1. The control of organismal aging by skeletal muscle

Liam C. Hunt, Fabio Demontis

As with most tissues, homeostatic balance within skeletal muscle inevitably declines with age. In addition to a reduction in muscle mass and functional capacity, muscle aging is associated with increased mortality and a higher risk of developing several age-related diseases such as cancer, metabolic syndrome, Alzheimer's and Parkinson's disease. Here, we discuss how muscle-derived signals and age-related changes in skeletal muscle initiate inter-tissue communication and systemically affect organismal aging. In addition to preserving muscle function, interventions to counter muscle aging may prevent age-related deterioration of tissues distal from the muscle and extend healthy lifespan.

Introduction

As early as the fourth decade of life in humans, skeletal muscle homeostatic balance begins to decline, leading to the loss of muscle mass and function with age (sarcopenia). Many local and systemic factors contribute to sarcopenia [1]. At the cellular level, atrophy of muscle cells (myofibers), disorganization of sarcomeres (the contractile apparatus of muscle), defects in mitochondria and other organelles, transcriptional and proteomic changes, dysfunctional protein homeostasis, oxidative damage of macromolecules, and metabolic defects contribute to the decline in muscle function (Figure 1). In addition to these intrinsic changes, systemic changes in anabolic and catabolic endocrine factors, denervation of motor units, changes in myofiber types, and reduced vascular capacity also lead to sarcopenia.

[Keywords]
skeletal muscle aging, metabolism, myokine signaling, inter-tissue communication, lifespan

While muscle function declines with increasing age, there are indications that regimens that maintain muscle function into old age can also decrease the incidence of several age-related diseases and increase lifespan. For example, dietary restriction in specific instances may increase lifespan and can also attenuate transcriptional changes in muscle caused by aging in mice [2]. Physical exercise can minimize or slow the progression of sarcopenia into old age [3] and may also have the potential to extend lifespan [4]. In addition, epidemiological studies in humans have shown that decreased muscle strength is associated with mortality from all causes and with a higher risk of developing several age-related diseases, including cancer, metabolic syndrome, dyslipidemia, Alzheimer's and Parkinson's disease [1) 5)].

Taken together, these reports suggest that there are

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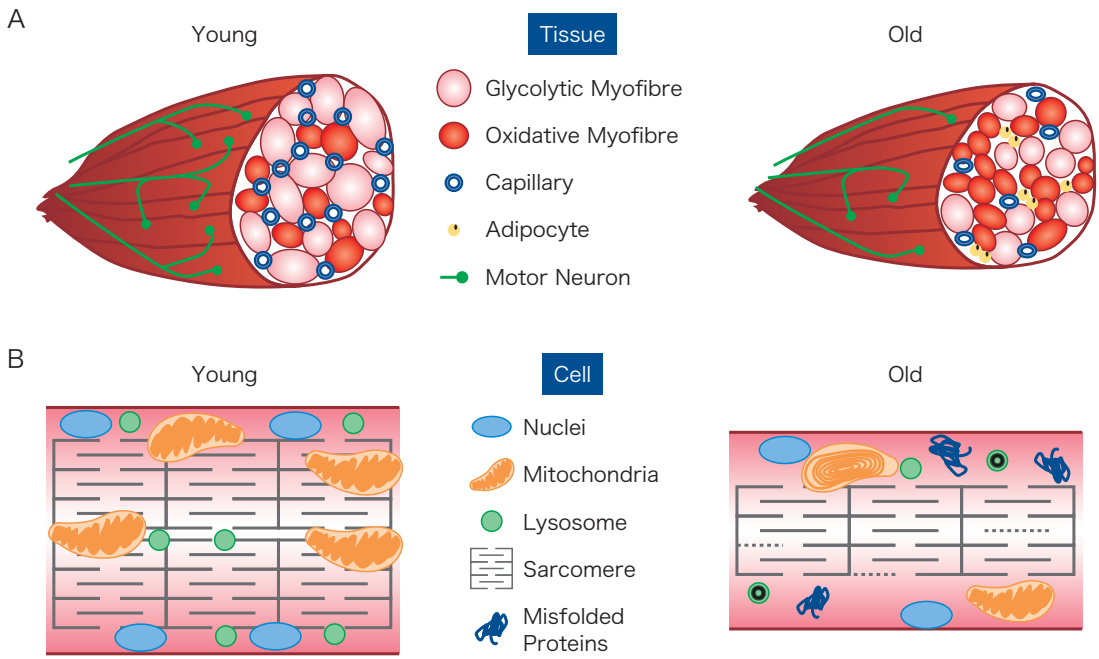

