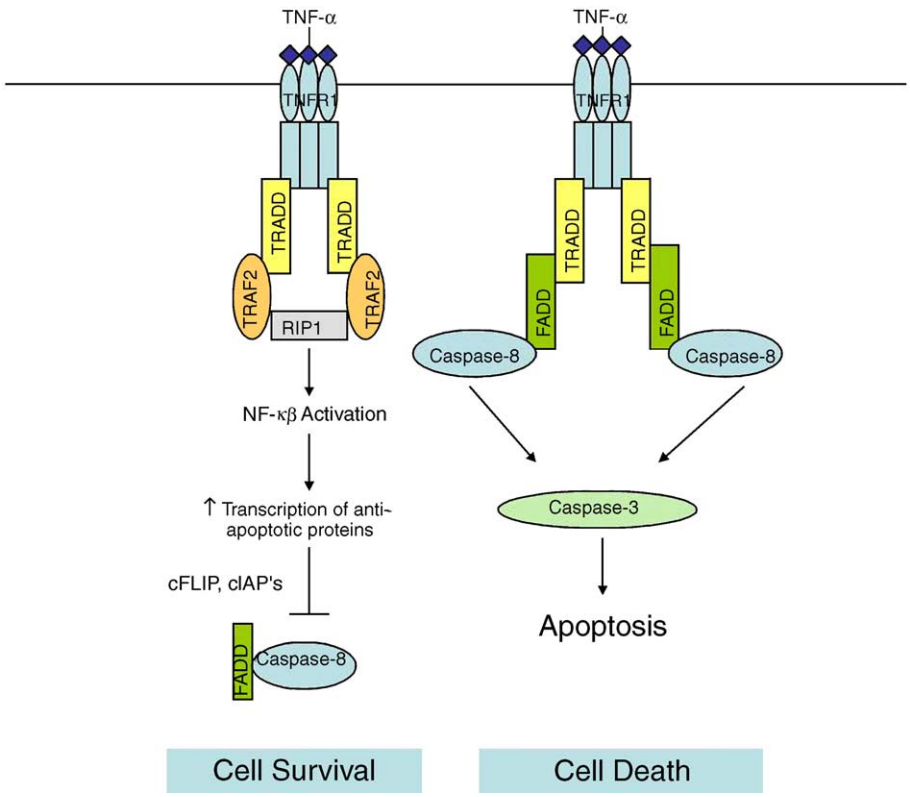

Fig. 2. Apoptotic and antiapoptotic signaling via TNF-α. Recruitment of TRADD, TRAF2 and RIP1 to TNFR1 leads to NF-κB activation. Nuclear factor-kappa B activation results in transcription of antiapoptotic proteins, such as cFLIP and cIAPs, which favors cell survival. Recruitment of TRADD, FADD, caspase-8 to TNFR1 leads to the activation of caspase-3, thereby resulting in cell death.

challenged by a changing environment such as occurs in vivo with disease states and aging.

