Review

Muscle and bone, two interconnected tissues

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ABSTRACT

As bones are levers for skeletal muscle to exert forces, both are complementary and essential for locomotion and individual autonomy. In the past decades, the idea of a bone–muscle unit has emerged. Numerous studies have confirmed this hypothesis from in utero to aging works. Space flight, bed rest as well as osteoporosis and sarcopenia experiments have allowed to accumulate considerable evidence. Mechanical loading is a key mechanism linking both tissues with a central promoting role of physical activity. Moreover, the skeletal muscle secretome accounts various molecules that affect bone including insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (FGF-2), interleukin-6 (IL-6), IL-15, myostatin, osteoglycin (OGN), FAMSC, Tmem119 and osteoactivin. Even though studies on the potential effects of bone on muscle metabolism are sparse, few osteokines have been identified: Prostaglandin E2 (PGE2) and Wnt3a, which are secreted by osteocytes, osteocalcin (OCN) and IGF-1, which are produced by osteoblasts and sclerostin which is secreted by both cell types, might impact skeletal muscle cells. Cartilage and adipose tissue are also likely to participate to this control loop and should not be set aside. Indeed, chondrocytes are known to secrete Dickkopf-1 (DKK-1) and Indian hedgehog (Ihh) and adipocytes produce leptin, adiponectin and IL-6, which potentially modulate bone and muscle metabolisms. The understanding of this system will enable to define new levers to prevent/treat sarcopenia and osteoporosis at the same time. These strategies might include nutritional interventions and physical exercise.

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1. Introduction
Evidence from numerous studies has revealed for decades, that a close functional and developmental relationship exists between muscle and bone mass. According to the concept of the bone–muscle unit, a very strong relationship should exist between maximum muscle force and bone mass/geometry, and both tissues fulfill a common function, locomotion. "Indeed, if skeletal muscle was initially considered as a tissue whose primary function is to move objects against the force of gravity" (Vandenburgh et al., 1999), the sliding filament theory described the fact that contraction of myofilaments is at the heart of movements (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). Huxley (1975) then developed a 2 or 3 steps model and Cooke (2004) gathered information on the historical improvement of the muscle contraction model from 1972 to 2004. If muscle contraction is essential for motion, the skeleton (levers) is needed to exert forces (Campbell and Reece, 2004; Marieb, 1999). Regarding phylogeny, if arthropods are frequently described as the first land animals (MacNaughton et al., 2002; Schaefer et al., 2010), the emergence of land life and the diversification of organisms are believed to be partly due to the appearance of the skeleton (Volkmann and Baluska, 2006). The primary mechanical function of bones is to provide rigid levers (thanks to mineralization) for muscles to pull against, and to remain as light as possible to allow efficient locomotion (Törner, 1998), the mechanisms underlying such a relationship are still poorly understood and most of the biochemical interactions among the tissues and cells remain mostly unknown (Abreu et al., 2012). In recent years, a great number of scientific papers considered bone as a target of skeletal muscle secretory pattern but surprisingly, only a few mentioned the potential effects of bone on muscle metabolism. The objective of this review is thus to provide an update on the development of knowledge about the locomotor system.

2. Methodology
Computer-assisted searches of publications and reviews were conducted on PubMed database to identify pertinent papers published until 2014. Database was interrogated with the following keywords: locomotor system/function, musculoskeletal system, bone–muscle crosstalk, bone, skeleton, osteoporosis, cartilage, muscle, skeletal muscle, sarcopenia, osteoblast, osteoclast, osteocyte, osteokines, bone-secreted factors, myoblast, chondrocyte, tendon, tenocytes, tendon secreted factors, metabolism, formation, resorption, loss, atrophy, humoral factor, myokines, myostatin, endocrinology, secretory organ, bed rest, space flight/travel, mechanosensation, mechanical loading.
Moreover, relevant references mentioned in the previously identified papers were analyzed as well.

3. Is there a link between muscle and bone?
An essential component of the musculoskeletal system is the anchoring of the force-generating muscles to the solid support of the organism: the skeleton. Most of the available data provide evidence that muscle and bone closely interact. These observations led to the concept of the "Bone–muscle unit", this was evidenced phylogenetically by the lifelong linear association between total body bone mineral content (BMC) and lean body mass. This was the case in the study carried out on 1450 persons from 2 to 87 years by Ferretti et al. (1998). In the same way, in the Finnish Twin Cohort Study, lean mass was a better predictor of whole body bone mineral density (BMD) than fat mass (p<0.01) (Bogl et al., 2011). In addition, a clinical study performed in boys and girls during pubertal development showed that the increase in bone strength was preceded by the increase in muscle strength (Rauch et al., 2004). Jakowski et al. (2013) have shown that lean tissue mass accrual impacts adult bone strength.
Preclinical data strengthen the lessons from such clinical trials. Muscle bone crosstalk appears to manifest even before birth in mammals. Long bone shape and the joint is dependent on muscle contraction. In the absence of mechanical loads, the stereotypical circumferential outline of each bone is lost, leading to the development of mechanically inferior bones (Bren-Mattison et al., 2011; Sharir et al., 2011). Indeed, MyoD−/−/Myf5−/− (dd/ff) mice lack skeletal muscle, so they develop without any active movement in utero and die soon after birth. In the fetuses, long bones were found to be less mineralized and had altered morphological features. They also presented many more osteoclasts in the newly laid bone (Gomez et al., 2007). In a study were mice limb muscle were removed and replaced by implants of either minced skeletal muscle, nonlimb skeletal muscle, cardiac muscle, liver or nothing. Nodules of cartilage and bone were only induced by the three first ones (Zacks and Sheff, 1982). Finally, fracture healing was impaired by excision of a large muscle segment, while the diffusion of high molecular weight molecule from muscle appeared to enhance bone synthesis (Kaufman et al., 2008; Utvag et al., 2003). Liu et al. (2010) reviewed the potential role of muscle in bone repair and suggested that osteo-inducible cellular populations from muscle may directly be implicated in bone formation and healing.
In summary, various situations such as ageing or pathological states, as well as environmental factors (nutrition, physical activity/mechanical stress) may influence muscle and bone simultaneously. These observations lead to the concept of possible interactions between muscle and bone, which might be very important for understanding the physiology and pathophysiology of sarcopenia and osteoporosis. As a matter of fact, muscle/bone relationships include different levels of cross-talk: through systemic humoral pathways, but also at the cellular and molecular levels. The crosstalk can be bidirectional, i.e., from muscle to bone and...
from bone to muscle. This control scheme is even more complex as other players, among which adipose tissue, tendons, are involved in these regulation loops.

4. Understanding the relationship between muscle and bone

The formation of the musculoskeletal system represents an intricate process of tissue assembly involving heterotypic inductive interactions between tendons, muscles and cartilage.

4.1. Physiological observations

The action of muscle on the bone adaptive response is well recognized and has been modeled in the Wolff’s law and Frost’s mechanostat (Frost, 1987, 2003; Frost and Schonau, 2000; Li et al., 2012; Woo et al., 1981). According to those theories, mechanical loading is the most important determinant of the bone strength. There is existing preclinical and clinical evidence to show that low magnitude mechanical signals are anabolic to both bone and muscle (Chan et al., 2012; Muir et al., 2011) and this is why physical activities are known to impact both muscle and bone metabolisms, the final effect being activity dependent. Indeed, in an evaluation of the impact of different sports on BMD, the sedentary controls had the lowest bone density values which underlines the positive effect of sports on BMD (Egan et al., 2006). Comparison of football, running and controls showed that football enhances both BMD and muscle fiber number after 16 months training (Krustup et al., 2010). In the study carried out by Seabra et al. (2012) in 117 soccer players and 34 control subjects, at all body sites, muscle strength of knee extensors was associated with BMD. In a study performed in 7–9 year-old swimmers, gymnasts and controls, the authors also concluded that bone loading activities may lead to increased bone density among young girls (Cassell et al., 1996), the increase in BMD per unit increase in body weight being more among gymnasts than among swimmers and controls, while fat mass, percent body fat, and lean mass were less in gymnasts compared with swimmers and controls (all \( p < 0.05 \)). In a similar kind of trial, conducted in older women (18–24 years old) by Emslander et al. (1998), total body and femoral neck BMD were significantly higher in the study group that performed weight-bearing exercises than in control subjects.

