Mechanotransduction and Biomechanical Properties of Bone Tissue as a Basis for the Use of Intraosseous Osteopathic Techniques

Novoseltsev Svyatoslav Valeryevich[1*], Reshetnikov Alexey Germanovich[2], Vyunov Vladislav Dmitrievich[3], Suleymanov Timur Rasimovich[4], Nefedova Anastasia Maksimovna[5], Solomatov Petr Grigorievich[6]

[1,2,3,4,5,6]*Department of Sports Medicine and Medical Rehabilitation of the Institute of Clinical Medicine. N.V. Sklifosovsky Federal State Autonomous Educational Institution of Higher Education First Moscow State Medical University. THEM. Sechenov of the Ministry of Health of Russia (Sechenov University); E-mail: snovoselcev@mail.ru*

Abstracts: The development and maintenance of bone mass is critical to movement, health, and quality of life. Bone mass is regulated by various factors, activation of biologically active substances, enzymes, including changes in mechanical load. The osteocyte network provides a wide-ranging load monitoring "network" that penetrates every cubic millimeter of bone tissue. Deformities or trauma create a limitation that can cause asymmetric bone growth, such as plagiocephaly. A deep understanding of the cellular mechanisms responsible for bone remodeling as well as bone mechanotransduction is essential for the development of effective exercise, osteopathic techniques, and pharmaceutical strategies to increase and/or prevent bone loss. This review summarizes current data on the main molecular mechanisms involved in bone mechanotransduction, which provides insight into bone tissue, methods of working with it, and points of application for correcting various dysfunctions to promote bone growth in length. This process is carried out by increasing the proliferation of chondrocytes and the activity of growth plates. This contributes to the correction of such consequences of intraosseous dysfunctions as the development of anatomical differences in the size of the lower extremities in children.

Keywords: Mtorc1, Growth, Bone Mineral Density, Mechanical Loading, Mechanotransduction.

1. MAIN TEST OF THE ARTICLE

The osteon is the basic structural unit of bone tissue, which ensures its strength and stability. It consists of a central canal called the Havers' canal, which contains blood vessels and nerve endings whose function is to nourish and control the osteon. The canal is surrounded by osteocytes.

Osteocytes perform many functions, such as maintaining the metabolism and mineral composition of the bone matrix, regulating bone growth and development, and participating in the process of bone tissue renewal.

It is a continuous process that is able to maintain the mechanical strength of bone under conditions of constant microtraumatization thanks to osteoblasts, the cells responsible for the synthesis of the bone matrix, and osteoclasts, the cells that destroy old bone tissue [1].

Remodeling occurs due to all cells of the bone tissue, and the aggregate of these cells is called the Basic Multicellular Unit (BMU) [2].

The bone remodeling cycle lasts 200 days, but when the BME cell pool is imbalanced in thyrotoxicosis or primary parathyroidism, the cycle is reduced to 100 days, and in myxedema it increases to 1000 days [3].

Bone remodeling consists of five stages. At the first stage there is an activation of osteocytes (mechanical load is transformed into biological impulse) in a bone matrix thickness [4]. Factors attracting precursors of osteoclasts (cells of monocytic-macrophage series) are released, then there is activation of multinucleated osteoclasts themselves, which secrete metalloproteinases destroying a surface protein layer.

At the second stage (resorption), osteoclasts form resorptive lacunae, and calcium and phosphates enter the blood. Resorption lasts about 30-40 days [5].

The third stage consists of reversion. Osteoclasts change into preosteoblasts (cells from the mesenchymal sprout). And they in their turn to mature osteoblasts secrete molecules, organic matrix and regulators of mineralization - type I collagen, osteocalcin, osteonectin, osteopontin. As a consequence, mineralization of the matrix occurs due to the precipitation of calcium and phosphate coming from the blood [6]. The formation of bone tissue takes about 150-200 days and normally ends with the complete filling of the lacuna with new matrix [5].

Pathological processes can disrupt the filling of the lacuna, and this can lead to a loss of bone mass with each cycle of remodeling [7].

