BioFactors

Sarcopenia of aging: Underlying cellular mechanisms and protection by calorie restriction

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Abstract.

Sarcopenia, the loss of muscle mass and function, is a common feature of aging and impacts on individual health and quality of life. Several cellular mechanisms have been involved in the pathogenesis of this syndrome, including mitochondrial dysfunction, altered apoptotic and autophagic signaling, and, more recently, trace metal dyshomeostasis. Calorie restriction (CR) without malnutrition has been shown to ameliorate the age-related loss of muscle mass in a variety a species. Mechanisms of protection span from preservation of mitochondrial functional and structural integrity to mitochondrial biogenesis, reduction of oxidative stress, and favorable modulation of apoptotic and autophagic signaling pathways. Importantly, preliminary evidence indicates that moderate CR may promote muscle mitochondrial biogenesis in middle-aged human subjects. Further research is warranted to investigate whether CR may represent a safe and efficient strategy to delay the onset and mitigate the progression of sarcopenia in older adults.

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1. Introduction

The age-related loss of muscle mass and function, referred to as sarcopenia, is a universal characteristic of the aging process, documented in several species, from worms to humans [1]. In older individuals, compromised muscle function is highly predictive of falls [2], disability [3], and all-cause mortality [4]. Moreover, mobility disability resulting from muscle loss is associated with poor quality of life and increased social and health care needs in older adults [5]. The age-dependent loss of muscle mass is often accompanied by a parallel gain in fat mass, leading to a phenotype called "sarcopenic obesity" [6]. The coexistence of these conditions is thought to promote a vicious cycle in which the decline in muscle mass reduces resting metabolic rate and physical activity, leading to increased deposition of adipose tissue [6]. The accumulation of fat mass, in turn, accelerates the loss of muscle mass via the secretion of catabolic cytokines (*i.e.*, TNF-α) and insulin resistance.

The etiology of sarcopenia is complex and characterized by the contribution of multiple factors, including loss of α-motor neurons [7], increased contraction-induced injury [8], impaired satellite cell function [9], altered hormonal status (*e.g.*, decline of growth hormone and testosterone levels) [10], increased production of catabolic cytokines [11], inadequate nutrition [12], and decreased physical activity [10].

Histologically, the aged muscle is characterized by a decline of both the number and size of muscle fibers, with a preferential loss of type II (fast-twitch) fibers [13]. Increased fiber size variability and accumulation of nongrouping, scattered, and angulated fibers have also been described in old rodent muscles [14]. In addition, advanced age is associated with increases in the extracellular space and deposition of protein aggregates within the interstitial matrix [15]. The subsequent disruption of muscle architecture contributes to muscle fatigability and decreased force production observed with age [16]. Studies have shown that sarcopenic changes may be the consequence of accumulating oxidative damage to muscle constituents [17,18], which is thought to stem from altered mitochondrial function [19]. With regards to the actual mechanism responsible for the loss of muscle fibers, several reports have indicated that it may reside in a defective regulation of myocyte apoptotic signaling, as evidenced

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by the increased occurrence of myonuclear apoptotic DNA fragmentation in aged muscles [17,20–25]. In this context, it is noteworthy that mitochondria, besides their role as energy suppliers, are also centrally involved in the regulation of the apoptotic program [17].

Calorie restriction (CR) without malnutrition is currently considered the most powerful anti-aging intervention, owing to its ability of extending both mean and maximum lifespan in a variety of species. With regards to skeletal muscle, consistent evidence has shown that CR is able to retard the onset and impede the progression of sarcopenia by acting at several critical control points, ranging from mitochondrial function to oxidative stress, muscle architecture, and myonuclear apoptosis. In addition, recent experimental evidence from our laboratory suggests that CR might positively modulate autophagy in myocytes, a cellular housekeeping process that becomes dysfunctional over the course of aging (unpublished results).