Swimming exercise had no effect on BMD, even though it could significantly improve shoulder, back, and grip muscle strength. Consistent with those data, in the Duncan’s investigation, adolescent female runners had higher total body, femoral neck and leg BMD than swimmers (Duncan et al., 2002). A comparison of different weight bearing sports showed that BMD is consistent with the specificity of the stimulus (Heinemann et al., 1993). The same authors concluded from another study that “training including high strain rates in versatile movements and high peak forces is more effective in bone formation than training with a large number of low-force repetitions” (Heinemann et al., 1995). Actually, an increase in muscle mass impacts the interface of the two organs and stimulates local bone growth by stretching collagen fibers and periostium (Kaji, 2013). Targeting more specifically the impact of exercise on muscle, resistance and endurance training are known to induce distinct muscular adaptations (Tanaka and Swensen, 1998). Billette and Hoppeler (2003) demonstrated that extreme fiber type distributions was different in a 50m crawl swimmer (around 80% type II (fast-twitch) fibers in his vastus lateralis muscle) and in a professional cyclist who has about 80% type I (slow-twitch) fibers. In the same way, in a study based on strength training using a one leg model during 10 weeks followed by 12 weeks detraining in 6 men, muscle force production was shown to be increased in both legs after training and strength performance was decreased after the detraining period, also in both legs. Moreover, cross sectional areas were modified in the training leg: 21% increase in type Ila and 18% increase in type IIb (18%) fibers after training, and a decrease of 12% in type IIb fibers during detraining (Houston et al., 1983).

An additional fact underlying the importance of physical activity on both bone and muscle is that in obese adults under restrictive diet, the negative modifications in hip geometry parameters which were observed, disappeared when they were also subjected to physical exercise (Armamento-Villareal et al., 2012). What is important to notice is that both muscle force as well as muscle size, as surrogates of mechanical loading, correlate with BMD and BMC.

To summarize, physical activity and more importantly loading exercises can modulate the locomotor system. Nevertheless, bone is not only subjected to muscle contraction forces but also to gravitational loading (Jedex and Carlson, 2009). Even though, according to Robling (2009), a convincing body of data suggests that muscle contractions are present, significant and capable of accounting for most of the adaptive responses, Jedex and Carlson (2009) stated, in their review, that the alternative gravitational loading can have a significant role in determining bone mass and morphology, depending on the specific activity, the location of the bone within the skeleton, and whether the bone is weight-bearing or not. As a matter of fact, bone remodeling appears to be sensitive to both external loads arising from gravitational loading as well to internal loads generated by muscular activity. Indeed, vibrations applied at very low magnitudes may be sensed directly by transmittance of the signal through the skeleton in the absence of muscular activity (Jedex and Rubin, 2010). This is why numerous studies on the impact of space flight and bed rest on muscle and bone health emphasize the central role of gravity and therefore mechanical loading. The impact of resistive exercise on both muscle and bone was assessed in the long time bed rest (LTBR) study, which was concluded by underlining the importance of mechanical stimuli (Rittweger et al., 2005). In summary, mechanical loading, due to physical activity and/or to gravitational loading, has a central role in the bone–muscle unit and specifically in the determination of bone and muscle mass and structure. These adaptations are made in response to mechanosensation, whose cellular and molecular mechanisms are developed in the following paragraphs.

4.2. Cellular mechanisms

In both muscle and bone, sensed mechanical stimuli can be converted to biochemical signals and elicit synthesis or catabolism. Mechano sensing is the first step of mechanostressduction and is followed by the signal reception, transduction and transmission. The complex interconnected network of osteocytes is considered to be the site of signal transduction and transmission to the osteoblasts (Cowin, 2007) as osteocytes are the most mechanosensitive bone cells (Klein-Nulend et al., 1995). They transduce the loading mechanical signals and release signaling molecules to recruit osteoblasts or osteoclasts (Klein-Nulend et al., 2012a,b). Indeed, during hindlimb unloading, introducing exercise in order to elicit a minimum number of high-intensity muscle contractions allowed to suppress unloading-induced increases in sclerotin- positive osteocytes and to restore cortical bone formation rate (Macias et al., 2012). However, osteocytes might not be the only cell responsible for mechanosensation unless 30% osteocytes are sufficient. In fact, transgenic mice lacking 70–80% osteocytes subjected to unloading were shown to be resistant to bone loss. Nevertheless, after 14 days of reloading, bone formation rate corrected for bone surface was increased as in the control mice (Tatsumi et al., 2007). Besides, mechanical loading has been demonstrated to induce the expression of predictive markers (bone morphogenetic protein-2 (BMP-2), alkaline phosphatase (ALP) and type 1 collagen (Col1))
4 of the differentiated osteoblast phenotype in MC3T3-E1 cells (Lu et al., 2012). Kamel et al. (2010) compared the response of MLO-Y4 osteocytic cells, 2T3 osteoblasts and primary neonatal calvarial cells to pulsatile fluid shear stress. MLO-Y4 were more sensitive than 2T3 and primary neonatal calvarial osteoblasts to such a stress, β-catenin nuclear translocation and prostaglandin E2 (PGE2) production being more important.

4.3. Molecular mechanisms

To better understand the mechanism of mechanosensation, Li et al. (2012) investigated the impact of fluid shear stress on pre-osteoblastic MC3T3-E1 cells. Extracellular-signal-regulated kinase 5 (ERK5), a mitogen-activated protein kinase (MAPK) member, was rapidly phosphorylated and the cytoskeleton underwent reorganization upon this stress. In addition, Wnt/β-catenin signaling pathway seems to be one of the most important pathway activated by mechanical loading in osteocytes (estrogen receptor-α (ER-α) is involved in such a regulation) (Armstrong et al., 2007; Dallas et al., 2013), this signaling pathway being essential for bone cell viability and function and for skeletal integrity. Indeed, myotube conditioned media can protect osteocytes and osteoblastic cells from dexamethasone induced apoptosis, and this through the Wnt/β-catenin pathway for osteocytes (Jahn et al., 2012). In addition, in β-catenin haploinsufficient mice, deleting a single copy of β-catenin in osteocytes abolishes the anabolic response to loading (Javaheri et al., 2013) and trabecular bone in females was more severely affected. Lee et al. (2003) also found that bone adaptive response to loading was impaired in the absence of the ER-α isoform. Moreover, mice lacking low density lipoprotein receptor-related protein 5 (LRP5) exhibited impaired response to mechanical loading (Sawakami et al., 2006). However, in primary mouse osteoblasts and UMR-106 osteoblast-like cells, strain activated a cascade independently of Wnt/LRP5, involving insulin-like growth factor-1 (IGF-1) receptor (IGF-1R), ER-α and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)-dependent activation of β-catenin (Sunders et al., 2010). IGF-1 is effectively known to be increased in response to load. Raab-Cullen et al. (1994) and Tahimic et al. (2013) proposed a crosstalk between the IGF-1 and integrin signaling pathways.