2b. Receptor-mediated apoptotic and antiapoptotic signaling

Cytokines can induce apoptosis in some cell types via their interaction with specific receptors of the TNF receptor (TNFR) superfamily (Fig. 2). Tumor necrosis factor α is a cytokine that can elicit a broad spectrum of responses. Exposure of cells to TNF-α most commonly causes activation of nuclear factor-kappa B (NF-κB) and activating protein-1, resulting in the expression of genes involved in cell survival and acute and chronic inflammatory responses [29]. However, TNF-α is also capable of inducing apoptosis. Tumor necrosis factor α signals via two membrane receptors: TNFR1 and TNFR2. These receptors are homologous in their extracellular domains but are structurally different in their cytoplasmic domains. Tumor necrosis factor receptor 1 contains a death domain, whereas TNFR2 does not. Tumor necrosis factor receptor 1 mediates signaling for both apoptosis and cell survival, whereas TNFR2 mostly transduces signals favoring cell survival. Ligand binding to TNFR1 can induce apoptosis in an effector cell via the activation of procaspase-8, which cleaves and activates procaspase-3 initiating the caspase cascade. Alternatively, binding of TNF-α to TNFR1 can induce a proinflammatory/antiapoptotic response mediated through the transcription factor NF-κB. Recent evidence suggests that cytokines can increase the levels of antiapoptotic proteins in mitotic cells. For example, a recent article by Micheau and Tschopp [30] shows that the stimulation of NF-κB and subsequent transcriptional activity of NF-κB determines the cells' fate. Specifically, cells accommodating defective NF-κB signals (resulting in low quantities of antiapoptotic proteins) undergo TNF-α-induced apoptotic elimination. The presence and/or recruitment of adaptor proteins to the cytoplasmic domain of TNFR1 determine the outcome: caspase activation resulting in apoptosis or NF-κB activation resulting in cell survival. Adaptor proteins include TNF receptor-associated death domain (TRADD), TNF receptor-associated factor-2 (TRAF2), receptor interacting protein-1 (RIP1) and fas-associated death domain (FADD). To activate NF-κB, thereby promoting cell survival, TRADD associates with TNFR1 and is able to recruit RIP1 and TRAF2 to form a TNFR1-TRADD-RIP1-TRAF2 complex. IκB kinase (IKKγ) is recruited to the TNF-R1 complex where it becomes activated. Once activated, IKKγ phosphorylates inhibitor of κB (IκB-α), an inhibitor of NF-κB, which targets it for eventual degradation and allows NF-κB to translocate to the nucleus with consequent

expression of inflammatory and antiapoptotic proteins. To induce apoptosis upon ligand binding to TNFR1, TRADD can instead recruit RIP1 and FADD, forming a TNFR1-TRADD-RIP1-FADD complex leading to procaspase-8 activation. Apoptosis mediated via TNFR1 can be inhibited by cFLIP, ARC and cIAPs, which interferes with the activation of procaspase-8 [31].

Once apoptosis is initiated via caspase-8, the release of cytochrome *c*, and other proapoptotic proteins such as Smac/Diablo and Omi/HtrA2, from the mitochondria and activation of the mitochondrion-mediated signaling may occur, but is downstream from caspase-8 activation. Active caspase-8 cleaves Bid, which then stimulates Bax and Bak activity resulting in cytochrome *c* release. Some cell types require activation of the mitochondrion-mediated signaling via Bid to execute apoptosis and others do not.

Tumor necrosis factor α can induce apoptosis by more than one pathway, although the most widely accepted pathway involves TRADD, FADD and caspase-8 [29]. Evidence for an alternative pathway comes from the residual apoptotic response observed when FADD-deficient cells are exposed to TNF-α [32]. Activation of the TNFR1 receptor can also lead to the up-regulation of inducible nitric oxide synthase (iNOS), resulting in the excessive production of nitric oxide (NO), which can lead to apoptosis.

3. Role of TNF-α signaling in muscle wasting

Skeletal muscle may be exposed to a subtle but chronic inflammatory milieu as well as undergo molecular adaptations during aging, which may contribute to the progressive process of sarcopenia. An elevated inflammatory milieu appears to play a contributing role in the increased risk of morbidity and mortality and the decline in physical performance with age [33,34]. The aging-associated inflammation affecting skeletal muscle includes elevated circulating levels of TNF-α as well as the local expression of TNF-α by skeletal muscle [35]. Most of what is known about the effects of TNF-α on skeletal muscle was shown using in vitro models and in vivo models of cachexia, which is muscle wasting secondary to a disease condition characterized by a clinically significant elevation of circulating TNF-α. Much less is known about the role of TNF-α in the process of aging, which is characterized by a much more subtle increase in circulating levels of TNF-α. The effects of TNF-α on skeletal muscle have shown to depend on the differentiated status of the muscle cells and the physiological conditions. Specifically, it appears that TNF-α affects myoblasts differently than fully differentiated multinucleated myofibers. Furthermore, TNF-α differentially affects the various fiber types, type I vs. type II. Physiological conditions altering TNF-α signaling may include the concentration of circulating TNF-α, among other cytokines and hormones, and the adaptations that occur within skeletal muscle during the aging process. These possibilities are discussed below.

