Bone remodeling in the context of cellular and systemic regulation: the role of osteocytes and the nervous system

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Abstract

Bone is a dynamic tissue that undergoes constant remodeling. The appropriate course of this process determines development and regeneration of the skeleton. Tight molecular control of bone remodeling is vital for the maintenance of appropriate physiology and microarchitecture of the bone, providing homeostasis, also at the systemic level. The process of remodeling is regulated by a rich innervation of the skeleton, being the source of various growth factors, neurotransmitters, and hormones regulating function of the bone. Although the course of bone remodeling at the cellular level is mainly associated with the activity of osteoclasts and osteoblasts, recently also osteocytes have gained a growing interest as the principal regulators of bone turnover. Osteocytes play a significant role in the regulation of osteogenesis, releasing sclerostin (SOST), an inhibitor of bone formation. The process of bone turnover, especially osteogenesis, is also modulated by extra-skeletal molecules. Proliferation and differentiation of osteoblasts are promoted by the brain-derived serotonin and hypothetically inhibited by its intestinal equivalent. The activity of SOST and serotonin is either directly or indirectly associated with the canonical Wnt/β-catenin signaling pathway, the main regulatory pathway of osteoblasts function. The impairment of bone remodeling may lead to many skeletal diseases, such as high bone mass syndrome or osteoporosis. In this paper, we review the most recent data on the cellular and molecular mechanisms of bone remodeling control, with particular emphasis on the role of osteocytes and the nervous system in this process.

Key Words
- bone
- bone remodeling
- osteocytes
- osteoblasts
- osteoclasts
- nervous system
- osteogenesis
- bone resorption
- sclerostin
- serotonin
- Wnt/β-catenin pathway
- bone diseases

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Role of osteoclasts and osteoblasts in bone remodeling

The bone is composed of extracellular matrix and bone cells at various stages of differentiation (Baron 2003, Pogoda *et al*. 2005). Moreover, it is filled with a network of blood vessels, nerve fibers, and bone marrow.

Maintenance of adequate bone mass, appropriate mechanical properties, and integrity of the skeleton requires constant bone remodeling. This process involves simultaneous resorption and formation of the bone,

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contrary to bone modeling (formation), which is associated with an increase in bone mass and strength but excludes resorption (Boyce & Xing 2008). This results in the repair of skeletal microinjuries on one hand and adaptation of the skeleton to constant mechanical load associated with everyday activities on the other. Bone growth (modeling) is the most intensive in the childhood and adolescence, before reaching full maturity. Uninterrupted osteogenesis in this period is a prerequisite for normal mechanical properties of the bones and their resistance to various pathologies, e.g., osteoporosis.

The phenomenon of bone turnover reflects activity of the two cellular populations: osteoclasts involved in the resorption of bone tissue and osteoblasts that form the new bone (Harada & Rodan 2003, Teitelbaum & Ross 2003). Bone remodeling takes place in the so-called basic multicellular unit, consisting of a group of osteoclasts forming the cutting cone in the front and a group of osteoblasts forming the closing cone behind. The latter associates with blood vessels and the peripheral innervation (Frost 1969, Elefteriou 2008; Fig. 1). Appropriate communication between bone cells and the cells of other organs is a prerequisite for maintaining homeostasis of the bone remodeling process. Recent studies of rare skeletal diseases identified a number of cellular and molecular mechanisms involved in the control of the bone remodeling process (Johnson 2015). We currently know that a shift toward excessive bone formation may result from a genetically determined disorder of sclerostin (SOST) synthesis in osteocytes, e.g., leading to van Buchem disease (van Lierop *et al*. 2013), or from mutation of the gene coding for LDL-receptor-related protein 5 (LRP5), a co-receptor involved in the Wnt/β-catenin pathway. The latter pathway is particularly involved in bone formation, and thus its disruption leads to high bone mass disease (Boyden *et al*. 2002). Enhanced bone resorption, associated with the overactivity of osteoclasts, leads to osteoporosis, a frequent disease of older patients, especially postmenopausal women, characterized by increased bone fracture risk (Komoroski *et al*. 2014). However, a complete inhibition of bone resorption is equally unfavorable, as the overactivation thereof. Osteopetrosis is another consequence of osteoclast dysfunction (Albers-Schönberg 1904, Mellibovsky *et al*. 2004) mainly caused by the presence of mutations in the cells. The above-mentioned evidence points to a