2. CR mitigates age-related mitochondrial functional decline and oxidative stress in skeletal muscle

Mitochondrial functional decline is considered as a central mechanism driving the aging process [19]. Given the high reliance of skeletal myocytes on ATP provision, the impact of mitochondrial functional loss is particularly pronounced in skeletal muscle, leading to impaired strength and endurance [26]. Past experimental evidence has indicated that mitochondrial oxidative phosphorylation capacity declines in muscles over the course of aging [27,28]. Notably, deletions and point-mutations in mitochondrial DNA (mtDNA) have been found to accumulate in aged muscles and to colocalize with electron transport chain (ETC) abnormalities and fiber atrophy [29–31]. MtDNA is particularly vulnerable to oxidative damage because of its proximity to the ETC (the main cellular source of oxidants) and the lack of protective histones [26]. Moreover, because of the compactness of mitochondrial genome (*i.e.*, lack of introns), each mutation is likely to affect gene integrity. Furthermore, the efficiency of the main pathway for the removal of small mtDNA base modifications (base excision repair, BER) declines at different levels with aging [32]. Mitochondrial production of reactive oxygen species (ROS) has been shown to increase in skeletal muscle over the course of aging [33], which may be responsible for the accumulation of mtDNA mutations. The relevance of mtDNA damage to sarcopenia is evidenced by decreased activity of complex I, and IV of the ETC observed in aged skeletal muscles of various species [29,30,34–36]. In contrast, content of complex II (succinate dehydrogenase), which is entirely encoded by nuclear DNA, increases in muscle fibers from old animals, probably as a result of compensatory upregulation of mitochondrial biogenesis. Notably, fibers harboring high levels of mtDNA deletions and ETC abnormalities often display morphological aberrations, including segmental atrophy, fiber splitting, and breakage [29–31,37]. Importantly, CR was shown to reduce the prevalence of mtDNA deletion mutations as well as the abundance of fiber displaying ETC abnormalities in old laboratory rodents [38,39].

Preservation of mitochondrial structural and functional integrity by CR is believed to result from the attenuation of oxidative damage promoted by the dietary intervention. In this context, it has been reported that CR reduces mitochondrial proton leak and ROS generation in skeletal muscle [27,40–42] and increases the expression of genes involved in ROS scavenging functions [43]. Additionally, it has been reported that CR may alter mitochondrial membrane fatty acid composition, making it more resistant to lipid peroxidation and less prone to proton leak [44,45]. Lass et al. [46] have also demonstrated that CR counteracts the age-associated increase in superoxide anion radical generation, lipid peroxidation, and mitochondrial protein damage in murine skeletal muscle. Moreover, Drew et al. [27] reported reduced levels of oxidative damage to mtDNA in the gastrocnemius muscle of old CR rats compared with ad libitum fed (AL) controls. Interestingly, in a very recent study, 6-month 25% CR increased skeletal muscle mitochondrial biogenesis and reduced DNA damage in healthy, middle-aged, overweight human subjects [47]. The increase in mitochondrial number may be interpreted as positive adaptation promoted by CR, as a larger mitochondrial mass imposes a reduced workload per unit mitochondria, thus limiting oxidant generation [48].

In conclusion, mitochondrial dysfunction appears as a prominent contributing factor to age-related muscle atrophy. CR limits the severity of mitochondrial alterations with age by inducing positive adaptations, which ultimately result in the maintenance of healthy mitochondria with high bioenergetic capacity and reduced propensity toward oxidant production (Fig. 1).

3. CR hinders muscle tissue iron accumulation with age

Iron (Fe) is an essential metal required for the proper functioning of many cellular processes including oxygen and electron transport, drug metabolism and steroid and DNA biosynthesis [49,50]. It is a cofactor for many enzymes because of its ability to fluctuate between the ferric (Fe^{3+}, oxidized) and ferrous (reduced, Fe^{2+}) state. Approximately 70% of total body Fe is associated with hemoglobin, with most of the remaining Fe stored in the liver as ferritin or as myoglobin in muscle cells. Fe deficiency is a high prevalent condition among older adults, contributing to the development of anemia and its detrimental consequences. However, evidence is accumulating indicating that age-related tissue Fe overload may be involved in the pathogenesis of several degenerative conditions, including Alzheimer's disease [51], Parkinson's disease [52], and possibly sarcopenia [18,53–56].

Fe is a highly redox active metal capable of converting oxidant intermediates, such as hydrogen peroxide into harmful free radical species (*e.g.*, hydroxyl radical). Additionally, Fe has been shown to catalyze the nitration of tyrosine residues resulting in protein damage [57]. Because of the potential toxicity of Fe, its homeostasis is strictly regulated by a multitude of sensors, transporters, and storage proteins [58].