3a. Effects of TNF-α on skeletal muscle in vitro and in vivo models of cachexia

The catabolic effects of TNF-α on skeletal muscle is evident. Animals infused with TNF-α exhibit significant muscle wasting. Furthermore, genetically altered TNFR1 knockout mice implanted with cachexia-inducing tumors, which produce TNF-α, exhibit significantly less muscle wasting compared to wild-type mice with tumor burden [36]. These data suggest that TNF-α does indeed induce significant muscle wasting in vivo, and that signaling via TNFR1 may be responsible for producing these effects. The muscle wasting in response to TNF-α is likely resulting from activation of apoptotic mechanisms and/or enhanced myofibrillar protein degradation [36–38].

Chronic exposure of skeletal muscle to TNF-α can cause apoptosis in both myoblasts and myofibers. Therefore, TNF-α causes existing differentiated muscle fibers to degenerate and at the same time limit the ability for regeneration via proliferation and fusion of myoblasts. Myoblasts exposed to chronically elevated TNF-α undergo apoptosis and show procaspase-8 cleavage [39]. It appears that the initial effects of TNF-α on myoblasts are cleavage and activation of procaspase-8 as well as activation of NF-κB, which favors survival and counteracts the apoptotic signal. Continued exposure to TNF-α leads to the inactivation of NF-κB, possibly due to cleavage by the active caspases, which alters the balance of the survival and death signals resulting in apoptosis of the myoblast [39]. Inhibition of NF-κB via a dominant inhibitory IκB-α-containing adenoviral vector accelerates the apoptotic response to TNF-α [39]. Caspase-8 inhibition significantly reduces TNF-α-induced apoptosis. The data suggest that in the condition of an elevated cytokine milieu such as cachexia, TNF-α exposure results in the activation of caspase-8 and inactivation of NF-κB causing apoptosis of myoblasts, thereby decreasing the potential for myofiber regeneration or hypertrophy.

Differentiated myofibers appear to be more resistant to TNF-α-induced apoptosis than undifferentiated myoblasts [38,40]. Data suggest that myoblasts undergoing differentiation dramatically increase TRAF2 expression, which confers protection against TNF-α-induced apoptosis, presumably via enhanced activation of NF-κB signaling [40]. Although TNF-α-induced activation of NF-κB in myoblasts appears to be protective against apoptosis and degeneration, in contrast, activation of NF-κB appears to be responsible for wasting of differentiated myotubes exposed to TNF-α [38]. This suggests that inhibition of NF-κB would prevent muscle wasting. In differentiated myofibers, TNF-α-induced apoptosis may be mediated by iNOS up-regulation and/or to other mechanisms [41]. The iNOS gene has been shown to be a molecular target of NF-κB. Inducible nitric oxide synthase up-regulation can lead to the excessive production of nitric oxide, as much as 1000× increase, resulting in protein degradation and/or apoptosis [42]. Excessive NO production can result in the formation of peroxynitrite ($ONOO^-$) via its

Table 1
The effects of TNF-α on pro- and antiapoptotic signaling in undifferentiated myoblasts and differentiated myotubes in vitro

Model	Stimulus	Signaling	Time required for response	Apoptosis	Ref.
C2C12 myoblasts	TNF-α (20 ng/ml)	Caspase-8 activation Jnk1 and Jnk2 activation	2–6 h 15 min	Yes (48 h) Yes (48 h)	[39]
C2C12 myoblasts	TNF-α (20 ng/ml) and NF-κB inhibition			Yes (24 h)	[39]
C2C12 differentiated myotubes	TNF-α (1–10 ng/ml)	NF-κB binding	15–30 min	No (72 h)	[37]
Primary rat myotubes	TNF-α (1–10 ng/ml)	NF-κB binding	15–30 min	No (72 h)	[37]
C2C12 differentiated myotubes	TNF-α (1–6 ng/ml)	Protein degradation	72 h time point	No (72 h)	[38]
C2C12 differentiated myotubes	TNF-α (1–6 ng/ml) and NF-κB inhibition	No protein degradation	72 h time point	No (72 h)	[38]
C2C12 differentiated myotubes	TNF-α (100 ng/ml)	10× increase in NF-κB activation (compared to myoblasts)	8 h time point	Not determined	[40]

