



UvA-DARE (Digital Academic Repository)

The role of osteocytes in bone mechanotransduction

Santos, A.M.; Bakker, A.D.; Klein Nulend, J.

Published in:
Osteoporosis International

DOI:
[10.1007/s00198-009-0858-5](https://doi.org/10.1007/s00198-009-0858-5)

[Link to publication](#)

Citation for published version (APA):

Santos, A., Bakker, A. D., & Klein-Nulend, J. (2009). The role of osteocytes in bone mechanotransduction. *Osteoporosis International*, 20(6), 1027-1031. DOI: 10.1007/s00198-009-0858-5

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <http://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

The role of osteocytes in bone mechanotransduction

A. Santos · A. D. Bakker · J. Klein-Nulend

Published online: 2 April 2009

© The Author(s) 2009. This article is published with open access at Springerlink.com

Introduction

Bones are subjected to a variety of mechanical loads during daily activities. In the nineteenth century, Julius Wolff proposed that bones adapt their mass and 3D structure to the loading conditions in order to optimize their load-bearing capacity, and that this process is driven by mechanical stress [1]. For the past centuries, an increasing number of theoretical and experimental results reveal that osteocytes are the pivotal cells orchestrating this bio-mechanical regulation of bone mass and structure, which is accomplished by the process of bone remodeling [2–5]

Osteocytes are terminally differentiated cells of the osteogenic lineage that are derived from mesenchymal precursor cells. A number of molecules have been identified as important markers of osteocytes, such as matrix extracellular phosphoglycoprotein [6] sclerostin [7], dentin matrix protein-1 [8], and plex protein [8]. The osteocytes are the most abundant cells in adult bone and are constantly spaced throughout the mineralized matrix. Mature osteocytes have a characteristic dendritic cell shape, with processes radiating from the cell body through the canaliculi in different directions. These processes form an intercellular network through gap and adherent junctions with surrounding osteocytes, the cells lining the bone surface and bone marrow. Through this unique 3D network, osteocytes are

anatomically placed in a prime position not only to sense deformations driven by stresses placed upon bone, but also to respond with passage of signals to the neighboring cells [9].

For more than a decade now, it is known that the osteocytes are very sensitive to stress applied to intact bone tissue [10–16]. Computer simulation models have shown that mechanosensors lying at the surface of bone, as osteoblasts and bone lining cells do, would be less sensitive to changes in the loading pattern than the osteocytes, lying within the calcified matrix [3]. Interestingly, targeted ablation of osteocytes in mice disturbs the adaptation of bone to mechanical loading [16].

Osteocytes as key players in the process of bone mechanotransduction

It is currently believed that when bones are loaded, the resulting deformation will drive the thin layer of interstitial fluid surrounding the network of osteocytes to flow from regions under high pressure to regions under low pressure [17, 18]. This flow of fluid is sensed by the osteocytes which in turn produce signaling molecules that can regulate bone resorption through the osteoclasts, and bone formation through the osteoblasts, leading to adequate bone remodeling [17, 18]. This concept is known as the fluid flow hypothesis. Evidence has been increasing for the flow of canalicular interstitial fluid as the likely factor that informs the osteocytes about the level of bone loading [2, 5, 17, 18]. Nevertheless, Vatsa and colleagues [19, 20] proposed that if osteocytes could sense matrix strains directly, the cell shape, cytoskeletal alignment and distribution of adhesion sites in osteocytes *in situ* would bear alignment to the mechanical loading patterns. Indeed, it was shown that the

Data presented at the Third Meeting on Bone Quality, France, 24–25 June 2008: Bone Ultrastructure.

