Delgado-Calle J, Riancho JA
Departamento de Medicina Interna - Hospital Universitario Marqués de Valdecilla-IFIMAV-Universidad de Cantabria - Santander

Cellular and molecular mechanobiology of bone tissue

Correspondence: José A. Riancho - Departamento de Medicina Interna - Hospital Universitario Marqués de Valdecilla-IFIMAV-Universidad de Cantabria - Av. Marqués de Valdecilla, s/n - Santander 39008 (Spain)
e-mail: rianchoj@unican.es

Date of receipt: 12/02/2013
Date of acceptance: 01/03/2013

Working FEIOMM scholarship to attend the 34rd Congress of the ASBMR (Minneapolis 2012).

Summary
Several data support the concept that skeletal homeostasis, repair and adaptation to daily life depend on mechanically-induced signals that promote appropriate responses of bone cells. This review considers the cells that are responsive to mechanical signals within the bone environment, and the molecular mechanisms involved in mechanotransduction, the process by which cells convert mechanical stimuli in biochemical signals and subsequently modify biological activity. Understanding the cellular and molecular mechanisms underlying bone responses to mechanical loads will positively impact current knowledge on basic bone biology and pathophysiology and will likely contribute to the development of new interventions to improve bone strength.

Key words: *osteocytes, mechanical adaptation, mechanotransduction, mechanosensors.*

Introduction

The skeleton is a strong, hard and tough organ which is formed by specialised connective tissue which is characterised by having an extracellular calcified bone matrix in which are embedded different types of cells which give functionality to the tissue. In general, it is possible to attribute four basic functions to bones. On the one hand they have a structural function, providing internal support to the body and protecting vital organs. They also have a role in locomotive function as a result of the interaction between the bones, the muscles and the joints. On the other hand, they are responsible for the production of certain essential components for the differentiation and survival of the haematopoietic mother cells. Finally, bones are an important store of calcium and phosphates, both their deposit and their mobilisation contributing to the maintenance of mineral homeostasis. For some time now, advances in the understanding of bone biology have suggested that bone may also be considered as a key endocrine organ, capable of participating in the regulation of different physiological processes such as energy metabolism or reproduction[1].

Taking into account the important role which this tissue plays in the physiology of the organism, it is of vital importance that both its composition and its mechanical resistance are maintained throughout life. Hence, bone is constantly renewed by a process known as bone remodelling[2], which replaces old bone with new. This renovation takes place through the balanced action, coordinated in time and space, of the osteoblasts and the osteoclasts[3,4]. It is possible that part of this process occurs at the specific points of the bone which require renovation[5,6], although it is thought that most of it happens randomly, resulting in a complete renovation of the skeleton approximately every 10 years.

Bone is a tremendously dynamic tissue, its structure and size changing from birth until consolidation in adulthood. Furthermore, bone has the capacity to change to adapt to new functional demands which may arise in an individual on a day to day basis. Hence, besides remodelling, there is another process, called bone modelling, which allows bones to acquire their normal shape and structure, and which modify them at certain points through the action, independent and not linked, of the osteoblasts and osteoclasts[7]. Bone modelling can take place during the growth phase, or even in adulthood, to change the shape of bone in response to mechanical load, a process known as mechanical adaptation[8]. It is known that daily physical stimulus of the skeleton induces an anabolic effect in bone tissue, facilitating the maintenance bone mass and reinforcing resistance in those areas which receive a greater mechanical load[9]. A clear example of this effect is seen in the forearm of tennis players, which show an increase in bone mass up to 10% in the arm which wields the racquet[10]. And contrarily, a reduction in physical demands, such as prolonged periods in bed, space voyages or situations of paralysis or relative immobility provoke losses in the quantity and quality of bone, and as a consequence, an increase in the risk of fracture[11]. The main objective of this work is to offer a general vision of the types of cells and the molecular mechanisms responsible for regulating the adaptive response of bone to its physical environment.