At the final stage, osteoblasts turn into osteocytes and cover cells on the bone surface. In this resting state, BME remain until the next cycle of remodeling [8].

Bone has two parts: spongy and compact. In the compact part, remodeling occurs in tunnels (havers systems) [9]. The duration of the remodeling cycle in compact bone is shorter than in cancellous bone and is about 120 days [9].

About 30% of trabecular bone and about 3% of compact bone undergo remodeling in the human body each year [10].

Biomechanical properties of bone tissue play an important role in its function and ability to withstand mechanical loads. They are determined not only by its composition, but also by its architecture. The main mechanical properties of bone tissue are *strength, stiffness, and elasticity*.

Bone tissue consists of organic and inorganic components. Organic components include collagen, proteoglycans, and other proteins that give it flexibility and strength. Inorganic components are minerals, such as calcium and phosphorus, which give bone hardness and rigidity.

The architecture of bone tissue can be altered by various factors, such as training, injury, or disease. For example, when exercising, bones are subjected to increased stress, which contributes to their density and strength. However, with osteoporosis, bones become more brittle and prone to fractures. This is due to mechanotransduction processes.

Bone tissue *mechanotransduction* is the process by which mechanical signals are converted into biological responses in bone tissue. A series of secondary biochemical signaling events then occur to propagate the signal within the cell and to other sensory/effector cells. Efforts to understand the signaling pathways involved in mechanical signal propagation have revealed many changes in the mechanically stimulated osteocyte/osteoblast, including changes in gene expression, protein and lipid modifications. This process plays an important role in the regulation of bone remodeling, bone adaptation to mechanical loading, and maintenance of bone mass.

1.1. Ion channels

Many mechanosensitive tissues in the body are regulated by ion channels. Ion channels are pore-forming proteins that cross the plasma membrane and allow ions to flow in and out of the cell based on an electrochemical gradient. Their activity (opening and closing) can be controlled by various mechanisms; among them are changes in voltage across the membrane, biochemical ligands, or physical stimuli such as mechanical perturbation of the membrane. It has been experimentally confirmed that ion channels play a crucial role in the process of mechanical transmission in bone [11,12]. For example, $Gd3^{+}$ is a potent blocking agent for mechanosensitive ion channels, and pretreatment of bone cells with $Gd3^{+}$ before mechanical loading stimulation results in loss of subsequent expression of the mechanical transmission marker [13,14].

More recently, a family of transient potential (Trp) channels has been investigated regarding their role in mechanotransduction of bone cells [15,16]. In particular, TrpV4, a receptor that is sensitive to mechanical perturbations (primarily cell edema) and osmolarity in other tissues [17]. The Trpv4 channel has been shown to be sensitive to shear stress in other cell types as well, so its role in bone as a mechanosensor is promising. Another Trp channel that has received considerable attention in mechanotransduction of bone cells is TrpP1, also known as polycystin-1 (encoded by the Pkd1 gene).

1.2. Receptors associated with G-protein

G-protein-coupled receptors (GPCRs) represent the largest family of cell surface receptors and are activated by various ligands, including neurotransmitters, hormones, small peptides, local cytokines, amino acids and fatty acids, among others [18]. More than a decade ago it was demonstrated that fluid flow activates G-proteins in osteoblasts and that pharmacological prevention of G-protein activation prevents the normal response to fluid shift [19]. Interestingly, fluid shift stress leads to a conformational change in two GPCRs, the parathyroid hormone receptor 1 (PTH1R) and the bradykinin B2 receptor, in osteoblast MC3T3 cells and endothelial BAEC cells [20,21]. It has also been reported that the responsiveness of the energy transfer signal can be modulated by membrane fluidity (e.g., modulation of membrane stiffness), indicating that these GPCRs can be direct sensors of membrane mechanical perturbation. Although mechanotransduction may or may not involve either of these two specific receptors, the data indicate that other GPCRs, more important for the mechanotransduction response, may undergo similar conformational changes upon mechanical stimulation of the cell. Although bone cells possess the G-protein mechanism necessary to activate this pathway, verification of this mechanoreceptor mechanism in bone cells *as such* would require similar experiments in bone-specific models, which have not yet been performed.