A. Santos · A. D. Bakker · J. Klein-Nulend (✉)
Department of Oral Cell Biology,
ACTA, University of Amsterdam and VU University Amsterdam,
Research Institute MOVE,
Van der Boechorststraat 7,
1081 BT Amsterdam, The Netherlands
e-mail: j.kleinnulend@vumc.nl

cell shape and distribution of actin and paxillin staining in osteocytes of mouse tibiae and calvariae were orientated accordingly to the respective mechanical loading patterns applied in these bones, suggesting that osteocytes might be able to directly sense matrix strains in bone [19, 20]. In accordance with these results, Wang and colleagues [21] developed a theoretical model that predicts that integrin-based attachment complexes along the osteocyte cell processes would amplify small tissue level strains. It was shown that osteocyte cell processes are directly attached to canalicular projections in the canalicular wall via $\alpha\text{v}\beta\text{3}$ integrins [21]. The theoretical model predicts that the tensile forces acting on these integrins are <15 pN. Axial strains caused by actin microfilaments on fixed integrin attachments are an order of magnitude larger than the radial strains in the previously proposed strain amplification theory [21]. In vitro experiments indicated that membrane strains of this order are large enough to open stretch activated cation channels [21], thus theories regarding shear stress within lacunae and osteocyte signaling need further investigation.

Osteocyte structures involved in mechanosensing: cell processes, cell body, and cilia

Up to now it has not been determined which of the osteocyte cell parts are most important for the function of the osteocyte as mechanosensor. It has been suggested that fluid flow over dendritic processes in the lacunar–canalicular porosity can induce strains in the actin filament bundles of the cytoskeleton that are more than an order of magnitude larger than tissue level strains [22]. Vatsa and colleagues [23] developed a method which enabled the quantification of mechanically induced intracellular nitric oxide (NO) production of the cell body and the cell process in single MLO-Y4 osteocytes using DAR-4M AM chromophore [23]. NO released by nitric oxide synthase (NOS) is a known early mediator of the response of osteocytes to mechanical loading and it mediates the induction of bone formation by mechanical loading in vivo [24, 25]. In single osteocytes, mechanical stimulation of both cell body and cell process resulted in up-regulation of intracellular NO production [23]. These results indicate that both cell body and cell process might play a role in mechanosensing and mechanotransduction in bone [23]. In addition, it was shown that a mechanically stimulated single osteocyte can communicate the information of the local mechanical stimulus to the other cells in the vicinity independent of intercellular connections, suggesting that this communication occurs via extracellular soluble factors [9]. Furthermore, an alternative mechanosensing structure has been proposed, i.e., osteocytes project a single cilia from their

cell surface [26]. This structure can translate fluid flow stimuli into a cellular response, indicating that primary cilia might act as a mechanosensitive structure within the osteocyte [27].

The role of the cytoskeleton in mechanosensing

Lately, evidence is emerging highlighting the crucial role of the cytoskeleton as a structure that is highly responsive to external physical and chemical stimuli. The cytoskeleton is involved in processes such as mechanosensing and largely determines the material properties of the cell (i.e., stiffness). It is known that the effect of stresses applied at different rates at an object is largely determined by the material properties of that object. Low magnitude ($<10\mu\epsilon$) and high frequency (10–100 Hz) loading can stimulate bone growth and inhibit disuse osteoporosis, while high loading rates have been shown to increase bone mass and strength after jumping exercises in middle-age osteopenic ovariectomized rats [28]. For bone cells, Bacabac and colleagues [29–31] have shown that the production of signaling molecules in response to an in vitro fluid shear stress (at 5 and 9 Hz) and vibration stress (5–100 Hz) correlated with the applied stress rate [29–31]. The faster the stress was applied, the stronger the observed response of the cells [32], suggesting that the bone cellular response to loading and mechanical properties of the cell are related, which implies that the response of bone cells to loading is related to cytoskeletal properties. The same group developed a novel application of two-particle microrheology, for which a 3D in vitro system was devised to quantify the forces induced by cells on attached fibronectin-coated probes (4 μm). The frequency at which the cells generate forces on the beads is related to the metabolic activity of the cell [33]. With this device and using NO production as a read-out, the material properties of round suspended MLO-Y4 osteocytes and flat adherent MLO-Y4 osteocytes were characterized. Osteocytes with round suspended morphology required lower force stimulation in order to show an increase in NO production, even though they were an order-of-magnitude more elastic compared to flat adherent cells [34]. Apparently, elastic osteocytes seem to require less mechanical forces in order to respond than stiffer cells [34]. In contrast, flat adherent MLO-Y4 osteocytes, primary chicken osteocytes, MC3T3-E1 osteoblasts, and primary chicken osteoblasts all showed a similar elastic modulus of less than 1 kPa [33]. This indicates that differences in mechanosensitivity between osteocytes and osteoblasts might not only be directly related to the elasticity of the cell, but also to other cell-specific properties, i.e., the presence of receptors or ion channels in the membrane, or how cells change their material properties in relation to deformation.

