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Mechanical Coupling of Bones and Muscles

The musculoskeletal system is extremely complex. Contrary to the reductionist view that it is made up of only bones and muscles, the system is actually composed of bones, skeletal muscles, nerves, blood vessels, tendons, ligaments, cartilage, joints, and other connective tissues.

Historically, the association between bones and muscles has been primarily seen as a mechanical coupling where one tissue, skeletal muscle, actively applies the load and another tissue, bone, receives

the load and serves as the attachment site. This physiologic association supports the locomotion, protection, and the shape/form of animals. In recent years, we have begun to appreciate that the relationship between muscle and bone goes beyond this mechanical coupling; that these two tissues also function at a higher level through crosstalk signaling that is important for their function.

The tight coupling between skeletal muscle and bone in animals begins during embryonic development with the formation of the paraxial mesoderm, which subsequently gives rise to somites that become muscles, bones, tendons, and other tissues [1]. The development of each of these tissues will be discussed later in the chapter. It is important to mention at the outset that as the skeleton develops, it has been postulated that muscle contraction in the fetus contributes to skeletal growth and development and that skeletal adaptations in early postnatal life are driven by changing mechanical forces [2, 3]. Although exercise across all ages has proven beneficial, clearly peak bone mass accrual during pre-pubertal growth is dramatically affected by exercise, or physical activity, and to a lesser extent thereafter [4]. Peak bone mass is achieved by both men and women by their third decade of life, with men having obtained more bone mass due to hormonal and other influences (i.e. diet, genetics, etc.). Human females begin to lose bone mass with a rapid loss phase occurring 2–3 years before menopause and continuing until 3–4 years after the last

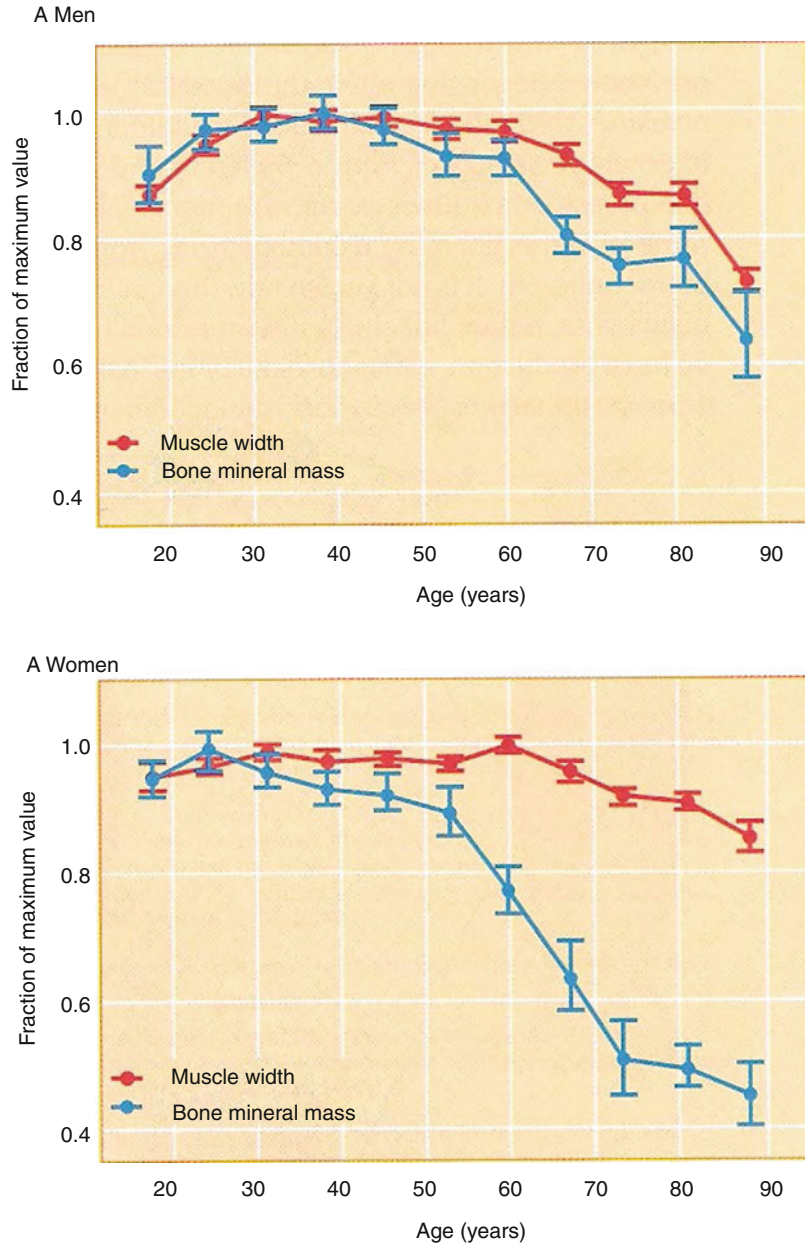
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Fig. 4.1 Aging-related changes in bone mineral mass of the radius and muscle width in the forearm in adult men and women (From Novotny et al. [7])



menses [5], then experiencing a more gradual but steady decline. Males do not experience this same dramatic decline, but rather a gradual, steady bone loss beginning in their 50s and 60s [6] (Fig. 4.1). Individuals with reduced bone mass, as seen in osteoporosis, often also develop the reduced muscle mass and function, a condition known as sarcopenia. However, declines in bone mass do not fully explain sarcopenia, nor does

muscle atrophy fully explain the totality of osteoporosis. This seeming mystery may be due in part to the use of bone mass and muscle mass as the predominant measures for osteoporosis and sarcopenia. The assessments of bone quality and muscle function might better reflect the physiological basis for these diseases. However, these metrics are not in place to date, especially in the clinical arena.

The mechanical coupling of skeletal muscle and bone is visible. Bone adjusts its mass and architecture to changes in mechanical load and as contraction of skeletal muscle is essential for locomotion, it is obvious that muscle contractions apply load to the bone. This mechanical perspective implies that as muscle function declines, the result would be decreased loading of the skeleton, leading to a reduced bone mass. However, as noted above, the inability to account for totality of the changes in the musculoskeletal system based upon mechanical considerations, while important, is only a part of this major complex problem. For example, it is not possible to explain the development of osteoporosis based solely upon the presence of sarcopenia (and vice-versa). These observations imply that beyond the mechanical coupling there must also be a biochemical coupling.

As we will discuss in this chapter, it is now fully evident that muscle and bone produce factors that circulate and act on distant tissues, thus fulfilling the classical definition of endocrine action. Surprisingly, it has only been recently that the action they have on one another has begun to be under careful investigation. Perhaps this oversight derives from the bias of mechanical coupling and, until recent years, a lack of technology, testing abilities, and bioinformatics techniques. However, understanding this apparent endocrine crosstalk and biochemical coupling is an exciting new avenue of research with tremendous clinical potential.

Beyond Mechanical Coupling: Bone-Muscle Developmental and Genetic Coupling

Considering their common embryonic development while forming from somites originating from the paraxial mesoderm, the intimate relationship and close coupling between the tissues that comprise the musculoskeletal system is therefore unquestionable [3]. Bone and muscle cells share a common mesenchymal precursor and their shared experience of organogenesis occurs through a firmly orchestrated network of genes and proteins during intrauterine

development. In fact, some of our own bias to accept a purely mechanical coupling between these two tissues derives from embryology. For example, it is the contraction of muscles in the developing embryo that contributes to the process of skeletal development itself and clearly to the growth of the skeleton, as well as a host of adaptations in early postnatal life that result from the changing mechanical forces [2, 4]. Furthermore, even post-development, the immediate results of muscle load can be discerned on bones, since they adjust their shape and mass to changes in load, and such load can be easily interpreted as coming from muscle contractions. We are obviously not suggesting that contracting forces do not play a major role, but rather, there are a number of additional influences to consider.

While universally accepted that bone and muscle develop embryologically as a unit, the underlying environmental cues that drive skeletal mass and other properties are regulated by a complex set of genetic factors. Heritability studies have estimated that from 40 to 80 % of the major skeletal phenotypes and muscle traits are related to genetics [8–11]. Thus, given the genetic and developmental closeness of these two tissues, it seems highly likely that there would be some degree of shared genetic components underlying some of their phenotypes. The identification of these shared (i.e., pleiotropic) genetic cues are essential to promote the discovery of the molecular/biochemical coupling that exists between muscle and bone, and serve as evidence that bone and muscle interact beyond mechanical.

Genome Wide Association Studies (GWAS)

Over the past decade, GWAS have produced several candidate gene regions that show association with variations in a number of different human bone phenotypes and muscle traits. Even limiting the focus to a small sampling of the bone muscle phenotype GWAS [12–20] and bone phenotype GWAS [21–29] reported in recent years evidence of the powerful genetic coupling between these

tissues. An interesting question that arises is how mechanical forces during development interact with genetic signals. For example, do fetuses that have stronger intrauterine contractions produce genetic signaling that in turn favors the development and formation of stronger bones and muscles and a healthier musculoskeletal system? Or, are there specific genetic signals that lead to bones and muscles of higher mass and strength? Or, is it a combination of both possibilities?

Due to the development of new bioinformatics and statistical models, bivariate GWAS has been used to identify pleiotropic candidate genes/single-nucleotide polymorphisms (SNPs); regions concomitantly associated with traits in both bone and muscle [13, 30–34]. These recent bivariate GWAS based studies, including both bone and muscle phenotypes, have revealed a short list of several novel potentially-pleiotropic candidate genes such as *GLYAT* [13], *HTR1E*, *COL4A2*, *AKAP6*, *SLC2A11*, *RYR3* and *MEF2C* [31], *PRKCH* and *SCNN1B* [30]; *HK2*, *UMOD* and two microRNAs *MIR873* and *MIR876* [34].

The *MEF2C* gene encodes a potent transcription factor (myocyte enhancer factor 2C) that was originally thought to be involved in cardiac and skeletal muscle development and was also used as a marker of myogenic cells in the somites [35]. Intriguing data has recently emerged with the development of the osteocyte specific mouse model of *Mef2C* deletion. These mice display increased bone density through a complex mechanism involving reduced *Sost* expression and increased osteoprotegerin (OPG) expression resulting in a reduced receptor activator of nuclear factor κ B ligand (RANKL)/OPG ratio, and reduced osteoclastogenesis [36]. Therefore, the important roles of *MEF2C* in cardiac and skeletal muscle development, as well as in adult bone mass regulation, support the concept of shared genetic determinants in bone and muscle and in this case even beyond, since cardiac muscle is also part of this *MEF2C* loop.

Another of the suggestive genes that emerged from the GWAS analyses was *METTL21C*, which is highly expressed in muscle. Interestingly, it has been reported that there may be an association between the *METTL21* family of proteins and

inclusion body myositis with Paget's disease of bone [37–39]. The role of *Mettl21c* in myogenesis was explored using the murine skeletal muscle cell line, C2C12. By silencing the activity of *Mettl21c* through the process of siRNA transfection, we demonstrated a significant decrease in C2C12 myogenesis as evidenced in the reduced fusion index, smaller myotube cell area, and decreased calcium release from the sarcoplasmic reticulum when compared to negative controls (Fig. 4.2).

To study the impact of *Mettl21c* on bone cells, the osteocyte cell line MLO-Y4 was used. We found that a partial knockdown of *Mettl21c* in MLO-Y4 cells led to an increased dexamethasone-induced cell death. These findings further support the concept that bones and muscles share genetic determinants, and that it is through the modulation of specific factors that lead to the phenotypic and functional effects (Fig. 4.3).

