PERSPECTIVES

Exercise and the Skeleton: How It Works and What It Really Does

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Abstract

Exercise and nutrition are among the most commonly advocated lifestyle measures to improve skeletal health. A variety of exercise regimens has emphasized the beneficial role of speed, strength, power, endurance and coordination in improving bone mass and structure, and the effects of these regimens vary depending on age and are maximized during childhood and adolescence. Exercise influences the skeleton primarily by its direct impact on bone and by improving muscle mass and strength, which exerts further strains on the skeleton. These strains are sensed by mechanoreceptors, primarily on osteocytes, whose nature remains incompletely understood but which ultimately transduce the mechanical signals into biological signals, a process that involves the Wnt-β-catenin canonical signaling pathway. This biological signal is able to trigger bone remodeling by directing osteoblast activity and osteoclastic resorption. The role of microcracks in the mechanotransduction pathway and in the initiation of bone repair remains to be elucidated. Moreover, exercise induces changes in circulating levels of hormones such as growth hormone (GH) and insulin-like growth factor (IGF)-1, which exert anabolic effects on muscle and bone. A better understanding of the potential and limitations of the effects of physical activity on the skeleton, and of the molecular mechanisms mediating these effects, will lead to the development of new bone anabolic agents. *IBMS BoneKEy*. 2010 July;7(7):235-248.
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Introduction

Bone has two main functions as a metabolic and structural tissue. The metabolic demands on the skeleton are regulated largely through calciotropic hormones whereas the structural functions of bone are determined primarily by genetic factors and adaptation mechanisms to its loading environment. From an historical point of view, it is important to note that the work of Wolff concentrated on the principle that static stressing of bone provoked alterations in shape, whereas Wilhelm Roux was the first to propose in 1881 a dynamic concept of functional adaptation by which the skeleton optimizes its structure to meet mechanical demands (1). Frost then developed the concept of the "mechanostat" describing the piezoelectric properties of the bone and other signaling mechanisms through which bone strength is increased where it is most needed. The importance of bone adaptation to exercise is such that osteoporosis can be defined as a failure of the adaptive response to maintain the structure needed to withstand daily loading (2).

The beneficial effects of exercise on the mechanical properties of bone tissue are determined by the structure (macroarchitecture and microarchitecture) and the intrinsic quality of bone (the latter involving mainly hydroxyapatite and collagen content, which will not be the focus of this review). The loads applied on bone tissue during exercise can be divided into four categories: axial compression, bending, shearing and twisting. Each of these forces will affect bone tissue differently. For example, compression applies a homogenous strain on the surface of a transverse section of bone whereas twisting applies a high strain on the external surface but no significant forces on the internal

surface of bone. During exercise there is a combination of these different forces, making it difficult to predict the resulting effects on bone structure. However, several animal and human studies have shown that exercise influences both modeling and remodeling of the cortical and trabecular bone compartments. Loading increases periosteal deposition and slows endosteal resorption or causes endosteal deposition depending upon the skeletal location. Strains produce modeling mostly in the proximal region of limb bones (3;4), changing bones' geometry as evaluated by the total tissue volume, cortical or endocortical cross-sectional area, and moment of inertia. In rodents, exercise also increases bone volume fraction (BV/TV) as a result of increasing trabecular number and thickness, and decreasing trabecular separation (5-7). Similar effects have been reported in humans by measuring apparent trabecular bone parameters with QCT, pQCT or MRI (8;9). Finally, there is abundant evidence that mechanical properties are improved in response to loading, mostly through increases in periosteal apposition and particularly in the young (10;11) (see below).

Interestingly, bone turnover changes may tend towards bone formation in response to exercise of moderate intensity, but towards bone resorption with intensive training (12). In the latter case, Haversian (intracortical) bone remodeling could be increased, particularly in the distal region of the weight-bearing bones where stresses and strains are higher, creating microdamage. This in turn would induce osteocyte apoptosis that may initiate Haversian remodeling (13). The true nature of the message transmitted by osteocyte apoptosis is still unknown (this is one of the new interests in the field of mechanical bone signaling) (14). This remodeling process produces a transient increase in porosity, leaving the bone insufficiently strong to resist further strains produced by repeated loading, which would be a mechanism for stress fractures.