5. Understanding the cross-talk between muscle and bone

5.1. Muscle to bone

Hamrick proposed that muscle secretion of local growth factors can stimulate bone formation independently of mechanical loading and that both mechanical and biological stimuli function syner-

Fig. 1. Molecular interactions between bone, skeletal muscle, cartilage adipose tissue and tendons. The secreted molecules from muscle, bone cartilage, tendons and adipose tissue are represented by circles. The arrows outline regulation pathways. V, ε, λ and ω symbolize the effect of FAM5C, Tmem119, osteoglycin and osteoactivin respectively on the expression of Runx2, ostein, ALP, Col1 and OCN.

Akt protein kinase B; ALP, alkaline phosphatase; BMP-2, bone morphogenetic protein-2; Col1, type I collagen; DKK-1, Dickkopf-1; ERK1/2, extracellular-signal-regulated kinase 1/2; ERK5, extracellular-signal-regulated kinase 5; FGF-2, basic fibroblast growth factor; GPRC6a, G protein-coupled receptor 6a; IGF-1, insulin-like growth factor-1; IGF-BP-2, IGF binding protein 2; IGF-BP-5, IGF binding protein 5; Ihh, Indian hedgehog; IL-6, interleukin-6; IL-15, interleukin-15; IGF, leukemia inhibitory factor; MAPK, mitogen-activated protein kinase; OCN, osteocalcin; PGE2, prostaglandin E2; PI3K, phosphatidylinositol 3-kinase; Runx2, Runx-related transcription factor 2; TGF-β, transforming growth factor β.
gistically (Hamrick et al., 2010). Indeed, blood flow to the limb is proportional to muscle mass and ischemia delays fracture healing in mice (Kaji, 2013; Lu et al., 2007), but muscle is not only a vascular supply for bone as fasciocutaneous tissue, a more vascularized system, was less effective in fracture healing than muscle in mice (Harry et al., 2009). In fact, the muscle secretome consists of several hundred secreted peptides and this provides a conceptual basis and a whole new paradigm for understanding how muscles communicate with other organs, including bones. Investigation of the C2C12 cells secretome during muscle differentiation allowed to identify more than 600 proteins, including growth factors, cytokines and metallopeptidases (Henningsen et al., 2010). In a similar study, 27 secretory proteins with a minimum of two tryptic peptides were identified (Chan et al., 2007). Some of them are involved in extra-cellular matrix remodeling, cellular proliferation, migration and signaling. A putative network of proteins involving matrix metalloproteinase 2 (MMP-2), osteonectin, and cystatin C that all interact with the transforming growth factor β (TGF-β) signaling has thus been postulated to contribute toward a functional role in the myogenic differentiation program. Those myokines were defined as “cytokines or other peptides that are produced, expressed and released by muscle fibres” (Pedersen and Febbraio, 2012), even though they are not muscle-specific (Hamrick, 2012). Many of those proteins produced by skeletal muscle are dependent upon contraction; therefore, physical inactivity probably leads to an altered myokine response, which could provide a potential mechanism for the association between sedentary behavior and many chronic diseases (Fig. 1).

5.1.1. Growth factors

IGF-1 which positively regulates osteoblast function, is, mainly produced in the liver but expressed in multiple extrahepatic tissues including bone and skeletal muscle (Huang et al., 2007; Ohlsson et al., 2009; Schiaffino and Mammucari, 2011). IGF-1 is known to activate IGF–1R which acts through the PI3K/Akt and the MAPK/ERK pathways (Bikle and Wang, 2011; Tahimic et al., 2013). It has been shown that IGF-1 is localized to the muscle–bone interface of the mouse forelimb (Hamrick et al., 2010) and even if IGF-1 is expressed locally by osteocytes, some investigations provided evidence that muscle IGF-1 can modulate bone metabolism (Lean et al., 1996; Sheng et al., 2013). Indeed, during discrete muscle atrophy, electroporation and ectopic expression of IGF-I and/or sonic hedgehog (Shh) within the gastrocnemius/soleus muscle, not only attenuated the decrease of muscle fiber area, muscle mass and muscle mass density that normally occurs but inhibited parameters of osteopenia within the tibia and fibula associated with hindlimb unloading as well (Alzghoul et al., 2004). Moreover, localized overexpression of human IGF-1 in mice muscles caused muscle mass increase and cortical bone parameters enhancement (Banu et al., 2003). Female knockout (KO) hepatocyte-specific IGF-1 transgenic (KO-HIT) mice, which exclusively express IGF-1 in the liver, showed lower trabecular bone volume per total volume (%BV/TV) than controls (p = 0.09), and decreased trabecular number (Tb.N) in the distal femoral metaphysis (Elis et al., 2011). This can be caused by the lack of bone IGF-1 but possibly by the lack of muscle IGF-1 as well. IGF binding protein 5 (IGFBP-5) can stimulate osteoblast activity and therefore enhance bone formation in O VX mice, at least in part, via an IGF-1-independent mechanism (Andress, 2001; Coenen Schinke et al., 1999; Hoeflich et al., 2007; Kaji, 2013; Miyakoshi et al., 2001). As a matter of fact, 6 IGFBP regulate the interaction of IGF-1 with its receptor (Ohlsson et al., 2009; Rajaram et al., 1997). IGFBP-2 binds to IGFs and prevents its fixation to IGF receptors (Rajaram et al., 1997). Relative appendicular skeletal muscle mass (ASM) (which is associated with a number of bone parameters in women like trabecular volumetric bone mineral density at the femoral neck and spine) has been shown to be significantly inversely related to serum IGFBP-2 levels (Lebrasseur et al., 2012). Indeed, high IGFBP-2 circulating concentrations were associated with lower BMD in men and women (Amini et al., 2007).

Besides, basic fibroblast growth factor (bFGF or FGF-2) and its receptor are to be localized to the muscle–bone interface and periosteum of the mouse forelimb, respectively (Hamrick et al., 2010). It was shown that mechanically induced, sarcolemma wound-mediated FGF-2 release is an important autocrine mechanism for transducing the stimulus of mechanical load into a skeletal muscle growth response (Abraham et al., 1986; Clarke and Feeback, 1996; Clarke et al., 1993). Even if further investigations are needed, FGF-2 could be an osteoinducer factor released by muscle. Intravenous or intravenous injections of FGF-2 stimulated bone formation in ovariectomized rats (Liang et al., 1999; Nakamura et al., 1998). BMP-2 combined with low doses of FGF-2 (16, 80 and 400 ng) increased osteoinductive activity while high doses of FGF-2 (2, 10 and 50 μg) were inhibitors (Fujimura et al., 2002). This was consistent with the data published by Nakamura et al. (2005) showing that in vitro, low doses of FGF-2 (0.1 and 1 ng/mL) when pooled with BMP-2, increased the expression of BMP receptor (BMPR)-1B, phosphorylated Smad1, Noggin, and osteocalcin (OCN), contrary to high doses (10 and 100 ng/mL) Smad6 and Runt-related transcription factor 2 (Runx2/cbfa1) mRNA increased dose dependently as well and proliferation was enhanced with high doses of this growth factor (Fig. 2).

5.1.2. Myokines

As reported by Pedersen and Febbraio (2012) muscle is acknowledged to function in an endocrine manner to modulate other biological targets including bone (but the liver, pancreas, adipose tissue as well). Indeed, its secretion products, termed myokines are involved in the muscle–bone crosstalk and may also indi-
rectly impact bone through actions on other tissues (Pedersen and Febbraio, 2012). They include interleukins (IL-6, IL-7, IL-8, IL-15), leukemia inhibitory factor (LIF), brain-derived neurotrophic factor (BDNF), irisin, follistatin-like protein 1 (FSTL-1), muscle, and myostatin (DiGirolamo et al., 2013).