Bone cells sensitive to mechanical stimulus

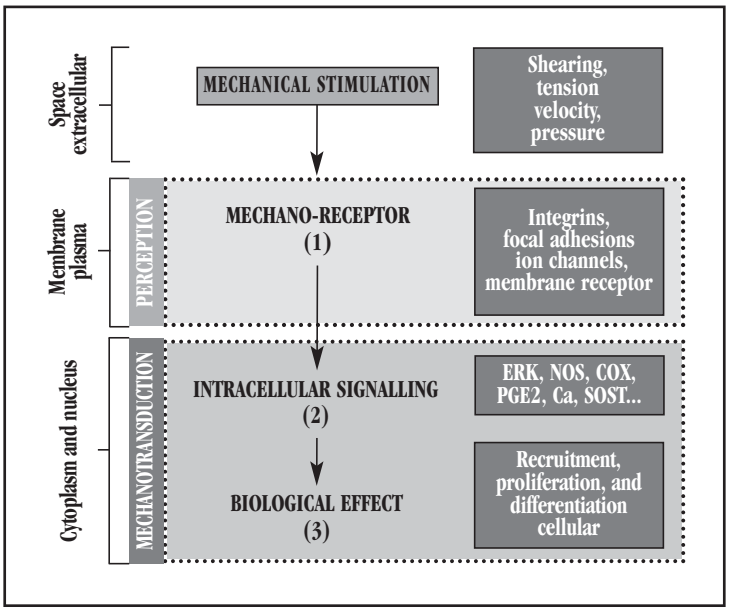
The process of mechanical adaptation requires that the cells are capable of detecting the mechanical signals and transforming them into biological signals, a phenomenon known as mechanotransduction. Ultimately it will be these signals which will direct the necessary changes in the bone architecture. The mechanisms responsible for the response to the physical stimuli in bone are still little understood, but everything points to the fact that there are various types of bone cells involved. It is possible that both the osteoclasts, and the mesenchymal progenitors, osteoblasts and osteocytes are capable of perceiving or being affected by mechanical stimuli coming from the environment. To what extent the responses which occur in each of these types of cells is the result of direct or indirect mechanisms is something which is not totally clear at present. But in any case, it seems evident that the interactions between all these cells are key to the regulation of the recruitment, proliferation and differentiation of the osteoblasts and osteoclasts, events which will ultimately determine the changes in the bone tissue.

Due both to their disposition and abundance in bone (90% of all osteoblast cells), as well as the network of canaliculi which connect them themselves and with other bone cells, the osteocytes are considered to be the main cells charged with mechanotransduction[12]. Hence, Tatsumi et al. observed that the specific elimination of the osteocytes and their dendritic processes in rats blocked bone loss induced by the absence of mechanical stimulation, supporting the essential role of these cells in mechanotransduction[13].

It is worth mentioning that osteocytes are found deeply embedded within the bone matrix. This suggests that these cells may be exposed to a wide range of stimuli which may include tension, shearing, changes in pressure or flow of fluids[14]. Furthermore, certain characteristics of these stimuli, such as their magnitude or frequency, may also fundamentally influence the cell response. Given the intrinsic characteristics of bone tissue, high magnitude mechanical stimuli deriving from daily activity generate relatively small deformations (0.1% deformation from the original state). On the other hand, the skeleton is continuously subject to stimuli of very low magnitude (deformations <0.0005% from the baseline position) and high frequency (10-50Hz), a product of the constant muscular contractions required to maintain posture[15]. In most of these cases, the stimuli are incapable of acting directly on the cells embedded in the matrix. It is thought rather that these stimuli indu-

ce changes in the interstitial fluid which flows in the extensive network of canaliculi which connect the osteocytes. The movement of fluid within this system may be influenced by mechanical stimuli in the environment and generate shearing forces, changes in the velocity or pressure exerted on the bone cells, which would be capable of activating a whole battery of membrane receptors which will be those responsible for initiating the cascade of intracellular signalling which direct the biological responses need to respond to a certain mechanical stimulus. In addition to being the receptive medium, this system of canaliculi contributes to the amplification and distribution of the signal to adjacent cells. There is a range of experimental evidence which supports this idea. On the one hand, a flow of fluid has been observed around the osteocytes in the tibias of rats which have been mechanically stimulated[16]. Similarly, Price et al. have shown how there is a movement of fluid in the canaliculi in response to certain mechanical stimuli[17]. That said, it is important to mention that the possibility cannot be completely excluded that the osteocytes are responding directly to the pressure coming from the mineralised matrix after the physical stimulus[18,19]. It is even possible that the same stimulus may provoke the simultaneous appearance of a number of these forces. Although the composition of the mineralised matrix and the functional interactions between the cells which are embedded in them are becoming better understood, the mechanisms which underlie the perception and the subsequent transduction of these physical signals are for the moment the object of intense scientific debate.