Figure 1 Age associated changes in skeletal muscle tissue (A) and cells (B)
In old muscle compared to young, a loss of mass occurs with a decrease in the diameter of myofibers, particularly the fast glycolytic myofibers. There is a fiber type switch that consists in a decrease in the proportion of fast glycolytic myofibers with a coincident increase in oxidative fibers. Decreased capillary density, intramuscular adipocyte accumulation, and reduced innervation of muscle by motor units are also observed. At the cellular level, decreased myofiber size in old age is associated with accumulation of damaged contractile proteins and disorganized sarcomeres (indicated by dashed lines), dysfunctional mitochondria, and a reduction in the number of nuclei compared to young muscle. Accumulation of lipofuscin bodies and dysfunctional lysosomes are linked to the accumulation of misfolded proteins and insufficient organelle turnover.

associations between muscle health and lifespan. Recent studies in model organisms (discussed below) confirm this hypothesis and provide direct evidence that preventing age-associated changes specifically in skeletal muscle can increase healthy lifespan.

1 Skeletal muscle-specific genetic interventions that modulate lifespan

Direct links between muscle function and lifespan have become apparent by utilizing the powerful genetic tools available in the fruit fly, *Drosophila melanogaster*, through skeletal muscle-targeted transgene overexpression and RNA interference (RNAi).

In this organism, Demontis and Perrimon have established the importance of skeletal muscle autophagy and protein homeostasis in the systemic regulation of aging and lifespan [6]. Overexpression of the nutrient- and stress-sensing transcription factor *FOXO* (forkhead box O) and the eukaryotic translation initiation factor 4E binding protein (*4E-BP*) in skeletal muscle promoted autophagy and prevented accumulation of polyubiquitinated protein aggregates with age in *Drosophila* skeletal muscle. Moreover, *FOXO/4E-BP* overexpression specifically in skeletal muscle prevented aggregate accumulation also in the retina, brain and adipose during aging, indicating that FOXO/4E-BP signaling in muscle can systemically affect aging in other tissues. In this instance, *FOXO* overexpression in muscle reduced release of insulin-like peptides from the secretory cells of the fly, thus limiting insulin signaling peripherally and increasing 4E-BP activity systemically. Overexpression of *FOXO/4E-BP* also reduced food intake, glycemia, and age-associated motor defects. Altogether this research indicates that FOXO/4E-BP signaling in skeletal muscle regulates autophagy cell-autonomously and non-autonomously,

possibly via the release of muscle-derived signals.

Skeletal muscle has great influence on systemic metabolism due to the large proportion of body mass which consists of muscle (around 40–50%), and the energy demands of contracting muscle. In support for a role of muscle function and metabolism in controlling lifespan, Katewa and colleagues have demonstrated that inhibiting muscle activity through genetic wing ablation or by mechanical clipping of wings prevented the lifespan extension caused by dietary restriction [7]. Furthermore, decreasing the levels of acetyl-CoA carboxylase (*ACC*), an enzyme involved in fatty acid synthesis, by RNAi specifically in the muscle prevented the lifespan extension caused by dietary restriction. Importantly, this effect was not seen with *ACC* RNAi in neuronal cells or the fat body (a *Drosophila* tissue related to the adipose tissue and liver), indicating a key role of skeletal muscle in sensing dietary interventions that regulate lifespan.