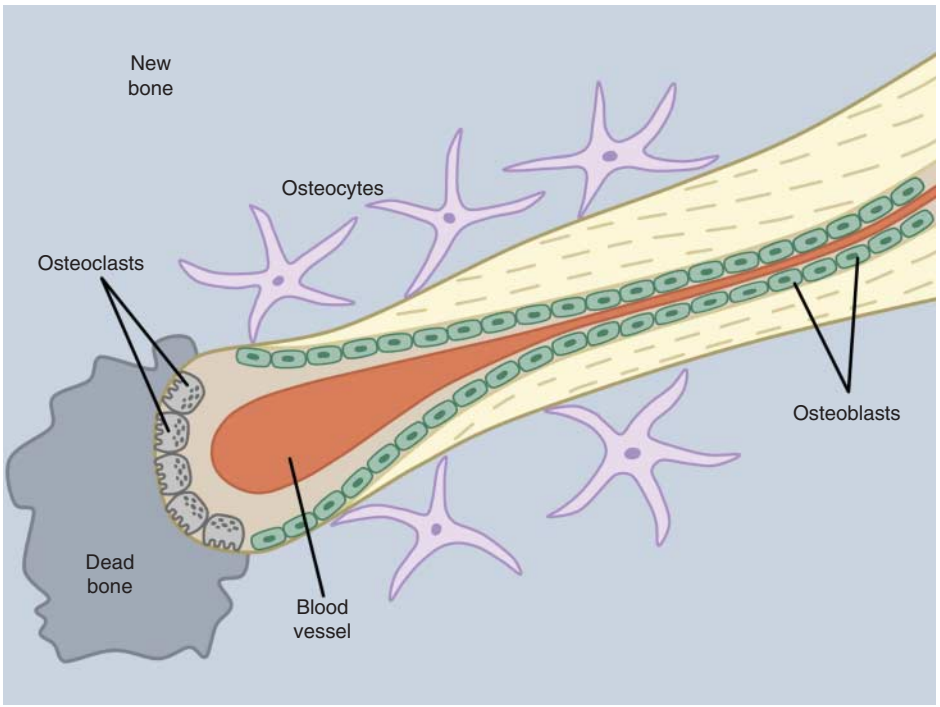

Figure 1
The process of cortical bone remodeling. The cutting cone, built of osteoclasts, moves forward and resorbs either a dead bone or a damaged/old bone matrix. The blood vessel delivers nutrients and growth factors for osteoblasts to lay down a new bone. Original artwork, based on microphotographs made by the authors and data from Parra-Torres *et al*. (2013). A full colour version of this figure is available at http://dx.doi.org/10.1530/JME-15-0067.

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significant role of physiological bone remodeling in the normal functioning of the skeleton and a whole body. Furthermore, the knowledge of cellular and molecular mechanisms involved at various stages of the bone remodeling process frequently constitutes the basis for pharmacotherapy of skeletal disorders. Identifying novel molecules and other intercellular mechanisms will give us a better understanding of how bone remodeling is controlled and should facilitate the development of new approaches to control bone remodeling and bone mass in a variety of pathological conditions.

The cells involved in bone remodeling, i.e., osteoclasts and osteoblasts (part of which finally differentiate into osteocytes at further stages of the process), originate from multipotent stem cells of the bone marrow (Del Fattore *et al*. 2010). The cells that give rise to osteoblasts and osteoclasts are mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) respectively (Del Fattore *et al*. 2010). These two cellular populations can be distinguished, e.g., on the basis of specific surface proteins expressed on their plasma membranes. MSCs are *inter alia* characterized by the surface expression of, e.g., CD73, CD90, and CD105 molecules, whereas HSCs express membrane receptors, such as CD34, CD45, and CD14 (Feisst *et al*. 2014). However, it is worth mentioning that Dominici *et al*. (2006) listed many other so-called minimal criteria, which besides surface markers characteristics, enable a more detailed definition of MSCs.

Osteoclasts are multinucleated cells, related to the cells of macrophage–monocyte and dendritic lineages (Ott 2004, Hirvonen *et al*. 2013). Osteoclasts are the first cells observed at the site of bone remodeling. Then, osteoblasts migrate to the area of resorption, fill it with a new bone matrix and control mineralization thereof (Ott 2004, Hirvonen *et al*. 2013; Fig. 1).