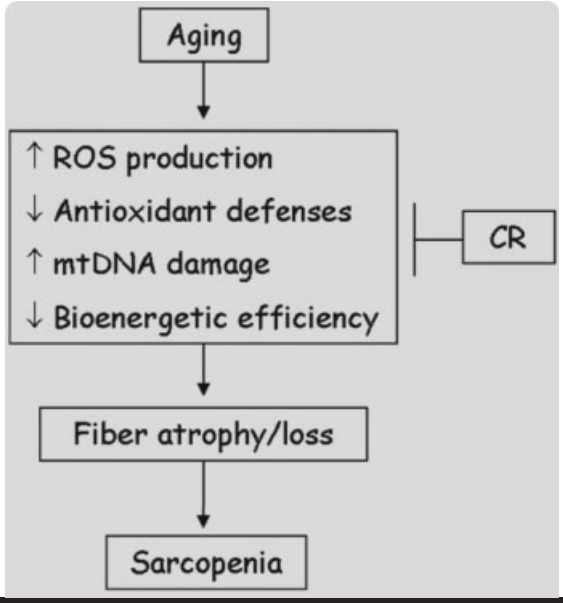

Fig. 1. Age-related muscle mitochondrial dysfunction results from multiple contributing factors and plays a prominent role in the development of sarcopenia. Calorie restriction preserves mitochondrial function in the aged muscle, thus mitigating fiber atrophy and loss of muscle mass.

Excess Fe has been associated with muscular atrophy due to disuse [18] and aging [18,54,55], likely through the exacerbation of oxidative damage to muscle constituents. The importance of Fe overload in muscle atrophy was first demonstrated by Kondo et al. [59], who reported mitigation of oxidative damage and muscle mass loss following administration of the Fe chelator deferoxamine to hind limb immobilized rats. Importantly, Hofer et al. [18] demonstrated that Fe accumulated in atrophied rather than normal fibers, suggesting a causal relation between Fe overload and loss of muscle mass. Furthermore, a recent study from our laboratory showed that advanced age was associated with increased Fe content within skeletal muscle mitochondria [56]. Notably, Fe accumulated to a higher extent in subsarcolemmal than in intermyofibrillar mitochondria and impacted on mtRNA oxidation and permeability transition pore (mPTP) opening susceptibility [56]. Importantly, mPTP opening can lead to cell death via necrosis or apoptosis [60]. Therefore, facilitation of permeability transition by Fe overload may represent a crucial mechanism underlying skeletal myocyte loss with age.

Evidence on the effects of CR on Fe accumulation with aging is scarce and conflicting. CR has been shown to mitigate Fe accumulation and oxidative damage in kidneys of aged rats [61]. Conversely, in the same study, old animals subjected to dietary restriction displayed higher Fe levels in liver and brain compared with age-matched *ad libitum* fed controls [61]. Further, CR was found to exacerbate the age-related accumulation of chelatable and non-heme Fe in mouse liver and kidney [62]. The same study reported no CR protection against age-dependent Fe accumulation in heart, striatum, hippocampus, midbrain, and cerebellum. Likewise, Borten et al. [63] showed that CR was unable to prevent age-related Fe accumulation in rat dorsal hippocampus of rats.

With regard to skeletal muscle, Xu et al. [54] recently reported an amelioration of age-associated accumulation of Fe and nucleic acid oxidative damage in the gastrocnemius muscle of CR rats. Interestingly, preservation of iron homeostasis by CR was positively correlated with forelimb grip strength, suggesting that Fe accumulation in aged muscle may contribute to the loss of function. The lack of a general consensus regarding the impact of CR on Fe homeostasis may result from tissue and species-specific effects of the dietary intervention. Additionally, the literature is void of studies investigating the effect of CR on Fe transport and storage mechanisms in skeletal muscle. In conclusion, the available evidence points toward a detrimental effect of age-related Fe accumulation in skeletal muscle, which appears to impact on both muscular mass and function. Mitigation of muscle Fe overload by CR may therefore be regarded as an additional means whereby the dietary intervention protects against sarcopenia.