With regards to interleukins, IL6 was indeed considered as a myokine involved in the muscle–bone crosstalk (Hamrick, 2012). In a recent clinical study, in which 38 postmenopausal women with osteopenia were given bisphosphonate and calcitriol, baseline serum IL-6 was negatively correlated to both handgrip strength and lumbar BMD. Even if both factors were no more correlated to IL-6 level after the 6-months treatment, all 3 parameters evolved significantly: IL-6 decreased whereas lumbar BMD and handgrip strength increased (Park et al., 2013).

The IL-8 chemokine, a myokine mainly produced by macrophages and endothelial cells and myotubes, exerts marked chemotactic activity towards leukocytes, in addition to being an angiogenic factor. Muscular IL8 mRNA levels are enhanced by exercise and its production is induced by IL-6 (Pedersen and Febbraio, 2012).

The cytokine IL-15 isoform with a 48 amino acids signal peptide is mostly expressed by skeletal muscle and functions as a secretary signal peptide, the shorter 21 amino acids signal isoform being preferentially expressed in tissues such as testis and thymus (Grabstein et al., 1994; Tagaya et al., 1997). Transgenic mice with higher circulating levels of IL-15 were characterized by a higher BMC (Quinn et al., 2009). Leukemia inhibitory factor (LIF) could also be a good myokine candidate as it is expressed by different cells including myoblast and osteoblast (Malaval and Aubin, 2001) and because its secretion by myoblasts was enhanced by exercise, which could impact periosteum osteoblasts (Broholm et al., 2008; Sims and Johnson, 2012).

BDNF, which serves a key role in maintaining the population of muscle progenitors in adult muscle, has receptor in osteoblasts and chondrocytes (Griesbeck et al., 1995; Mousavi and Jasmin, 2006). Its biological effect on the skeleton is quite complex as it has a positive effect on bone cells in vitro, while deletion of central BDNF expression in mice results in increased bone mass and white adipose tissue (Camerino et al., 2012).

Irisin is a newly discovered exercise-mediated myokine which regulates energy metabolism (Bostrom et al., 2012). It is a transmembrane protein localized in skeletal muscle which is induced by exercise. Irisin promotes osteoblast differentiation through the Wnt-β-catenin pathway and inhibits osteoblast differentiation by suppressing the receptor activator of nuclear factor-kappa B ligand (RANKL)/nuclear factor of activated T cells (NFAT) c1 pathway (Zhang et al., 2013). In postmenopausal women with low bone mass, circulating irisin levels were associated with previous osteoporotic fractures (Anastasiaklis et al., 2014).

Musclin is a novel skeletal muscle-derived secretory factor. Its protein sequence seems to be identical to that of osteocrin, a molecule expressed in osteoblasts which disappeared after birth (Moffatt and Thomas, 2009; Nishizawa et al., 2004). Musclin expression is tightly regulated by nutritional changes and its physiological role could be linked to glucose metabolism. It is down-regulated by FoxO1 (Yasu et al., 2007), a key modulator of the ability of the skeleton to function as an endocrine organ (regulation of glucose metabolism) (Rached et al., 2010) and to inhibit osteoclastogenesis (Bartell et al., 2014). Growth differentiation factor-8 (GDF-8) or myostatin, a member of TGF-β superfamily, has been identified specifically in developing and adult skeletal muscle (McPherron et al., 1997), where it is a negative regulator of growth as GDF-8 null mice display bigger muscle, and myostatin overexpression is responsible for muscle loss (Giannesini et al., 2013; Zimmers et al., 2002). Myostatin not only impacts muscle mass but also muscle performance (Giannesini et al., 2013). Moreover, knocking out myostatin significantly increased femoral and lumbar spine BMD in mice (Hamrick, 2003; Montgomery et al., 2005).

Ten years ago, Zimmers et al. (2002) hypothesized that myostatin had a role in the preservation of appropriate tissue mass. In skeletal muscle, myostatin binds to activin receptor type IIB (ActRIIB) which leads to the regulation of the myogenic genes (Huang et al., 2011; Langley et al., 2002). A treatment with the ActRIIB-Fc fusion protein, which binds myostatin, caused a dose-dependent augmentation in N-terminal propeptide of type 1 procollagen (PINP) serum levels in mice and BMD increase (Chiu et al., 2013). Myostatin, in muscle cells, can activate the ERK1/2 cascade, modulate the Wnt pathways (repression of Wnt4 for example) and stimulate the secretion of transforming growth factor-β 1 (TGF-B1) (Steelman et al., 2006; Yang et al., 2006; Zhu et al., 2007). Moreover, in vitro, myostatin expression is under the control of TGF-β1 and IGF-1, through the activation of calcium-dependent transcription factors such as nuclear factor of activated T cells (NFAT) and cAMP response element binding protein (CREB) (Valdes et al., 2013; Zhu et al., 2007; Zuloaga et al., 2013). As these signaling pathways can be activated in bone cells, these results seem promising for the regulation of the bone by muscle. However, according to Arounleut et al. (2013), the role of myostatin on mature and intact bone could be limited. Indeed, the impact on bone accretion can be elicited via muscle, and to date whether myostatin action on bone is direct or indirect remains unclear (Buehling and Binkley, 2013). To our knowledge, nothing has been published on the effect of myostatin on cultured osteoblasts or osteoclasts. Myostatin impact seems limited to the control of the osteogenic differentiation of mesenchymal stem cells during mechanical stimulation or fracture healing (Cho et al., 2002; Elkassawawy et al., 2012; Hamrick et al., 2007). Myostatin mRNA expression in mice tibia is undetectable before fracture, and highly expressed the day after (Cho et al., 2002) and although myostatin deficiency increases muscle mass and bone strength, it does not prevent muscle and bone catabolism with unloading (Hamrick et al., 2007).

5.1.3. Other molecules

Using fibroblast ossification progressor disorder in which muscle tissue is gradually replaced by bone to search for muscle-derived bone anabolic factors, Tanaka et al. identified osteoactivin (OGN), a protein produced in myoblastic cells which enhances bone formation parameters in osteoblasts, FAMSC, whose role in bone remains unknown and Tmem119, whose expression increases during osteoblast differentiation (Kaji, 2013; Tanaka et al., 2012a,b,c). Stable overexpression of OGN in MC3T3-E1 cells was shown to suppress the expression of Runx2 and Osterix mRNA and stimulate that of ALP, Col1, and OCN, the levels of Col1 and β-catenin proteins, mineralization, as well as Smad3/4-responsive transcriptional activity. The enhanced levels of Col1 mRNA by OGN in osteoblasts were suggested to involve ERK1/2 (Tanaka et al., 2012b).

In the same way, conditionized medium from C2C12 cells overexpressing FAMSC was associated with increased levels of osterix, ALP and OCN mRNA in MC3T3-E1 cells, while suppressed-FAMSC expression inhibited those parameters (Tanaka et al., 2012c). In Tmem119-overexpressing C2C12 cells, protein levels for osteoblast differentiation markers such as Runx2, Osterix, and ALP, as well as ALP and OCN mRNA were elevated and mineralization was induced as compared to what was observed in empty vector-transfected cells (Tanaka et al., 2012a). Moreover, Tmem119 was shown to stimulate the differentiation of myoblasts into osteoblasts through the induction of BMP-2 downstream of Runx2 and Osterix, among other pathways, and to suppress the differentiation of myoblasts into myotubes.