Figure 1. Transduction of the mechanical signal in bone tissue. The process of mechanotransduction converts the mechanical stimuli into a sequence of cellular events which are finally translated into a biological effect (e.g. an increase in cell proliferation, initiation of programmes of cell differentiation...). The transduction of the signal starts in the membrane, by means of different structures sensitive to changes in the mechanical characteristics which surround the bone cells (1). These receptors activate various intracellular pathways (e.g. ERK, ion flow, G proteins...) which ultimately provoke changes in the expression in certain key genes in the cell biology of bone (2). Variations in the levels of these genes ultimately modify the proliferation, differentiation and recruitment of bone precursors (3).

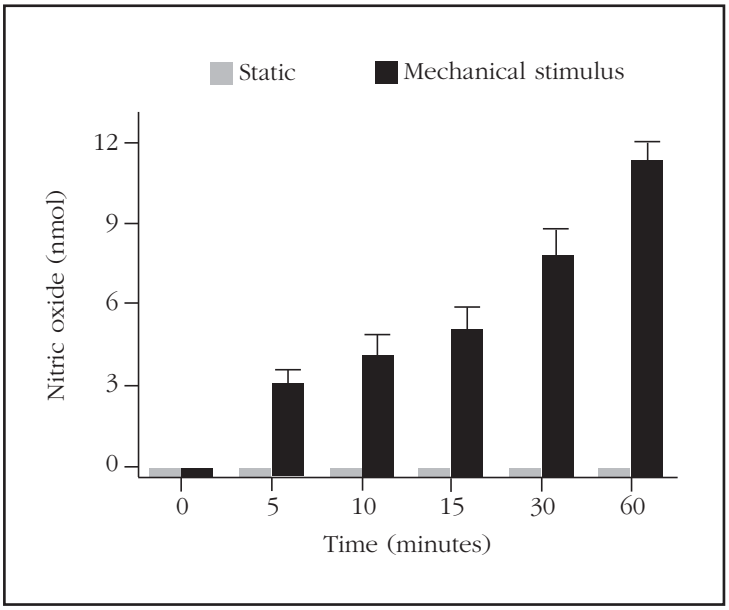


Transduction of the mechanical signal

The ability of the bone cells to perceive mechanical signals in their mineralised environment requires the presence of mechanoreceptors, in other words, molecules, protein complexes or biological structures capable of detecting changes in the different forces associated with mechanical load (e.g. pressure, fluid flow...). In theory, these structures should 1) connect the cells with the extracellular space, allowing it to "sense" the pressure provoked in the extracellular mineralised matrix, or 2) be situated in the plasmatic membrane to detect changes in the pressure or flow of fluid which surrounds these cells. Among the elements which have been postulated as being responsible are different integrins, focal adhesions, ciliary structures and different membrane proteins. In fact it has been shown experimentally how the structures capable of anchoring the cells in the extracellular space which surrounds them, such as the aforementioned integrins or focal adhesions, are necessary for the perception of mechanical stimuli[20,21]. Similarly, the canals sensitive to physical stimuli, such as the calcium canals or the connexins, also play an important role in the reception and subsequent transduction of the signal, generally allowing the entry or exit of different factors charged with mediating the cellular response to physical stress[22]. Finally, the mechanosensory organs, such as cilia are increasingly being seen to be important in this area. So much so that it has been suggested that the release of prostaglandin E2 (PGE2) after the perception of the mechanical signals may be, at least in part, regulated by these types of structures[23,24]. It has also recently been suggested that the cytoskeleton, which connects the cell interior with the extracellular surroundings may be a critical element in determining how the osteocytes "sense" these forces[25].