4. Modulation of skeletal muscle apoptotic signaling by CR

Growing evidence indicates that progressive elimination of myonuclei via an apoptosis-like process may represent a fundamental mechanism driving the onset and progression of sarcopenia [17,22,64–67]. Apoptosis is executed via specific signaling pathways, eventually leading to DNA fragmentation, nuclear condensation, proteolysis, membrane blebbing, and cell fragmentation, with formation of apoptotic bodies, which are then engulfed by macrophages or neighboring cells. Execution of apoptosis in skeletal muscle displays unique features, as myofibers are multinucleated. Therefore, apoptosis may result in the elimination of individual myonuclei (myonuclear apoptosis) and the relative portion of sarcoplasm, without demise of the entire fiber. With respect to the final executioner of cell death, two distinct pathways of apoptosis have been described, namely the caspase-independent and the caspase-dependent apoptosis. This latter pathway is carried out via sequential activation of cysteine-dependent, aspartate-specific proteases (caspases) [68]. The caspase-independent apoptotic pathway is executed via mitochondrial release of mediators (*e.g.*, apoptosis inducing factor, AIF, and endonuclease G, EndoG) that are capable of directly producing DNA-fragmentation [69]. Mitochondria are considered a key center for the induction and regulation of apoptosis. Noticeably, mitochondria can induce apoptosis in both a caspase-dependent and independent manner [69]. Upon apoptotic stimuli, mitochondrial outer membrane permeabilization can occur, followed by release of cytochrome *c*, which initiates the intrinsic pathway of apoptosis. Once in the cytosol, cytochrome *c* promotes oligomerization of apoptosis protease activating factor-1 (Apaf-1) in the presence of ATP/dATP. The resulting apoptosome activates caspase-9, which in turn engages caspase-3. This

latter is responsible for the proteolytic events and DNA fragmentation (via caspase-activated DNase, CAD). In addition, caspase-independent apoptogenic factors residing in the mitochondrial intermembrane space, such as AIF and EndoG, can be released into the cytosol, translocate to the nucleus and cleave DNA independent of caspase activation. Our laboratory has extensively investigated age-related changes in apoptotic signaling transduction pathways in skeletal muscle and their modulation by lifelong CR [23,70,71]. Our data indicate that CR is able to mitigate the majority of the apoptotic pathways involved in age-associated skeletal muscle loss. We reported that myocyte expression of procaspase-3 and cleaved caspase-3 as well as the extent of DNA fragmentation were elevated in the gastrocnemius muscle of aged rats and were significantly reduced by CR [23,70,71]. In addition, CR increased the cytosolic content of apoptosis repressor with a caspase recruitment domain (ARC) in the gastrocnemius muscle of old rodents [70]. Furthermore, expression levels of procaspase-12 were significantly lower in the gastrocnemius muscle of old CR rats compared with age-matched AL animals, indicating that CR also has the potential of attenuating sarcoplasmic reticulum stress-mediated apoptosis [70]. In the same study, we also reported a reduction in mitochondrial release of AIF in the plantaris muscle of CR rodents [70]. Recently, we found that CR also counteracted myocyte apoptosis induced by the death-receptor pathway triggered by TNF-α in aged rats [23,71]. Indeed, myocyte expression of TNF-α in the superficial vastus lateralis (SVL) [23] and gastrocnemius [71] muscles was increased in old rodents and prevented by the CR regimen. Furthermore, CR prevented the age-related elevation of cleaved caspase-8 levels, downstream of TNF-α [23,72]. As a result, apoptotic DNA fragmentation was significantly attenuated by the dietary restriction in the SVL and gastrocnemius muscles of old CR rats compared with age-matched AL controls.

Taken as a whole, our findings indicate that the proapoptotic environment taking place in aged skeletal muscle may be substantially attenuated by CR at several critical control points (Fig. 2). Additionally, preliminary data from our laboratory indicate that even mild CR (8%) might be effective in counteracting the age-related acceleration of myocyte apoptosis in rodents (unpublished results). Although mild CR may not maximize the potential benefits, this approach appears to be much more feasible for humans to maintain longterm.