These experiments allowed the discovery of more molecules such as osteoactivin, which stimulates bone remodeling (Sondag et al., 2013; Tanaka et al., 2012a). Osteoactivin is upregulated.
under unloading conditions in muscle and also expressed in osteo-
clasts and osteoblasts (Abdelmagid et al., 2008; Nikawa et al., 2004; Sheng et al., 2008) and its expression is regulated by BMP-2 through the Smad-1 signaling pathway (Abdelmagid et al., 2007). It enhanced osteoblast ALP activity and mineralization and OCN production and calcium deposition were inhibited by anti-osteocytic antibodies (Selim et al., 2003). An important fact is that osteoac-
tivin overexpression in C2C12 cells downregulated MyoD and myogenin protein levels and upregulated ALP protein level and activity, as well as Runx2 protein synthesis. This transdifferenti-
ation of myoblasts into osteoblasts took place under the control of focal adhesion kinase (FAK) signaling (Sondag et al., 2013).

Finally, other molecules have been identified as good candidates: osteonectin, FGF-21, follistatin-like-1, MMP-2, myocytes enhancer factor 2C (MEF2C) and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), BMP-1, osteocrin (also named musclin) but too little information is available for the moment.

5.2. Bone to muscle

The lacunocanicular system is connected to the vascular system so bone-secreted molecules can be released into the bloodstream (Ciani et al., 2009; Dallas et al., 2013). Both osteoblasts and osteo-
cytes were shown to secrete osteokines. A recent study revealed that bone marrow mesenchymal stromal cells stimulate myoblast proliferation through vascular endothelial growth factor (VEGF) (Sassoli et al., 2012).

5.2.1. Osteocyte-secreted molecules

MLO-Y4 osteocyte-like cell conditioned medium, which was shown to contain 3 osteocyte-secreted factors, PGE2, sclerostin and monocyte chemotactic protein-3 (MCP-3), was able to accelerate myogenic differentiation of C2C12 myoblasts and this was linked to significant modifications in intracellular calcium homeostasis (Mo et al., 2012). PGE2 can activate the β-catenin pathway via the stimu-
lization of PI3K in osteocyte in response to fluid shear stress (Kamel et al., 2010; Kitase et al., 2010) and it has been shown that inhibiting PI3K in skeletal muscle derived stem cells can increase the expres-
sion of Runx2 and Osterix and enhance mineralization, even if ALP activity is decreased (Payne et al., 2010). Moreover, MLO-Y4 cells exposed to mechanical loading produced various factors, such as IGF-I, vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), which may regulate muscle growth (Juffer et al., 2012; Kaji, 2013).

Besides, pulsating fluid flow upregulated gene expression of Wnt3a, c-jun, connexin 43 and CD44 in MLO-Y4 osteocytes and downregulated gene expression of Wnt5a and c-jun in MC3T3-E1 osteoblasts. This was suppressed by the Wnt antagonist secreted frizzled related sequence protein 4 (sFRP4), suggesting that load-
ing activates the Wnt canonical pathway through functional Wnt production, but also by the nitric oxide (NO) inhibitor L-NAME (Santos et al., 2009). In C2C12 cells, Wnt3a was found to enhance the expression of myoD and myogenin and the size of the myotubes and therefore to promote myogenesis (Romero-Suárez et al., 2011). Sclerostin which is a suppressor of the canonical Wnt/β-
catenin signal which is mainly produced by osteocytes but also by osteoblasts and impacts osteoblastic differentiation (Semenov et al., 2005; van Bezoijen et al., 2007; Winkler et al., 2003) has thus been identified as a factor which could link bone to muscle during exercise (Kaji, 2013), even if Mo et al. (2012) showed that it did not impact C2C12 myogenesis. As a matter of fact, during weight loss in obese adults, the increase in sclerostin levels which occurs in volunteers subjected only to diet was prevented by adding exer-
cise (Armamento-Villareal et al., 2012). In rats, hindlimb unloading increased sclerostin-positive osteocytes. Adding resistance training led to a reduced number of these cells but not to the control level (Macias et al., 2012).

5.2.2. Osteoblast-derived molecules

OCN is an osteoblast-derived hormone, which has a unique known receptor: G protein-coupled receptor (GPRC6a). GPRC6a mechanisms are inhibited by the embryonic stem cell phosphatase (Esp) tyrosine phosphatase (Karsenty and Oury, 2012; Oury et al., 2011). An indirect effect of OCN on muscle is that it improves sensitiv-
ity to insulin (Lee et al., 2007). Moreover, intermittent injections of OCN conducted to the increase of mitochondria number and area in mice, which is said to increase energy expenditure (Ferron et al., 2012). Karsenty and co-workers presented new results on the impact of OCN on muscle during the 2012 ASBMR meeting (Abreu et al., 2012). Using ocn−/−, esp−/− and gprc6a−/− female mice, they provided evidence of a modulation of muscle mass, function and regeneration by OCN. Indeed, ocn−/− and gprc6a−/− mice had a reduced muscle mass, whereas esp−/− mice had increased muscle mass. To test muscle function, running experiments were conducted; and running distance, maximum speed and time on treadmill were all reduced in OCN null mice. Moreover, a specific osteoblast specific knockout of OCN also exhibited reduced muscle function.

On another hand IGF-1 can be produced by bone cells (osteoblasts as well as osteocytes) and it is well known for its activation of the PI3K/Akt pathway in skeletal muscle (Schiaffino and Mammucari, 2011). To identify the specific role of bone IGF-1 relative to systemic IGF-1, IGF-1 was deleted in cells expressing collagen type I c.2. This led to a reduction of 70% in bone IGF-1 whereas serum levels were identical to that of controls (Govoni et al., 2007).

A fivefold decrease in IGF-I expression was also observed in muscle even if Collα1 and iCre expression were much lower compared to bone. In osteocyte-IGF-1 conditional KO mice, IGF-1 mRNA expres-
sion was significantly reduced by 65% in bones and by 59% in muscles as well (Sheng et al., 2013), although the reduction in muscle was not significant, and no differences in muscle fiber mor-
phology, size or number of nuclei was observed. Tahimi et al. (2013), in a recent review, also concluded that muscle and bone share similar IGF-1 responsive gene networks as osteoprogenitor igf-1r (Istres-Cre-igf-1r) and cartilage (Cart1gfr1) conditional KO displayed both impaired muscles and skeleton.

“FGF23 is a 251 amino-acid peptide synthesized by osteocytes and osteoblasts in response to high phosphate intake, hyper-
phosphatemia or an increase in serum calcitriol concentration. It regulates systemic phosphate levels, vitamin D metabolism and α-klotho expression through a novel bone–kidney axis (Quarles, 2012). Klotho, acts as a plasma soluble co-receptor of FGF23 to modulate skeletal muscle differentiation, through down-regulation of insulin/IGF-I signaling (Kido et al., 2012). Moreover, Klotho also regulates smooth muscles as it mitigates the effects of phosphate and FGF23 on contractility the vessel wall via increased NO production (Six et al., 2014).”