The search for the "mechanoreceptor" in bone continues, and progress has been made on several fronts. The molecule or mechanism that is at the forefront of mechanoreception-the protein that converts a physical signal into a biochemical signal-remains elusive. It is also possible that no single mechanism is responsible for triggering the entire event; numerous signaling systems in biology exhibit redundancy. While signal reception mechanisms are still being developed, much more progress has been made in mechanically stimulated second messengers, which are essential for mechanically induced changes in bone mass. These mechanisms are described below.

1.3. Mechanically stimulated second messengers in bone

Once a mechanical signal is received by the local bone cell population and converted into the original biological signal, a series of secondary biochemical signaling events must occur to propagate the signal within the cell and to other sensory/effector cells. Efforts to understand the signaling pathways involved in mechanical signal propagation have revealed many changes in the mechanically stimulated osteocyte/osteoblast, including changes in gene expression, protein and lipid modifications (such as phosphorylation), protein degradation, intracellular translocation events, release of secreted factors, and changes in cell shape and size. It is important to understand which of these are crucial for mechanical transduction and which are merely auxiliary events that have few functional consequences for the mechanical transduction process.

1.4. Prostaglandins

One of the earliest pathways identified for involvement in bone cell mechanotransduction is the cyclooxygenase (Cox)/prostaglandin (PG) pathway. PG are powerful and short-lived (sec/min) signaling molecules derived from arachidonic acid (AA) that act in an autocrine/paracrine manner [22]. In a multistep process, intracellular cyclooxygenase (Cox) enzymes convert AA to PGG2 and then to PGH2, after which various PG synthases generate specific PGs, including PGE2 [22]. PGs are released from the cell in response to a series of stimuli, where they can then bind to specific GPCRs. Vigorous exercise, such as jumping, causes the immediate release of PGE2 from lower limb bone tissue [23]. In rodents, mechanical loading regulates mRNA and Cox2 protein levels (Cox isoform excreted), whereas the constitutive isoform (Cox1) remains unchanged [24,25]. The importance of PGE2 signaling has been demonstrated *in vivo* by depleting the intracellular PGE2 pool before mechanical loading. Pharmacological inhibition of Cox1 and Cox2 by indomethacin treatment or selective inhibition of Cox2 alone by

NS-398 treatment was found to reduce the osteogenic response to load conducted several hours after inhibitor administration [26]. This result was confirmed *in vitro* using fluid shear and stretch, where PGE2 levels are easier to measure from cell cultures [27,28]. The mechanism of PGE2 release from mechanically stimulated cells is controversial and may involve the discovery of large, pore-forming hemicagins by connexin-43 [29] or the purinergic protein complex P2X7[30,31]. Once released, PGE2 binds in an autocrine or paracrine manner to Ep heptagel receptors (Ep1-4), which mediate its effects [32,33]. Thus, *in vivo* and *in vitro* experiments indicate a prominent role for prostaglandins in bone cell mechanotransduction, but the mechanism by which PGE2 is released and which receptors are important for its paracrine/autocrine effects are unclear.

1.5. Nitrous Oxide

Similar to skeletal muscle, another pathway activated by mechanical stimulation in bone is nitric oxide signaling [34]. Nitric oxide (NO) is a free radical and as such can spread freely through the plasma membrane. NO is formed from the amino acid L-arginine by one of the three isoforms of nitric oxide synthase (NOS). *In vitro*, NO is released from mechanically stimulated osteoblasts and osteocytes [35].

