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REVIEW

Morphological and Functional Attributes of Musculoskeletal Aging

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ABSTRACT

The purpose of this study was to review the pertinent aspects of musculoskeletal aging and provide an update of the current literature on the subject. The study comprises a descriptive review of recently published papers retrieved from major electronic databases. Manuscripts published in English within last 10 years were included in this review. We preferentially reviewed the information that is recent and constitutes a major advance in the field of musculoskeletal aging. The major emphasis was placed on discussing the current concept of aging and the evolution of term referred to as a degenerative condition, delineating the morphological versus functional implications of aging skeletal muscle, bone, articular cartilage, vasculature, and outlining the role and effectiveness of current of senolytic strategies to mitigate the process.

INTRODUCTION

Aging can be generally considered as a universal process of molecular and physiological changes that progressively accumulate over time to confer a greater susceptibility to disease and subsequently death. Systemic aging not only occurs at varying rates amongst different people, but the rate of aging can also vary across individual organ systems and tissue structures for each person [1]. With a focus on the extension of not only lifespan, but also healthspan, it is thus deemed important by researchers in the field of aging to understand unique aging processes in tissues, and why aging is not a homogeneous process. In this descriptive review, we will focus on the age-related changes of the musculoskeletal system in the context of current models of the general mechanisms of aging. Furthermore, we will also discuss potential therapies that may offer future physicians an improved method of treating and/or preventing age-related degeneration at both cellular and functional levels.

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The Concept of Age

The way we think about aging has changed over the past few decades. Aging was once defined as a purely chronological decline and loss of function. While the passage of time is still positively correlated with the symptoms of aging, today, interest is increasingly placed on the role of biological aging [2,3]. Individual variation in the rate of aging on a population scale is easily evidenced by marked differences between the shortest lived and longest-lived members of the population. The presence of disease states, such as the progerias, markedly shorten life span and are accompanied by symptoms classically associated with old age. Comparatively, there is an increasing prevalence of centenarians, who are chronologically out-aging the majority of the population [4].

There are several accepted hallmarks of aging [2,3,5], which have been previously reviewed and are outlined in Table 1, all of

Table 1. The Hallmarks of Aging.

The Hayflick Limit: The Hayflick Limit describes a predetermined number of cellular divisions, dependent upon telomere length, that a cell completes before it becomes senescent or undergoes apoptosis.
Epigenetics: Changes occur to the epigenome that are induced by dysregulation of DNA repair mechanisms. DNA repair circuits damaged over time contribute to the changes in gene expression observed in aging cells, and this process proceeds as a feed-forward cycle.
Increasing Disorganization/Dysregulation of the Nucleus and Nuclear Transport Systems: Evidenced by the laminopathies, such as Hutchinson-Gilford progeria, which lead to aged phenotypes.
Misregulation of Transcription Factors: Genes that increase lifespan are overseen by transcription factors that fail to localize to the nucleus with age, leading to decreased activation of highly conserved pathways.
Decreases in Proteostasis with Age: Largely connected to a dysregulation of the Unfolded Protein Response and autophagy pathways with increasing age. Downregulation of protein translation is known to increase lifespan, possibly due to activation of mTOR signaling pathways, and an increased ability of the cell to maintain proteostasis.
Mitochondrial Dysfunction: The cell is less able to clear damaged organelles such as mitochondria via decreases in autophagy, damaged mitochondria also have less oxidative capacity.
Cytoskeletal Damage: Induced by toxins, reactive oxygen species and Ultraviolet rays, among other stressors. Increased cytoskeletal damage is evidenced by age related pathologies such as Alzheimer's disease and other neurodegenerative diseases.
Geometric Changes in the Cell Membrane: Induced by cytoskeletal changes, impairing cell function.
Senescence Associated Secretory Phenotype (SASP): Cells that become senescent release inflammatory proteins that damage cells in their vicinity, a component of inflammaging.

which cause damage in aging cells. However, some tissues are more susceptible than others to the effects of one or more of these mechanisms. Inflammaging, a newly coined term, refers to the involvement of chronic low-grade inflammation due to progressive overactivation of the innate immune system as contributory mechanism for age-related aberrations [6,7].