Finally, the canonical (i.e., Wnt/β-catenin) pathway plays a major role in bone metabolism. Its increases bone mass through a number of mechanisms including renewal of stem cells, induc-
tion of osteoblastogenesis, and inhibition of both osteoblast and osteocyte apoptosis. It also promotes osteoblast proliferation and mineralization by increasing the OPG/RANKL ratio, while its role on osteoclasts remains unknown (Kubota et al., 2009). As a matter of fact, changes in IL-6 and the OPG/RANKL system may elicit systemic responses in muscle inflammation and repair processes (Phillippou et al., 2009). This may explain why Wnt signaling plays an essential role during embryonic muscle development and in the mainte-
nance of skeletal muscle homeostasis in the adult. Wnt7a/Fzd7 signaling stimulates skeletal muscle growth and repair by inducing the symmetric expansion of satellite stem cells through the planar...
6. Cartilage, the third agent

Bone and muscle form a unit but are also part of an organism. Cartilage is a good candidate for a larger system as it shares the mesenchymal stem cell origin with bone and muscle and is located in close proximity, which allows a possible paracrine communication.

Cartilage has a central role in locomotion. In each limb, joints allow the flexibility necessary for body motion and locomotion (Campbell and Reece, 2004). This is why Rittweger (2008) suggested that joint-size is the ‘third agent’ of the bone–muscle unit and proposed appropriate peak joint forces and adapted size of the joint as leads to pursue research. As sarcopenia and osteoarthritis share common pathological pathways, treating the 2 diseases at the same time is promising (De Ceuninck et al., 2013). Moreover, Wnt BMP, TGF-β and MAPK signaling pathways are thought to participate in the bone–cartilage crosstalk (Hopwood et al., 2007; Sharma et al., 2013).

Chondrocytes cultured together with C2C12 muscle cells as well as in muscle cell-conditioned medium had enhanced cartilage matrix production (Cairns et al., 2010a). Moreover, these chondrocytes were able to resist to IL-1β and TNF-α-induced cartilage damage (Cairns et al., 2010b). Therefore, such data allowed to think that muscle secrete molecules which play a role in chondrocytes metabolism.

As myostatin is expressed directly after fracture, the impact of this molecule on the recruitment of mesenchymal cells to promote chondrogenesis in early healing was proposed (Cho et al., 2002). Its receptor (ActRIIB) is expressed in cultured chondrocytes (Funaba et al., 2001). Myostatin treatment of bone marrow–derived mesenchymal stem cells induced a decrease in chondrogenic differentiation markers, while myostatin deficiency significantly increased the proliferation of epiphyseal growth plate chondrocytes by 35% (Elkasrawy et al., 2011). Dickkopf-1 (DKK-1), which is a regulatory molecule of the Wnt pathway, secreted, among other sources, by chondrocytes (Fedi et al., 1999; Oh et al., 2012), has been shown to contribute to bone loss. Anti-DKK-1 antibody were able to induce a dose-dependent decrease in the number of osteoclasts (Diarra et al., 2007). A third example is the Indian hedgehog (Ihh) which is expressed by chondrocytes during myogenesis (Bren-Mattison et al., 2011; Vorikamp et al., 1996). Ihh 5−/−Q10 mice exhibit an impaired osteoblast development in endochondral bones and reduced muscle mass, independently of bone length (Bren-Mattison et al., 2011; St-Jacques et al., 1999).

7. The adipose tissue is also involved in the control loop

The key role of adipose tissue on both skeletal muscle and bone cannot be ignored. Indeed, adipocytes, myoblasts and osteoblasts derive from the common mesenchymal stem cells (Migliaccio et al., 2011), adipose tissue is an endocrine tissue, an energy reservoir (Migliaccio et al., 2011) and produces aromatase, responsible for a key step in the biosynthesis of estrogens (Perel and Killinger, 1979).

This is why the role of fat in muscle–bone interactions is one of the main questions raised in a recent ASBMR workshop (Bonewald et al., 2013).

Leptin, which suppresses appetite, was the first adipokine identified (Friedman and Halaas, 1998; Zhang et al., 1994). Its impact on bone is still controversial, having a direct stimulatory effect on bone and an indirect opposite effect via the central nervous system (for review see (Naot and Cornish, 2014)). In skeletal muscle, leptin is capable of enhancing energy consumption through the activation of AMP-activated protein kinase (AMPK) (Pedersen and Febbraio, 2008; Trayhurn et al., 2011).

Numerous studies have been conducted on the impact of adiponectin and bone as well and the majority of reports have indicated a negative effect (Holecki and Wiecek, 2010; Naot and Cornish, 2014). Adiponectin has a metabolic protective effect on skeletal muscle, as it promotes glucose and fatty acids homeostasis (Liu and Sweeney, 2014) while adiponectin deficiency leads to insulin resistance (Karpe, 2013). IL-6, which is produced by skeletal muscle as mentioned above, is also secreted by adipose tissue (Migliaccio et al., 2011). Therefore, it has been qualified as an adipomyokine in a recent review (Trayhurn et al., 2011). Acute increase of IL-6 levels has a positive effect on skeletal muscle cell insulin sensitivity (Weigert et al., 2005). However, chronically increased IL-6 levels are detrimental as they are associated with insulin resistance (Trayhurn et al., 2011). Moreover, IL-6 is widely recognized as a bone resorption factor (Holecki and Wiecek, 2010).

Myostatin appears to be a key factor in the integrated physiology of muscle, fat and bone, as well. It is unclear whether it directly affects fat and bone, or indirectly via muscle, nevertheless, in parallel of increasing skeletal muscle mass and bone formation, inhibition of myostatin also decreases fat mass (Buehring and Binkley, 2013). Moreover, increased myostatin levels have been found in obesity and levels decrease after weight loss from caloric restriction.

Finally, the potential role of visfatin and resistin has also been discussed by Holecki and Wiecek (2010), Scotece et al. (2014) and Naot and Cornish (2014), but the authors concluded that more studies are needed before final conclusions can be drawn.

All those data explain why there is growing evidence for a negative impact of excess adipose tissue on bone metabolism, with a preferential differentiation of mesenchymal stem cells into adipocytes at the expense of osteoblasts and an enhanced production of pro-inflammatory cytokines (Cao, 2011). Identically, excess of adipose tissue alters skeletal muscle metabolism by increasing insulin resistance and lipotoxicity (Karpe et al., 2011).

8. Tendons

The musculoskeletal system consists not only of bone, muscle, cartilage, but also of connective tissues such as tendons and ligaments which form a specialized graded interface referred to as an insertion site, the enthesis (Lu and Thomopoulos, 2013). The mechanical abilities of such a structure facilitates joint motion as it far exceeds the capabilities of muscle contractile elements alone (Apostolakos et al., 2014; Roberts, 2002). In comparison to other mesenchymal lineages, the biology of tenogenic differentiation is barely understood (Noack et al., 2014). Nevertheless, formation of a mature myotendinous junction between a muscle and its site of attachment seems to be a highly regulated process that involves myofiber migration, cell–cell signaling, and culminates with the stable adhesion between the adjacent muscle–tendon cells (Li and Geisbrecht, 2012). Tendon formation requires myotomal FGF proteins, which depend upon Myf5 and Myod1. Sox5/Sox6 cartilage transcription factors also modulate tendon development (Brent et al., 2005). In case of improper establishment or maintenance of muscle–tendon attachment sites, a decrease in force generation during muscle contraction and progressive muscular dystrophies is observed. A stable myotendinous junction is maintained by the formation of the integrin-mediated hemiadherens junctions at the site of muscle–tendon attachment (the muscle-expressed αPS2/βPS integrin complex binds to Tig and Tsp while the tendon-expressed αPS1/βPS integrin complex binds to Laminin) (Brent et al., 2005).