Osteocytes and their central role in bone turnover

While recent studies centered on the mechanisms of molecular communication between osteoblasts and osteoclasts during the regulation of bone metabolism (Sanchez-Fernandez *et al*. 2008), the superior role of osteocytes entrapped inside small lacunae of a hard bone matrix in this process was mostly neglected. Despite being the largest fraction of bone cells (~95% of all cells present in the adult skeleton), for long osteocytes were believed to be no more than differentiated osteoblasts, 'buried' in a bone matrix (Dallas *et al*. 2013) and long lived albeit incapable of proliferation and metabolically inactive (Neve *et al*. 2012, Dallas *et al*. 2013). Therefore, most previous studies of osteocyte biology have focused around their morphology and topography within the lacunar–canalicular system, rather than around their function. Interestingly, osteocytes are not central cells exclusively for bone tissue. Owing to their involvement in systemic regulation of phosphate metabolism (Dallas *et al*. 2013), associated with the synthesis of fibroblast growth factor 23, they are postulated to be one of the most significant secretory cells of human body. A theory on the principal regulatory function of osteocytes on all stages of bone turnover process is still gaining a growing interest, and especially due to a significant clinical potential of osteocytes-derived SOST inhibition by specific antibodies (see 'The role of SOST in the regulation of bone growth and remodeling' below), these cells are now being extensively researched. Currently we know that osteocytes are the principal regulatory cells involved in the response of the bone to mechanical stimulation and control bone resorption and formation, modulating the function of osteoclasts and osteoblasts at a molecular level (Belanger *et al*. 1967, Kogianni & Noble 2007). This control is particularly possible due to a secretion of SOST, an inhibitor of osteoblast activity, and other osteocyte cytokines, e.g., receptor-activator of NFκβ ligand (RANKL) or osteoprotegrin, a stimulator or an inhibitor of osteoclast differentiation respectively. A significant role of osteocytes as principal bone mechanosensitive cells rely on their capability to detect variations in the level of strain and distribute signals leading to adaptive responses. This is possible due to the formation of a functional osteocytes-abundant syncytium and strategic location of these cells within bone (Turner *et al*. 2002).

Mechanically stimulated osteocytes can communicate with each other via gap junctions, narrow channels extending between nearby cells. Gap junctions allow the communication between cells via the transfer of small molecules and ions. Recently, York *et al*. (2015) have shown that at supraphysiologic strains, the inhibition of gap junctional intercellular communication led to increases in SOST expression relative to cells in which communication was present. This study indicated that the gap-junctional communication may play a significant role in regulating bone remodeling. As already mentioned, SOST is one of the most crucial proteins involved in the control of bone remodeling by the osteocytes.

Some studies have shown that mechanical loading can promote bone formation, e.g., through downregulation of SOST expression in osteocytes. For example, Tu *et al*. (2012), using a mouse model in which the human SOST is expressed in osteocytes and is not

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regulated by loading, have demonstrated that a reduction of SOST is necessary for the stimulation of the expression of Wnt target genes and the increase in bone formation induced by mechanical force. However, Morse *et al.* (2014), through the experiments on the *Sost*$^{-/-}$ and WT mice have recently shown that the increase in bone formation under mechanical loading is unrelated to the SOST-dependent mechanism.

Recent studies showed that the activity of osteocytes during bone remodeling is under tight control of hormones secreted by other endocrine glands, such as the parathyroid hormone (PTH) of the parathyroid gland, a regulator of bone resorption and formation, or gonadal estrogen, an inhibitor of osteocyte apoptosis (Khosla *et al.* 2012, Rhee *et al.* 2013).

Osteocytes were shown to communicate with osteoblasts in a paracrine manner. The ability of osteocytes to modulate osteoblast function is associated with the synthesis of previously mentioned SOST (Neve *et al.* 2012, Dallas *et al.* 2013), an inhibitor of bone formation. As a result of this interaction, the rate of bone formation slows down (Fig. 2). Similar effects on bone formation can be observed in the presence of Dickkopf-1 (DKK1) protein (Li *et al.* 2011). The role of SOST in the physiology and pathology of the bone is discussed under 'The role of SOST in the regulation of bone growth and remodeling' below. Osteocytes can also affect the osteoblasts by a secretion of prostaglandin E2 (PGE2), nitric oxide (NO), and ATP, which stimulate the activity thereof (Fig. 2).