Myostatin (*MSTN*), or growth and differentiation factor 8 (*GDF8*), is a member of the TGF- β superfamily and is a muscle-derived factor (myokine) that circulates in the blood, making it an attractive candidate for muscle-bone endocrine signaling [40, 41]. The deletion and/or mutations in myostatin lead to muscle hypertrophy or “double muscling” in animal models [42–47] and in humans [48]. This is a prime example of how a mutation assumed to be restricted to one tissue can lead to altered properties in another (Fig. 4.4). In this case, the loss of myostatin not only leads to muscle doubling, but also leads to a generalized increase in bone density and strength [49]. A question that could lead to major mechanistic insights is how does myostatin exert its effects on bone? Possible explanations include direct effects of mechanical loading of bone due to the increased muscle mass, indirect action by regulating hepatic production of IGF-1 [50], or some other unknown mechanism. While the IGF-1 and GH axis is an important mechanism to be pursued due to its effects on age-related changes in bone and skeletal muscle [51], a tantalizing possibility is that larger volume muscles might release relatively larger amounts of myokines, which in turn reach the bone cells via the canalicular system, exerting anabolic effects on bone.

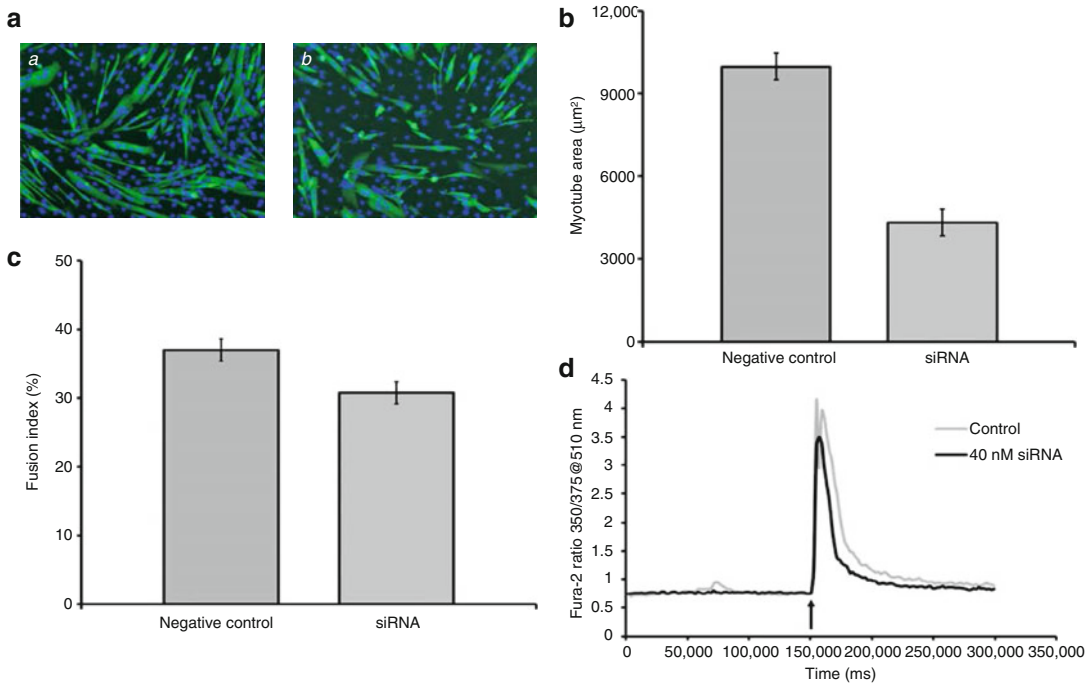


Fig. 4.2 Partial silencing of Mettl21c reduces fusion index, myotubecell area, and calcium release from the sarcoplasmic reticulum. **(a)** Representative fluorescence images of DAPI-stained nuclei (*blue*) and MHC antibody (*green*) of C2C12 at 3 days of differentiation after transfection: *(a)* negative control; and *(b)* Mettl21c-siRNA-treated C2C12 cells. **(b)** Summary data show that the Fusion Index in Mettl21c-siRNA-treated C2C12 cells decreased significantly compared to negative control ($p < 0.05$). **(c)** Summary data show that myotubecell area

in Mettl21c-siRNA-treated C2C12 cells drastically decreased compared to negative control ($p < 0.0001$). **(d)** Representative calcium transients induced by 20 mM caffeine (*arrow*) on C2C12 myotubes loaded with Fura-2/AM. In Mettl21c-siRNA-treated C2C12 myotubes at day 5 of differentiation, compared to negative control, the amplitude peak calcium response to caffeine was significantly decreased and the relaxation phase of the transient was shorter ($p < 0.01$). DAPI,4,6-diamidino-2-phenylindole; MHC,myosin heavy chain (From Huang et al. [38])

The possibility of this endocrine-like crosstalk, along with the promise of novel and impactful therapeutic interventions, has motivated research to be conducted in this area.

Disease Conditions with Multiple Tissue Effects

The endocrine interactions that are being discovered through the effects of bone cell secreted factors and myokines go well beyond the musculoskeletal unit. Recent findings have supported the interconnection of bone, muscle, and adipose tissue. The striking rise of chronic diseases such as diabetes, metabolic syndrome, and obesity seem to closely parallel the rise in the

prevalence of sarcopenia and osteoporosis, particularly in the elderly population [52]. The musculoskeletal system is the largest organ system of the body. If we embrace the concept that bone, tendon, and muscle all produce and secrete a myriad of factors, and that they influence not only each other but also multiple organs and overall body metabolism, it makes sense that when the musculoskeletal tissues become less effective with aging, organs throughout the body would also be effected. With fewer bone-derived factors (osteokines) and fewer myokines being secreted, it is logical to expect changes in fat metabolism, as well as kidney function, and even testosterone levels; thereby translating into multiple organ effects that are normally interpreted as “aging consequences”. This new view not only

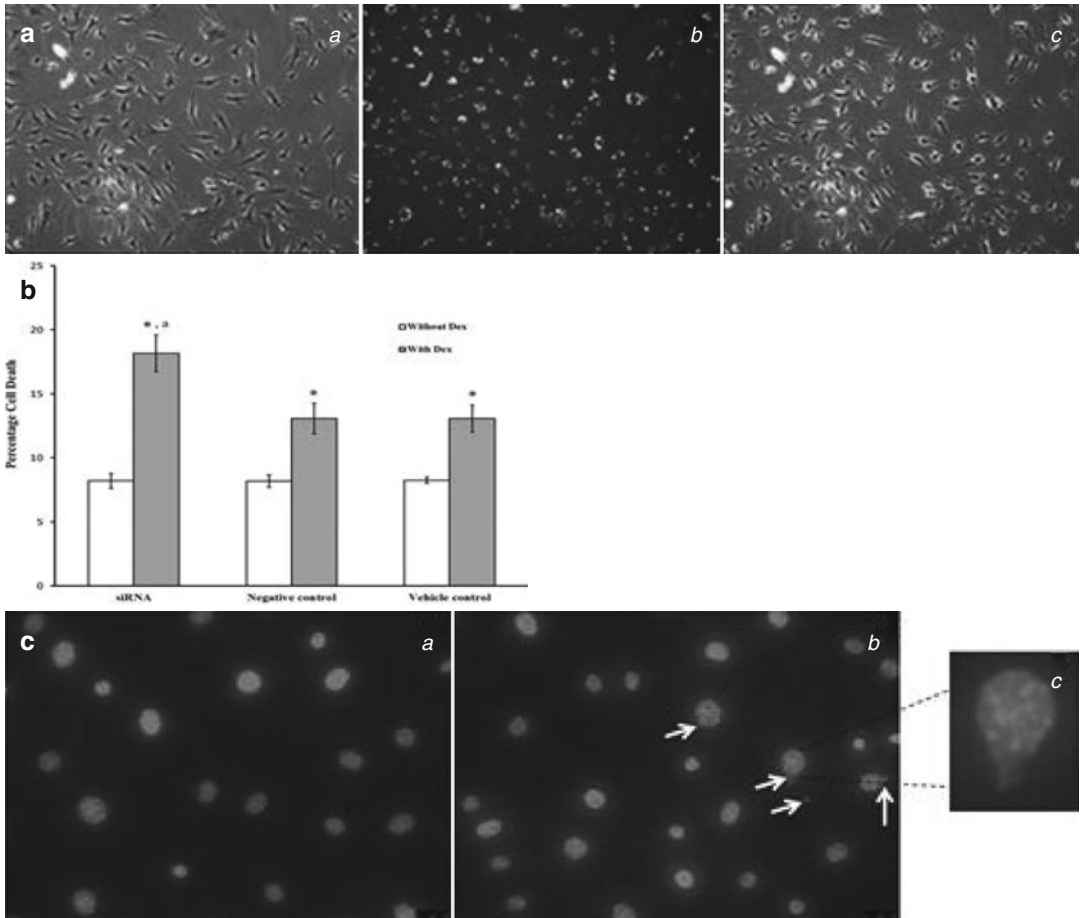


Fig. 4.3 Partial knockdown of Mettl21c in MLO-Y4 osteocytes increased cell death induced by dexamethasone. (a) Representative image of MLO-Y4 osteocytes 24 h after transfection of All Star negative control siRNA (200 nM); (a) phase contrast image; (b) fluorescent image of tagged siRNA; both images were taken from the same area; (c) merging of (a) and (b). (b) Summary data of cell death in MLO-Y4 cells transfected with Mettl21C siRNA, with and without Dex treatment. Cell death was detected 48 h after

treatment by trypan blue exclusion assay (Dex increased cell death under all conditions; and Mettl21c knockdown induced cell death levels higher than Dex alone, $p < 0.05$). (c) The nuclear fragmentation assay shows: (a) absence of nuclear blebbing in control MLO-Y4 cells as compared with (b) enhanced blebbing in the siRNA-treated MLO-Y4 osteocytes; (c) the enlarged image clearly shows the blebbing process in siRNA-treated MLO-Y4 osteocytes. Dex, dexamethasone (From Huang et al. [38])

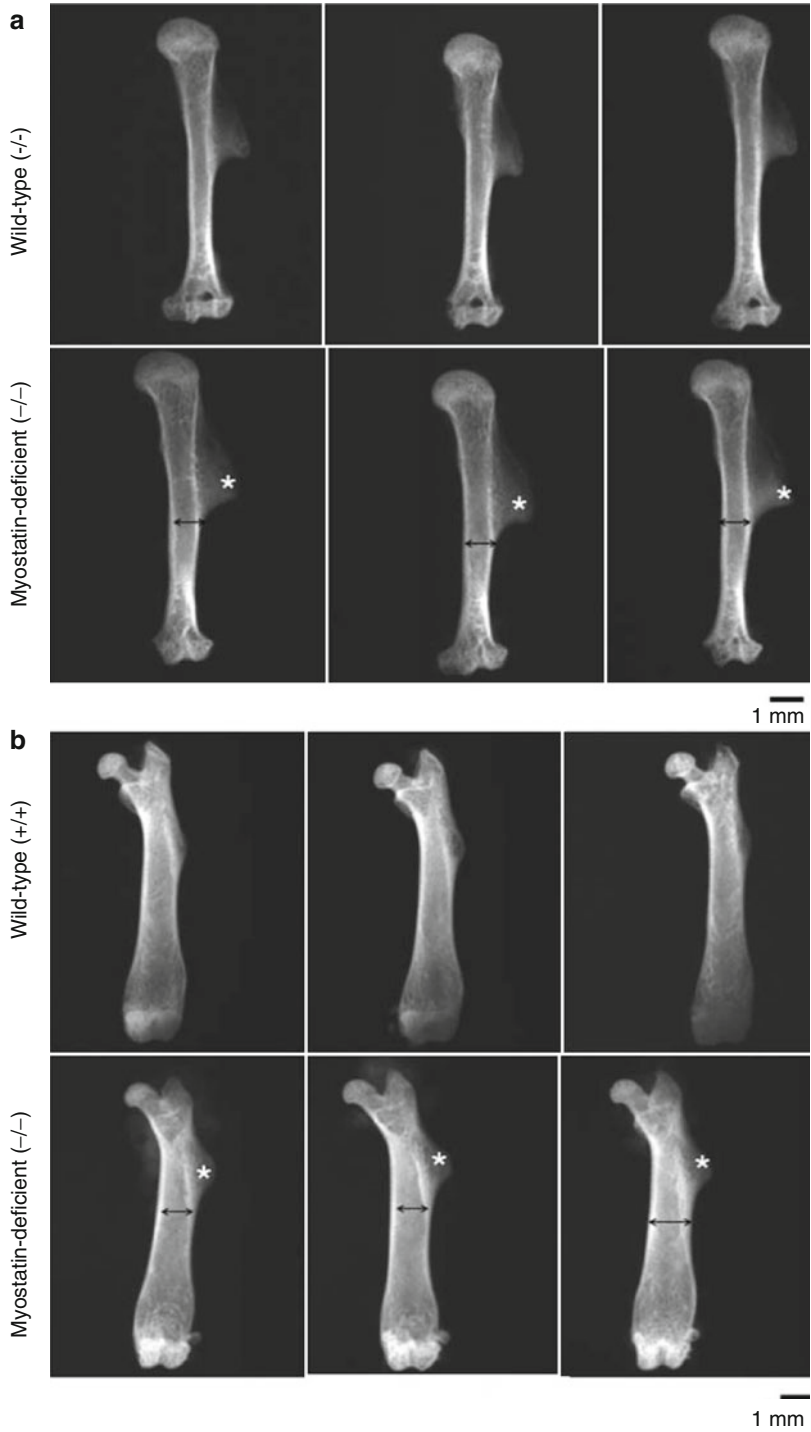
helps to explain some of the multiple organ decline in function, but could also lead to new therapies related to the endocrine function of bone and muscle.