Other studies have also shown an important role for energy-sensing in muscle. AMPK (adenosine monophosphate activated protein kinase) is a kinase that responds to low ATP levels and stimulates fatty acid uptake and oxidation in muscle. Forced activation of AMPK by transgenic overexpression specifically in skeletal muscle increases lifespan in *Drosophila*, while *AMPK* RNAi elicits converse effects [8]. Taken together, studies in *Drosophila* indicate that stress-, nutrient-, and energy-sensing in skeletal muscle regulate lifespan and systemic aging.

Studies in mice also indicate the importance of skeletal muscle in lifespan determination. Overexpression in muscle of the cytoplasmic variant of phosphoenolpyruvate carboxykinase (*Pepck*), an enzyme involved in gluconeogenesis, allows mice to live up to 2 years longer than wild-type counterparts, and sustains reproductive capacity to an older age [9]. Importantly, *Pepck* transgenic mice display a large increase in mitochondrial density within the myofibers. Coincident with this potential for increased oxidative capacity, the mice have an outstanding ability to perform wheel running for longer periods and at higher speeds than controls. These changes lead to reduced body mass and decrease adiposity, despite increased food intake, emphasizing the important systemic effects of exercise, muscle metabolism, and oxidative capacity.

While the aforementioned study suggests that increased mitochondrial activity can counteract aging, there is also evidence that a reduction in mitochondrial activity via mitochondrial uncoupling can increase lifespan. Specifically, transgenic overexpression of uncoupling protein 1 (*Ucp1*) in skeletal muscle increased median lifespan while also decreasing body mass and adiposity, and increasing body temperature in mice (likely through increased substrate utilization with decreased ATP production, leading to heat dissipation) [10]. *Ucp1* transgenic mice also had increased signaling through pathways typically activated in response to exercise, including up-regulation of sirtuin deacetylase activity, decreased acetylation of peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1 α), and increased phosphorylation of AMPK. **Table 1** reports some of the muscle-specific genetic interventions that have been shown to affect organismal aging and lifespan in *Drosophila* and mice. Taken together, studies in model organisms indicate the role of skeletal muscle-specific genetic and metabolic interventions in modulating whole-body metabolism and systemic aging.

2 Indirect effects of muscle mass and metabolism

At rest, the metabolic rate per unit mass of skeletal muscle, when considered as a single tissue, is relatively small compared to other energy demanding tissues such as the brain, heart, and kidneys. However, due to the proportionally large mass of skeletal muscle relative to total body mass, skeletal muscle can account for as much as 27% of whole body resting energy expenditure [11]. Considering that muscle is frequently utilized and not at rest, skeletal muscle has the potential to contribute greatly to energy expenditure. Thus skeletal muscle mass and metabolism can greatly impact whole-organism metabolism.

Examples of the influence skeletal muscle mass can have over whole organism metabolism are shown in several mouse models whereby muscle mass is increased either by overexpression of an anabolic transgene or knock-out of an endogenous muscle growth inhibitor (such as IGF-1 and myostatin respectively, which are discussed in more detail later). These mice in addition to having increased muscle mass also typically show decreased fat mass and an improved ability to tolerate diets rich in fat and carbohydrates [12]. The decrease in fat mass is often correlated with the degree to which muscle mass is increased in the experimental protocol, indicating that muscle mass is a

Table 1 Summary of some skeletal muscle-specific genetic interventions that modulate lifespan