interaction with superoxide anion. $ONOO^-$ is a highly reactive molecule that can nitrate proteins and lipids within the cell, rendering them inactive and can enhance their degradation by the ubiquitin–proteasome system [43–45]. Furthermore, inhibition of NF-κB may prevent muscle wasting because it has been shown that TNF-α-induced activation of NF-κB in differentiated myofibers results in the expression of components of the ubiquitin–proteosome system leading to enhanced myofibrillar protein degradation and atrophy [46]. Indeed, components of the ubiquitin–proteosome system are up-regulated in cachectic muscle [47]. In summary, muscle wasting in cachectic conditions is likely due to apoptosis of myoblasts and differentiated myofibers, although via different mechanisms, as well as atrophy of remaining differentiated myofibers due to up-regulation of the ubiquitin–proteosome system (see Table 1).

3b. Adaptations of TNF-α signaling in aging skeletal muscle in vivo

The role of TNF-α in the aging process of skeletal muscle is not as clear as has been shown with that in cachexia. However, it is rationale to hypothesize that age-related molecular adaptations associated with TNF-α signaling may contribute to sarcopenia. Evidence shows that molecular adaptations may be fiber-type dependent, which in part may explain the differential effects of aging on types I and II muscle fibers. Type II muscle fibers appear to be more susceptible to age-related apoptosis compared to type I muscle fibers [35]. The superficial vastus lateralis (SVL), which is primarily composed of type II muscle fibers, shows evidence of age-related molecular alterations favoring apoptosis. Aged SVL is characterized by elevated protein levels of FADD and cleaved caspase-8 consistent with increased DNA laddering evident of apoptosis [35]. Cleaved caspase-3 is also elevated in aged gastrocnemius, although it is not known if this adaptation is fiber-type specific because this muscle is composed of both types I and type II muscle fibers [17]. It is not clear if apoptosis induced via caspase-8 in type II fibers involves activation of the mitochondrial-mediated signaling pathway. Alway et al. [19] have shown that Bax increases, Bcl-2 decreases and caspase-9 activity is elevated with age in the plantaris muscle, consisting of mostly type II myofibers, of rats. In contrast, although shown in a mixed fiber muscle, cytosolic cytochrome *c* decreases in the gastrocnemius muscle of aged rats as well as an increase in the mitochondrial content of ARC [17]. The soleus muscle, composed primarily of type I muscle fibers, did not show the same age-related alterations. Instead, the aged soleus muscle is characterized by molecular alterations favoring susceptibility to NF-κB activation, rather than increased susceptibility to apoptosis, with elevated protein levels of IKKγ, IκB-α and p65 [35]. Tumor necrosis factor receptor 1 protein levels are not affected by age in either muscle [35]. Downstream targets of NF-κB include XIAP, an inhibitor of apoptosis, components of the ubiquitin–proteosome system and iNOS. X-linked inhibitor of apoptosis protein is up-regulated in the gastrocnemius muscle of aged rodents [17]. Again, because the gastrocnemius contains both types I and II muscle fibers, it is not known whether the up-regulation of antiapoptotic proteins with age is specific to a particular fiber type. In contrast, there is no evidence of up-regulation of the ubiquitin–proteosome system or iNOS in aging muscle [48,49]. Also, evidence of enhanced NO production in aging muscle is scarce [50,51].