1.6. mTORC1

mTORC1 is a conserved serine/threonine kinase and is a major regulator of cell growth, in part regulating mRNA translation and hence protein synthesis, with the potential to regulate both translation efficiency (i.e. mRNA translation rate) and translational capacity (i.e. ribosome number) (for review see [36]. To date, there is some evidence that mTORC1 does play a role in bone growth and, more specifically, in the regulation of bone length. For example, several studies have shown that the mTORC1 inhibitor, rapamycin, inhibits long bone growth in young rodents, possibly by directly inhibiting chondrocyte differentiation and/or indirectly by inhibiting growth plate angiogenesis, resulting in reduced chondrogenesis [37-41]. Thus, mTORC1 may play a vital role in long-term bone growth by providing a mechanism for mitogens and nutrients to stimulate bone growth by increasing growth plate activity. Importantly, recent data also implicate mTORC1 in mechanically induced cartilage growth [42]. In particular, it has been recognized that mechanical activation of mTORC1 is necessary for cell proliferation, chondrogenesis, and cartilage growth during embryonic bone development [42]. Other evidence suggests that mTORC1 not only regulates chondrocytes but also regulates osteoblasts. For example, rapamycin has been shown to inhibit the proliferation and differentiation of preosteoblast cells by partially inhibiting the expression of cyclins A and D1 and the Runx2 transcription factor, respectively [43]. In addition, rapamycin has been reported to inhibit erythropoietin-induced osteoblast differentiation in some preosteoblast cell lines [44]. These data suggest that mTORC1 may play an important role in the regulation of bone mass, in part by modulating the abundance of osteoblasts. This is also supported by a recent study that implicated mTORC1 in the Wnt signaling pathway, which enhances postnatal bone mass by increasing the number and activity of osteoblasts [45]. Specifically, in this study, induction of Wnt7b expression either during embryonic development or in the first month of postpartum resulted in a profound increase in bone mass that was associated with an increase in the number of osteoblasts [45]. In addition, it was found that Wnt7b (and Wnt3a) activated mTORC1 signaling *in vitro* and *in vivo* via the non-canonical PI3K/Akt pathway and that induced deletion of the mTORC1 component, Raptor, markedly reduced the Wnt7b-induced increase in osteoblast activity and bone mass [45]. Interestingly, rapamycin analogues (e.g., everolimus) appear to inhibit osteoclast survival and activity, suggesting that mTORC1 activation may also increase bone resorption [46]; however, the Wnt7b-induced increase in mTORC1 signaling and bone mass was not associated with changes in bone resorption [45]. These exciting data suggest that mTORC1 activation can indeed play a role in the regulation of bone mass by stimulating an increase in the number and activity of osteoblasts.

1.7. Wnt alarm system

More recently *Wnt signaling pathway* (integration site associated with Wingless) has been identified as a major intermediate player in mechanotransduction of bone cells [47]. The family of secreted Wnt glycoproteins consists of 19 different Wnt genes (in humans), protein products of which can activate several signaling pathways [48]. Wnt signaling includes secreted Wnt activating receptor complex, which stimulates a number of phosphoproteins [49].

And ultimately there is an increase in osteogenesis and a decrease in resorption, and as a consequence, mechanical loading activates transcription mediated by a number of proteins both *in vivo* and *in vitro* [50,51]. Notably, osteocytes appear to be the first cells to demonstrate transcriptional activity of proteins after loading [52], suggesting that Wnt signaling in osteocytes may be a sensor cell response pathway.

1.8. IGF-1 signaling

In contrast to skeletal muscle, in which IGF-1 is apparently not required for mechanoinduced increase in mTORC1 signaling and skeletal muscle mass, mechanotransduction of bone cells requires IGF-1 signaling for the anabolic effects of loading. For example, IGF-1 is known to play an important role in embryonic bone development [53,54] and early studies have also shown a potential role for IGF-1 in the regulation of bone mass in response to changes in mechanical loading. In particular, studies performed in mechanically stimulated osteocytes *in vitro as well as in* mechanically stimulated rat vertebra and tibia *in vivo* showed increased expression of IGF-1 mRNA after exposure to increased loading [55]. Furthermore, IGF-1 administration was shown to increase the proliferation of osteoblasts *in vivo* [56] and that transgenic overexpression of IGF-1 in osteoblasts led to an increased response to mechanical loading *in vivo* in mice [57].

Taken together, these data suggest that IGF-1 does play a crucial role in the mechanical regulation of bone mass. However, the downstream effects of activated IGF-1 are less clear. IGF-1 is clearly important for mechanical signaling in bone cells, with some evidence suggesting that IGF-1 can regulate Wnt and PG signaling in response to increased mechanical loading. However, more studies are needed to further investigate other possible IGF-1-mediated signaling pathways.