In this system, two metalloproteinasises, the matrix metalloproteinasises (MMPs) and a disintegrin-like, metalloproteinasise domain
with thrombospondin motifs (ADAMTSs), belonging to the family of Zn(2+)-dependent endopeptidases involved in the catabolism of several components of the ECM, such as collagens, proteoglycans, fibronectin and many others, also play an important role (Sbardella et al., 2014). Upregulation of MMP-9 or downregulation of tissue inhibitor of metalloproteinases (TIMPs), lead to disordered collagen expression and disrupted tendon function (impaired potential to respond to mechanical loading), resulting in result in chronic injuries (Davis et al., 2013). In another hand, MMP-9 is important for osteoclast resorption and formation (Cackowski et al., 2010). Moreover, periostin may belong to the factors contributing to the development of tenogenic tissue (Noack et al., 2014). Recent studies have also implicated transforming growth factor β (TGFβ) signaling as a major regulator of tendons (Pryce et al., 2009).

Consequently, the dependence of tendon differentiation on the interaction with muscle represents a convergence of function in both systems. This is why injuries and degenerative conditions in tendons and ligaments represent almost half of the musculoskeletal injuries treated in orthopedic clinics. Consequently it is fundamental to better understand the trade in this zone of interactions between different tissues (including bone).

In summary, the musculoskeletal system, which comprises muscles, tendons and bones (and even fat), represents a fascinating example in which the accurate assembly of distinct cell types is crucial for the efficient movement, as well as for the stability, of the entire organism.

9. Lessons to be learned from specific physiological situations

The exposome of a person includes: internal (metabolism, endogenous circulating hormones, body morphology, gut microflora, inflammation, lipid peroxidation, oxidative stress and ageing), specific external (lifestyle factors, physical activity, diet, infectious agents, environmental contaminants) and general external (education, financial status, psychological and mental stress...) parameters that change over time (Wild, 2012). This is why specific situations lead to exacerbated metabolisms and allow to better understand systemic biology based on interactions between the different tissues and organs of the body.

9.1. Ageing

Ageing is associated with a decline in muscle and bone functions leading to sarcopenia and osteoporosis, respectively. Sarcopenia is defined by the age-related alteration of muscle mass and function (Bijlsma et al., 2012; Borrie, 2009; Dardevet et al., 2012), while osteoporosis is “a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture” (Bijlsma et al., 2012; Conference Report, 1993; Kanis et al., 2008; Klibanski et al., 2001). Recent studies have reinforced the connection between these two chronic age-related pathologies. They share common dysregulation patterns: decline in serum estrogen, androgen, IGF-1, increased level of pro-inflammatory cytokines (IL-6 and TNF-α) and possible common genes modifications (reduced Akt levels have been related to both type I muscle fiber atrophy and osteoporosis (Joseph et al., 2005; Karakis, 2011; Terracciano et al., 2013). Moreover both sarcopenia and osteoporosis may result from reduced physical activity, and therefore lower mechanical loading which appeared during ageing (Booth et al., 2012). Indeed, in the KuoPi osteoporosis risk factor and prevention study, the osteoporosis fracture prevention study and the STRAMBO study, grip strength was shown to be an indicator of osteoporosis (Rikkonen et al., 2012; Sjoblom et al., 2013; Szulc et al., 2013). Moreover, in another clinical study involving 1397 Korean men, sarcopenia was associated with lower T-scores at the lumbar spine, total femur and femur neck and importantly, with impaired quality of life (Go et al., 2013). This is why a new tool has even been defined under the term dysmobility syndrome to diagnose both pathologies (Binkley et al., 2013). Interestingly, we have recently shown that ovarioectomy, a classical model of menopause, alters both bone and muscle metabolisms in rats (Tagliaferri et al., 2014). Ovarian ablation is associated with decreased bone mineral density and impaired microarchitecture. In skeletal muscle, a reduction in the protein synthesis rate associated with a decline in MuRF1 gene expression, a ubiquitin ligase involved in protein degradation, is observed, suggesting a reduced muscle protein turnover.

As a matter of fact, an important consequence of locomotor disability is falls and fractures. Falls are generally due to multiple factors: vitamin D deficiency, impaired cognition, strength, balance and gait, multiple medical prescriptions, and environmental hazards (Fraix, 2012; Tinetti, 2003). Fall-related fractures can be caused by the reduction in muscle mass and strength or by osteoporosis itself as usually when BMD is reduced, relatively higher mechanical load is exerted on trabecular bone in osteopenic and osteoporotic subjects (Borrie, 2009; Bukhari, 2009; Kanis, 1994; Kanis et al., 1994; Ma et al., 2013; Oden et al., 2012). Falls and fractures lead to the reduction of independence and an impaired quality of life (Tappenden et al., 2012).

9.2. Nutrition facts

Sarcopenia and osteoporosis are often associated with malnutrition which leads to a vicious circle promoting the frailty situation. According to Agarwal et al. (2013), 23–60% of hospitalized elderly patients are malnourished. Besides, based on a Spanish study, 25.4% of community-dwelling older adults are at risk of malnutrition, while 4.3% are undernourished (Cuervo et al., 2009). In Australia, in such a community, 38.4% are at risk of undernutrition, and 4.8% malnourished (Visvanathan et al., 2003).

If the primary role of diet is to provide sufficient nutrients to meet the metabolic requirements of an individual, there is an emerging rationale to support the hypothesis that, by modulating specific target functions in the body, it can help to achieve optimal health. Regarding osteoporosis prevention, the main goal is to provide enough bioavailable amounts of constitutive elements such as proteins and calcium (Coxam et al., 2008; Nieves, 2013). The nutritional strategy for preventing sarcopenia is based on protein intakes (Bauer et al., 2013; Deutz et al., 2014; Walrand et al., 2011). Besides, because calcium is critical to achieve optimal peak bone mass and modulate the rate of bone loss associated with ageing and because it is the most likely to be inadequate in terms of dietary intake, every strategy targeting an improvement of bioavailable calcium is very interesting. Indeed, if it is inadequate during growth, the full genetic program for skeletal mass cannot be achieved, then, if calcium intake is not enough to offset obligatory losses, acquired skeletal mass cannot be maintained (Heaney, 2003) given the small metabolic pool of calcium, circulating concentration is mainly maintained at the expense of skeletal compartment, i.e., from an increased bone resorption (Cashman, 2002). In fact, given its low absorbability, the prospect of finding substances that might improve its bioavailability has enticed many scientists. Vitamin D can help to optimize its absorption (Klibanski et al., 2001; Lee et al., 2013). This is why, many metaanalysis showed that vitamin D in addition to calcium can reduce the risk of hip fracture (Bischoff-Ferrari et al., 2012; Boonen et al., 2007). Interestingly, those nutrients, especially proteins and more recently vitamin D, have been acknowledged to be essential for muscles as well. Protein intake is associated with less lean mass loss in people aged from age 70 to 79 years old (Houston et al., 2008), while protein quan-
tity but also quality can prevent muscle loss progression (Boirie, 2009; Gryson et al., 2013). Importantly, calcium absorption was enhanced when dietary protein increases from 10 to 20% of energy in healthy postmenopausal women (Hunt et al., 2009), and calcium seems essential to the beneficial effect of protein on bone health (Mangano et al., 2014). As far as vitamin D is concerned, better lower-extremity function is associated with 25-hydroxyvitamin D concentrations between 40 and 94 nmol/L whereas, physical performance being impaired below 50 nmol/L (Bischoff-Ferrari et al., 2004; Wicherts et al., 2007). At least 60 nmol/L has to be achieved in serum to reduce the risk of falling (Bischoff-Ferrari et al., 2009).