Here, we will focus on the mechanisms of aging that are most prevalent in musculoskeletal cells. There are many cell types that contribute to the health and stability of the musculoskeletal system. However, examining the two hallmark cells of bone and muscle osteocytes and myocytes, along with their corresponding stem cell precursors, mesenchymal stem cells and satellite cells, gives basic insights into the mechanisms of aging of this system. General changes seen in the musculoskeletal system are outlined in Figure 1, and provide a framework with which to appreciate the cellular changes discussed in detail in this review.

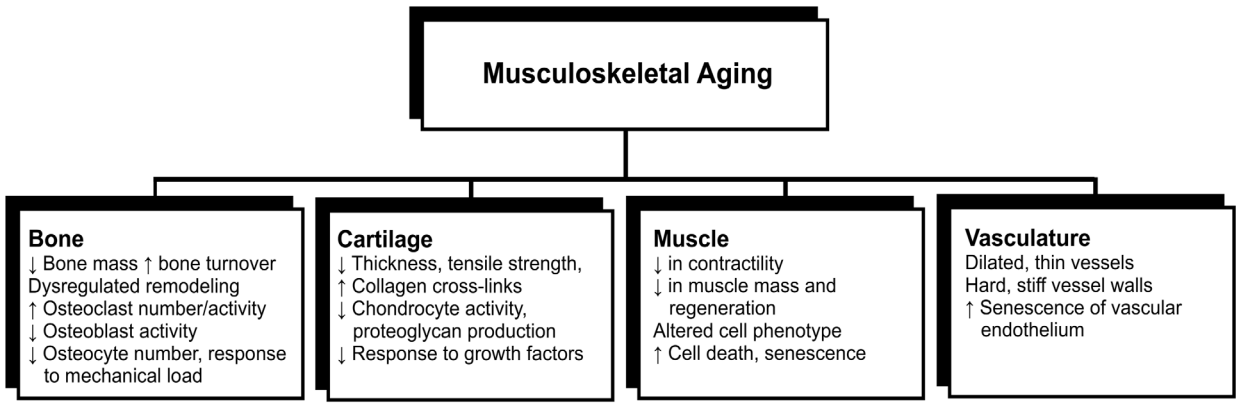

Figure 1. The musculoskeletal system components and their aging mechanisms.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) originate in a variety of tissues and are known precursors of osteogenic, adipogenic and chondrogenic lineages [21,22]. Relevant to bone homeostasis, MSCs have long been the named precursors of osteoblasts that terminally differentiate into osteocytes. Osteogenesis in response to injury or normal homeostatic bone resorption and replacement is reliant on the recruitment of these stem cells [8-11]. With age, there is a greater propensity of MSCs to differentiate into adipocytes. Several mechanisms have been identified as contributors to this process: increased dysregulation of transcription factors, increased production of microRNAs that serve to repress gene expression, decreased autophagy and accumulation of abnormal laminin A protein—all of which are proposed to stem from age related epigenetic changes and damage from ROS [11].

Additionally, as MSCs age, they secrete more exogenous vessels containing internal cellular material, continuously activating the innate immune response. They also secrete increased amounts of pro-inflammatory cytokines, furthering their indication as a driving factor in inflammaging. Thirdly, MSCs show a decreased expression of osteogenic gene products, contributing further to decreased new bone formation in

in the bone marrow niche. Finally, these cells also skew towards a myeloid lineage with age, and have been implicated in the age-related development of myeloid cancers [23].

The viability and therapeutic promise of stem cells has long been a source of excitement for clinicians. The latest stem cell therapy attempt showing some promise in reviving mesenchymal stem cells involves exposing aged tissues to a youthful circulation via heterochronic parabiosis. Evidence suggests that a factor in young circulation is able to reactivate the β-catenin/Wnt signaling system, restoring the ability of old MSCs to differentiate into osteoblasts, aiding in fracture repair [19].

Emerging evidence also indicates the discovery of the human skeletal stem cell (HSSC). This cell differs from the more general mesenchymal stem cell in that it can only produce bone, cartilage and stroma [21]. HSSCs undergo expansion in response to acute skeletal injury, which implicates possible therapeutic use. Since it is newly described, further study is needed to understand their function in correlation with age, and to determine if they have therapeutic potential for aged patients in the setting of acute injury.