The Unique Case of Fracture Healing

An intriguing and well documented observation that cannot be ignored in the context of bone-muscle interactions is the observation that in

open fractures, where muscle injury is also extensive or where muscle atrophy develops, fracture healing is significantly impaired [53–56]. Rodent models of fracture have supported the concept that muscle secretory activity aids in the process of fracture healing. For example, a significant difference (i.e., lesser healing) was found in rats with fractured femurs when their quadriceps muscles had also been paralyzed by botulin injections [57]. In mice with tibial open fracture, Harry et al., reported that when the fracture area

Fig. 4.4 Radiographs demonstrating increased shaft diameter and increased muscle attachment site at the deltoideus crest in humerus (a), and the third trochanter in femur (b) in *Myostatin*^{-/-} mice compared to wild type. Notice the extension of the articular surface towards the neck of the femur (b) (From Elkasrawy and Hamrick [49])



had been covered with muscle flaps, bone repair was significantly improved [58]. The clinical significance of these findings cannot be overstated

since these findings have also been reproduced in humans with open tibial fractures [59] (Fig. 4.5 [58]; Table 4.1 [59]).

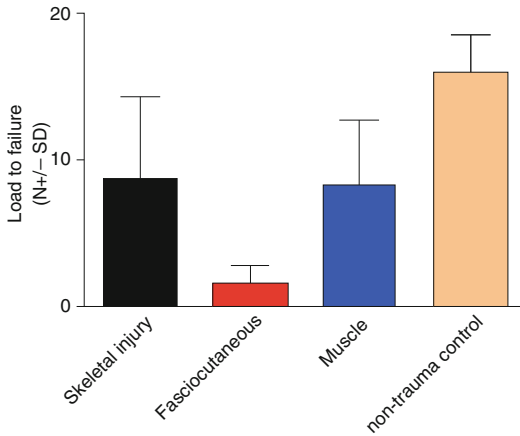


Fig. 4.5 Load at which failure occurred (N), through callus in the fracture groups, and un-fractured cortex in the non-trauma control group, at 28 days post fracture. Bars show standard deviations (SD) (From Hayutin et al. [128])

Table 4.1 Recorded union, delayed union and non-union in-between the study and control groups [58]

	Groups A and B ^a (tribial fracture+ compartment) [12, 37, 42, 60–71]	Control group (tibial fracture) [9, 72–75]
No. of cases with available data (%)	238/245, 97.14 %	418/426, 98.12 %
Union (%)	131, 55.04 %	339, 81.10 %
Delayed (union/non union)	107, 44.96 %	79, 18.90 %

^aBoth groups are presented together due to the absence of adequate data of the healing process differentiated per age groups

Taken together, these studies strongly suggest that even under conditions where substantial mechanical forces are not being produced, muscles have the intrinsic biochemical capacity to secrete factors that stimulate growth and repair. It is almost as if muscles could function as a second periosteal layer as recently proposed by Little and colleagues [76, 77]. Yet another line of evidence derives from the documented observation in humans that fracture healing is improved upon the stimulation of the affected bone with pulsed electromagnetic stimulation (PEMS) [78]. Our groups have recently demonstrated that PEMS

enhances myogenesis of C2C12 myoblasts [79]. Therefore, it is possible that the improved healing observed with PEMS on bone might be attributable to direct effects on bone cells and to indirect effects of myokines. The idea of circulating factors playing a role in these processes gains further strength in light of the studies conducted by Hamrick and colleagues showing that exogenous myostatin treatment accelerates bone and muscle healing [80].

Bones and Muscles as Endocrine Organs

Anatomic proximity of the musculoskeletal system along with the physiologic roles in mechanical support and body movement has, as mentioned, defined the connection between bones and muscles historically. In fact, so physiologically reasonable is the mechanical relationship between bones and muscles that the consideration of other possible links between the tissues has in some ways been encumbered. In the 1960s, an interdisciplinary group hosted by the University of Utah began to convene annually and compiled an impressive body of evidence in support of the biomechanical relationship between bones and muscles. As a result, a new paradigm emerged that included the mechanostat model, which purported that bone strength and density was largely a function of imposed mechanical forces [81]. That model, together with the Utah paradigm continued to influence for decades research conducted on bone and muscle physiology.

However, a key aspect to the model remained nearly dormant until recent years. Even the staunchest supporters of the model admitted to the likelihood of non-mechanical agents locally and systemically affecting the skeletal architecture [81]. Nevertheless, the vast majority of investigative efforts related to the bone-muscle unit focused on the impact of mechanical loads. Basic research has experienced in recent years an explosion of technical advancements. These new techniques, equipment, cell lines and transgenic animal models provide now increased opportunities to learn more about both the mechanical and

the biochemical relationship between bones and muscles. Additional experimental approaches include the study of specially created cell lines and the development of transgenic animal models. To expand the body of knowledge related to factors secreted by bones and muscles, researchers look for evidence of the impact such factors have throughout the body.

Perchance the easiest way to see the endocrine nature of skeletal muscles is to look to one of its major functions; which is to sequester glucose from the blood stream. Skeletal muscles are essential for the maintenance of body glucose homeostasis as demonstrated by the fact that by having a larger volume of muscles and/or being more active physically a person can help prevent diabetes type II [82–84]. While literally working as an organismal sink for glucose, skeletal muscles can simultaneously undergo catabolic reactions that lead to the release of amino acids, which travel via the blood stream to find in the liver the proper conditions for gluconeogenesis. Under certain conditions, lipids can serve as another source of energy for skeletal muscles. Lipids are also emerging as important signaling molecules; a significant point that will be discussed later in this chapter.

Bone as an Endocrine Organ

A century ago, the predominant understanding of bone physiology was that there were primarily two types of bone cells: osteoblasts and osteoclasts. At the time, it was widely accepted that skeletal homeostasis relied on the function of these two bone cells; bone formation for osteoblasts, and bone resorption for osteoclasts. The primary influences on these two bone effector cells were believed to come from hormones and dietary calcium. Although osteocytes were identified and even recognized to be the most abundant type of bone cell, they were believed to be inert, serving primarily as structural components of the bone matrix. Since then, the dynamic and crucial role of osteocytes has become known. It was in the early 1990s that support for the endocrine function of this important bone cell began

to gain momentum. Osteocytes are connected to one another via gap junctions, and it was a focus on this area that provided researchers with insight into the biochemical nature of bone cells. In 1992, Marotti and his team of researchers at the University of Modena, Italy, published their findings and working hypothesis on the metabolic activity of osteocytes. Using transmission electron microscopy (TEM), they demonstrated changes in the skeletal microstructure that occur with aging, as well as evidence of osteocyte modulation of osteoblast activity [85]. Three decades earlier, Dr. Marshall Urist had demonstrated the osteogenic properties of bone by implanting a decalcified and lyophilized sample of bone into host tissue [86]. His experiments revealed that factors present in the sample of bone cells led to neovascularization and bone deposition into the host tissue. It was with the advent of technical advancements that the factors could begin to be identified and studied. Urist recommended the name Bone Morphogenic Protein (BMP) for the factor responsible for new bone formation in his trailblazing experiments. Since then, BMP has come to refer to a group of growth factors implicated in the morphogenesis of tissues throughout the body.

Klein-Nulend and her team observed that bone cells secreted prostaglandins in response to mechanical stress induced in pulsating fluid flow experiments [87]. Research findings continue to support the significant role of prostaglandins in bone homeostasis, particularly the E and F series of prostaglandins [60, 88–91].

Research performed in the early part of this century provided evidence that in addition to their function as a sensory and responsive cell, osteocytes also serve to help regulate bone density through the secretion of sclerostin, a protein that inhibits bone formation [92]. Their work tested the hypothesis that the dysregulation in bone formation resulted from phenotypes observed in osteosclerosis patients. This hypothesis was further supported through genetic testing and the development of transgenic mice with increased sclerostin production and low bone mass. Since these explorations into the biochemical nature of bone cells, continued research by a number of biomedical scientists including Bonewald,

Johnson, Dallas, Karsenty, and Yamashita continue to provide evidence in support of osteoblast/osteocyte-secreted factors that impact not only bone homeostasis but also distant tissues such as the brain, heart, kidney, prostate, and muscle.

Fibroblast growth factor 23, or FGF23, is a bone-derived protein that has been identified as integral to vitamin D metabolism and the regulation of systemic phosphate levels [93]. In their 2012 review, Bonewald and Wacker discussed FGF23 expression in osteocytes and its role in cardiovascular health [94]. This has found additional support from research performed on transgenic mice phenotypes. Although the exact pathways are not fully understood, it appears that osteocyte expression of FGF23 is under the influence of molecules such as DMP1, PHEX, and MEPE [95]. Osteocalcin, which is also known as bone gamma-carboxyglutamic acid-containing protein (BGLAP) is a protein found, as the name implies, in bone and dentin. Osteocalcin helps to provide structure and has been shown to also play a part in energy metabolism, calcium ion homeostasis, and male fertility [96]. It was postulated more than 20 years ago, that bone cells are the primary source of osteocalcin [97], and recent advances in genetic engineering have helped support that idea [75, 96, 98, 99]. Osteocalcin, along with other hormone-like substances secreted by bone cells, are now thought to interact with substances from the liver and adipose tissue in a way that may predispose individuals to obesity, diabetes, non-alcoholic fatty liver disease, and osteoporosis.

The impressive list of bone derived factors continues to grow, and includes: ATP, calcium, DKK1, DMP1, FGF23, MEPE, Nitric Oxide, OPG, osteocalcin, prostaglandins (particularly PGE₂), RANKL, sclerostin, and SOST. These factors represent a myriad of biochemical structures ranging from simple organic molecules to complex proteins, all of which help illustrate the diversity and far-reaching impact of bone as an endocrine organ.

Muscle as an Endocrine Organ

Skeletal muscle represents the majority of muscle tissue in the body and is so named for its

functional connection and vicinity to the skeletal system. Also called striated muscle because of its appearance, skeletal muscle is under the control of the somatic nervous system and is responsible for voluntary movement, facial expressions, postural support, and respiratory expansion. Skeletal muscle develops from myogenic precursor cells and myoblasts. Myoblasts are small, mononucleated cells capable of either entering the cell cycle and proliferating, or fusing with other myoblasts to form myotubes. As myoblasts fuse and begin to form myotubes, they enlarge and take on an elongated shape (Fig. 4.6). Muscle cell proliferation and differentiation occur in the embryonic and early stages of development, and continue throughout the lifespan. Skeletal muscle is a dynamic tissue, whose cells undergo myogenesis repeatedly as muscles regenerate in response to injury [100, 101].