Li *et al.* (2012) exposed MLO-Y4 osteocyte-like cells to low-intensity pulsed ultrasound (LIPUS) stimulation *in vitro* and studied the effects of LIPUS-stimulated MLO-Y4-conditioned media (LIPUS-osteocyte-CM) on MC3T3-E1 preosteoblast proliferation and differentiation. MC3T3-E1 cells cultured in LIPUS-osteocyte-CM exhibited a significant inhibition of proliferation and an increased alkaline phosphatase (ALP) activity. LIPUS also enhanced secretion of PGE2 and NO in MLO-Y4. The authors thus

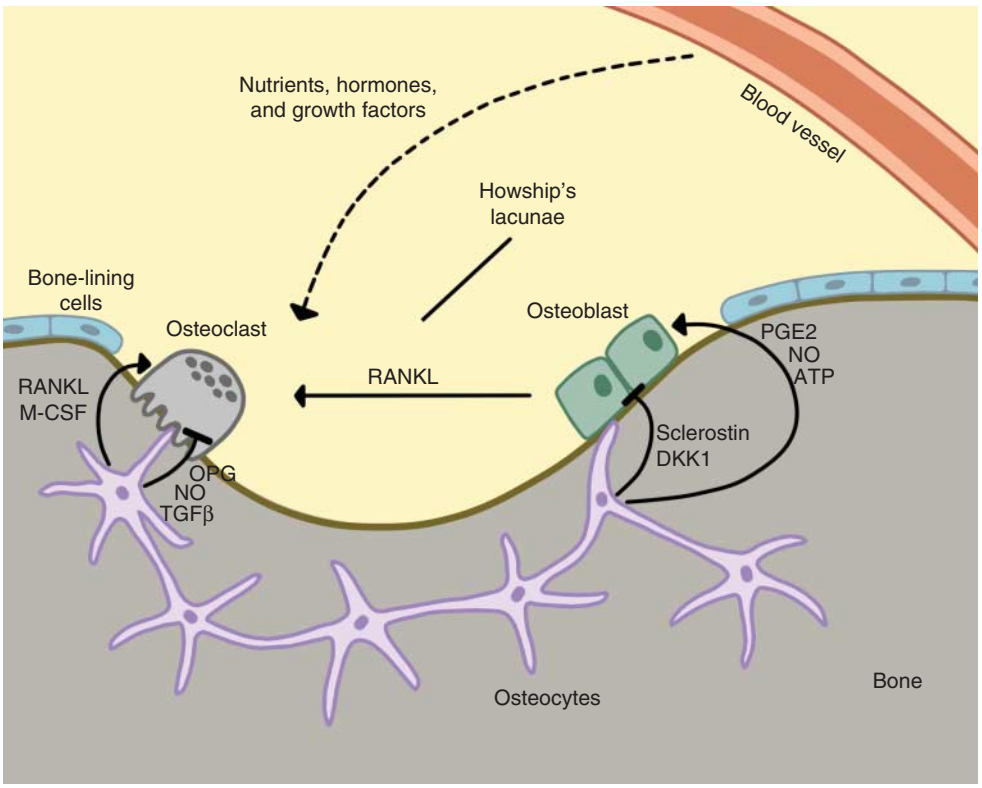

Figure 2

Interaction of bone cells: osteocytes, osteoblasts, and osteoclasts during the bone remodeling. Osteocytes activate osteoclasts via RANKL and M-CSF and inhibit their activity via OPG, NO, and TGFβ. Osteocytes-derived PGE2, NO, and ATP stimulate, whereas sclerostin or DKK1 decrease osteoblasts activity. Osteoblasts interact with osteoclasts through RANKL. Bone-lining cells support the process of bone turnover. RANKL, receptor-activator of NFκβ ligand; M-CSF, macrophage colony stimulating factor; OPG, osteoprotegerin; NO, nitric oxide; TGFβ, transforming growth factor beta; PGE2, prostaglandin E2; DKK1, Dickkopf-1. Original artwork, data from Kular *et al.* (2012), Zuo *et al.* (2012), and Parra-Torres *et al.* (2013). A full colour version of this figure is available at http://dx.doi.org/10.1530/JME-15-0067.