Species	Target in muscle	General effects	Muscle effects	Lifespan effect	Reference
Mus musculus	・UCP1 overexpression	・Reduced body mass ・Reduced adiposity ・Increased body temperature	・Increased SIRT deacetylase activity ・Decreased PGC-1 α acetylation (activation) ・Increased AMPK phosphorylation (activation)	・Increased median lifespan	10
Mus musculus	・PEPCK (cytosolic) overexpression	・Reduced body mass ・Increased food intake ・Reduced adiposity ・Reduced insulin levels ・Hyperactivity and improved running performance	・Increased intramyofibrillar mitochondrial density ・Increased triglyceride content and number of adipocytes	・Increase in maximum lifespan up to 2 years longer than control	9
Drosophila melanogaster	・FOXO/4E-BP overexpression	・Non-autonomous reduction in the accumulation of polyubiquitinated protein aggregates during aging ・Reduced glycemia	・Increased autophagy ・Cell-autonomous reduction in the accumulation of polyubiquitinated protein aggregates ・Reduced age-associated motor deficits	・Increased median lifespan	6
Drosophila melanogaster	・AMPK RNAi ・AMPK overexpression			・Increased lifespan with AMPK transgenic and decreased with RNAi	8
Drosophila melanogaster	・ACC RNAi		・Decreased fatty acid oxidation	・Decreased median lifespan ・Prevented the increase in median lifespan due to dietary restriction	7

major determinant of metabolic homeostasis.

Even without increased muscle mass, metabolic expenditure by skeletal muscle can still influence systemic metabolism. This was elegantly demonstrated in the previously mentioned study whereby overexpression of *UCP1* in muscle systemically decreased body mass by increasing energy expenditure [10]. Additionally, a number of other studies have demonstrated that perturbation of skeletal muscle metabolism can affect metabolism in other tissues. For example, muscle-specific inactivation of GLUT4, a protein that allows glucose transport into muscle, reduced insulin stimulated glucose uptake by 92% in muscle but also reduced insulin stimulated glucose uptake in adipose tissue and suppressed glucose production in the liver [13]. Administering phloridzin to prevent hyperglycemia restored glucose uptake in the adipose tissue but not in the skeletal muscle lacking GLUT4. This indicated that the insulin resistance induced in other tissues was caused indirectly by glucose toxicity due to elevated circulating glucose. Thus, the ability of skeletal muscle to uptake and utilize energy substrates can indirectly modulate whole-organism metabolism and have systemic effects.

Secondarily to energy utilization, muscle may also communicate systemically through the release of metabolites. Muscle is a major site of lactate production through the process of anaerobic glycolysis [14]. Lactate released by muscle into the circulation can be uptaken by the liver where it may undergo gluconeo-