In vitro, 1α,25-dihydroxyvitamin D3 has been shown to enhance mineralization in osteoblasts and to promote myogenic differentiation (Garcia et al., 2011; van Driel et al., 2006). Finally, leaving those well studied nutrients aside, the concept of a healthy diet providing adequate amounts of other constituents (including various potent micronutrients or specific fatty acids) deserves mention for both targets (Barilario et al., 2013; Boirie et al., 2014; Coxam et al., 2008; Cruz-Jentoft et al., 2014; Nieves, 2013; Puel et al., 2007; Wauquier et al., 2009; Welch, 2014).

9.3. Space flight and weightlessness

Trappe (2009) made a connection between bone and muscle loss caused by microgravity over several months and by ageing over decades. The most common example of alteration is the atrophy of muscle and the loss of bone during space flight (Léblanc et al., 2000).

In 9 crewmembers with an exercise prescription, both soleus and gastrocnemius volume decreased (~15 ± 2% and ~10 ± 2%, respectively; peak power being 32% lower (p < 0.05), after 6 months aboard the International Space Station. Furthermore, there was a 12–17% shift in myosin heavy chain phenotype of the gastrocnemius and soleus with a slow-to-fast fiber type transition (Trappe et al., 2009). In the same way, after a 5 (n = 3) or 11 (n = 5) days space travel, mean cross-sectional fiber areas (measured on vastus lateralis biopsies) were 11–36% smaller and there were 6–8% fewer type I fibers (Edgerton et al., 1995). Similar results were observed in human soleus biopsies (Widrick et al., 1999). The impacts of microgravity on muscle: atrophy and decline in peak force have been summarized in Fitts’ review (Fitts et al., 2001). In vitro embryonic avian muscle cells protein synthesis rate decreased, without any impairment of protein degradation, after 9–10 days aboard the Space Transportation System (Vandenburgh et al., 1999). This was speculated to be exacerbated in vivo as altered circulating levels of factors such as growth hormone, glucocorticoids, or anabolic steroids, were likely to also occur in such a situation.

With regards to the skeleton, space travel also slowed down bone formation (Doty, 2004; Lang et al., 2006; Wronska et al., 1987), while histological indexes of bone resorption were normal in rats, even though biochemical markers of resorption were elevated during space flight (McCarthy, 2011; Wronska et al., 1987). An in vitro study carried out in osteoclasts from murine bone marrow confirmed that bone resorbing cells and their precursors are direct targets for microgravity (Tamma et al., 2009). Moreover, mechanisms of osteoclastogenesis, which was doubled for osteoclasts subjected to modeled gravity (mXg) in rotary cell culture system for 24 h compared to normal gravity control (Xg), have been studied (Sambandam et al., 2010). Space travel bone loss (increased resorption coupled with decreased formation) is actually similar to that observed in postmenopausal osteoporotic women (McCarthy, 2011). An important fact is that the severity of bone demineralization and muscle atrophy generally increases with space flight duration (Meck et al., 2009).

Long-term bed rest has similar impacts on the locomotor system and is used to simulate the physiological changes which may occur in the microgravity environment (Allner and Tesch, 2004; Droppert, 1993). Head-down tilt bed rest is another model, also used as a space flight model (Morgan et al., 2012; Spector et al., 2009; Wang et al., 2012).

Indeed, bed rest decreases muscle force production as well as skeletal muscle volume and muscle cross sectional area (Belavy et al., 2009; Bloomfield, 1997; Stuempfe and Drury, 2007). Skeletal muscle is even impaired at the cellular level: fiber size and diameter are reduced, as is capillarity (Krasnoff and Painter, 1999). In the same way, Ferrando et al. (1995) measured a significant decrease in segmental thigh muscle volume (approximately 3%, p < 0.05) after 7 days of bed rest. In another experiment conducted by Ferrando et al. (1996), inactivity resulted in a loss of body protein which was predominantly due to a decrease in muscle protein synthesis both at the skeletal muscle and whole body levels. Protein breakdown did not change significantly.

As a matter of fact, bone is also affected by immobilization, its mineral density being reduced (Krasnoff and Painter, 1999). Such loss in bone mass could be explained by removal of the compressive forces on the skeleton (Morgan et al., 2012; Stuempfe and Drury, 2007; Wang et al., 2012). According to the short time bed rest clinical trial conducted on 12 subjects by Morgan et al. (2012), a 20% increase in urinary markers of bone resorption was observed (p < 0.001). In the same way, in an eight weeks bed rest trial performed on 20 healthy men (Berlin Bed-Rest Study), increase in the bone resorption marker collagen type 1 cross-linked C-telopeptide (CTX) was observed within 3 days (Armbrecht et al., 2010). Consequently, under conditions of disuse, a decline in bone strength is preceded by a decline in muscle mass (Burr, 1997; Lloyd et al., 2013). The coupling between diminished bone mass and muscular strength increases the risk of fractures (Stuempfe and Drury, 2007). Furthermore, Bloomfield (1997) pointed out that muscle mass and strength are regained weeks or months before bone mass, which also contributes to the risk of fractures.

Those experimental conditions help to understand the mechanisms underlying those processes of both osteopenia and sarcopenia and to set up new strategies (resistive exercise, electrostimulation, nutrition) to limit astronauts’ muscle and bone losses (Armbrecht et al., 2010; Belavy et al., 2009; Brooks et al., 2010; Mayr et al., 1999; Rittweger et al., 2005; Smith et al., 2012).

10. Conclusion

Numerous studies have reinforced the bone–muscle unit hypothesis. Mechanical and metabolic relations have been identified. Skeletal muscle is thought to control bone mass and mineral density by mechanical loading which promotes bone metabolism through the activation of signaling pathways, triggered, in majority, in osteocytes. Moreover, osteoinducer (IGF-1, IGF-2, IL-15, OCN, FAMSC, Tnem119, osteoactivin) and osteinhibitor (IL-6 and myostatin) myokines have been identified in skeletal muscle cells.

Besides, bone cells have been found to secrete PGE2, Wnt3a, OCN and IGF-1, which are thought to have myoinducer properties, and sclerostin which could impair muscle metabolism. The key question of who from the muscle or bone predominates on this system of regulation remains intact. In this light, the “muscle-predominant” concept based on the myostatin signalling pathway has emerged (DiGirolamo et al., 2013). The roles of cartilage adipose tissue and tendons are not to be forgotten has they can potentially affect bone and muscle and can in turn be affected by these tissues. Indeed, chondrocytes secrete DKK1, an osteoinhibitor molecule and IHH which can promote both muscle and bone metabolisms. Adipocytes can modulate bone and muscle via the secretion adiponectin, leptin and IL-6. Moreover, myostatin seems to play a central role in cross-linking these tissues, as it can negatively impact muscle, bone...
The muscle–bone unit.

Fig. 3. The muscle–bone unit.

and cartilage and promote adipose tissue. Other metabolic mechanisms such as inflammation are involved in the development of osteoporosis and sarcopenia, pathologies affecting bone and muscle respectively. Studies on space-flight and bed-rest allowed the identification of mechanisms underlying the processes of both osteoporosis and sarcopenia. The beneficial impact of physical exercise and a balanced diet, including key ingredients as proteins, calcium, vitamin D and various potent micronutrients or specific fatty acids, seems essential (Fig. 3).

The involvement of all those parameters reinforces the concept of pleiotropy, a basis of systemic biology and the critical need for investigation, simultaneously targeting all those tissue together with more complete environmental exposure assessment.

Further investigations are needed to fully understand the mechanisms involved in the cross-talk between bone, skeletal muscle, tendons, adipose tissue and even blood vessels, in order to develop new strategies to counteract the development of both osteoporosis and sarcopenia.

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