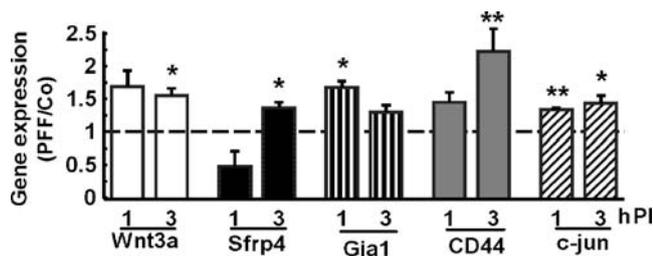


Fig. 1 Mechanical loading by pulsating fluid flow up-regulates gene expression of Wnts, Wnt antagonist, and Wnt target genes in MLO-Y4 osteocytes. One hour of PFF followed by 3 h of post-incubation without PFF (PI) up-regulated mRNA expression levels of Wnt3a and the antagonist SFRP4. One hour of PFF followed by 1 to 3 h of post-incubation without PFF increased mRNA expression of the target genes connexin-43, c-jun, and CD44. Values were normalized for GAPDH, PBGD, HPRT, and 18s and expressed as mean±SEM of PFF-treated-over-control ratios of three to six independent cultures. PFF pulsating fluid flow, Co control, SFRP4 secreted frizzled related protein 4, Gja1 connexin-43, CD44 CD44 antigen, PI post-incubation without PFF. Significant effect of PFF, * $p < 0.05$; ** $p < 0.01$

Key signaling molecules in mechanotransduction: NO, prostaglandins, and Wnt

An important step in the chain of events leading to adaption of bone to mechanical loading is the transduction of physical stimuli into biochemical factors that can alter the activity of the osteoblasts and osteoclasts. An important early response to mechanical loading is the influx of calcium ions. The calcium release may occur directly via mechanosensitive ion channels in the plasma membrane which induce release of calcium from internal stores [18, 35–39]. Calcium release can also occur indirectly via the opening of hemichannels (un-apposed halves of gap junctions) that result in release of ATP and NAD^+ , which in turn raise the intracellular calcium levels amplifying the wave propagation of calcium [40, 41]. The rise in intracellular calcium concentration is necessary for activation of calcium/calmodulin-dependent proteins such as NOS. The activation of phospholipase A_2 results a.o. in the stimulation of arachidonic acid production and prostaglandin E_2 (PGE_2) release mediated by the enzyme cyclooxygenase (COX) [37]. It has been shown in vitro that pulsating fluid flow (PFF) stimulates within minutes the release of NO and prostaglandins PGE_2 and PGI_2 from osteocytes, while osteoblasts were less responsive and osteoprogenitor cells were the least responsive [42–44]. Moreover, COX-2, one of the known isoforms of COX, can be induced by mechanical loading in vitro [45]. Again, osteocytes were much more responsive than osteoblasts and osteoprogenitor cells. After a 15-min treatment with PFF, osteocytes exhibited a three-fold increase of COX-2 messenger RNA (mRNA) expression while the other two cell populations showed no increase [46]. Moreover, in osteocytes, the

induction of COX-2 was sustained up to 1 h after mechanical loading was ceased. These results suggest that as bone cells mature, they increase their capacity to produce prostaglandins in response to fluid flow [47], either by direct response to load or by increased expression of COX-2 after cessation of the mechanical stimuli. Because induction of COX-2 is a crucial step in the induction of bone formation by mechanical loading in vivo [47], these results provide direct experimental support for the concept that osteocytes, the long-living terminal differentiation stage of osteoblasts, function as the “professional” mechanosensors in bone tissue.