The process that leads to muscle contraction begins when acetylcholine (ACh) is released by a motor neuron across the synapse at the neuromuscular junction. Motor neurons originate in the central nervous system and the cell bodies of these neurons are located in the spinal cord. The neuronal fiber (axon) projects outside the spinal cord to directly or indirectly control muscles. At the muscle level, nerve-ending terminals spread and innervate each muscle fiber within a given skeletal muscle. A membrane called the sarcolemma covers each muscle fiber, and within each muscle fiber are thousands of sarcomeres, which are the functional units of contraction. The sarcomere is composed of thick myofilaments called myosin, and thin myofilaments called actin. The neuromuscular junction is a synapse with the terminal end of the motor neuron on one side and the motor end plate of a skeletal muscle fiber on the other. Release of ACh from the motor neuron causes stimulation of a muscle fiber through the exchange of sodium and potassium ions. This leads to the generation of an action potential that spreads along the sarcolemma and is transmitted into the interior of the muscle fiber by structures called transverse tubules, or T-tubules. T-tubules are juxtaposed to the calcium ion storage units, the sarcoplasmic reticulum (SR). As the action potential travels along the T-tubule, it causes the voltage-sensitive receptor named

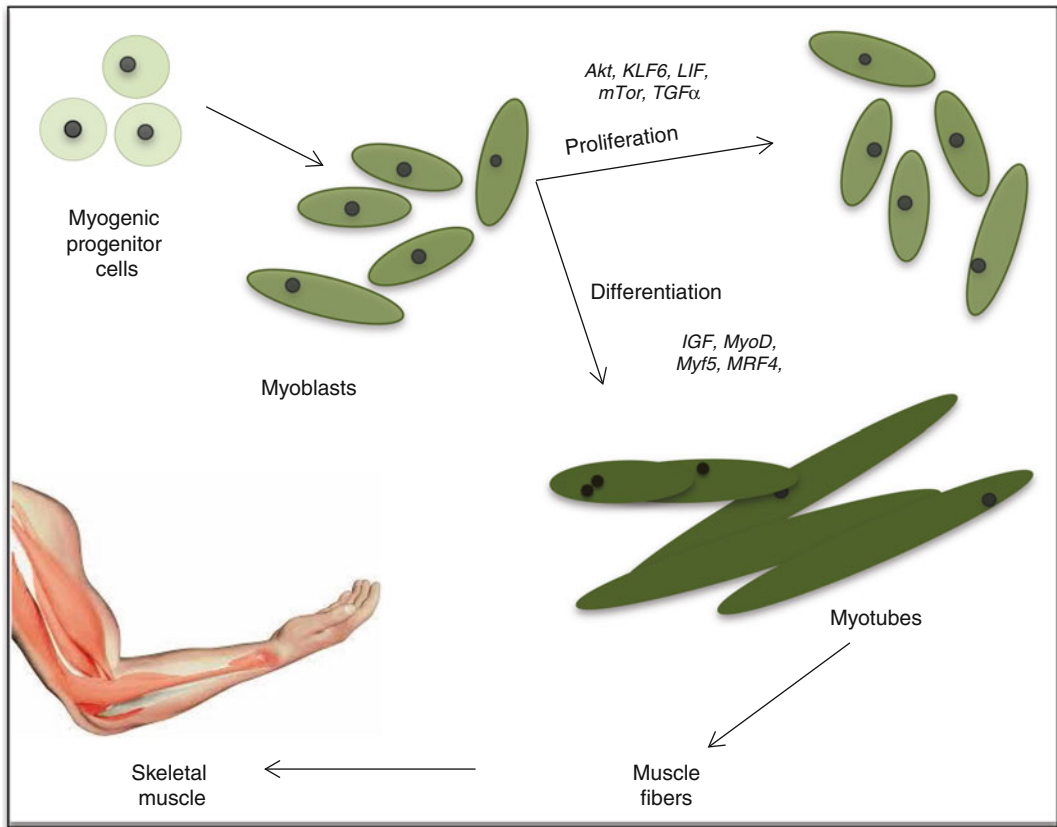


Fig. 4.6 Skeletal muscle cell myogenesis model (From Isaacson Dissertation – Myogenesis Model)

Dihydropyridine Receptor (DHPR) to change shape, and it is this allosteric modification of the DHPR that allows it to physically interact with the largest known mammalian channels, the Ryanodine Receptors (RyR) precisely located on the surface of the membrane of the SR. This DHPR-RyR contact, leads to the opening of the RyR, which brings about the release of calcium from the SR into the cytosol of the skeletal muscle cell. This rise in cytosolic calcium causes the binding sites on the actin filament to be exposed, allowing myofilaments heads to bind. The myosin filaments pull the actin filaments in, resulting in a shortening of the sarcomere. It is the shortening of sarcomere throughout the muscle fibers that causes muscle contraction. The process by which the electrical stimulation, or excitation is transferred into a mechanical contraction is called the excitation-contraction coupling (ECC) and is fundamental to skeletal muscle physiology [102–104]. The ECC is the cellular and

molecular reason that we can execute from very fine controlled movements to the lifting of several hundred kilograms of weight.

The functional role of skeletal muscle to move and support the body has long been realized; it has only been recently that endocrine-like function of skeletal muscle has begun to be appreciated. Research associated with skeletal muscle secreted factors began primarily in relation to those produced in response to injury. For example, several myokines, prostaglandin, IL-6, and LIF, have been shown to enhance the myocyte differentiation after injury [105–108]. Muscle regeneration is an ongoing phenomenon throughout the life span, and provides an excellent opportunity for investigation into the endocrine function of this organ, as well as hope for targeted interventions to slow the process of muscle wasting. Two additional factors secreted by injured skeletal muscle are TGF α and TGF β 1 [106, 109]. These myokines

have an inhibitory effect on muscle cell proliferation and differentiation.

In addition to raising serum levels of IL-6, exercise has been found to induce a six-fold increase in mRNA of Chemokine CXC motif ligand-1 (CXCL-1) and a 2.4-fold increase in serum CXCL-1. A functional homolog for IL-8, murine CXCL-1, belongs to a group that has gained attention for its role in inflammation, chemotaxis, angiogenesis, neuroprotective activity, and tumor growth regulation and is associated with a decrease in visceral fat [110].

Obesity and type 2 diabetes have both reached epidemic proportions worldwide. As the body of knowledge related to myokines continues to expand, researchers are investigating the role of certain myokines, or more accurately the lack thereof, because of limited exercise and the possible connection to these chronic diseases [110]. Recently, a new myokine brought hope for the development of molecules to target fat tissue accumulation, since irisin was shown to regulate the conversion of 'bad' (white) fat into 'good' (brown) fat that is essential for thermogenesis in mice [111]. Since the original publication, 49 papers have been published on the effects of irisin. A recent study by Park [112] concluded that irisin might be directly associated with a higher risk of cardiovascular diseases and metabolic syndrome in humans, suggesting that augmented secretion of irisin by either adipocytes or muscle cells might occur to overcome an underlying irisin resistance, similar to the hyperinsulinemia seen in insulin resistance associated with obesity [112].

The list of myokines continues to grow, and includes IL-8, which has been shown to increase angiogenesis [113]; IL-5, which is an anabolic factor being investigated for its role in muscle-fat crosstalk; IL-7, which is being studied for its impact on satellite cells during myogenesis [114]; and brain-derived neurotrophic factor (BDNF) [115]. With a deeper understanding of skeletal muscle as an endocrine organ comes the promise of innovative approaches to the prevention and treatment of diseases and disorders throughout the body.

Interplay Between Bones and Muscles as Endocrine Organs

The conditions are determined in utero for the connection between bones and muscles, as they share a common mesenchymal precursor and experience organogenesis through a tightly orchestrated network of genes during intrauterine development. As mentioned, their anatomic proximity lends credence to the hypothesis that bones and muscles influence each other in a paracrine nature. Evidence of such a relationship exists as pathologic conditions are studied; specifically, some of the bone stress syndromes where inflammation localized to the muscle area underneath the periosteal region spreads into the bone itself. These situations support the paracrine relationship hypothesis, suggesting inflammatory molecules from adjacent muscle fibers may penetrate into this region of the bone. Another powerful clinical example of this paracrine relationship is the aforementioned muscle flap application to compounded bone fractures. The effect of this therapeutic approach is significantly faster healing for these fractured bones. Although the specific molecular mechanism of action is not completely understood, the introduction of muscle flaps has been used as a successful therapeutic approach to treat chronic osteomyelitis and to accelerate the healing of bone fractures [116]. These mechanisms might display further importance for bone and muscle healing after musculoskeletal injury.

Experiments performed using osteocyte and muscle cell lines have revealed that PGE₂ secretion from osteocytes is more than 1000 times greater than PGE₂ secretion from muscle cells. This excess amount of PGE₂ from osteocytes could interplay with injured muscles, which would aid in muscle regeneration and repair. Intriguingly, recent *in vitro* studies have provided support for a role of osteocyte secreted PGE₂ in aiding with the process of myogenesis [89]. While these studies were originally performed with the myogenic cell line C2C12, as shown in Fig. 4.7, PGE₂ signaling is also a potent stimulator of myogenic differentiation in primary myoblasts/myotubes.

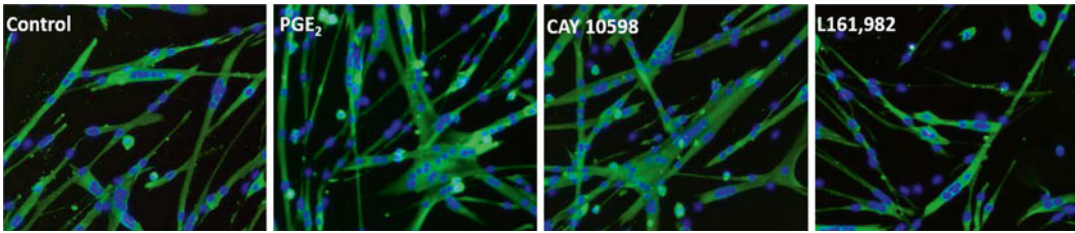


Fig. 4.7 PGE₂ signaling modulates myogenesis of primary myoblast/myotubes. Representative images following treatment with PGE₂, EP4 agonist CAY10598, and EP4 inhibitor L161,982 in 5-month old mouse primary myotubes, showing the same pattern previously published in C2C12 cells in Mo et al. [89]. Ingreen, myosin heavy chain, MHC and

inblue: DAPI. Unpublished results by the Brotto Lab (Mo and Brotto Unpublished Results, 2015). It is evident that PGE₂ and CAY 10598 promote myogenesis while L161,982 inhibitsit. PGE₂ is a major osteokine secreted by osteocytes and one of the mediators of the bone-muscle crosstalk [89] (From Mo and Brotto Unpublished Results, 2015)

Recently developed transgenic animal models provide an excellent opportunity for researchers to gain further insight into this bone-muscle crosstalk, as in the case of the myostatin-deficient mouse. Myostatin, which was discussed earlier with regard to its pleiotropic characteristics, was discovered in the late 1990s to be a potent inhibitor of muscle growth. It is expressed during development and in adult skeletal muscle, serving as an important negative regulator of skeletal muscle growth [45, 117]. Myostatin appears to decrease myoblast proliferation. The myostatin-deficient mouse model has increased muscle size and strength, with individual muscles weighing significantly more than wild type mice [118]. Hamrick used this myostatin-deficient mouse model to investigate the effects of increased muscle mass on bone mineral content and density. He and his team found that although a consistent correlation was not found in all regions of the skeletal system, there was increased cortical bone mineral density in the distal femur and an increased periosteal circumference along the humerus [49, 119–121]. Another group used the same myostatin-deficient mouse model to look at the impact of the chronic loss of myostatin on multiple organ systems and found that it appeared to preserve bone density [122]. From a contrasting perspective, Zimmers investigated the effects of myostatin overexpression in an animal model and observed a profound loss of muscle and fat, mimicking the presentation seen in chronically ill

patients and commonly referred to clinically as cachexia [118]. Further research into the disruption of myostatin is a worthy direction in an effort to preserve muscle mass in patients with chronic diseases.