Once the stimuli have been perceived, they should be transformed into biological signals which promote changes in cell activity, such as phosphorylation, translocation of transcription factors or changes in gene expression. Among the mediators which join the perception of the signals through the aforementioned structures and these

Figure 2. Production of nitric oxide in response to mechanical stimulus. The application of a pulsing flow directly on the cell membrane of osteoblast cells (HOS-TE85) induces a marked secretion of nitric oxide into the medium (black bands), these increasing with the duration of the stimulus. On the other hand nitric oxide was not detected in those cells which were not mechanically stimulated (white bands).
nmol=nanomoles



effectors are found different kinases, receptors associated with the G protein and second messengers such as calcium or cyclical AMP[8,14] (Figure 1).

In spite of the fact that not all the molecular mechanisms which mediate the transduction of the signal are completely understood, we now have a better idea of which factors are ultimately in charge of modulating the activity of the different types of cells. Thus it is known that mechanical stimuli provoke changes in the expression of certain target genes such as sclerostin, Wnt ligands, nitric oxide synthases or prostaglandins, among others[26-29]. Although there may be various molecules involved, the effect of the mechanical load on the bone is characterised principally by a reduction in the expression of sclerostin on the part of the osteocytes[26,30,31]. Sclerostin is a powerful inhibitor of bone formation, which inhibits the signalling of the Wnt ligands by bonding with LRP-type co-receptors[32]. In support of the significant role being given to sclerostin in this process, it has been observed that rats deficient in this gene are resistant to loss of bone mass in the hind limbs induced by the absence of mechanical stimulation[31]. However, in spite of the important role of this molecule in the adaptation of bone, hardly anything is known about what are the mechanisms which provoke the reduction in its expression in response to mechanical stimulus. Hence, our group has shown in *in vitro* experiments that the lowering of transcriptional levels of SOST may be, at least partly, mediated by the production of nitric oxide[33] (Figure 2). It has recently been suggested that the estrogens may also be involved in the modulation of the transcriptional levels of this gene in response to mechanical stimuli[34]. Although, as has already been mentioned, the response is mainly led by the levels of sclerostin, it seems that it is not possible to discount the idea that there are various molecules and signalling pathways involved in mechanical adaptation. In fact, the production and subsequent signalling mediated by PGE2, as well as the role of nitric oxide and the synthesis of Wnt ligands, also appear to play a significant role in the formation of bone promoted by mechanical forces[27,28,35].

Conclusion

The combination of mechanisms which underlie mechanical adaptation are even today little understood. The wide range of physical stimuli to which cells may be subjected, as well as the diversity of biological responses and the possible interactions between the different types of cells involved in the process increase exponentially the complexity of studying the mechanisms involved. The use of animal models has helped advance understanding of the mechanobiology, although on occasion the results are difficult to interpret, mainly due to the impossibility of isolating other biophysical components of the load applied, or because of the difficulty in the choice of an appropriate mechanical stimulus. There have been various advances in this field achieved through the use of *in vitro* techniques, since these provide greater control of the different factors which may have an influence on the response. However, these experiments eliminate the natural environment of the bone in which the mechanosensory cells are found. It seems, therefore, that it will be necessary to approach its study from various experimental angles, combining the investigation of individual molecules in certain types of cells, with functional studies in animals. Although not mentioned earlier, since it was not the objective of this review, it is nevertheless important to take into account role that muscle, and in particular the factors produced by this tissue, may have in mechanical adaptation[36].

There is no doubt that the study and understanding of the molecular mechanisms which regulate the capacity of bone to respond to functional demand may lead to the development of new and more effective therapeutic strategies for musculoskeletal disorders , covering a wide range from the establishment of optimum physical exercise regimes to medicines which take advantage of the main signalling pathways involved in mechanical adaptation.

Financing: Jesus Delgado-Calle has a predoctoral fellowship IFIMAV. The authors declare no conflict of interest.