The Influence of Age on the Skeletal Response to Exercise in Humans

A quick overview of published data on exercise and bone in rodents and humans shows mainly beneficial effects (15;16). However a closer look reveals discordant results particularly depending on the age of practice (Fig. 1) (17). A number of published studies indicate that the most effective period during which to observe a larger increase of bone mineral mass with exercise is during adolescence, especially during the pre-pubertal years (18;19). Hence a prospective study in 120 children aged 8 years showed that 3 jumps of 61 cm per week over the course of 7 months doubled the percent change of bone mineral content (BMC) at the femoral neck and spine compared to a control group (20). Jumping was also effective in children aged 13 years in improving bone accrual in a sex-specific manner (21). Girls practicing gymnastics exhibit higher bone mineral density (BMD) and bone diameter at the midshaft tibia and femur compared to girls playing football (22). As summarized in a recent review, physical activity should include activities that generate high ground-reaction forces, such as jumping, skipping and running and last at least 7 to 20 months, to increase bone mass by about 2% at weight-bearing sites (femoral neck, spine) (23). Greater spinal BMC and apparent BMD (aBMD), as well as trabecular volumetric density and bone strength in the peripheral skeleton, are also observed in adult elite gymnasts (24), suggesting that the effects of exercise on bone mass growth are retained in adults, providing a true benefit as they reach peri-menopause. However, it has been reported that more than half of the BMC gain (+3.5% after 7 months of exercise versus controls) induced by jumping in pre-pubertal boys and girls (25) was lost over 8 years, that is, even before these subjects reached peak bone mass. To understand this phenomenon, the 'Achilles tendon of the exercise effect,' it should be kept in mind that 80% of peak bone mass is genetically determined (26;27). Hence an initially greater bone mass (just after the intervention) may subsequently be remodeled to ultimately reach its genetically determined target.
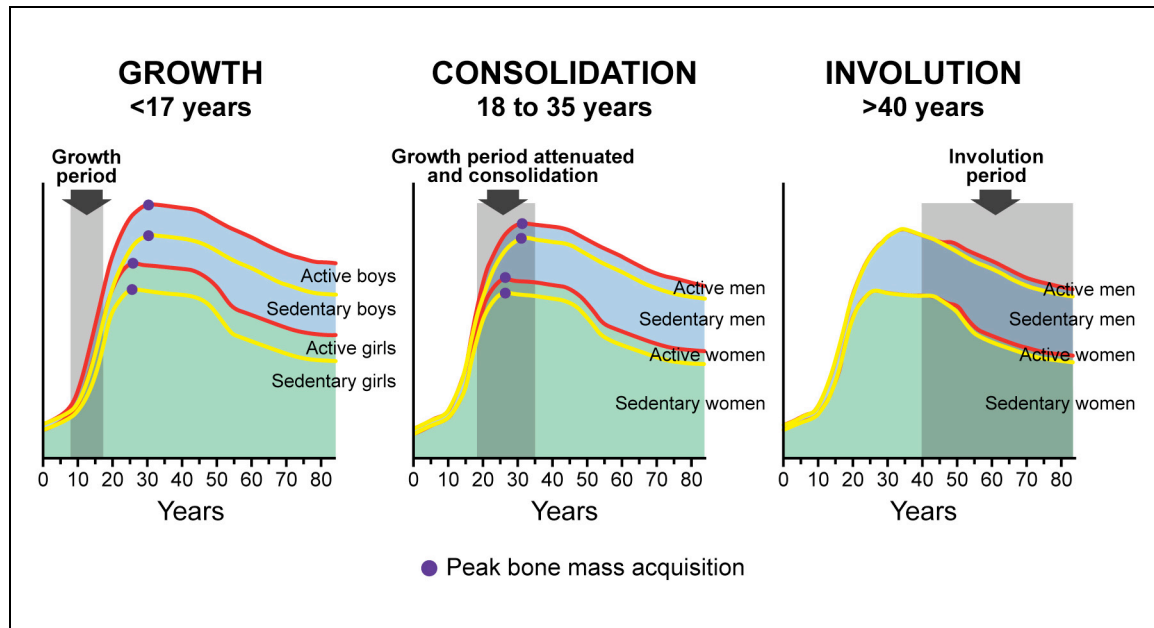
Fig. 1. Schematic description of the effect of physical activity on bone mass throughout life. The red curve represents a continuous exercise effect; otherwise a punctuated effect of exercise could be attenuated over time. The yellow curve represents a sedentary person engaging in normal activities (such as walking). Adapted from Blanchet C, Chaire L, Chagnon A, Thibault G. Activité physique et santé osseuse. Avis du comité scientifique de Kino-Québec. Québec: Gouvernement du Québec, Ministère de l'Éducation, du Loisir et du Sport; 2008:1-42.