Skeletal Myocytes

Skeletal myocytes contain the functional contractile units of muscle tissue, and thus maintaining their efficacy and integrity are of utmost importance in the quest to lengthen healthspan. Comparing skeletal muscle from 20-29 year-old women to that of 65-71 year-old women, a marked shift in gene expression was observed. Proteins binding pre-mRNAs were upregulated while energy metabolism genes were downregulated, consistent with the information theory of aging and the decrease in metabolism observed with age [24]. Increased levels of DNA damage are also seen in myocytes, hypothesized to be due to the increased metabolic load and mechanical stresses associated with contractile function [25,26].

Protein aggregates increase with age in a variety of tissues. However, a decline in proteostasis is one of the most notable features of myocyte aging [24,26]. With age, the crosstalk between protein synthesis and protein degradation is disturbed. Mechanistically, these declines in crosstalk occur in the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal system [27]. While both are important to proteostasis, the UPS has been estimated to decline in function by 60%, comparing young mice to old, a markedly greater decline than that seen in the autophagy-lysosomal system [27,28]. There is some indication that damage to the proteasome itself due to modification by reactive oxygen species contributes to the failure of this system [28].

Mechanical damage to contractile proteins is expected and unavoidable, reinforcing the need to maintain protein recycling in myocytes. Notably, exercise, improved autophagy and decreased pro-apoptotic markers in 70-year-old participants [29,30]. Sarcopenia, the most notable disease of aged muscle, is described by this proteasome imbalance, in which an overall shift towards protein degradation is seen, along with the build-up of non-functional proteins. Resistance exercise in particular is the preferred mode of increasing the anabolic tendency in skeletal muscle, shifting the cellular proteomic response towards protein synthesis. Endurance exercise on the other hand, evidences ability to activate several intracellular pathways that all converge on improving mitochondrial

quality and biogenesis, improving skeletal muscle quality [30].

Another stark mark of aging myocytes is the build-up of intra and extracellular lipids that occurs with old age [25]. Associated with this lipid accumulation is the preferential loss of Type II glycolytic fibers as opposed to Type I oxidative fibers [31,32]. Type II fibers are less able to oxidize the lipids and thus they are preferentially overwhelmed and undergo apoptosis. Lipotoxic effects manifest by activating cellular stress mechanisms that lead to proinflammatory cytokine signaling, which in turn activates stress mechanisms in surrounding myoctyes [31]. Additionally adipose tissue in aged individuals contains the highest load of senescent cells, and its increase with age in skeletal muscle is also associated with poor muscle function. Senescent cells release a milieu of inflammatory cytokines, and thus, this extracellular adipose tissue further contributes to increased myocyte apoptosis [33,34].

Satellite Cells

Satellite cells are necessary for proper muscle fiber repair and hypertrophy. However, the number of satellite cells in human muscle decreases with age [35]. These cells accumulate DNA damage over time that leads to a senescent phenotype, thereby decreasing the number of functional cells. Additionally, increased signaling in the p38αβ MAPK pathway and IL-6-JAK/STAT3 pathway contribute to the tendency of aged satellite cells to perform symmetric divisions as opposed to asymmetric divisions [36]. In an effort to ameliorate the loss of muscle satellite cells; resistance exercise in particular, increases the number of satellite cells in Type II muscle fiber niches in the elderly mirroring levels seen in younger adults [30]. Moreover, aged individuals with a lifelong history of endurance exercise show more active satellite cell induced remodeling, than their counterparts [30].

Increases in secretion of inflammatory proteins with age may contribute to the depletion of these stem cells [2,3,8]. Inflammatory markers are only one of the many extrinsic factors that affect the viability of satellite cells. Recently, increasing interest is focusing on the distance between satellite cells and capillaries, as it is known that muscle vascularization decreases with age and impairs the ability of satellite cells to function [35,37].