Bibliography

1. Karsenty G. Bone endocrine regulation of energy metabolism and male reproduction. C R Biol 2011;334:720-4.
2. Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. J Biol Chem 2010;285:25103-8.
3. Hadjidakis DJ, Androulakis II. Bone remodeling. Ann N Y Acad Sci 2006;1092:385-96.
4. Sims NA, Gooi JH. Bone remodeling: Multiple cellular interactions required for coupling of bone formation and resorption. Semin Cell Dev Biol 2008;19:444-51.
5. Bonewald LF. The amazing osteocyte. J Bone Miner Res 2011;26:229-38.
6. Cardoso L, Herman BC, Verborgt O, Laudier D, Majeska RJ, Schaffler MB. Osteocyte apoptosis controls activation of intracortical resorption in response to bone fatigue. J Bone Miner Res 2009;24:597-605.
7. Teti A. Bone Development: Overview of Bone Cells and Signaling. Curr Osteoporos Rep 2011;9:264-73.
8. Price JS, Sugiyama T, Galea GL, Meakin LB, Sunters A, Lanyon LE. Role of endocrine and paracrine factors in the adaptation of bone to mechanical loading. Curr Osteoporos Rep 2011;9:76-82.
9. Suva LJ, Gaddy D, Perrien DS, Thomas RL, Findlay DM. Regulation of bone mass by mechanical loading: microarchitecture and genetics. Curr Osteoporos Rep 2005;3:46-51.
10. Ducher G, Prouteau S, Courteix D, Benhamou CL. Cortical and trabecular bone at the forearm show different adaptation patterns in response to tennis playing. J Clin Densitom 2004;7:399-405.
11. Vandamme K, Holy X, Bensidhoum M, Deschepper M, Logeart-Avramoglou D, Naert I, Duyck J, Petite H. Impaired osteoblastogenesis potential of progenitor cells in skeletal unloading is associated with alterations in angiogenic and energy metabolism profile. Biomed Mater Eng 2012;22:219-26.
12. Bonewald LF, Johnson ML. Osteocytes, mechanosensing and Wnt signaling. Bone 2008;42:606-15.
13. Tatsumi S, Ishii K, Amizuka N, Li M, Kobayashi T, Kohno K, Ito M, Takeshita S, Ikeda K. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. Cell Metab 2007;5:464-75.
14. Thompson WR, Rubin CT, Rubin J. Mechanical regulation of signaling pathways in bone. Gene 2012;503:179-93.
15. Huang RP, Rubin CT, McLeod KJ. Changes in postural muscle dynamics as a function of age. J Gerontol A Biol Sci Med Sci 1999;54:B352-7.
16. Knothe Tate ML, Steck R, Forwood MR, Niederer P. In vivo demonstration of load-induced fluid flow in the rat tibia and its potential implications for processes associated with functional adaptation. J Exp Biol 2000;203:2737-45.
17. Price C, Zhou X, Li W, Wang L. Real-time measurement of solute transport within the lacunar-canalicular system of mechanically loaded bone: direct evidence for load-induced fluid flow. J Bone Miner Res 2011;26:277-85.
18. Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, Cullen DM, Zhao W, Li C, Kharode Y, Sauter L, Babij P, Brown EL, Hill AA, Akhter MP, Johnson ML, Recker RR, Komm BS, Bex FJ. Wnt/beta-catenin signaling is a normal physiological response to mechanical loading in bone. J Biol Chem 2006;281:31720-8.
19. Bonivtch AR, Bonewald LF, Nicolella DP. Tissue strain amplification at the osteocyte lacuna: a microstructural finite element analysis. J Biomech 2007;40:2199-206.
20. Batra N, Burra S, Siller-Jackson AJ, Gu S, Xia X, Weber GF, DeSimone D, Bonewald LF, Lafer EM, Sprague E, Schwartz MA, Jiang JX. Mechanical stress-activated integrin alpha5beta1 induces opening of connexin 43 hemichannels. Proc Natl Acad Sci U S A 2012;109:3359-64.