Nutrition may influence the effect of exercise in children and adults. Calcium supplements in prepubertal girls have been shown to interact with their level of physical activity on bone mass gain (28). Moreover, exercise had no effect in children with a low calcium intake (452.8 mg/day), a mild effect in those with moderate intake (762.9 mg/day) and a greater effect in those with a high calcium intake (1073 mg/day) (29). Similar observations have been reported for protein intake and exercise on BMD and microarchitecture in young boys (30), which could be explained by a dual stimulation of insulin-like growth factor (IGF)-1 production by proteins and exercise.

The effects of exercise in adults are less prominent. In a meta-analysis pooling 14 studies, exercise resulted in a slightly higher BMD at the spine (weighted mean difference 0.016 g/cm^2, CI, 0.005 to 0.027, p=0.005) (31). In older populations there are no published, randomized, prospective studies demonstrating positive effects of exercise on BMD. The lack of clear exercise effects in this population may have several explanations. First is the difficulty in the elderly of practicing exercise with an impact of sufficient intensity to have a bone-forming effect (see below) (32). Moreover, a lack of motivation and fatigue limit compliance and the effectiveness of conventional exercise training. Second, lower muscle mass and strength (sarcopenia) in the elderly limits the strain exerted on the skeleton (33). Finally, the number of osteocytes and/or their sensitivity to mechanical strain may decline with age. The osteogenic potential of mesenchymal stem cells also declines with age as a result of changing expression of Wnts and PPARs, with a related increase of bone marrow adipogenicity (34;35). Nevertheless, despite no positive effect on BMD, several studies have demonstrated a reduction of fracture rate in elderly people practicing an activity, probably by preventing falls (36-38).

Mechanisms of Exercise-Related Bone Modeling/Remodeling

Exercise influences the skeleton by three main mechanisms: a direct impact on bone

that is translated into biological signals by mechanoreceptors; an indirect impact by improving muscle mass and strength that secondarily stimulates these mechanoreceptors; and an indirect impact by inducing changes in hormone levels (such as calciotropic hormones, leptin, etc.) and local factors (Fig 2).
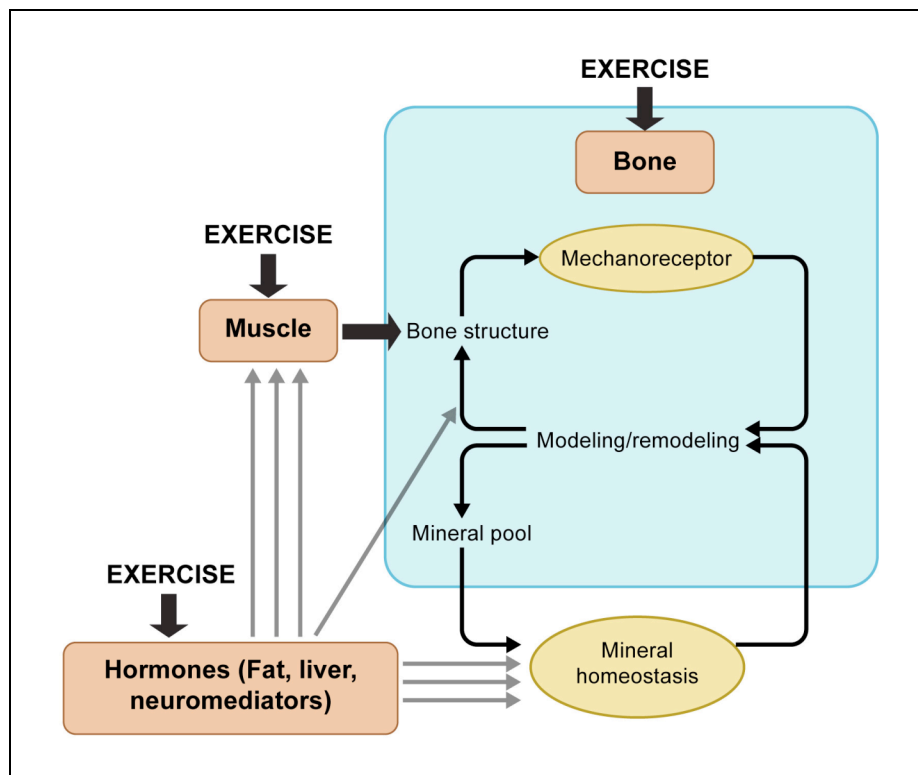