Autophagy is necessary for the maintenance of stemness. As seen in other cell types, decreases in the functionality of cellular autophagy mechanisms with age contributes to the development of senescence in satellite cells [35,36]. Senescent satellite cells are highly implicated in age related muscle disease, namely sarcopenia [30]. Furthermore, with age, the extracellular matrix in the stem cell niche becomes dense, depleting satellite cell numbers. Exercise promotes the reorganization of the extracellular matrix, as well as helps to reactivate protective autophagy mechanisms [30,39]. Calorie restriction, is a long studied and reliable way to increase beneficial autophagy in cells, including but not limited to, satellite stem cells [30].

Lastly, parabiosis has been proposed as one method of regenerating the function of satellite cells. However, previous reviews cited mixed results of these studies [36]. GDF11, a member of the TGF-β family, is a factor whose prevalence decreases with age. Initially thought to rescue satellite cells, newer evidence postulates that it

contributes to muscle wasting [35,40-42]. Future regenerative efforts are now focusing on the therapeutic benefit of senolytic drugs [43-45].

The Vascular Connection

Aging of the vasculature is of particular interest to those in the field of aging as it is a causative factor of the development of both cardiovascular and cerebrovascular diseases [46]. However, reduction in microvasculature integrity in the musculoskeletal system is one of the most important contributors to aging and age-related pathologies in this organ system [37,47-51].

Murine models of bone aging closely approximate bone aging in humans. An age-related decline in arterial capillaries in a murine model correlates directly to the age-related decrease in arterial perfusion seen in the human femur [15,48]. In bone, a decrease in the integrity of the lacuno-canalicular system, which impairs the delivery of signaling molecules required for osteocyte induced activation of osteoblasts and osteoclasts, is enhanced by this degeneration of microvasculature [10]. It is important to consider the effects this loss of microvasculature has on the stem cell microenvironment. Different types of blood vessels in bone contribute to microenvironments that are able to support respective subsets of mesenchymal stem cells [52]. Disruptions in blood flow, as seen in the aged femur, have shown to decrease angiogenesis and osteogenesis, indicating a highly pertinent role of blood flow in the development of osteoporosis, as well as contributing to the disturbance of these stem cell microenvironments [53].

In comparison to younger subjects, aging skeletal muscle tissue also shows a marked decrease in capillary density and blood flow in older subjects [54,55]. As noted previously, higher distances between muscle capillaries and satellite cells impair their ability to repair muscle fibers [37,50]. Along with capillary density decreases, age-dependent changes in properties of endothelial vessels as well as increased endothelial cell apoptosis in skeletal muscle, impair normal satellite cell function [55,56]. Among alterations in signaling mechanisms, decreases in available nitric oxide and increased levels of vasoconstrictors are observed with age [51]. These, as well as other, vasodilatory disturbances at the commencement of exercise contribute to the reduction of exercise induced benefits in the elderly [56,57].

Identification of decreased HIF1 expression as a marker of vascular cell senesce, and its implication in plaque formation has drawn increasing attention towards the dysregulation of this signaling system with age. Age-related impairment leads to decreased response to limb ischemia and decreased angiogenesis, as well as decreased wound healing. Pharmacological stabilization of this system is proposed to extend lifespan by 30-50% [58]. However, the HIF system performs conflicting roles in the vasculature, overexpression possibly contributing to cancerous phenotypes, thus early therapeutic successes using this target should be viewed with a critical eye [58].

Recent advances in the understanding of angiogenesis promoting signaling pathways show great promise in reversing and restoring the capillarization ability in the elderly. Older adults that perform regular exercise have a more normal endothelial cell expression profile, pointing to further benefits of exercise therapy [58]. Researchers, taking a different mechanistic approach, supplemented elderly mice with nicotinamide mononucleotide (NMN), a precursor

molecule of NAD+, and saw activation of a SIRT1-H2S signaling pathway. These mice demonstrated increases in capillary density as well as a two-fold increase in endurance capacity, making them functionally and physiologically comparable to their younger counterparts [55].