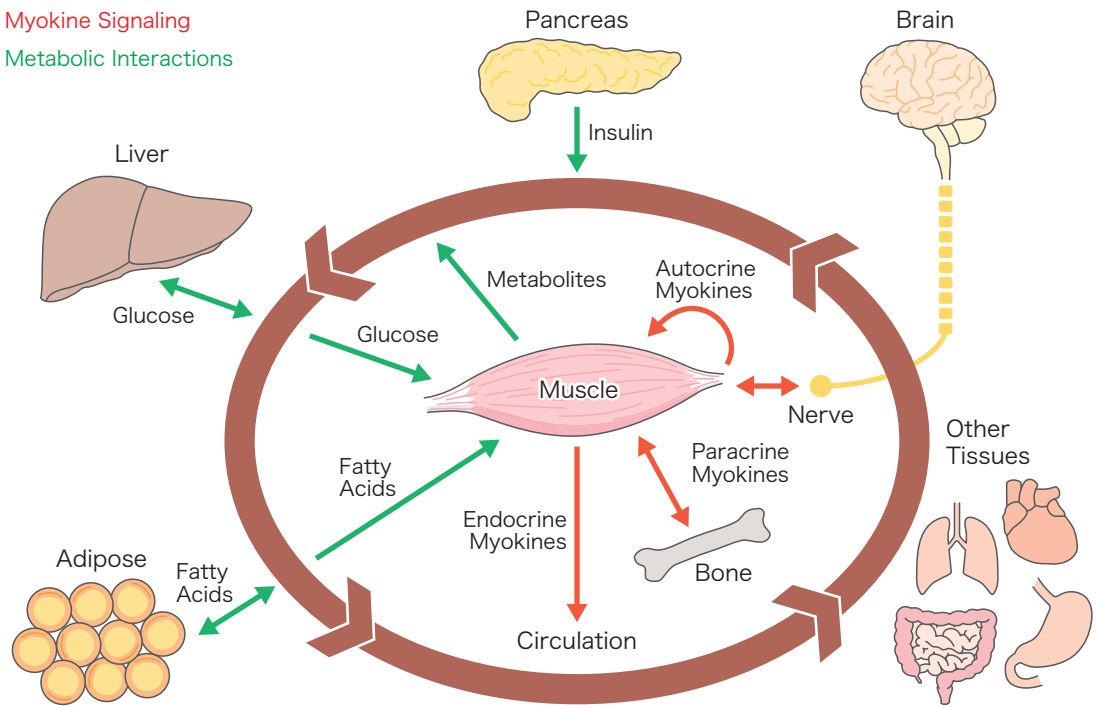

Figure 2 Skeletal muscle may interact with other tissues during aging via several mechanisms
Myokine signaling and metabolic cross-talk are highlighted by red and green arrows, respectively. The utilization of energy substrates, namely fatty acids and glucose, by muscle can indirectly affect metabolic homeostasis in the adipose, hepatic, and pancreatic tissue, and alter systemic insulin sensitivity. Metabolites produced by the muscle may also signal systemically after release into circulation. Muscle may interact with nerve and bone via direct interaction and/or the release of paracrine factors, while autocrine factors act on the muscle itself. Endocrine factors produced by muscle in response to specific stimuli, such as exercise and nutrient-sensing, are released into circulation and modulate cellular homeostasis in a variety of target tissues. Skeletal muscle may contribute to organismal aging and lifespan determination via these inter-tissue interactions.

genesis in a process known as the Cori cycle. Circulating lactate has been shown to induce insulin release [15] while also increasing insulin resistance and suppressing glycolysis [16]. Age-related changes in the metabolome are observed in the muscles of aged mice with a shift towards increased levels of metabolites deriving from fatty acid metabolism and, to a lesser extent, glucose metabolism [17]. Possibly, age-related changes in the levels of metabolites released by muscle may play a role in the systemic regulation of aging. Muscle is also an important site of protein metabolism, responding to low levels of nutrition by breaking down cellular proteins and releasing amino acids systemically. Conversely, amino acid uptake in muscle increases in response to insulin and muscle activity. Thus, muscle has an important role in regulating plasma levels of amino acids, which may regulate the function of peripheral tissues during aging. Taken together, the uptake and release of energy substrates and metabolites by the muscle from and to circulation indicates the important role of muscle in influencing metabolic and cellular homeostasis peripherally (Figure 2).

3 Direct inter-tissue communication via myokines

Skeletal muscle has been gaining increasing recognition as an endocrine tissue with the capacity to produce growth factors and cytokines that are released into circulation. Muscle may also interact with adjacent muscle and non-muscle cells, such as the motor neurons innervating the muscle, the endothelial cells

of the vasculature, intramuscular adipocytes, and the bone to which the muscle is associated. Growth factors and cytokines released from skeletal muscle have been termed myokines and can act as autocrine, paracrine and endocrine factors on several target tissues, including the pancreas, adipose tissue, and liver (**Figure 2**). Although some myokines are almost exclusively expressed in skeletal muscle, others are also expressed in other tissues.