21. Santos A, Bakker AD, Zandieh-Doulabi B, Blieck-Hogervorst JM, Klein-Nulend J. Early activation of the beta-catenin pathway in osteocytes is mediated by nitric oxide, phosphatidyl inositol-3 kinase/Akt, and focal adhesion kinase. Biochem Biophys Res Commun 2010;391:364-9.
22. Zhang Y, Paul EM, Sathyendra V, Davison A, Sharkey N, Bronson S, Srinivasan S, Gross TS, Donahue HJ. Enhanced osteoclastic resorption and responsiveness to mechanical load in gap junction deficient bone. PLoS ONE 2011;6:e23516.
23. Malone AM, Anderson CT, Stearns T, Jacobs CR. Primary cilia in bone. J Musculoskelet Neuronal Interact 2007;7:301.
24. Malone AM, Anderson CT, Tummala P, Kwon RY, Johnston TR, Stearns T, Jacobs CR. Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism. Proc Natl Acad Sci U S A 2007;104:13325-30.
25. Klein-Nulend J, Bacabac RG, Bakker AD. Mechanical loading and how it affects bone cells: the role of the osteocyte cytoskeleton in maintaining our skeleton. Eur Cell Mater 2012;24:278-91.
26. Robling AG, Niziolek PJ, Baldridge LA, Condon KW, Allen MR, Alam I, Mantila SM, Gluhak-Heinrich J, Bellido TM, Harris SE, Turner CH. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. J Biol Chem 2008;283:5866-75.
27. Galea GL, Sunters A, Meakin LB, Zaman G, Sugiyama T, Lanyon LE, Price JS. Sost down-regulation by mechanical strain in human osteoblastic cells involves PGE2 signaling via EP4. FEBS Lett 2011;4:2450-4.
28. Fox SW, Chambers TJ, Chow JW. Nitric oxide is an early mediator of the increase in bone formation by mechanical stimulation. Am J Physiol 1996;270:E955-60.
29. Vatsa A, Smit TH, Klein-Nulend J. Extracellular NO signalling from a mechanically stimulated osteocyte. J Biomech 2007;40 Suppl 1:S89-95.
30. Papanicolaou SE, Phipps RJ, Fyhrie DP, Genetos DC. Modulation of sclerostin expression by mechanical loading and bone morphogenetic proteins in osteogenic cells. Biorheology 2009;46:389-99.
31. Lin C, Jiang X, Dai Z, Guo X, Weng T, Wang J, et al. Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling. J Bone Miner Res 2009;24:1651-61.
32. Li X, Zhang Y, Kang H, Liu W, Liu P, Zhang J, et al. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. J Biol Chem 2005;280:19883-7.
33. Delgado-Calle J, Riancho JA, Klein-Nulend J. New insights into human SOST mechanotransduction: role of nitric oxide. J Bone Miner Res 2012;27 Suppl 1. Disponible en: http://www.asbmr.org/Meetings/AnnualMeeting/AbstractDetail.aspx?aid=51d4e88b-f79d-47e2-a15b-134f0c57b52e.
34. Galea GL, Meakin LB, Sugiyama T, Zebda N, Sunters A, Taipaleenmaki H, et al. Estrogen receptor alpha mediates proliferation of osteoblastic cells stimulated by estrogen and mechanical strain, but their acute down regulation of the Wnt antagonist Sost is mediated by Estrogen Receptor beta. J Biol Chem 2013; en prensa.
35. Sunters A, Armstrong VJ, Zaman G, Kypta RM, Kawano Y, Lanyon LE, et al Mechano-transduction in osteoblastic cells involves strain-regulated estrogen receptor alpha-mediated control of insulin-like growth factor (IGF) I receptor sensitivity to Ambient IGF, leading to phosphatidylinositol 3-kinase/AKT-dependent Wnt/LRP5 receptor-independent activation of beta-catenin signaling. J Biol Chem 2010;285:8743-58.
36. Juffer P, Jaspers RT, Lips P, Bakker AD, Klein-Nulend J. Expression of muscle anabolic and metabolic factors in mechanically loaded MLO-Y4 osteocytes. Am J Physiol Endocrinol Metab 2012;302:E389-95.