Functional Changes with Age

Functional measurements continue to be the most reliable predictors of mortality in the elderly [9]. Gait speed, grip strength, and repetitive sit-stand motions are the most widely used. Grip strength in particular has shown to be a highly reliable predictor of future disability, mobility decline, and mortality [59]. Grip strength in isolation of gait speed and chair stand tests is postulated to have equal predictive value in people aged [60,66-76]. In a cohort of people ages 60-70, higher gait speeds are predictive of lower levels of functional decline in both men and women. Conversely, low gait speeds in people over the age of 65 are predictive of increased disability and mortality. Changes in gait speed can be detected as early as 40 years of age and may warrant the implementation of lifestyle changes and other therapeutic interventions to prevent early decline in function [61-63]. Interventions such as the implementation of progressive resistance training with high intensities show the most promise for improving gait speed in older adults [64].

Declines in muscle strength, decreases in appendicular lean mass, as well as bone mineral density, are all observed with age [9,61]. Loss of strength of muscle contraction contributes to a decline in mechanical force able to be placed on bone, leading to a cyclical decline in functionality of both tissues [61]. Declines in muscle strength and bone strength vary among individuals, due to both genetic, sex and lifestyle factors [62,65]. In men, sedentary behaviors are associated with decreased mitochondrial function in skeletal muscle, contributing to functional decline. Adversarially, regular exercise showed protective effects against reductions in appendicular lean mass, sarcopenia and abdominal adiposity [66]. Additionally, long term smoking is associated with accelerated loss of bone density, and men reporting a healthier diet had less signs of disability at the age of 80 and above [62].

Declines in musculoskeletal function in women are largely attributed to the loss of estrogen that occurs after menopause. Thus, musculoskeletal studies are largely separated by gender. However, a less sedentary lifestyle is also associated with improvements in bone health in postmenopausal women [63]. Because declines in bone density and muscle mass begin as early as 40 years of age, the prevention of musculoskeletal aging is more intelligently implemented in young and middle-aged populations. When it comes to the type of physical activity most beneficial in this age range, moderate to very high intensity exercise showed great promise in decreasing the amount of bone density lost, as well as increasing postural stability and muscular strength [67].

Musculoskeletal Diseases with a Degenerative Etiology

There are a multitude of musculoskeletal diseases that result from a gradual degeneration of function, the two most prominent in aged individuals being osteoporosis and sarcopenia. At the cellular level, we have explored what processes contribute to these degenerative phenotypes. At the population level, it is estimated that women under 50

generally have normal bone mineral density, but by the age of 80 years, 35% are osteoporotic [8]. Today, 1 in 3 women and 1 in 5 men will suffer an osteoporotic fracture [68]. Additionally, sarcopenia is estimated to be present in 18% of men over the age of 65, rising to 30% by the age of 80, with a >50% prevalence in those over 80 [8,69]. Some argue that osteoporosis, as well as sarcopenia and its related dynapenia, are underdiagnosed in the United States.68 With an increasingly aging population, proper understanding of these two conditions is necessary for diagnosis, treatment and prevention of these diseases.

Generally speaking, osteoporosis is "a skeletal disorder characterized by compromised bone strength predisposing a person to increased risk of fracture"[69]. Decreases in estrogen after menopause is the main risk factor for the development of osteoporosis. With a continuously aging population, osteoporosis is increasingly seen in men, as they begin to lose androgens at more advanced ages [71,72]. The pathophysiology of osteoporosis is characterized into four categories—defects in the microarchitecture of bone, poor intrinsic material properties of bone, defective repair of microdamage and excessive bone remodeling [73]. All of which can be understood in the context of cellular aging, as discussed previously.

Muscle tissue is known to affect the rate of aging of other tissues in the body. Some have even gone as far as to label it the sentinel tissue of aging. Apart from the contractile functions discussed previously, it functions in regulating global glucose metabolism, modulating insulin sensitivity and as an endocrine organ via secretion of myokines [74]. Therefore, the detection and amelioration of muscle aging such as seen in sarcopenia, has wide implications. Sarcopenia has long been determined an age-related disease, with its development dependent on the degeneration of muscle tissue function and integrity. Sarcopenia differs from disuse atrophy in that it involves both hypoplasia and a decrease in fiber size [75]. Loss of signaling hormones such as testosterone with age and loss of neural innervation also contribute to the development of sarcopenia, but were not explored in this review [69]. Cellular contributions to the sarcopenic phenotype, as discussed previously, have huge implications in the future of clinical treatment strategies.