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concluded that increased secretion of PGE2 by the osteocytes may play a role in a communication between LIPUS-stimulated MLO-Y4 and MC3T3-E1 cells.

Moreover, osteocytes modulate function of osteoclasts, either stimulating them to bone resorption or inhibiting their resorptive activity. Whereas some of the molecules synthesized by osteocytes, such as the macrophage colony stimulating factor and RANKL, promote osteoclast differentiation, the other, e.g., the proteins of the transforming growth factor beta (TGFβ) superfamily and endothelial NO synthase, were shown to inhibit maturation of these cells (Pfeilschifter *et al*. 1988, Heino *et al*. 2002, Loveridge *et al*. 2002; Fig. 2).

Studies by Nakashima *et al*. (2011) and Xiong *et al*. (2011) demonstrated that osteocytes act as a major source of RANKL to promote osteoclastogenesis in adult bone remodeling. The knowledge on the bone cell interaction that is affected during the process of bone remodeling may help with the understanding of the bone pathophysiology at the tissue level and generate novel strategies targeting bone diseases. For example, it is currently well known how to inhibit RANKL (linking preosteoblasts and preosteoclasts) with denosumab (anti-RANKL antibody) (Leder *et al*. 2015) and SOST (linking osteocytes and osteoblasts) with, e.g., blosozumab to reduce bone resorption or increase bone formation (e.g., in osteoporosis) respectively (McColm *et al*. 2014) (see 'The role of SOST in the regulation of bone growth and remodeling' below).

Furthermore, recent findings point to an important role of osteocyte apoptosis in the regulation of bone turnover. Local skeletal microinjuries, e.g., related to bone fracture, induce osteocyte apoptosis; this stimulates osteoclasts and then osteoblasts to bone remodeling (Heino *et al*. 2009, Schaffler *et al*. 2014). Cardoso *et al*. (2009) showed for the first time that the blockade of osteocyte apoptosis completely inhibits the resorptive activity of osteoclasts. Activation of osteoclasts and osteoblasts may also result from the osteocyte necrosis caused by a mechanical stress and resultant inflammation (Heino *et al*. 2009, Schaffler *et al*. 2014). Torreggiani *et al*. (2013) showed that osteocytes not only regulate function of osteoblasts but also may dedifferentiate to the latter cells. This may happen whenever regeneration of an injury requires massive migration of osteoblasts and proliferation thereof. The ability of osteocytes to dedifferentiate was documented in a murine model, both *in vitro* and *in vivo*. According to Torreggiani *et al*. (2013), this phenomenon may be associated with exposure of osteocytes to external environment, e.g., during mechanical injury of the bone with disintegration of the surrounding tissues (due to bone fracture or surgical intervention, e.g., joint replacement or stabilization of fracture with metal screws). The external environment is an important source of various signals that may induce a transformation of osteocytes into proliferating osteoblasts, thus enabling healing of large bone defects. However, identification of the exact molecular mechanisms involved in the process of osteocyte dedifferentiation requires further research. Explanation of this phenomenon would undoubtedly have serious implications for novel strategies of regenerative medicine, based on an easy access to proliferating osteoblasts originating from terminally differentiated osteocytes. This is especially promising if the dedifferentiated cells could be obtained in an *in vitro* culture.

Bone remodeling in the context of systemic regulation: role of nervous system

Bone remodeling is a complex process modulated by a number of factors taking place simultaneously at many sites. Not only various substances released in an auto-, para- or endocrine manner control the activity of cells involved in the remodeling process; nowadays we know that also the CNS plays a vital role in this process acting, e.g., via an array of neurotransmitters and neuropeptides. Rich vascularization and innervation of the skeleton constitute the main source of various growth factors, neurotransmitters, and hormones regulating function of the bone also through the cooperation with other organs and tissues. Bone metabolism and remodeling are to a large extent controlled by, e.g., serotonin or a brain-derived neurotrophic factor (Camerino *et al*. 2012, Masi 2012), a brain-derived neurotransmitter and growth factor, respectively. Synthesis of these factors is in turn under strong influence of hormones, e.g., leptin released from the adipose tissue (Masi 2012).