Another family of molecules that very recently has been identified as mediator of the adaptive response of bone to mechanical loading is the Wnt family of proteins. Wnts belong to a family of secreted glycoproteins and have been associated with the adaptive response of bone to mechanical loading [48–50]. Inactivating mutations in the human low-density lipoprotein receptor-related protein 5 (LRP5) were shown to cause osteoporosis, while gain-of-function mutations in the LRP5 co-receptor increased Wnt signaling resulting in higher bone mass [48–50]. Although evidence is accumulating that Wnts are involved in the regulation of bone mechanical adaptation, it is unknown which cells produce Wnts in response to mechanical loading. Santos and colleagues [51] have shown that 1 h of pulsating fluid flow (0.7 ± 0.3 Pa, 5 Hz) up-regulated mRNA expression of Wnt3a as well as the Wnt antagonist SFRP4 in MLO-Y4 osteocytes at 1 to 3 h after cessation of the fluid flow stimulus (Fig. 1). These results suggest that osteocytes in vitro are able to respond to fluid shear stress by modulation of mRNA expression of molecules involved in Wnt signaling. Importantly, PFF also up-regulated gene expression of known Wnt target genes such as connexin 43, c-jun, and CD44 in MLO-Y4 osteocytes indicating that

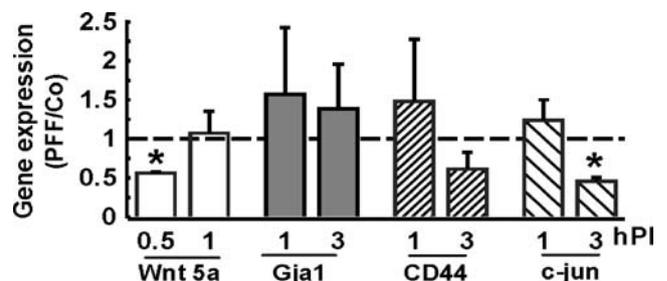


Fig. 2 Mechanical loading by pulsating fluid flow down-regulates gene expression of Wnts and Wnt target genes in MC3T3-E1 osteoblasts. One hour of PFF followed by 0.5 h of post-incubation without PFF (PI) down-regulated mRNA expression levels of Wnt5a and the target gene c-jun. Values were normalized for GAPDH, PBGD, HPRT, and 18s and expressed as mean±SEM of PFF-treated-over-control ratios of three to six independent cultures. PFF pulsating fluid flow, Co control, SFRP4 secreted frizzled related protein 4, Gja1 connexin-43, CD44 CD44 antigen, PI post-incubation without PFF. Significant effect of PFF, * $p < 0.05$

mechanical loading activated the canonical Wnt signaling pathway (Fig. 1). The response to PFF was different in MC3T3-E1 osteoblasts (Fig. 2), i.e., the expression of most Wnt-related genes, including Wnt5a and c-jun, was down-regulated in response to PFF which underscores the specificity of the mechano-response of osteocytes in terms of Wnt expression. Mechanical loading might thus lead to Wnt production by osteocytes thereby driving the mechanical adaptation of bone [51].

Osteocytes as conductors of bone remodeling and orchestrators of osteoblast and osteoclast activity