As mentioned in a previous section, osteocalcin serves as a splendid example of bone as an endocrine organ [123]. This osteoblast-derived factor, circulating levels of which increase with exercise, binds to the GPRC6A receptor, affecting distant adipocytes and pancreatic β cells. Interestingly, osteoblasts also naturally express the osteo-testicular phosphatase gene (*Esp*), which inhibits the function of osteocalcin [69]. With this information in mind, it is of specific interest to the discussion of bone-muscle crosstalk that the *Gprc6a* knockout mouse displays the phenotype of decreased muscle mass, while the *Esp* knockout mouse has increased muscle mass. Through these observations, it can be proposed that osteocalcin, a known bone cell factor, may play a role in the regulation of muscle mass. This is the type of knowledge that promises a deeper understanding of sarcopenia; as osteocalcin could be a target for the development of therapies to prevent, delay, or slow the progression of this highly prevalent disorder associated with aging. If this knowledge is useful for sarcopenia, it is possible that it may also be useful for its associated disorder, osteoporosis.

The endocrine communication that continues to be revealed through effects of factors derived

from these tissues is not limited merely to a bone-muscle connection. There is a growing awareness that the factors secreted from tissues throughout the body impact the overall health of the individual. The significance of this dynamic interrelationship is becoming more apparent with the aging of the world's population and the concomitant rise of chronic diseases such as obesity, diabetes, and metabolic syndrome. According to the Centers for Disease Control and Prevention, the prevalence of obesity in the United States among adults 65 years of age and older is nearly 35 %; translating into more than 8 million older adults [52]. The American Diabetes Association website reports that nearly 25 % of adults aged 60 and over have diabetes, and it is also becoming clear that the prevalence of metabolic syndrome increases with age [62]. Recognizing the overwhelming implications for public health posed by sarcopenia alone, a distinguished team of researchers called for increased research investigating the factors involved in the pathogenesis of sarcopenia more than a decade ago [124]. Data from many studies published around that same time began to suggest that sarcopenia influenced the development of other chronic conditions such as cardiovascular and metabolic diseases. Since then, more evidence has been uncovered that sarcopenia is often linked to dyslipidemia, insulin resistance, and hypertension as well as a decline in immunologic function [71, 125].

As the understanding of the role of bones and muscles as endocrine organs, and insights into bone-muscle crosstalk begin to be translated into meaningful and innovative therapeutic approaches, unprecedented advances will be achieved in the fight against chronic diseases such as obesity, diabetes, osteoporosis, and sarcopenia.

Musculoskeletal Diseases: The Special Cases of Osteoporosis and Sarcopenia

People are living longer than any time in history due to the many advances in healthcare. In 1900, the life expectancy was only 47 years. By 1930, it increased to 60 years, and by the early 2000s, the

life expectancy from birth had risen to more than 75 years. As the life expectancy climbs, there is a parallel increase in the percentage of the population aged 65 and older. Again, looking just back in time to 1900, only 4 % of the population was 65 and older, but that percentage nearly tripled to 13 % by 2008, and is projected to increase to an unprecedented 22 % by 2030 [126]. This has been reported as the largest demographic shift in history by experts in fields from finance to sociology [127, 128]. Many factors have contributed to this increased life expectancy, including the development of vaccines and antibiotics, improved nutrition, and processes to better the accessibility of clean water to more of the world's population.

Unfortunately, this trend of increasing age is accompanied by a corresponding increase in disability as aging adults experience a decline in physical functioning. The decline in functional reserve has been well documented throughout scientific and popular literature [129]. Nearly 300 diseases and injuries appear on the Global Burden of Disease (GBD) list, and a staggering 289 of those are known to cause disability. In the 2010 Global Burden of Disease (GBD) Study, researchers reported that the years lived with disability (YLD) per 100,000 people remained relatively constant over the years, until recently. With the increasing population of those who are 65 years of age and older, the YLD numbers have dramatically increased [130]. The seemingly undeniable fact of mortality is that aging is associated with the decline in function of nearly every system in the body along with the development of chronic conditions. Chronic diseases and disorders have a tremendous impact on individual health and healthcare expenditures, which has motivated much research into the prevalence of chronic conditions. Reports align and reveal that as many as 82 % of the older population in the U.S. has one or more chronic health conditions [131–133].

One of the body systems that experiences significant changes with aging is the musculoskeletal system. According to the 2010 Global Burden of Disease Study (GBD), musculoskeletal diseases are the second greatest cause of disability, affecting billions of people worldwide [130].

Disability leads to injury and a decline in function and independence. Rubenstein and Josephson reported that one in three community-dwelling older adults falls each year [134]. Fall-related injuries were responsible for more than two million Emergency Department visits and nearly 600,000 hospitalizations among older adults in 2009 [135, 136]. One of the most frightening injuries for an older adult to experience is a hip fracture. Ninety percent of hip fractures are the result of a fall, and the mortality rate 1-year post hip fracture is an astounding 25 %. It would seem the fear surrounding this injury is justified as only one in two older adults that experience a hip fracture return to their baseline level of activity [136]. There are many risk factors for falls, but primary among them is muscle weakness. The associated morbidity and mortality, especially hip fractures, is greatly increased among older adults, and is a significant health risk for those with osteoporosis. In addition to other chronic diseases and medication use, the decline in musculoskeletal health and function is a growing problem [74, 137, 138]. Loss of muscle mass and strength not only increases the individual's risk of falls, it impacts quality of life. Depp and Jeste reported that in the majority of cases, the very definition of "successful aging" is predicated by the absence of disability [73].

The musculoskeletal changes that occur not only impact the aging individual, they create a profound economic burden. Looking at sarcopenia alone, the healthcare costs in the year 2000 were \$18.5 billion, which represented 1.5 % of the nation's total direct healthcare costs that year [139]. That percentage would translate into more than \$40 billion a decade and a half later. Drawing from the 1995 Report of the National Osteoporosis Foundation [140], and adjusting for inflation and other factors to make the dollar values consistent, the same authors reported costs associated with osteoporotic fractures in the year 2000 to be \$16.3 billion. Included in this price tag is the cost of inpatient care, nursing home care, outpatient care, emergency room visits, radiology services, orthopedic medical supplies, and outpatient medications. Even with all these aspects factored in, these costs may be conservative, considering that

the United States Center for Disease Control and Prevention website, updated in September of 2013, reports the direct medical costs associated with fall-related injuries among the older adult population was \$30 billion in 2010, and is projected to climb to nearly \$55 billion by the year 2020 [141].

Osteoporosis

Osteoporosis is the most common metabolic bone disorder and is characterized by the progressive loss of bone mass. Bones are in a constant state of destruction and rebuilding, and in young, healthy individuals, the balance between bone formation and resorption is maintained. The decrease in bone density that has come to be known as osteoporosis appears to be the result of a growing imbalance of these two processes. The body of knowledge surrounding osteoporosis is growing as it is now recognized as the most common metabolic bone disease in the United States.

Evidence exists that the phenomenon of age related bone loss has afflicted mankind for centuries [142]. An English surgeon, Sir Astley Cooper was one of the first to document the changes he observed in bones of older adults: "With respect to the neck at the thigh-bone, a very principal cause of non-consolidation by bone is the advanced age at which it becomes obnoxious to fracture through that peculiar change which the part undergoes at this period of life without any apparent cause, but which renders it incapable of sustaining the superincumbent weight, and even in continuity insufficient to maintain its function; therefore it may be fairly supposed, when broken incompetent to set up a restorative action" [70]. The term, *osteoporosis*, is attributed to the French pathologist, Jean Lobstein, who used the term in a paper describing autopsy findings of holes in bones associated with fragility. In retrospect, it seems likely that he was actually using the term to describe osteogenesis imperfecta rather than osteoporosis [143].

Many definitions of osteoporosis have been offered over the years, with the most widely accepted being an operational definition based

upon bone mineral density (BMD). And, the most widely validated measure of BMD is dual energy X-ray absorptiometry (DXA). According to the World Health Organization (WHO) criteria, osteoporosis is defined as a bone mineral density (BMD) that falls greater than or equal to 2.5 standard deviations (SD) below the average value for young healthy women [144]. In recent years, there has been a growing debate about the use of DXA scan results. This diagnostic measure has proven to have high specificity, but low sensitivity; which means that a patient with a T-score below -2.5 has a high risk of fracture, but the patient with a T-score greater than -1.0 , or within normal limits, is not immune to fracture. Clinicians and researchers have learned that many factors must be considered in determining the risk of an osteoporotic fracture in the aging patient.

Women are at greater risk of osteoporosis than men. Additional risk factors for osteoporosis include increased age, Caucasian or Asian ethnicity, postmenopausal status, late menarche or early menopause, low peak bone mass, a family history of osteoporosis or low trauma fracture, low dietary calcium, vitamin D and vitamin K, low levels of physical activity, smoking, excessive alcohol intake, and the use of certain medications such as steroids, anti-convulsants, immunosuppressants and heparin [72, 145–148]. Health care providers must consider all of these as they contemplate preventive and treatment options for their aging patients.

Treatment plans for those diagnosed with osteoporosis are related to these identified risk factors. The initial approach includes lifestyle modifications such as increased physical activity if possible, smoking cessation, and decreasing alcohol intake [149]. Beyond these lifestyle changes to reduce the risk of injury, treatment goals are aimed at slowing or stopping bone loss and/or facilitating bone formation. Supplementations often recommended include calcium, vitamin D, and, in some cases, hormone replacement therapy [150]. The primary pharmacologic intervention are antiresorptives. These pharmacological approaches are reviewed in depth in Chap. 12 of this textbook.

Sarcopenia

Skeletal muscle accounts for 38 and 31 % of the total body weight in men and women, respectively, and represents the largest single organ in the human body [151]. Thus, it is reasonable that the age-related anatomical and physiologic changes in skeletal muscle have a significant impact on the overall health of the individual. Irwin Rosenberg first proposed the term ‘sarcopenia,’ in 1988 to describe age related muscle wasting [152]. The term derives from the two Greek words, *sarx* (flesh) and *penia*, (loss). Because all individuals experience muscle wasting with age, the prevalence of sarcopenia with age is essentially 100 %. However, Rosenberg and others recognized that in many, the muscle loss that accompanied aging happened at a seemingly accelerated rate and contributed significantly to disability. In 1988, a group of researchers and clinicians convened for a meeting in Albuquerque, New Mexico to discuss various measurements used to assess the health and nutritional status of the elderly population. It was in a summary report following that historic meeting that Rosenberg first coined the term, ‘sarcopenia.’ He stated at that time his motivation for coining the term was to draw attention to this all too common disabling physiologic phenomenon. With a name came an increase in research conducted into the process and effects of age-related skeletal muscle wasting. And, in a reciprocal fashion, as the body of knowledge related to sarcopenia grows and develops, so grows the acceptance of sarcopenia as a condition with specific and measureable signs and symptoms. It is now recognized that sarcopenia has significant implications for the lives of individuals, for the nation and for the world.

In the mid-1990s, the simple measurement used to identify sarcopenia among older adults was the upper-arm circumference. Even with this crude method of identification, researchers began to see a correlation between the presence of sarcopenia and the older adults’ mortality risk [153, 154]. In 1998, Baumgartner and his team suggested a modified approach to determining whether the muscle mass in an older adult was within normal limits, or whether it reflected a state

of compromised health, i.e. sarcopenia. From the research he and his colleagues were conducting on older adults in New Mexico, Baumgartner defined sarcopenia as a height adjusted to muscle mass of two standard deviations (SD) or more below the mean of a young reference population. With this as his measurement, he demonstrated the increasing prevalence of sarcopenia with aging. The prevalence of sarcopenia in the New Mexico Elder Health Survey was 14 % in those 65–69 years of age, compared with greater than 50 % in those 80 years of age and older [61]. Other researchers in the field of gerontology adopted this measurement standard in their investigations of age-related loss of muscle mass. The consistent use of measurements allows for more meaningful comparisons of research findings between studies.