Bone is a richly innervated tissue. The topography of nerve fibers within the bone, especially those containing neuropeptides, is presently a subject of extensive research. Neuropeptides released from the bone nerve fibers were identified as enzymes, sensory, sympathetic, and glutaminergic peptides (Elefteriou 2005, Masi 2012). These neuropeptides play the roles of neurotransmitters and immunomodulators, acting in a paracrine manner, i.e., in the close vicinity of their source nerve endings (Ma *et al*. 2013). All bone cells express receptors for such neuropeptides as calcitonin gene-related protein (CGRP), substance P (SP), vasoactive intestinal peptide (VIP), and *N*-methyl-D-aspartate (Masi 2012). This constitutes an indirect proof for involvement of the CNS in the control of all of the biological processes, including those taking place

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in the bones, such as bone remodeling. The results published by Ma *et al.* (2013) suggest that neuropeptides, such as SP, CGRP, VIP, neuropeptide Y (NPY), or tyrosine hydroxylase, may stimulate proliferation of osteoblasts *in vitro*. Moreover, they were shown to promote the activity of ALP and synthesis of osteocalcin in osteoblasts, additionally facilitating communication between these cells via gap junctions. Mei *et al.* (2014) indicated that SP (at the range of concentration of 10^{-9}–10^{-8} M) added to *in vitro* cultures of the mouse MC3T3-E1 cell line significantly upregulated the expressions of osteoblastic genes: collagen type 1, ALP, osteocalcin, and Runx-2. Moreover, SP (10^{-8} M) promoted the transfer of β-catenin into the nucleus. The effects of SP treatment were inhibited by the neurokinin-1 antagonist and DKK1, see 'The role of Wnt/β-catenin pathway in bone metabolism, and factors controlling this signaling pathway' below. These findings suggest that SP may enhance differentiation of MC3T3-E1 cells via regulation of the Wnt/β-catenin signaling pathway. Also the work by Fu *et al.* (2014) indicated the osteoblastic differentiation and promotion of the angiogenic ability of rat bone marrow-derived MSCs under SP treatment via the Wnt signaling pathway.

Furthermore, the findings reported recently by Baldock *et al.* (2014) point to a significant role of NPY in the prevention of stress-induced bone loss. In their murine model-based study, these authors showed that NPY can directly regulate central and peripheral noradrenergic neurons, activating their receptors Y2, and thus preventing the release of noradrenaline. This mechanism likely protects us against the bone loss resulting from a stress-induced release of catecholamines.

The hypothalamus and brain stem are the structures of the CNS that likely play particularly important roles in the regulation of bone turnover (Driessler & Baldock 2010). The hypothalamus and its semipermeable blood–brain barrier turned out to be one of the most potent intrinsic regulators that integrate not only the signals from peripheral tissues but also the internal signaling pathways of the brain.

Cerebral and intestinal serotonin and its role in the bone turnover

Pathways involving molecules synthesized outside the skeleton are equally important as the intrinsic SOST-based mechanisms of the bone growth control (see 'The role of Wnt/β-catenin pathway in bone metabolism, and factors controlling this signaling pathway' below). One of the most important extra-skeletal biologically active compounds regulating bone remodeling is serotonin (5-hydroxytryptamine); serotonin receptors are expressed on the plasma membranes of osteoblasts, osteoclasts, and osteocytes (de Vernejoul *et al.* 2012).

Mammalian serotonin is synthesized in the CNS and peripheral nervous system, whereby it acts as a neurotransmitter and hormone respectively (Goodrich *et al.* 1980, De-Miguel & Trueta 2005, Kode *et al.* 2012). As serotonin does not penetrate across the blood–brain barrier, these two pools act independently from each other. Serotonin synthesized in the brain (corresponding to 5% of the entire serotonin pool) stimulates proliferation of osteoblasts indirectly, via hypothalamic neurons. Binding to the 5-hydroxytryptamine 2c (HTR2c) receptors on the plasma membrane of hypothalamic neurons, serotonin inhibits the transmission of sympathetic stimulation and inactivates type 2 beta adrenoreceptors present in osteoblast membranes (Fig. 3). In turn, the intestinal equivalent of cerebral serotonin, synthesized by the enterochromaffin cells and representing ca. 95% of all body serotonin, inhibits the activity of osteoblasts, acting via the HTR1b; the latter receptor inactivates the protein kinase A signaling pathway that involves CREB transcription factor (Yadav *et al.* 2008, 2009; Fig. 3).