The final step of the mechanical signal transduction pathway towards bone remodeling is the transmission of molecules produced by osteocytes to the effector cells, i.e., osteoblasts and osteoclasts. Considering the close physical proximity of osteocytes to local osteoblasts and periosteal fibroblasts, it is highly plausible that soluble factors produced by osteocytes act in a paracrine manner to affect these cells. Thus, soluble mediators may regulate the properties of neighboring bone cell populations including their proliferation and differentiation. It has been shown that treatment of osteocytes with mechanical loading by PFF produce the most potent conditioned medium that inhibits osteoblast proliferation and stimulates alkaline phosphatase activity as compared to conditioned medium produced by osteoblasts and periosteal fibroblasts [52]. In addition, the fact that the osteocyte-conditioned medium regulates the properties of both osteoblasts and periosteal fibroblasts in a conserved NO-dependent mechanism lends support to the hypothesis that the osteocyte is an orchestrator of different cell populations in bone in response to mechanical loading [52]. Tan and colleagues [53] have shown that osteocytes subjected to mechanical loading by PFF inhibit osteoclast formation and resorption via soluble factors. The release of these factors was at least partially dependent on activation of an NO pathway in osteocytes as a response to fluid flow. The osteocyte appeared to be more responsive to fluid flow than the osteoblast and periosteal fibroblast regarding the production of soluble factors affecting osteoclast formation and bone resorption. This suggests a regulatory role for osteocytes in osteoclast formation and bone resorption during bone remodeling such as occurs after application of a mechanical load [53].

Conclusions

Understanding the role of osteocytes in bone mechanosensation and the consequence for bone metabolism and turnover is of vital importance. During the last decade,

molecular mechanisms and pathways involved in osteocyte mechanosensation have been identified and expanded significantly. It remains to be determined what makes osteocytes more responsive to shear stress than osteoblasts and what role the cell body, cell processes, and even cilia may play in this response.

The osteocyte likely orchestrates bone remodeling in the adult skeleton by directing both osteoblast and osteoclast function. New discoveries with regards to the cellular mechanisms underlying the process of mechanical adaptation of bone may lead to potential therapeutic targets in the treatment of diseases involving impaired bone turnover, e.g., osteoporosis or osteopetrosis.

Acknowledgments The Dutch Program for Tissue Engineering (DPTE) supported the work of A. Santos (DPTE grant # V6T6744). The Research Institute MOVE of the Vrije Universiteit supported the work of A.D. Bakker.

Conflicts of interest None.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

1. Wolff JD (1892) *Das Gesetz der Transformation der Knochen*. A Hirschwald, Berlin
2. Cowin SC, Moss-Salentijn L, Moss ML (1991) Candidates for the mechanosensory system in bone. *J Biomed Eng* 113:191–197
3. Mullender MG, Huijkes R (1995) Proposal for the regulatory mechanism of Wolff's law. *J Orthop Res* 13:503–512
4. Mullender MG, Huijkes R (1997) Osteocytes and bone lining cells: which are the best candidates for mechano-sensors in cancellous bone? *Bone* 20:527–532
5. Klein-Nulend J, Van der Plas A, Semeins CM et al (1995) Sensitivity of osteocytes to biomechanical stress in vitro. *FASEB J* 9:441–445
6. Gowen LC, Petersen DN, Mansolf AL et al (2003) Targeted disruption of the osteoblast/osteocyte factor 45 gene (OF45) results in increased bone formation and bone mass. *J Biol Chem* 278:1998–2007
7. Balemans W, Ebeling M, Patel N et al (2001) Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). *Hum Mol Genet* 10:537–543
8. Feng JQ, Ward LM, Liu S et al (2006) Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat Genet* 38:1310–1315
9. Vatsa A, Smit TH, Klein-Nulend J (2007) Extracellular NO signalling from a mechanically stimulated osteocyte. *J Biomech* 40:S89–S95
10. Skerry TM, Bitensky L, Chayen J et al (1989) Early strain-related changes in enzyme activity in osteocytes following bone loading in vivo. *J Bone Miner Res* 4:783–788
11. El-Haj AJ, Minter SL, Rawlinson SCF et al (1990) Cellular responses to mechanical loading in vitro. *J Bone Miner Res* 5:923–932
12. Dallas SL, Zaman G, Pead MJ et al (1993) Early strain-related changes in cultured embryonic chick tibiotarsi parallel those associated with adaptive modeling in vivo. *J Bone Miner Res* 8:251–259