Nearly all of the studies conducted prior to 2005 were cross-sectional, and focused on a correlation between sarcopenia and decreased muscle mass and the associated functional impairment leading to physical disability [124, 155–158]. At least one study in the early 2000s looked at the impact of muscle size and strength over time and reported a less than 5 % change in strength attributable to a corresponding change in muscle size [159]. In an 8-year follow up to the Cardiovascular Health Study, Janssen et al. [139] reported a 27 % increased risk of developing disability with sarcopenia when compared with individuals with normal muscle mass. Interestingly, at the beginning of that same study, the reported likelihood of having disability was 79 % greater in those with severe sarcopenia than in those with normal muscle mass. The longitudinal analysis was three times smaller than the cross-sectional analysis, reported at the baseline. This underscores the importance of not drawing conclusions too early in an investigation of data, and suggests that the sarcopenia-associated risk of functional impairment and physical disability reported in cross-sectional studies of older adults in the early 2000s may have been overestimated.

Questions surrounding a universally accepted definition of sarcopenia continue as clinicians and researchers search for specific age-related musculoskeletal changes that correlate most strongly with the risk of disability. Evidence is growing that

the rate at which muscles become weaker is much faster than the rate at which they become smaller [160]. Research findings are supporting that it is the loss of muscle strength, even more than the loss of muscle mass, that carries the greatest risk of disability in the aging adult [102, 160, 161].

Some researchers refer to the ‘bone-muscle unit’ in deference to observations that bones respond to varying levels of mechanical strain imposed by muscle mass and strength. The varying levels of mechanical strain appear to be modulated primarily by hormonal effects systemically, citing gender differences over time as evidence [62]. There is undeniably much evidence in support of the strong correlation between bone and muscle strength [163, 164].

A deeper understanding of the physiological relevance of these bone and muscle endocrine properties may serve to bridge the gap between the mechanical and biochemical theories of bone-muscle interaction. A feasible way of interpreting the role of these interactions is that they may serve to sense and transduce biomechanical signals such as unloading, loading, inactivity, or exercise, and even perhaps the translation of systemic hormonal stimulation into effective biochemical signals. Another way of interpreting and bridging these two theories is that one specific form of interaction could work as a priming for the other, in that, the physical effects of contraction on bone cells may prime these cells for the simultaneous, consecutive or ulterior effects of a secreted molecule. The growing evidence of a mismatch between changes in muscle mass and muscle strength that accompany muscle unloading also lends support to the biochemical communication between tissues [66]. The suggestion that in addition to mechanical force, other factors contribute to increasing muscle strength came more than three decades ago. In their work with isometric training, McDonagh and colleagues made experimental observations that led them to postulate “that the increase in the force of maximal voluntary isometric contraction must be related to factors other than the force-generating capacity of the muscle fibres themselves” [165].

In his work with the New Mexico Elder Survey, Baumgartner observed that, as many as 15 % of

individuals with sarcopenia are also obese. The sarcopenic-obese older adult captured his attention because in his cross-sectional study examining older adults in the New Mexico Aging Process Study, he found this subsector of the elderly population to be at especially high risk of physical disability [166]. The decline in both lean muscle mass and bone density poses an even greater risk to independence and safety of the older adult. The appreciation of this risk has inspired research into this phenomenon. Findings of such research suggest an increase in catabolic cytokines such as interleukin-6 (IL-6), as well as inflammatory markers such as C-reactive protein and sedimentation rate [157, 167]. Interestingly, many of these same factors observed in the development of obesity are secreted by adipocytes [168, 169].

In 2004, Baumgartner and his team set out to replicate as a longitudinal study, the findings of their 2000 cross-sectional study, based upon the same cohort of individuals in New Mexico [170]. Looking at the problem over time, they found that neither sarcopenia alone nor obesity alone increased the older adult's risk of functional impairment when compared with those with a normal body composition. Sarcopenic-obese individuals, however, had a 2.5 times greater risk of functional impairment [170]. A partial explanation for this could be related to the decreased resting metabolic rate that corresponds to the reduced skeletal muscle mass of sarcopenia [171].

Since the mid-2000s, several researchers have investigated the combination of sarcopenia and obesity, specifically concerned with the risk for physical disability. The findings of some researchers support an increased risk of physical disability in sarcopenic-obese older adults [172, 173], while the findings of other researchers do not support this notion [65, 174]. Of interest is the recognition that between research groups, and even within some research groups, conflicting results are obtained with regard to the question of sarcopenic-obesity and the increased risk for physical disability. It is possible that these conflicting results are the consequence, in part, of having no single definition of sarcopenia that is widely accepted within the research and clinical arenas. This experience should provide ample motivation to come to a consensus on the matter.

Attempts to understand the cause of an age related loss of muscle mass and strength have predominantly focused on the loss of skeletal muscle fibers, especially type II fibers. In recent years researchers have begun to delve into a wide variety of mechanisms involved in the pathophysiological mechanisms of sarcopenia including nutritional factors, activity levels, alterations in protein metabolism, and the impact of changing levels of hormones. Walrand and colleagues published a review that shed light on what many had begun to observe; suggesting that sarcopenia impacts the development of other chronic conditions such as cardiovascular and metabolic diseases [175]. Sarcopenia is beginning to be linked to dyslipidemia, insulin resistance and hypertension as well as a decline in immunologic function [71, 125].

Gaining momentum is yet another element to this issue; that age-related decreases in muscle strength result from a combination of loss of muscle mass (atrophy) and reduced muscle specific force (i.e., muscle force per unit of cross-sectional area), suggesting reduced muscle quality. Data is gathering to show that it is principally the weakness that accompanies sarcopenia, not the loss of muscle size *per se* that contributes to disability [62, 159, 161]. It is possible that the disproportionate loss of force and power compared to loss of muscle mass originates inside the muscle fibers themselves, due to defects on the excitation-contraction coupling (ECC) process that ultimately lead to reduced availability of calcium to be released during each cycle of contraction-relaxation [66, 102, 176]. Reflecting on the physiology behind muscle contraction, it is reasonable to postulate that factors released from distant tissues could influence the steps leading up to skeletal muscle contraction. The mismatch observed between muscle mass and strength, might at least in part, be explained by other tissue factors that influence the EC coupling, such as reduced calcium release from the sarcoplasmic reticulum (see Fig. 4.8).

It has also been noted that store-operated calcium entry is reduced in aged muscle cells. In a recent review, the argument is made that there is most likely a link between dysfunctional intracellular calcium homeostasis, the newly discovered modulatory genes, and biochemical

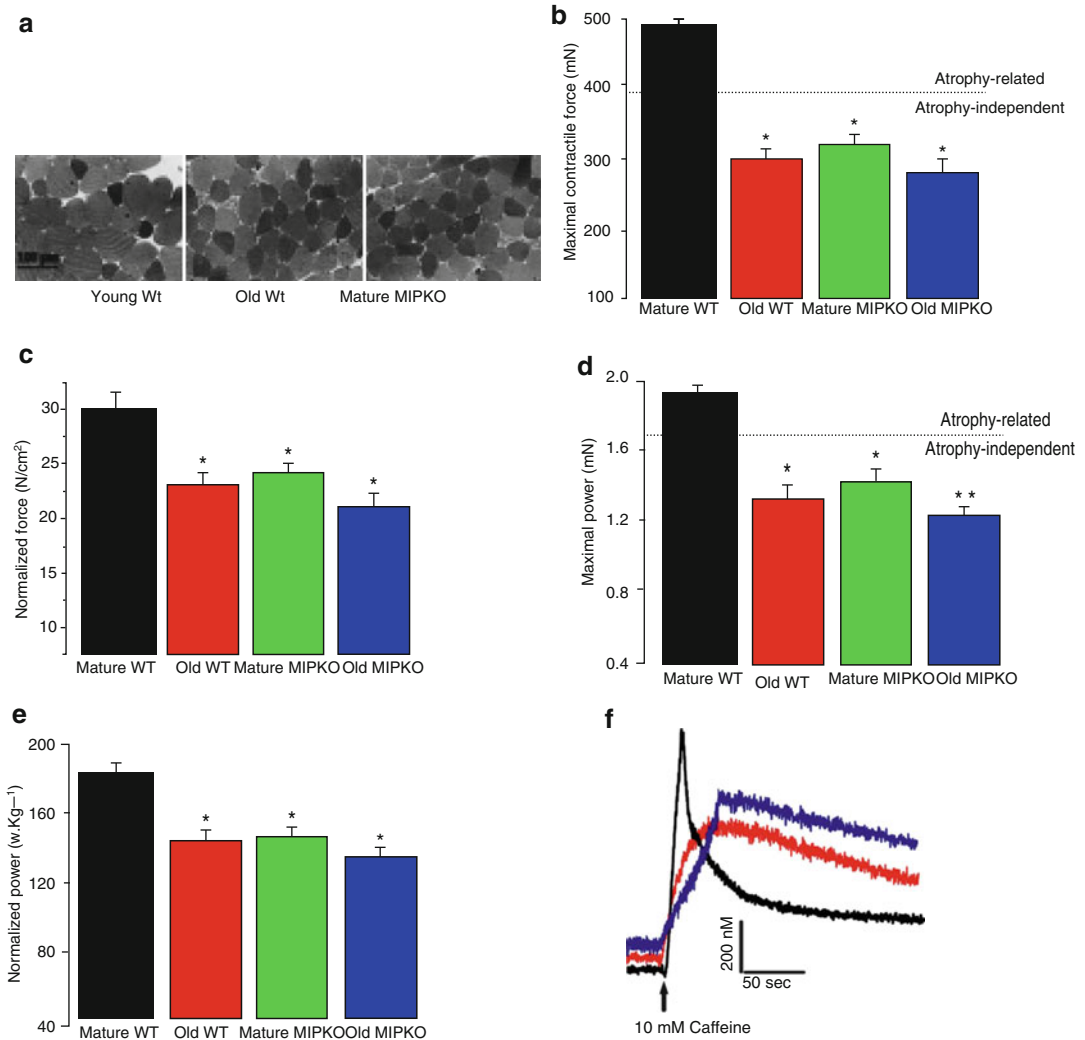


Fig. 4.8 Evidence for muscle atrophy, decreased contractile force, and reduced power in skeletal muscles suggested similarity from old WT and MIPKO mice. In all figures, the black bars are mature, wild type mice, the red bars are old, wild type mice, the green bars are mature MIPKO mice, and the blue bars are old, MIPKO mice. (a) Typical Toluidine blue-stained cross sections of EDL muscles from young Wt, old Wt, and mature MIPKO mice. The cross-sectional areas of old Wt and MIPKO cells are significantly reduced compared with those of the young Wt. (b) Maximal contractile force in EDL muscle for each genotype. Atrophy (decrease in muscle cross-sectional area) can explain ~1/2 of the drop in total force (note the dotted horizontal line), but does not account for

the complete decrease in contractile force. (c) Data from b, except that force is normalized per cross-sectional area (N/cm²). This figure illustrates the atrophy-independent component of contractile dysfunction. (d) Maximal power in EDL muscle from all four animal models. (e) Data from panel D was normalized per cross sectional area of muscles. It shows that a significant drop in power is atrophy-independent. Data is the average ± SE of 24 EDL muscles from 12 mice for each genotype. * indicates a significant difference (p < 0.01) between the control muscles and a particular genotype. ** indicates a significant difference (p < 0.01) between the old MIPKO mice and the old Wt and mature MIPKO mice (FROM Romero-Suarez et al. [176])

factors secreted by other body tissues, which is illustrated in Fig. 4.9.