Senolytic Drugs: The Pharmacological Clean-Up Crew

Senescence is seen in multiple cell types of the musculoskeletal system, most notably in stem cells and invasive adipose tissue. These cells not only resist apoptosis but secrete damaging and inflammatory proteins. They also express a unique marker known as p16Ink4a. Pioneer senolytic drugs target this marker and cause the cells to become susceptible to apoptosis again. These drugs have shown to alleviate symptoms of aging such as atherosclerosis, limited mobility and frailty in progeroid and normally aged mice [76]. It has been noted that senolytic drugs not only decrease bone resorption but either do not effect or actually enhance bone formation in a mouse model. Suggestive of a more effective treatment of osteoporosis than current therapies [45].

Two modes of treatment currently being explored include the genetic INKATTAC approach, which serves to restore apoptotic ability to senescent p16Ink4a positive cells. As well as, intermittent senolytic

therapy with Dasatinib and Quercetin (referred to as D+Q). In clinical trials, D+Q has been shown to improve several aspects of healthspan in preclinical models of aging and chronic disease [15]. Early evidence showing an in vivo reduction in senescent cells when studying pulmonary fibrosis [77]. Most recently, a trial targeting senescent cells to improve the skeletal health of older adults has begun. Female participants over 70 years of age will be given either D+Q therapy, Fisetin therapy or placebo therapy for twenty weeks. Primary end points will measure bone turnover markers. This trial is currently underway and is set to end in 2023 [78]. Pending the results of this trial further trials are still warranted to assess safety and long-term efficacy.

High numbers senescent cells in the adipose tissue of older women is correlated with physical disability [34]. In a recent study, older mice treated with senolytic drugs were able perform endurance exercise at the level of their young counterparts [45]. This suggests the ability of senolytic drugs to alleviate physical muscular weakness in older populations. While this therapy would be transformative for many age-related diseases not limited to the musculoskeletal system, it is noted that successful trials in mice have often failed in human studies due to the complexity of human biology and unforeseen side effects [76].

Mentioned briefly in this review in connection to the effects of supplementation on increasing microvasculature regeneration, NMN is an exciting development in the treatment/amelioration of age-related phenotypes. The implications of NMN supplementation on the regulation of altered NAD metabolism that is seen in many aged cells, is broad. Notable to this review, it has been implicated in reversing osteogenic inhibition in steroid induced osteoporosis as well as normal aging bone marrow via its effects on sirtuin pathways [79]. Recently published evidence from a 10-week, randomized, placebo-controlled, double-blind trial in postmenopausal women with prediabetes who were overweight or obese, showed no changes in muscle grip strength or muscle mitochondrial activity after NMN supplementation, but did show improvements in insulin sensitivity, signaling and muscle remodeling [80].

Nicotinamide Ribonucleotide (NR), another NAD precursor molecule, is also being explored in clinical trials. In a six-week randomized, double-blinded, placebo-controlled, crossover intervention study with 13 healthy overweight or obese men and women, NR supplementation showed no effect on insulin sensitivity or signaling but did produce a significant increase in fat-free body mass and increased sleeping metabolic rate [81]. Further clinical trials on the full range of effects on NAD metabolism and signaling, are necessary and exciting avenues for the future of these therapies and their ability to modulate musculoskeletal disease processes.

CONCLUSIONS

Deeper understanding of the mechanisms that contribute to age related pathologies aid in the understanding of why age-related pathologies develop and how they can be treated. This review briefly overviews the mechanisms of aging in musculoskeletal cells, and the associated vasculature. As well as the bigger picture clinical context of these cellular processes. Hopefully, this framework assisted in understanding why gross changes in function and pathologies occur.

Important to note is the connection of all of these cells and processes to the immune system. Immune cells are known to have crosstalk with stem cells and may affect tissue regeneration. Fibroblasts and general connective tissue changes also occur in muscle tissue with age and contribute to the aged phenotype. Additionally, the endocrine crosstalk between bone and muscle via myokines and other factors was not explored.

Optimistically speaking, ongoing clinical trials as well as future research into senolytic drugs and NMN supplementation will provide a framework for the development of rejuvenating therapies, and change the way we treat age-related disease.

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