5. CR stimulates autophagy: Possible implications for sarcopenia

Oxidative damage to lipids, proteins, and DNA, especially in postmitotic tissue of an aged organism, may be severe and ultimately lead to apoptotic or necrotic cell death. However, when the damage is less severe, autophagy-mediated cell survival may prevail [72]. Autophagy literally means "self-eating" and is a vital cellular process by which intracellular components are degraded within lysosomes [73,74]. There are three classifications of autophagy: (a) microautophagy, in which lysosomes directly take up cytosol, inclusions, and organelles for degradation; (b) chaperone-mediated autophagy, in which soluble proteins with a particular pentapeptide motif are recognized and transported across the lysosomal membrane for degradation; and (c) macroautophagy, in which a portion of cytoplasm including subcellular organelles is sequestered within a double membrane-bound vacuole that ultimately fuses with a lysosome [75]. Macroautophagy (subsequently referred to as autophagy) is the primary cellular pathway for degradation of long-lived proteins and organelles, and, importantly, the only mechanisms so far attributed to the degradation of dysfunctional and damaged mitochondria. It becomes apparent that autophagy is critical to overall cellular health, because in some postmitotic tissues, progressive accumulation of damaged intracellular components and potential lack of autophagic response eventually result in cell death and loss of tissue function. Accordingly, proper initiation and execution of autophagy have been associated with life-span extension in worms and flies [76–78]. A decline in autophagic activity during normal aging has been described for invertebrates and higher organisms [79–82], with the concomitant accumulation of damaged cellular components, such as undegradable lysosome-bound lipofuscin, protein aggregates, and damaged mitochondria [83]. However, it is the efficacy of autophagy within a specific tissue or organ that affects cellular homeostasis. Because the regulation and degree of autophagy are highly organ dependent [84], it seems reasonable to assume that age-related changes in autophagy are organ-specific as well. Although the autophagic activity in liver declines with age [81,82], data from our laboratory suggest that autophagy is maintained in heart and skeletal muscle of aged rats ([85] and unpublished data). Yet, the efficacy of autophagy in heart and skeletal muscle might not be sufficient to cope with the age-related magnitude of cellular damage. The consequences of autophagy dysregulation in skeletal muscle have mostly been studied with respect to myopathies, such as Pompe and Danon disease, in which myofiber morphology is altered and muscle function impaired [86,87]. However, the effect of age on the regulation of autophagy in skeletal muscle and the role of autophagy in sarcopenia have yet to be fully characterized.

Autophagy is a highly regulated process, with multiple signaling pathways controlling the induction as well as the formation and maturation of the autophagic vacuoles [88,89]. Autophagy is suppressed by amino acids and growth factors such as insulin [88,89]. However, dietary restriction is a potent inducer of autophagy in many species [84,85,90,91]. Autophagy may in fact play an important role in mediating CR's beneficial effects, as, for instance, reduced activity of autophagy genes in *C. elegans* suppressed the lifespan extension promoted by inherent dietary restriction [77]. During CR, autophagy is induced via at least two of the signaling pathways: activation of phosphoinositide 3-kinase class III through complex formation with Beclin 1 and downregulation of the nutrient-sensor mitochondrial target of rapamycin (mTOR) (Fig. 3) [92–94]. Notably, mTOR deficiency extends lifespan in worms [95], but whether this effect is, at least in part, due to increased autophagy has yet to be confirmed.

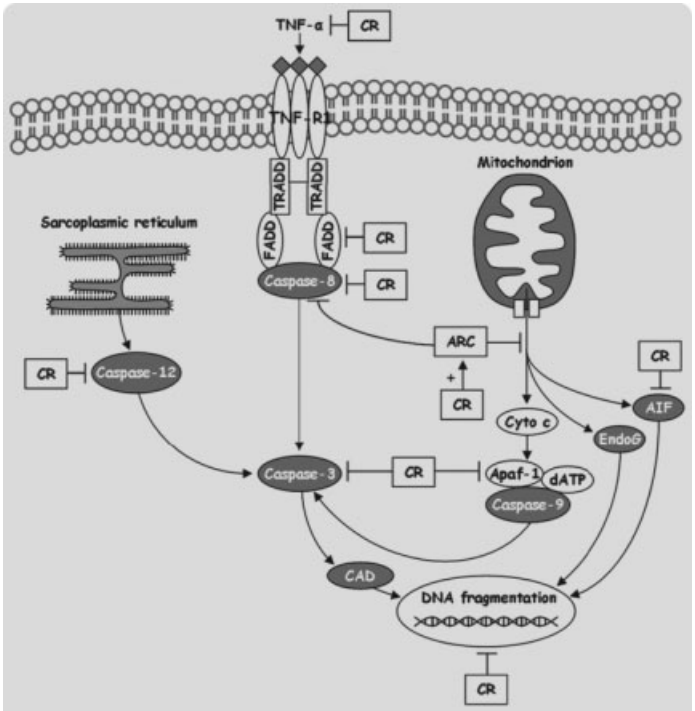

Fig. 2. Overview of the apoptotic pathways involved in age-related myocyte elimination and their modulation by calorie restriction.