Additional health risks experienced by sarcopenic older adults include insulin resistance and

the development of type 2 diabetes mellitus. Srikanthan, Hevener, and Karlamangla conducted a study to investigate the relationship between sarcopenia, obesity and age-related insulin

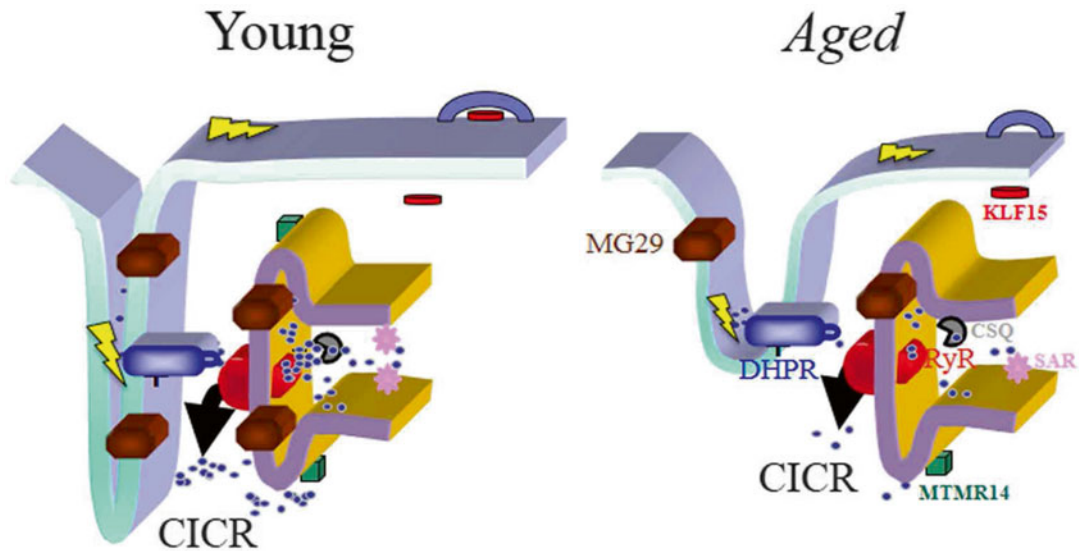


Fig. 4.9 Schematic drawing of the triad junction, the chief site of the E-C coupling process in skeletal muscles. The predicted localization of the four genes/proteins emphasized in this review article is shown and they are represented in different colors along with the Dihydropyridine Receptor (DHPR), the Ryanodine Receptortype1 (RyR1) and Calsequestrin (CSQ). In young muscles E-C coupling is effectively maintained through coordinated actions of the E-C coupling machinery and the optimal participation of MG29, MTMR14, SAR, and KLF15. Their concentration and/or effectiveness is reduced with aging, which associates with structural changes of the triad junction itself. Together these biochemical and morphological changes contribute to the reduced coupling between depolarization of the sarcolemma and contraction due to the reduced calcium release capacity of aged muscles. In summary, “E-C coupling quality” is reduced in aged muscles, and becomes a key factor to reduced muscle quality during aging. The steps of the E-C coupling process are described in detail in the text. In skeletal muscles, depolarization of the sarcolemma and its invaginations (t-tubules) represented by the lightning bolt in yellow color alters the configuration of the DHPR, which modifies its interaction with RyR1, leading to the dominant type of calcium release in skeletal muscle (depolarization-induced calcium release, DICR).

resistance. In their cross-sectional analysis of the National Health and Nutrition Examination Survey III (NANES III), they concluded that sarcopenia, independent of obesity, is associated with compromised glucose metabolism [177]. In support of that analysis, another study conducted around the same time concurred that type 2 diabetes was associated with an increased risk of sarcopenia [178]. A relationship between diabetes and sarcopenia certainly makes physiologic

This initial release phase can be further amplified by a secondary mechanism, calcium-induced calcium release (CICR), the main release mechanism in cardiac muscles. The structural deformation as well as the lack of organized triads is a hallmark of aged muscles and also common in other diseases covered in this article. Not detailed in this figure is the process of calcium entry or re-entry, store-operated calcium entry (SOCE), responsible for continual refilling of the sarcoplasmic reticulum (SR). SOCE is also reduced with aging, which we have postulated contributes to sarcopenia and to the un-matching between muscle mass and muscle contractile force during aging, since force/power decrease significantly more than the observed decrease in muscle mass. We foresee that new generations of drugs could be developed to specifically target the different steps of E-C coupling in disease states to increase efficiency of Ca²⁺ handling. F. Altered Ca²⁺ homeostasis is present in muscle fibers from old Wt and MIPKO mice. Original traces representative of caffeine-induced Fura-2 Ca²⁺ transients in mature Wt (*black trace*), old Wt (*red trace*), and mature MIPKO FDB muscle fibers (*blue trace*). Examples shown are representative of 6–12 muscle fibers from 3 mice, and data were normalized to the intracellular Ca²⁺ concentrations in nM (From Manring et al. [102], doi:10.3389/fphys.2014.00037. eCollection 2014. Review)

sense from the perspective that skeletal muscle represents the largest target tissue for insulin-mediated glucose uptake. A decline in muscle mass with aging is, therefore, associated with a decrease in sites for glucose uptake, which would be further exacerbated by a decline in physical activity. Along with this, data supports an increase in triglycerides with aging, which have been indicted both in age-related mitochondrial damage and with blocking of ability of insulin to

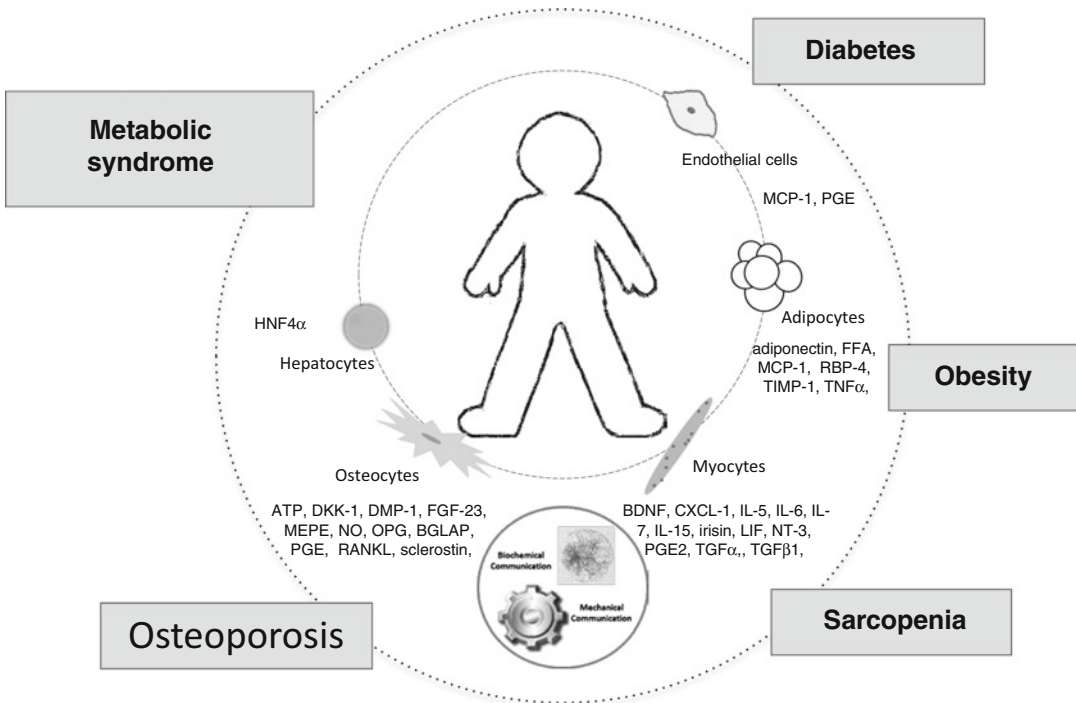


Fig. 4.10 Bone-muscle crosstalk, interactions with other tissues, and impact on chronic diseases. This original drawing illustrates the concept that interactions among different tissues throughout the organism are abundant and much more complex than previously realized. In this larger context, bone–muscle cross talk remains both physiologically and pathologically relevant but is also seen as being affected by other tissues of the body. At the center of this figure is the outline of an individual, the patient. The smaller circle, closest to the patient, lists cells discussed in the text, along with factors they are known to secrete. The dashed line connecting these cells indicates

that they are connected biochemically through the impact that their secreted factors have on one another. The larger circle surrounding the patient lists a number of conditions and diseases impacted by the biochemical interactions between cells listed and others. Special significance for multi-tissue/organ cross talk is revealed by pathological conditions such as obesity, diabetes, and metabolic syndrome. The dotted line of this larger circle indicates the developing understanding that these conditions and diseases impact one another. These conditions seem to directly influence sarcopenia and osteoporosis as detailed in the text (From Isaacson and Brotto [182])

facilitate glucose entry into the muscle cell. All of these phenomena contribute to an increase in blood glucose. Insulin is a potent anabolic hormone that impacts glucose, protein and lipid metabolism, and may play a significant role in all of this as well. It facilitates glucose uptake, inhibits hepatic glucose uptake and triglyceride production, inhibits skeletal muscle protein synthesis and inhibits adipose tissue lipolysis [179]. Recognizing this relationship, research conducted by Lee et al. provided data supporting a direct relationship between insulin resistance, the loss of lean muscle mass and the gain of fat mass in men aged 65 and older [180]. The chronic complications of diabetes mellitus affect systems throughout the body; including bones. Individuals

with type 1 diabetes mellitus have lower bone mass density, with impaired bone formation believed to be the primary cause [181]. Patients with type 1- or type 2-diabetic patients experience hypercalciuria during times of glycosuria. This increased loss of calcium has been hypothesized to contribute to impaired bone quality observed with diabetes, although the direct effects of this loss of calcium on skeletal muscle function remains elusive. As more is understood about these chronic conditions, the connections between them are becoming undeniable. Recognizing these connections and conducting research from this multifactorial perspective will deepen understanding and further the development of meaningful interventions (Fig. 4.10).

The Role of Cartilage, Ligaments, and Tendons in Bone-Muscle Crosstalk

Crosstalk in MSK Development

As previously mentioned in this chapter, tissues of the musculoskeletal (MSK) system have been traditionally studied as individual and autonomous entities, in spite of being functionally intimately associated. This is especially true for bones, muscles, and tendons, which are commonly referred to as the *musculoskeletal unit*. This interrelationship is more noticeably characterized by a biomechanical interdependence, which is essential for body movement. Tendons, in most cases, connect muscles to bones, as muscles contract and shorten, they pull tendons, which in their turn pull bones, resulting in movement. This way, a weakness in any component should imply in loss of functionality of the whole unit. In addition to bones, muscles, and tendons, other tissues are also part of the MSK system, such as ligaments, which are responsible for bone to bone connection and essential for joint stability, and cartilaginous tissues. At the articular ends of bones, hyaline cartilage is closely associated with bone tissue as they form cartilaginous joints.

More recently, the concept that the communication between MSK tissues may extend beyond their biomechanical relationship has been gaining momentum as novel evidence in this direction is acquired through new research. In this case, MSK tissues would *talk* by exchanging chemical factors, in a paracrine or even endocrine way, that is, factors produced by one tissue would exert a determined effect on another tissue of the MSK system. Earlier in the chapter, we presented evidence about this biochemical crosstalk between bones and muscles. However, what about other tissues of the MSK system? Is it reasonable to expect that they also communicate via chemical factors? Or, alternatively, do they merely fulfill an essentially mechanical function?

In order to answer these intriguing questions, first we turn our attention to the development of the MSK system, where the importance of a

crosstalk, biomechanical and biochemical, has been demonstrated. The events that characterize MSK embryologic development are quite complex and the objective of this section is not to present a comprehensive review of the topic, rather we will solely focus on findings that point to a biochemical crosstalk between tissues of the MSK system during development. Furthermore, we will restrict our discussion to vertebrates, more specifically using limb development as a model [184]. Muscles, tendons, and bones progenitor cells originate from different somites (i.e., divisions) of the mesoderm, respectively, the myotome, the syndetome, and the sclerotome. Two regions critical for the development of the MSK unit (muscle-tendon-bone): the myotendinous junction and the entheses, are specialized regions representing the muscle-tendon and tendon-bone interfaces, respectively. Correct assembly of these regions, which requires close interactions between these tissues, is crucial for the proper functioning of the MSK system.