Decades ago it was postulated that the osteocyte was the best candidate for the sensor cell type for several reasons [58]. First, osteocytes are evenly distributed throughout cortical and trabecular bone, even in areas of mineralized matrix devoid of vessels. Consequently, a network of osteocytes provides a large-scale "network" of load monitoring that penetrates every cubic millimeter of bone tissue. Secondly, osteocytes are connected to each other and to the cells on the bone surface through long cellular connections. Osteocytes have a large number of these cellular connections that transmit information intercellularly, which facilitates rapid cellular communication [59,60]. Third, it is clear that osteocytes are not effector cells because they are immersed in the bone matrix and therefore are not capable of causing bone resorption quickly and in large quantities [61]. This very localized osteocyte activity, although potentially significant for the regulation of serum calcium levels, has almost no effect on bone size, shape and structural properties. Since the role of the osteocyte as an effector cell has been ruled out, it has historically been considered a sensory cell [62]. In addition to teleological arguments, experiments have confirmed the role of the osteocyte as the primary mechanosensory cell type in bone. Interestingly, a recent gene expression profile performed on purified, stream-sorted populations of osteocytes extracted from living mouse bone revealed a surprising number of highly expressed genes that are traditionally considered "muscle genes" [63]. Although this experiment was not performed under mechanically altered conditions, it is also worth considering that osteocytes may be more "muscular" than previously thought, and that some mechanisms of deformation sensation may overlap between the two cell types. Based on this idea, we can assume that there may be disorders in the bone that can be handled osteopathically. These disorders can be called intraosseous somatic dysfunction (SD) because the bone interacts with various anatomical components [64], so there are forces acting on the bone: mechanical, fluid, and neural.

The ossification process is individual and occurs at different periods of human life. After birth, some bones are divided into separate parts that are connected by cartilage tissue, and it takes time for the cartilaginous part of the bone to be completely replaced by bone [65]. During osteogenesis, the flexibility of the fibrous components of the neo-cartilaginous or webbed regions of the bone can be limited, which can lead to SD, or more specifically, to intraosseous dysfunction [64].

Deformities or injuries create the very limitation that can cause bone growth asymmetry as an example of plagiocephaly [64]. The possibility of influencing this deformity through osteopathic treatment has been shown in

recent studies [66,67].

Various factors can also influence intraosseous dysfunction: impaired blood circulation or innervation of the bone itself [68-71]. For example, nerve cell sprouting occurs at the site of a bone lesion, which can contribute to tissue hyperinervation [72]. This proves that bone immobilization after injury may play a role in the process of peripheral and central sensitization [73]. In other words, limitation of mobility generates not only mechanical, but also local and central neurological consequences. Age is irrelevant to intraosseous dysfunction because bone remodeling occurs regardless of age [74].

Considering that muscle gene expression is similar to that in bone tissue [63] and that bone is part of the fascial system [75,76], dysfunction at this level in the form of impaired metabolism and properties of some bone matrix components [77] may affect local and global biomechanical adaptation to movements in the biotensegrity system [78].

CONCLUSIONS

In conclusion, we note that the molecular mechanisms regulating mechanotransduction in bone are complex. Moreover, several mechanisms probably work in synergy.

Current data showed that mechanosensitive ion channels and G-protein-coupled receptors play a significant role in detecting changes in mechanical stress in bone mass. In addition, IGF-1, NO, PG and Wnt signaling are also implicated as second messengers in bone mechanotransduction.

A growing body of evidence suggests that mechanical bone stimulators play an important role in promoting bone growth in length by increasing chondrocyte proliferation and growth plate activity, but ossification processes are not ideal and intraosseous disturbances are possible. The question arises, can we with the osteopathic approach change the quality of bone, eliminate the flexibility limitations of the fibrous components of the neo-bony cartilage or membranous regions of bone? It is necessary to study the influence of intraosseous dysfunctions on osteogenesis processes after birth, as well as the possibility of correcting such consequences of intraosseous dysfunctions as the development of anatomical variation in the lower limbs in children.

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