The autophagic response in skeletal muscle is clearly inducible by CR [84]. Furthermore, findings from our laboratory indicate that the autophagic response to CR persists even at old age (unpublished data). Although the beneficial effects of autophagy on longevity and cellular homeostasis have found broad support, it is yet unclear whether upregulation of autophagy is always beneficial in skeletal muscle. It needs to be identified whether a shift in the activity of regulatory proteins and autophagy-dependent degeneration of cellular components might contribute to disruption of myocyte function and muscle atrophy, or whether autophagic removal of cellular waste overall promotes myocyte health. Recent studies on mouse skeletal muscle [96–98] revealed a pivotal role for the forkhead transcription factor FoxO3 and its downstream targets atrogin 1 and MuRF1 in proteasome-associated skeletal muscle atrophy. Furthermore, Mammucari et al. [98] and Zhao et al. [99] demonstrated a link between FoxO3 and autophagy, possibly via disuse or fasting-induced transcription of BNIP3 and LC3, leading to unfavorable loss of muscle mass due to autophagic proteolysis. On the other hand, a recent study by Willcox et al. [100] identified FoxO3a genotype as associated with longevity in humans.

On the basis of our findings in aging rat heart [85] and skeletal muscle (unpublished data) and the emerging evidence for autophagy as essential for cellular homeostasis, we suggest that autophagy may be one mediator of the beneficial effects of CR on the attenuation of sarcopenia. However, more work is required to conclusively define the role of autophagy in age-related conditions such as sarcopenia. Once the role of autophagy in aging muscle is characterized, interventions to modulate this complex cellular process may represent a promising therapeutic strategy to counteract the detrimental accumulation of waste material in aging muscle.

6. Conclusions and future perspectives

CR has been consistently shown to attenuate the rate of functional decline and loss of muscle mass that occur with age. Importantly, CR has been recently reported to mitigate the severity of sarcopenia in non-human primates [101]. Experimental evidence indicates that these protective effects stem from the ability of CR to reduce the incidence of mitochondrial abnormalities, attenuate oxidative stress, maintain the proper functioning of autophagy, and counteract the age-related elevation of proapoptotic signaling in skeletal muscle. Importantly, moderate reduction in calorie intake appears to protect against mitochondrial functional decline also in human skeletal muscle. However, several reports suggest that an excessive CR in humans may be accompanied by a number of adverse effects, such as weakness, loss

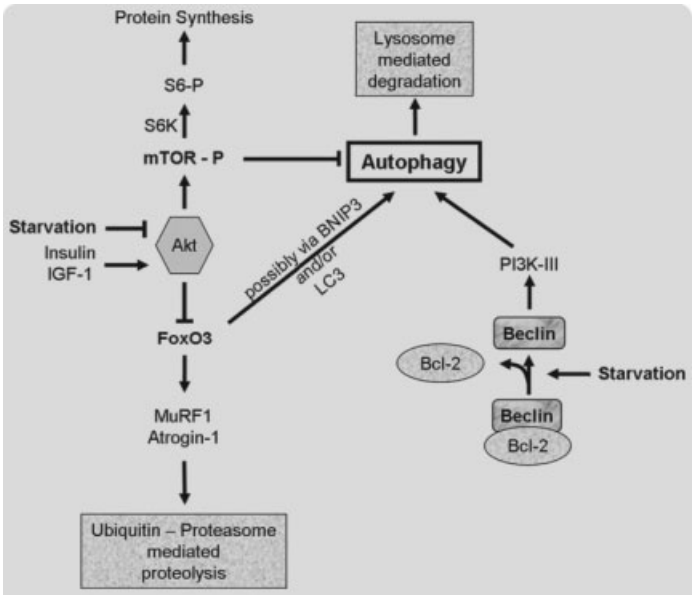

Fig. 3. Regulatory pathways of autophagy. Autophagy is suppressed by amino acids and growth factors such as insulin, which act through protein kinase B (Akt/PKB). However, when cells are starved for amino acids (Starvation), autophagy is activated via at least two possible signaling pathways: mTor and class III phosphoinositide 3-kinase (PI3K-III). Beclin interacts with and presumably activates the PI3K-III thereby promoting autophagy. This interaction can be inhibited by Bcl2 or Bcl-X_L, which directly interact with Beclin. Recently, FoxO3 transcription factor has been associated with increased ubiquitin-proteasome mediated proteolysis, as well as increased autophagy. FoxO is believed to stimulate autophagy via BNIP3 and LC3.

of libido, infertility, amenorrhea, osteoporosis, depression [102], which may limit its large-scale applicability to humans. However, recent data indicate that even a slight reduction in calorie intake (*i.e.*, 8% restriction) combined with voluntary exercise may retain the ability of counteracting sarcopenic changes in old rodents [15]. Future studies will have to investigate whether CR, alone or in combination with physical exercise, may represent a safe and efficient strategy to delay the onset and mitigate the progression of sarcopenia in older adults.

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