Experimental evidence has shown that, in addition to the most flagrant role of mechanical load, the biochemical communication between bone, tendon, and muscle is crucial to their proper development. For example, scleraxis (*Scx*), a tendon marker, regulates the genetic expression of bone morphogenic protein-4 (*Bmp4*) in tendon cells. When *Bmp4* expression was blocked in mice, it led to a partial loss of bone ridges [183], suggesting a role of factors secreted by tendon on bone ridge formation. In addition, syndetome-induction, which will initiate tendon formation in mouse embryos, is associated with fibroblast growth factors (FGFs), FGF-4 and FGF-6 in mice, and FGF-8 in chicken [64, 185], only expressed in the myotome. This induction is a consequence of the activation of *Scx* expression and other tendon markers [64, 186] (Fig. 4.11). Down-regulation of these tendon markers in muscle-less and aneural conditions was reverted by the administration of exogenous FGF4, demonstrating an unequivocal biochemical dependence of tendon formation from muscle [187]. On the other hand, muscle development, initially characterized by increased proliferation of myogenic cells with later adjustment to normal

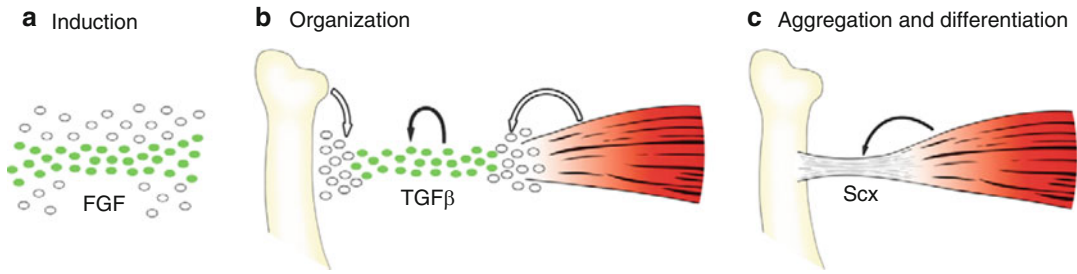


Fig. 4.11 Tissue interactions required for tendon progenitor induction in vertebrate embryos. Induction of tendon progenitors, identified as *Scx*-expressing cells, depends on a unique set of tissue interactions in different parts of the embryo. Each panel shows tendon progenitor distribution by whole-mount in situ hybridization (ISH) with an *Scx* probe. The line across each upper image shows the orientation of the section schematized beneath, which highlights the relevant tissue interactions (with tendon progenitors shown in green; muscle progenitors in red; cartilage in yellow). (a) Whole-mount *Scx*ISH on E10.5 mouse embryo and a schematic of a frontal trunk section, showing somite pairs (squares) and the neural tube (gray). Skeletal tissue derives from the sclerotome (Sc) of somites, whereas the musculature arises from the myotome (m). The tendon progenitors are found in the syndetome (S, green), a stripe of sclerotome cells at the junction between two adjacent myotomes. *Scx* expression

in syndetome cells is induced by FGFs secreted from the adjacent myotomes (arrows). (b) Whole-mount *Scx*ISH on E10.5 mouse limb bud and a schematized sagittal section through the limb bud. In the early limb bud, *Scx* expressed in mesodermal cells directly under the dorsal and ventral ectoderm. *Scx* expression at this stage depends on ectoderm (curved arrows) and not on a signal from the myoblasts or from pre-chondrogenic cells. (c) Whole-mount *Scx*ISH on E12.5 mouse limb and a schematized sagittal section through the autopod. In the differentiating autopod, *Scx* is expressed in subectodermal mesoderm along the differentiating skeletal elements. *Scx* expression along the differentiating digits can be induced by a signal from the skeletal condensations (straight arrow), and the sub-ectodermal position of the tendon progenitors suggests a role for the ectoderm (curved arrows) in tendon induction as well. A anterior, D distal, P posterior, Pr proximal, nt neural tube (From Schweitzer et al. [183])

morphology, happens because of controlled cell death modulated by local apoptotic factor(s). Interestingly, only muscle fibers that are not part of a stable myotendinous junction are affected, suggesting a role for tendon in muscle that belies morphogenesis. Retinoic acid, produced by both tendon and muscle, has been suggested as a potential apoptotic factor with an important role in mediating muscle apoptosis and muscle-tendon assembly [188].

Bone-Cartilage Crosstalk

Like other interactions between contiguous tissues of the MSK system, mechanical load is an integral part of their relationship and biomechanical crosstalk is indisputable. In this way, defective bone remodeling affects its mechanical properties, and, consequently, modifies how load is transmitted to cartilage, which ultimately results in altered local strains, and cartilage weakening and disease. Several studies have shown that initiation of osteoarthritis (OA) is preceded by increased osteoclastic activity and

subchondral bone resorption [68], but it is not clear if it happens only because of mechanical causes or if other factors are also involved. Despite this almost ubiquitous mechanical influence and the difficulty of removing the mechanical influence in this relationship, there is evidence that also points to a biochemical crosstalk between subchondral bone and cartilage in OA. In mice, over-expression of the EPHB4 receptor in osteoblasts was able to protect against OA induced by medial meniscus destabilization. In this case, OA was not initially related to a previously altered mechanical environment, and molecular changes in bone were able to deter OA development, which clearly suggests a role for factor(s) produced by bone in this process, and a bone-cartilage crosstalk [189]. There is also evidence that the hepatocyte growth-factor (HGF) may be part of the bone-cartilage crosstalk in OA. HGF expression and production in human subchondral osteoblasts are increased in OA, while the protein, but not the gene, can be detected in the articular cartilage, suggesting that

subchondral bone could be the origin of that HGF. Other potential players in the bone-cartilage crosstalk are RANK ligand (RANKL) and osteoprotegerin (OPG). Interestingly, though both molecules are produced by bone and cartilage cells, RANK receptor is expressed only in human OA chondrocytes [190]. How does this crosstalk happen? First, there is evidence that small molecules can easily diffuse between the bone marrow and the articular space, suggesting that, at least for small molecules, a direct exchange of biochemical factors between subchondral bone and articular cartilage is possible in a paracrine fashion [189, 191]. Second, during the pathogenesis of OA, vascular penetration in cartilage would expose the chondrocytes to cytokines and growth factors, such as VEGF, NGF, IL-1, IL-6, HGF, or IGF-1, from subchondral bone [192]. Overall, the demonstration of the bone-cartilage crosstalk indicates that targeting subchondral bone is a viable therapeutic approach in the OA treatment [193].

Tendon-Muscle Crosstalk

We have mentioned that during development there is strong evidence pointing to a biochemical crosstalk between muscle and tendon. Is there similar evidence post-development? Although the crosstalk between MSK tissues is a recently new research topic and the factors supposedly responsible for the crosstalk are yet to be identified, there are subtle but unquestionable indications that the relationship between muscles and tendons go beyond their biomechanical dependence.

Tendinopathies are common overuse injuries in sports and are often associated with aging. Tendons are difficult to heal, and most of the time the mechanical properties of the tissue are poorer when compared to the normal tissue. Clinicians have known for quite a long time that eccentric (i.e. lengthening contraction), not concentric (i.e. shortening contraction), training is the most effective conservative treatment to promote healing of tendon diseases, including Achilles and patellar tendinopathies [63, 194]. In eccentric training muscle contraction occurs at the same time that the muscles and tendons are stretching or remain stretched [63]. Despite overwhelming clinical

evidence, the mechanisms of how eccentric contraction improves tendon healing remain unknown. The answer may be in how the expression of growth factors, such as TGF β -1 and IGF-1, respond to eccentric and concentric training in tendons and muscles. Heinemeier et al. exposed rats to short-term strength training consisting of pure shortening (concentric), lengthening (eccentric), or static plantar extensors contraction. Strength training in the Achilles tendon, regardless of the type of contraction, increased the expression of TGF β -1 and IGF-1, even though the force produced was higher during the eccentric contractions. In the gastrocnemius muscle, there was similar upregulation of growth factors; however, the effect of lengthening was significantly greater than the effect of shortening [195]. The fact that muscle, but not tendon, responded differently depending on the type of strength training strongly suggests that if eccentric contractions have superior effect on tendon healing, it may be caused by one or more muscle factors produced during eccentric but not concentric contraction.

Transgenic mice are useful models to study the role of specific cytokines in different tissues. The *mdx* mouse is the classical animal model for Duchenne muscular dystrophy. Dystrophin is a cytoplasmatic protein that forms a complex that connects the cytoskeleton of the muscle fiber to the surrounding extracellular matrix. Without the dystrophin-complex, the sarcolemma becomes fragile and as the muscle fibers contract the muscle is damaged. As a result, *mdx* skeletal muscles are ~20 % weaker than normal muscles. Rizzuto et al. found that in dystrophin-deficient (*mdx*) mice, tendons are also affected by an increase in the number of dead cells, more energy dissipation during dynamic loading, and a significant loss of the elastic properties of the tissue. Furthermore, these functional changes suggest that *mdx* tendon experience similar alterations to those found in *mdx* muscles (reduction of viable cells and higher inflammatory response). It could be argued that a weaker muscle would cause a loss in tendon mechanical properties, but the similarity in response to missing dystrophin could also mean that either the protein has

a role in tendon homeostasis or that there is a paracrine communication between muscles and tendons [196].

The existence of a crosstalk between tissues of the MSK system, in development and adult life, are crucial for a better understanding of the biology of these tissues and may open the door to strategies that would improve the regeneration of these tissues, especially those that are difficult to heal.

Concluding Remarks and Future Directions

In the year 2000, United Nations member states signed the United Nations Millennium Declaration agreeing to work toward the achievement of eight Millennium Development Goals (MDGs) by the year 2030. Among these are the goals to improve health and combat disease. While major issues related to health care education and access, such as proper diet, exercise levels, and a host of social factors remain critical to be addressed, if humanity is to achieve the WHO health goals, all of us must do more. Even in face of all these challenges, however, humans are living longer due in large part to basic improvements in health conditions and reductions in absolute levels of poverty or misery.

Life expectancy in Japan has reached almost 83 years and the oldest human on record has lived to 122 years of age. Very simple metabolic and telomerase estimations give us the clue that humans could live to 125 years of age. This increasing life expectancy poses a unique set of challenges. On the one hand, there is the desire to live a longer and healthier life, but the cost seems quite high since morbidities and co-morbidities combine to afflict in particular older adults. Musculoskeletal diseases alone affect almost 2 billion humans in the planet and the large majority of them are older persons. The puzzle that is facing us is that while living a long life is desirable; it is really the quality of such life that matters the most.

As MSK scientists are trying to put the pieces of this puzzle together, they have come across some very interesting new ideas. There is no

doubt that bones-tendons-muscles come together in a unit of mechanical coupling where physical forces are constantly shaping the relationships between these tissues. What is relatively new is the “kine”; the endocrine-like role that these tissues play. It seems that the human body is significantly more endocrine than we had ever anticipated and the MSK players are no different from other parts of the body.

Thus, in this complex interaction of physical forces and secreted factors (myokines, osteokines, tenokines), these tissues interact both physically and biochemically in ways about which we are still learning. However, it is even more complex. Behind these interactions, there are complex genetic signaling pathways apparently shared among these tissues, and this pleiotropy might itself influence the way these tissues respond to physical and biochemical forces and factors.

In a world filled with uncertainty, there is one certainty; the MSK field is poised to bring many new developments and advancements as we improve our understanding of the MSK system and how different tissues interact and prime other in health and disease. If we learn for example to control or modulate the secretion or to mimic the effects of the “kines”, many MSK conditions could be treated or improved, including the twin diseases of aging, osteoporosis and sarcopenia. One last and very positive aspect of this field is that its basic research is continuing to push new boundaries of cell biological understanding, thus facilitating the development of new technologies that will benefit science and humankind in general.

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