Fig. 2. Schematic description of the effect of mechanical stimuli on the skeleton through structural adaptation of bone and the maintenance of physiological homeostasis by employing the mineral reservoir embedded in the bone structure. During exercise load is transmitted to the skeleton through direct stimulation of the bone mechanosensor and by indirect stimulation through dynamic muscle activity. Hormones from fat and the liver modulate loading by affecting bone and muscle growth as well as muscle performance, and act indirectly through potential changes in the mineral reservoir. Recent data also underscore a paracrine role of muscle factors on bone adaptation in response to loading as well as the importance of neurons' capacity to change their function, chemical profile, or structure in response to mechanical stimuli, improving skeletal modeling/remodeling responses. Adapted from Sievänen H. Hormonal influences on the muscle-bone feedback system: a perspective. *J Musculoskelet Neuronal Interact.* 2005 Jul-Sep;5(3):255-61.

Direct effects of exercise on bone

As recently reviewed by Rizzoli *et al*. (23), most of the exercises demonstrating a significant effect on bone mass acquisition are those with impact, whereas exercises such as swimming or bicycling exert no significant effects on bone. For this reason, studies on bone adaptation to exercise performed in the past 30 years have focused on the effects of direct mechanical stimuli on bone, which has led to three main observations. First, bone adaptive responses require dynamic rather than static

mechanical stimuli. Thus bone formation increases with the degree of bone deformation, however, up to a certain level of deformation the bone formation response plateaus (39). This is well-illustrated in animal studies using axial compression on the ulna or tibia showing that this relationship is not linear but rather more sigmoid in nature (40), and in human athletes. In the latter instance, those who perform triple jumps generating 20 G of force have higher limb BMD than gymnasts (10 G) and runners (3 G) (41). In a recent study, volumetric BMD of the proximal tibia

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increased with running distance to reach a plateau over 30 km/week, and was positively associated with peak acceleration (vertical acceleration expressed in G) in athletes running over 30 km/week (42). The second major observation is that extending the duration of skeletal loading does not yield proportional increases in bone mass as it does with the frequency of exercise. As loading duration is increased, the bone formation response tends to fade as the osteocytes and/or osteoblasts sensing the signals become desensitized. As a corollary, adaptive bone responses would be improved with brief, intermittent exercise. The third major observation is that the orientation and magnitude of stress on the skeleton during exercise must differ from the usual pattern of bone loading in order to produce a maximal stimulus on bone formation. This has been well-described by Lanyon *et al*. who write that "[t]he mechanically adaptive response is dominated not by the numerous cycles of 'normal' strain change engendered during the predominant activity but rather by far fewer cycles of relatively 'abnormal' strain changes produced during unusual loading situations" (43).

Mechanisms of mechanotransduction

Osteocytes, which represent up to 90% of all bone cells, seem to be the most appropriate cells to sense the magnitude and direction of mechanical strain: they are broadly distributed through both trabecular and cortical compartments, embedded in the calcified tissue and well-interconnected to other osteocytes, osteoblasts and osteoclasts through a network of canaliculi. Although it is widely accepted that the osteocyte is the cell responsible for sensing mechanical strain, there is a debate within the field as to whether or not this is detected at the cellular level and how this mechanical transduction is carried out. Osteocytes probably do not respond directly to mechanical strains, but rather respond to extracellular fluid waves in the osteocytic lacunae and canalicular system generated by the loading stimuli (44). Mechanoreceptors (strain-generated potential) present on osteocyte dendrites