It is not clear cut as to whether NF-κB signaling is enhanced with age. Despite some evidence supporting enhanced NF-κB signaling in type I fibers of aged skeletal muscle, direct evidence for increased activation and DNA binding of NF-κB is lacking [35,52,53]. There exists five different potential subunits in skeletal muscle (p65, p50, p52, Rel B, c-Rel) in which combinations of two make up the NF-κB transcription factor [54]. The heterodimer consisting of p65 and p50 is the most characterized and has different downstream targets than other potential heterodimers or homodimers. Most of the studies describing NF-κB activation and DNA binding in aging skeletal muscle have used assays that detect the p65 subunit of NF-κB [35,53]. It may be possible that activation of alternative subunits comprising the NF-κB transcription factors may be altered with normal aging and, thereby, provide protection against apoptosis in type I fibers. In summary, these data suggest that type II muscle fibers become more susceptible to apoptosis with

age, whereas type I muscle fibers may become more resistant to apoptosis, possibly through up-regulation of NF-κB signaling. The response and adaptations of skeletal muscle to TNF-α are variable and complex, seemingly dependent on conditions, such as TNF-α concentration among other factors, and muscle fiber type.

4. Effects of caloric restriction on TNF-α signaling in aging skeletal muscle

Caloric restriction is the only nongenetic experimental intervention known to consistently slow the intrinsic aging process [55,56]. Caloric restricting rodents 40% of the ad libitum diet, while maintaining adequate nutrition, increases maximum life span 30–40%. Caloric restriction slows the progressive decline in widespread organ function and attenuates the onset of age-related diseases, such as cancer, diabetes and Alzheimer's disease [55]. Caloric restriction also attenuates the age-associated increase in the inflammatory milieu, a possible contributing mechanism of action of the antiaging effects [11].

Evidence also suggests that CR has multiple beneficial effects on skeletal muscle function. Caloric restriction attenuates the decline in function [57], slows the loss of muscle mass and prevents the loss in fibers with age [35,58,59]. Life-long caloric restriction attenuates apoptosis and decreases the apoptotic potential in skeletal muscle, a likely mechanism contributing to the preservation of muscle fibers with age [17,35]. Life-long caloric restriction has been shown to attenuate the age-related adaptations in TNF-α signaling and therefore may be a contributing factor in preventing apoptosis of myofibers. Animals on a caloric restricted diet exhibit lower plasma levels of TNF-α [35]. In the SVL muscle, caloric restriction was shown to attenuate the age-related increase in protein levels of local TNF-α, FADD and cleaved caspase-8 [35]. In the gastrocnemius, caloric restriction prevented the age-related elevation in the apoptotic potential by attenuating the increase in procaspase-3, cleaved caspase-3 and AIF [17]. Cytosolic ARC was also elevated with caloric restriction, which may provide protection of myofibers from TNF-α-induced caspase-8 activation [17]. Although caloric restriction predominately affected apoptotic signaling in the SVL, NF-κB signaling was predominately affected in the soleus muscle [35]. Caloric restriction attenuated the age-related rise in IKKγ, IκB-α and p65. These data suggest that adaptations to life-long caloric restriction are not universal and differ in various types of skeletal muscle.

5. Conclusion

The loss of muscle mass with age is due to death of myofibers as well as atrophy of the remaining fibers. The mechanisms causing loss of fibers has not been clearly elucidated, but likely involves activation of apoptosis. An age-related increase in circulating levels of TNF-α and adaptations in TNF-α signaling in skeletal muscle may be a contributing stimulus for apoptosis and fiber loss. Age-related adaptations in TNF-α signaling appear to be fiber-type dependent. Type II fibers become more susceptible to TNF-α-induced apoptosis where type I fibers may become more resistant via enhanced NF-κB signaling. The data may explain the age-related transition in muscle fiber-type composition, which involves a decrease in the percentage of type II fibers.

Caloric restriction is the only nongenetic intervention that has been shown to consistently slow the intrinsic rate of aging in mammals. Life-long caloric restriction preserves muscle function in old age and attenuates the age-related loss in muscle mass. The apoptotic potential in skeletal muscle has been shown to be lower in caloric restricted animals compared to ad libitum counterparts, which may be an important mechanism by which myofibers are preserved. Of note, caloric restriction may have differing effects in muscle fiber types. Caloric restriction preferentially affects NF-κB signaling over signaling involved with TNF-α-induced apoptosis. In contrast, caloric restriction preferentially affects signaling involved with TNF-α-induced apoptosis over NF-κB signaling.

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