13. Lean JM, Jagger CJ, Chambers TJ et al (1995) Increased insulin-like growth factor I mRNA expression in rat osteocytes in response to mechanical stimulation. *Am J Physiol* 268:E318–E327
14. Forwood MR, Kelly WL, Worth NF (1998) Localization of prostaglandin endoperoxidase H synthase (PGHS)-1 and PGHS-2 in bone following mechanical loading in vivo. *Anat Rec* 252:580–586
15. Terai K, Takano-Yamamoto T, Ohba Y et al (1999) Role of osteopontin in bone remodeling caused by mechanical stress. *J Bone Miner Res* 14:839–849
16. Tatsumi S, Ishi K, Amizuka N et al (2007) Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab* 5:464–475
17. Cowin SC, Weinbaum S, Zeng Y (1995) A case for bone canaliculi as the anatomical site of strain generated potentials. *J Biomech* 28:1281–1297
18. You J, Yellowley CE, Donahue HJ et al (2000) Substrate deformation levels associated with routine physical activity are less stimulatory to bone cells relative to loading induced oscillating fluid flow. *J Biomech Eng* 122:387–393
19. Vatsa A, Breuls RG, Semeins CM et al (2008) Osteocyte morphology in fibula and calvaria—is there a role for mechanosensing? *Bone* 43(3):452–458
20. Vatsa A, Semeins CM, Smit TH et al (2008) Paxillin localisation in osteocytes—is it determined by the direction of loading? *Biochem Biophys Res Commun* 377(4):1019–1024
21. Wang Y, McNamara LM, Schaffler MB et al (2007) A model for the role of integrins in flow induced mechanotransduction in osteocytes. *Proc Natl Acad Sci USA* 104:15846–15941
22. Han Y, Cowin SC, Schaffler MB et al (2004) Mechanotransduction and strain amplification in osteocyte cell processes. *Proc Natl Acad Sci USA* 101:16689–16694
23. Vatsa A, Mizuno D, Smit TH et al (2006) Bio imaging of intracellular NO production in single bone cells after mechanical stimulation. *J Bone Miner Res* 21:1722–1728
24. Turner CH, Owan I, Jacobs DS et al (1997) Effects of nitric oxide synthase inhibitors on bone formation in rats. *Bone* 21:487–490
25. Chow JW, Fox SW, Lean JM et al (1998) Role of nitric oxide and prostaglandins in mechanically induced bone formation. *J Bone Miner Res* 13:1039–1044
26. Xiao Z, Zhang S, Mahlios J et al (2006) Cilia-like structures and polycystin-1 in osteoblasts/osteocytes and associated abnormalities in skeletogenesis and runx2 expression. *J Biol Chem* 281:30884–30895
27. Malone AM, Anderson CT, Tummala P et al (2007) Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism. *Proc Natl Acad Sci USA* 104:13325–13330
28. Nordstrom P, Pettersson U, Lorentzon R (1998) Type of physical activity, muscle strength, and pubertal stage as determinants of bone mineral density and bone area in adolescent boys. *J Bone Miner Res* 13:1141–1148
29. Bacabac RG, Smit TH, Mullender MG et al (2004) Nitric oxide production by bone cells is fluid shear stress rate dependent. *Biochem Biophys Res Commun* 315:823–829
30. Bacabac RG, Smit TH, Van Loon JJWA et al (2006) Bone cell responses to high-frequency vibration stress: does the nucleus oscillate within the cytoplasm? *FASEB J* 20:858–864
31. Mullender MG, Dijcks SJ, Bacabac RG et al (2006) Release of nitric oxide, but not prostaglandin E₂, by bone cells depends on fluid flow frequency. *J Orthop Res* 24:1170–1177
32. Bacabac RG, Smit TH, Mullender MG et al (2005) Initial stress-kick is required for fluid shear stress-induced rate dependent activation of bone cells. *Ann Biomed Eng* 33:104–110
33. Bacabac RG, Mizuno D, Schmidt CF et al (2006) Microrheology and force traction of mechanosensitive bone cells. *J Biomech* 39 (Suppl. 1):S231–S232