would in turn generate small changes in electrical charges, activate calcium channels, and/or stimulate the release of molecular mediators such as prostaglandin (through the activation of COX2) and nitric oxide (NO) that further communicate the mechanical signal to osteoblasts at the periosteum, and to osteoblasts and osteoclasts intracortically and at endocortical surfaces (45). Another possibility is that osteocytes, mesenchymal stem cells and/or osteoblast lining cells sense strain at their plasma membranes through stretch-activated ion channels that permit calcium flux, potentially initiating other intracellular responses (46) (Fig. 3). These biochemical signals have two primary functions: activation of transcriptional mechanisms regulating bone modeling/remodeling, and propagation of mechanical information to other osteocytes through paracrine effects (by NO and PGE2 secretion, for instance) and transmission of other chemical signals by gap junctions (2;47). Recent evidence in mice and from *in vitro* studies indicates that Wnt-LRP (48-50) signaling plays a major role in mediating mechanical loading (51). Mechanical strains in osteoblasts induce a rapid, transient accumulation of active β-catenin in the cytoplasm and its translocation to the nucleus inducing target genes such as *COX2* (52). Interestingly, the Wnt-β-catenin pathway also seems important in the mechanotransduction performed by the osteocyte. Bonewald *et al*. have demonstrated that fluid flow shear stress treatment of MLO-Y4 osteocytes results in increased phosphorylation of GSK-3β, β-catenin nuclear translocation, and changes in the expression of β-catenin target genes (such as those for sclerostin and dickkopf 1) (53). Other signaling pathways activated in response to mechanical loading, such as those involving Akt, may also crosstalk with the Wnt-β-catenin pathway (54). Consistent with these *in vitro* studies, *in vivo* loading of the ulna of the TOPGAL mouse results in activation of β-catenin signaling in osteocytes 1 hour after a single loading, whereas it was detectable on the bone surface only after 24 hours, suggesting an
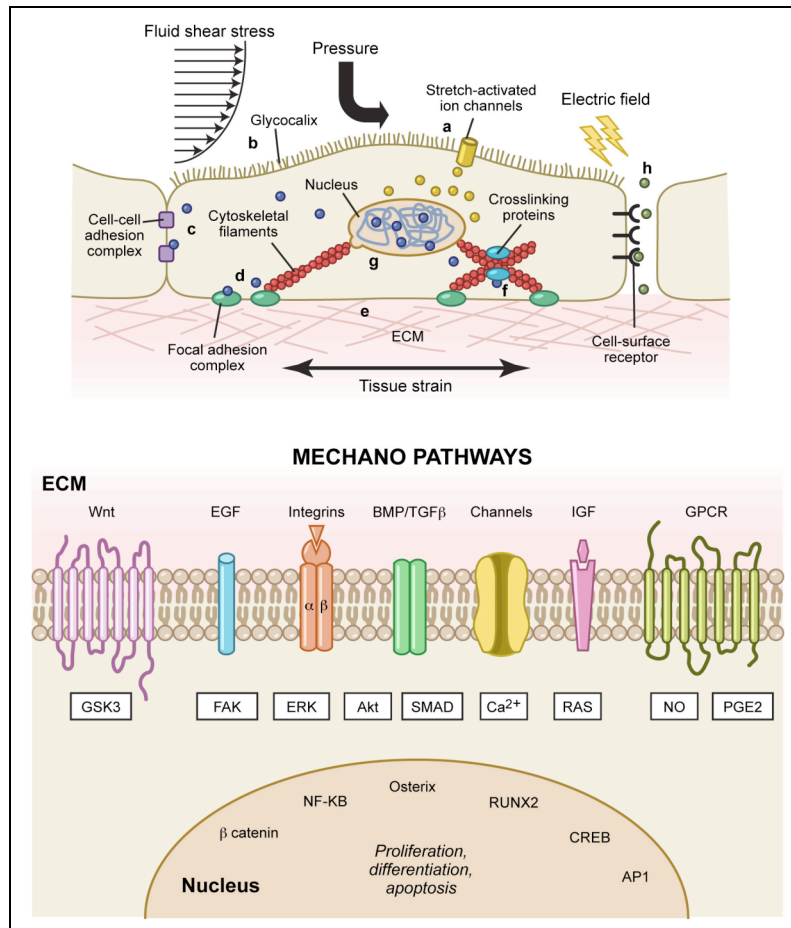
Fig. 3. Mechanotransduction. Osteocytes, osteoblasts, mesenchymal stem cells and many other cells have the capacity to response to mechanostimulators (fluid shear stress, pressure, electric fields and tissue strain). Several biological components have been proposed to act as mechanosensors: **a.** Stretch-activated ion channels in the plasma membrane open in response to membrane strain and allow the influx of calcium and other ions. **b.** Single cilia or the glycocalix, a layer of carbohydrate-rich proteins on the cell surface, can mediate mechanotransduction signaling in response to fluid shear stress by inducing the influx of calcium or increased intracellular cAMP, inducing COX2 and osteopontin signaling. **c**, **d.** Cell-cell junctional receptors or extracellular matrix (ECM)-cell focal adhesions allow cells to probe their environments, as is the case for the complex integrin-actin filaments that can activate several signaling pathways such as mitogen-activated protein kinase (MAPK)-focal adhesion kinase (FAK). **e.** Force-induced unfolding of ECM proteins, such as fibronectin, can initiate mechanotransduction signaling outside the cell. **f.** Intracellular strain can induce conformational changes in cytoskeletal elements such as filaments, crosslinkers or motor proteins, thereby changing binding affinities to specific molecules and activating signaling pathways such as the caveolin known to activate G protein signaling. **g.** The nucleus itself has been proposed to act as a mechanosensor. Intracellular deformations can alter chromatin conformation and modulate access to transcription factors or transcriptional machinery. **h.** Compression of the intercellular space can alter the effective concentration of autocrine and paracrine signaling molecules, such as nitric oxide (NO) and PGE2. In addition, changes in seven transmembrane domain G protein-coupled receptor (GPCR), lipid fluidity and even mitochondrial activity have been proposed as mechanosensors. These effects are often mediated through multiple, overlapping and crosstalking signaling pathways. The highlights of such cascades include the three modules of the MAPK family underscored by the activation of Ras, the Janus-activated kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, Rac activation, calcium (Ca^{2+}) and NO signaling. The convergence of these pathways results in the activation of select transcription factors such as β-catenin implicated in the Wnt/LRP pathway, the most important pathway both in osteoblasts and osteocytes. Adapted from Jaalouk DE, Lammerding J. Mechanotransduction gone awry. *Nat Rev Mol Cell Biol*. 2009 Jan;10(1):63-73, with permission from Macmillan Publishers Ltd.