Pedersen and Febbraio initiated the concept of skeletal muscle as a secretory organ and first adopted the term myokine after the discovery that increased levels of circulating interleukin-6 (IL-6) observed following exercise were derived from skeletal muscle [18]. Thus IL-6 is the prototypical myokine with a wide range of target tissues and effects associated. The acute spike in IL-6 following exercise locally increases glucose uptake and fatty acid oxidation in muscle, while systemically it promotes insulin release from pancreatic beta cells [19]. Because IL-6 is a pleiotropic cytokine with numerous effects on different tissues, some of which are considered positive and others negative to organismal health, it is difficult to predict how IL-6 may affect aging. Nevertheless, IL-6 is a myokine with the potential to affect organismal aging through inter-tissue cross-talk.

One of the most promising myokines to date is irisin [20], which is induced in skeletal muscle by endurance exercise through increased expression of PGC-1α, a transcriptional co-activator of the nuclear receptor PPAR-γ. In muscle, PGC-1α promotes mitochondrial biogenesis and switch to a slow-twitch myofiber type. In addition, PGC-1α induces the expression of FNDC5 (fibronectin domain type III containing 5 protein), a transmembrane protein that is proteolytically cleaved to release a soluble factor called irisin. Irisin then enters the circulation and induces the "browning" of adipose tissue, leading to an increase in its thermogenic properties. This study demonstrated that skeletal muscle can signal to adipose through myokines and that muscle can directly regulate systemic metabolism.

Insulin-like growth factor-1 (IGF-1) is another myokine produced in muscle in response to exercise. IGF-1 is an anabolic factor that promotes muscle growth and osteogenesis, both of which are important processes that decline during aging [21]. Muscle-produced IGF-1 can act as both an autocrine and paracrine factor, and muscle-restricted expression of IGF-1 in a mouse model of amyotrophic lateral sclerosis (ALS) not only improved muscle regeneration but also preserved neuromuscular junctions and improved survival of motor neurons in the spinal cord [22]. However, muscle-produced IGF-1 is unlikely to act as an endocrine factor as it is thought to act locally without entering the circulation.

In contrast to IGF-1, myostatin opposes muscle growth and it is therefore an important autocrine myokine. Myostatin loss leads to increased muscle mass and systemically increases insulin sensitivity and reduces adiposity [23]. Although increased muscle mass resulting from myostatin loss-of-function indirectly affects metabolic homeostasis, myostatin is detected in the circulation and may thus directly signal to other tissues expressing its receptor, such as the adipose [12].

In addition to a role in metabolic homeostasis, myokines may affect the progression of diseases that have increased prevalence in the aged. For example, it is well-known that exercise and muscle strength are inversely correlated with cancer progression [1) 5)] and myokines have been recently proposed to play a role in this regulation. For example, increased levels of oncostatin-M (OSM) in skeletal muscle and serum were observed following exercise, and serum from exercised mice inhibited the proliferation and increased apoptosis of breast cancer cells. A large proportion of this effect was directly attributable to OSM, based on the effect of blocking antibodies. Although it is currently unknown whether OSM can reduce tumor burden *in vivo*, this research identifies OSM as a novel myokine with possible anti-tumorigenic properties. Another potential anti-tumorigenic myokine induced by exercise is osteonectin/SPARC (secreted protein acidic and rich in cysteine). SPARC inhibits colon tumorigenesis by inducing apoptosis of colon mucosal cells [24] and this effect is lost in *Sparc* null mice. This research indicates that SPARC is a myokine necessary for exercise-mediated apoptosis of colon cancer cells.

Conclusions

The identification of factors deriving from muscle that cross-talk with peripheral tissues is a burgeoning area of study with great potential to influence research in a broad range of areas from metabolic and neurodegenerative disorders to cancer. Proteomic screening approaches have identified more than 200 proteins potentially produced and secreted by cultured human skeletal muscle [25]. More research is required to expose

the functions of these proteins as the majority is yet unknown. Although all myokines discussed have been associated with processes that could alter disease outcome and thus theoretically affect lifespan, direct effects on aging and lifespan have not been studied. The elucidation of these novel myokines and their functions will no doubt provide greater insight into the complexities of inter-tissue communication and the relationship between skeletal muscle, systemic aging, and lifespan.

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