34. Bacabac RG, Mizuno D, Schmidt CF et al (2008) Bone cell morphology, elasticity, and mechanosensing. *J Biomech* 41:1590–1598
35. Hung CT, Pollack SR, Reilly TM et al (1995) Realtime calcium response of cultured bone cells to fluid flow. *Clin Orthop Rel Res* 313:256–269
36. Hung CT, Allen FD, Pollack SR et al (1996) Intracellular calcium stores and extracellular calcium are required in the real-time calcium response of bone cells experiencing fluid flow. *J Biomech* 29:1411–1417
37. Hung CT, Allen FD, Pollack SR et al (1996) What is the role of the convective current density in the real-time calcium response of cultured bone cells to fluid flow? *J Biomech* 29:1403–1409
38. Ajubi NE, Klein-Nulend J, Alblas MJ et al (1999) Signal transduction pathways involved in fluid flow-induced prostaglandin E₂ production by cultured osteocytes. *Am J Physiol* 276:E171–E178
39. Chen NX, Ryder KD, Pavalko FM et al (2000) Ca(2+) regulates fluid shear-induced cytoskeletal reorganization and gene expression in osteoblasts. *Am J Physiol* 278:C989–C997
40. Goodenough DA, Paul DL (2003) Beyond the gap: functions of unpaired connexon channels. *Nat Rev Mol Cell Biol* 4:285–294
41. Genetos DC, Kephart CJ, Zhang Y et al (2007) Oscillating fluid flow activation of gap junction hemichannels induces ATP release from MLO-Y4 osteocytes. *J Cell Physiol* 212:207–214
42. Klein-Nulend J, Semeins CM, Ajubi NE et al (1995) Pulsating fluid flow increases nitric oxide (NO) synthesis by osteocytes but not periosteal fibroblasts—correlation with prostaglandin upregulation. *Biochem Biophys Res Commun* 217:640–648
43. Ajubi NE, Klein-Nulend J, Nijweide PJ et al (1996) Pulsating fluid flow increases prostaglandin production by cultured chicken osteocytes—a cytoskeleton-dependent process. *Biochem Biophys Res Commun* 225:62–68
44. Klein-Nulend J, Burger EH, Semeins CM et al (1997) Pulsating fluid flow stimulates prostaglandin release and inducible prostaglandin G/H synthase mRNA expression in primary mouse bone cells. *J Bone Miner Res* 12:45–51
45. Bakker AD, Klein-Nulend J, Burger EH (2003) Mechanotransduction in bone cells proceeds via activation of COX-2 but not COX-1. *Biochem Biophys Res Commun* 305:677–683
46. Westbroek I, Ajubi NE, Alblas MJ et al (2000) Differential stimulation of prostaglandin G/H synthase-2 in osteocytes and other osteogenic cells by pulsating fluid flow. *Biochem Biophys Res Commun* 268:414–419
47. Forwood MR (1996) Inducible cyclooxygenase (COX-2) mediates the induction of bone formation by mechanical loading in vivo. *J Bone Miner Res* 11:1688–1693
48. Gong Y, Slee RB, Fukai N et al (2001) LDL-receptor related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 107:513–523
49. Boyden LM, Mao J, Belsky J et al (2002) High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med* 346:1513–1521
50. Babij P, Zhao W, Small C et al (2003) High bone mass in mice expressing a mutant LRP5 gene. *J Bone Miner Res* 18:960–974
51. Santos A, Bakker AD, Zandieh-Doulabi B et al (2008) Pulsating fluid flow modulates gene expression of proteins involved in Wnt signaling pathways in osteocytes. *Trans 54th Ann Meeting Orthop Res Soc* 33: abstract # 0160
52. Vezeridis PS, Semeins CM, Chen Q et al (2005) Osteocytes subjected to pulsating fluid flow regulate osteoblast proliferation and differentiation. *Biochem Biophys Res Commun* 348:1082–1088
53. Tan SD, de Vries TJ, Kuijpers-Jagtman AM et al (2007) Osteocytes subjected to fluid flow inhibit osteoclast formation and bone resorption. *Bone* 41:745–751