earlier stimulation of canonical Wnt signaling in the osteocyte rather than in the osteoblast. It should also be noted that osteocyte-specific β-catenin-deficient mice are characterized by low bone mass (55). Fig. 3 illustrates the many mechanotransduction mechanisms possibly activated in response to exercise.

Skeletal effects of exercise through muscle

The strong association between muscle mass/strength and bone anabolism and catabolism during growth, development and aging highlights the important interaction of these two systems to optimize locomotion (56). The fact that peak rates of bone mineral acquisition are preceded by peak rates of muscle mass gain, whereas sarcopenia later in life is accompanied by bone loss, supports the idea that exercise programs aiming to improve muscle mass and strength would also stimulate bone formation (57). Several exercise programs, therefore, have been developed to stimulate both muscle and bone anabolism in postmenopausal women (58). It appears that high-intensity resistance training that improves strength and muscle mass also improves bone mass (59). Muscle contraction can activate bone mechanoreceptors (Fig. 2), particularly in the periosteum where tendons are attached. In fact, 70% of the tension exerted on bone depends on muscle contraction, which is therefore more important than body weight itself as a mechanical stimulus (as further demonstrated by bone loss during immobilization or paralysis) (33). This property is well-illustrated by the phenotype of myostatin-deficient mice, which present myofiber hypertrophy, high BMD in the spine and limbs as well as increased bone strength, mainly due to changes in bone geometry primarily at sites of muscle insertion. In contrast there were no changes in shape or cross-sectional area of the midshaft femur (60;61). Interestingly, the frequency of skeletal muscle contraction is higher (15-30 Hz) than the frequency of direct mechanical stimuli on bone (0-15 Hz), raising the possibility of synergistic effects on bone of exercises combining impact and muscle contraction (62;63). Again, this has been well-illustrated in mice lacking myostatin and performing exercise on a treadmill, in which case bone strength (represented by ultimate force, toughness and ultimate strain) was 25% higher than in normal mice (60). However the bone-muscle unit paradigm has several exceptions, such as in cycling or swimming where the bone response to exercise is lower compared to other activities, suggesting that there is less bone formation when gravitational forces are lower. For that reason no study has yet demonstrated unequivocally that either gravitational or muscle forces provide the dominant anabolic and anti-catabolic stimuli of exercise.

A potentially interesting new mechanism regulating the relationship between muscle and bone involves the nuclear receptor protein peroxisome proliferator-activated receptors (PPARs) β/δ. It has been shown that a PPAR β/δ agonist mimics the effects of exercise on muscle by increasing its oxidative metabolism and the switch of type II fast fibers responsible for rapid movements to type I slow fibers responsible for sustained activity (64-66). Transgenic mice over-expressing PPAR β/δ run faster and longer without myofiber hypertrophy and have been called 'marathon mice.' Preliminary data from our laboratory suggest that PPAR β/δ-deficient mice exhibit a low bone mass phenotype in relation to their low lean mass. Recently it was also shown that muscle can secrete growth factors such as IGF-1 and FGFs that would exert a paracrine role on periosteal cells (67). Muscle has also been suggested to contain a reserve of potential osteoprogenitor cells (68).

Exercise effects on bone through hormones

Several studies have shown that an acute bout of exercise can increase concentrations of anabolic hormones, such as GH/IGF-1, and FSH/LH/estrogen, across a wide age range (69). In particular, studies of estrogen by Lance Lanyon's group have provided evidence for a critical role of the estrogen

receptor in the mechanotransduction pathway (70;71). These hormonal changes are amplified by exercise patterns and intensity (see above).

Exercise modifies liver production of IGF-1 by modifying the flow of GH secreted by the pituitary gland. The rise in the amplitude and frequency of GH/IGF-1 production is explained by the stressing effects of exercise on serum glucose levels (hypoglycemia) (72;73). IGF-1 is also locally secreted by bone and muscle cells in response to the activation of mechanoreceptors (74) and IGF-I expression is increased in osteocytes and bone-lining cells within 6 hours of mechanical loading (75). Conversely, the low bone formation seen with disuse is due in part to a resistance to the effects of IGF-I on bone formation (76).

Parathyroid hormone (PTH) levels are also increased after maximal exercise independently of sex, age and training status (77-79). During physical exercise, the increase in PTH is due mainly to the calciuric effects of exercise, but other factors such as catecholamines and acidosis can also modify the secretion of PTH (80). One hypothesis is that the intermittent release of PTH produced by exercise is a systemic mediator of the anabolic actions on bone tissue (81). It was reported recently that variations in the circulating level of calciotropic hormones (PTH, vitamin D metabolites, and calcitonin) related to physical activity may modulate the bone tissue response to exercise (82). This is consistent with experimental evidence that administration of PTH before mechanical stimulation significantly increases the osteogenic response (83-85). This could be mediated by a PTH-stimulated production of PGE2 (86), and an increase in the activation of intracellular calcium, an important second messenger in the mechanotransduction cascade (see above) (83).

Adipokines, such as leptin, and enterokines, such as ghrelin, are also regulated by exercise (87;88). The role of the sympathetic nervous system, primarily β2 adrenergic receptor-mediated signaling, in the

regulation of bone turnover has also been reported (89-91), as has the role of other neuroendocrine factors (glutamate, NPY, AgRp) (92;93). Our preliminary observations in mice lacking the β2 adrenergic receptor indicate that their skeletal response to mechanical loading is unaltered, whereas the lack of the β1 adrenergic receptor attenuates the trabecular and cortical response to loading (94). The influence of adrenergic tone on bone metabolism in response to exercise, however, will be complex in consideration of adrenergic effects on fat tissue and metabolism (lipolysis) (89;95;96). An extensive review of β-adrenergic effects on bone and response to exercise has been published in recent years (89;95).

Bone vascularization has also been implicated in exercise effects on bone structure. Moreover, the skeletal response to treadmill exercise was blocked by an anti-VEGF neutralizing antibody (97), suggesting that neo-angiogenesis is indispensable for bone gain in these conditions.

In summary, exercise can effectively improve bone mass, structure and strength, provided it is administered early in life, and with sufficient loading intensity and frequency. Its ultimate effects on the prevention of osteoporosis, however, could be quite limited. A better understanding of the complex pathways transducing mechanical stimulation into bone formation, particularly at the muscle-bone functional unit, is leading to the development of novel pharmacological approaches, such as SARMs, myostatin antagonists and sclerostin antibodies, which in turn could mimic the effects of exercise on the skeleton.

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