Serotonin is synthesized from tryptophan; its production in the CNS (brain stem) is catalyzed by tryptophan hydroxylase 2 (TPH2), and the intestinal synthesis by TPH1. As mentioned previously, there is a tight association between the activity of serotonin and leptin, a hormone of adipocytes, during the control of the bone remodeling process. Cerebral synthesis of serotonin is believed to be regulated by leptin, a hormone of adipocytes. Apart from the bone remodeling, leptin is also involved in a number of other processes, e.g., body weight control. Leptin prevents enhanced osteogenesis resulting from the overproduction of serotonin in the brain stem in a mechanism of negative feedback loop (D'Amelio *et al.* 2012). The role of leptin in the control of bone formation is indirectly associated with the activity of hypothalamic neurons. The inhibitory effect of leptin on the synthesis of serotonin is associated with the activation of leptin receptors on serotoninergic neurons of the brain stem. Another factor contributing to the inhibitory effect of leptin on the process of bone growth is a protein, neuromedin U, a peptide synthesized by hypothalamic neurons and small intestinal cells, that plays an important role in the transmission of leptin signaling, acting downstream of leptin and inhibiting bone growth (Yadav *et al.* 2009, Driessler & Baldock 2010). Impaired synthesis of leptin results in serious structural skeletal disorders, e.g., an increase in the mass of cancellous bone

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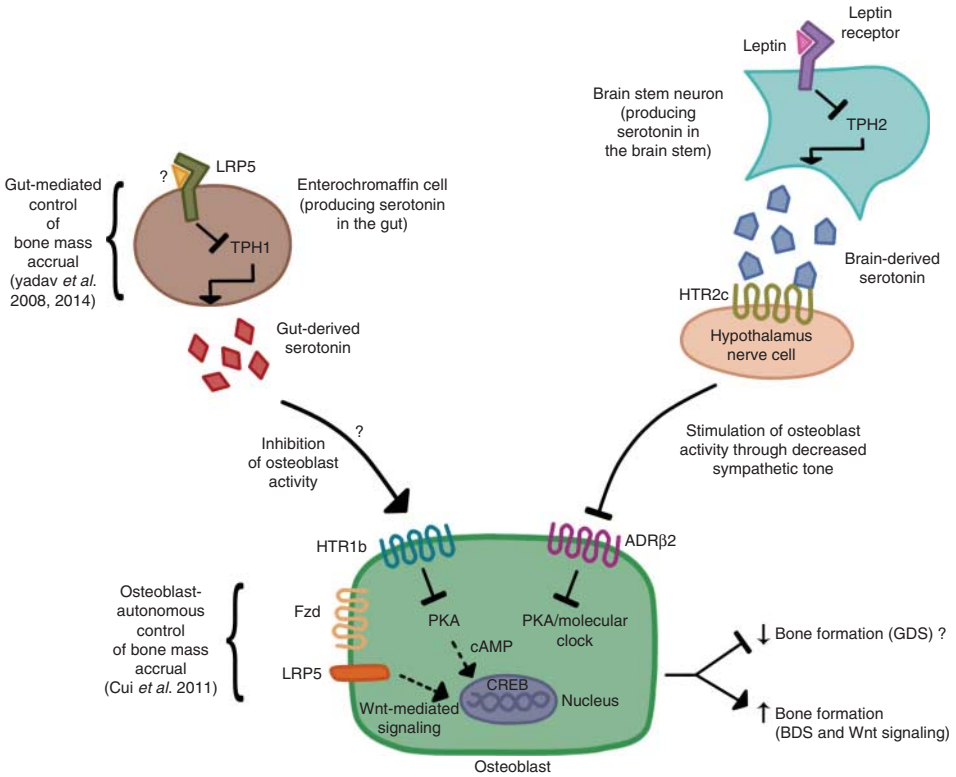

Figure 3

The mechanism of osteoblast activity regulation by brain- and gut-derived serotonin (BDS and GDS) respectively. GDS produced in the enterochromaffin cells by TPH1 enzyme inhibits osteoblast activity via the HTR1b, causing a reduced bone formation. Synthesis of GDS is decreased by LRP5 protein and its unknown ligand (Yadav *et al*. 2008, 2009). According to Cui *et al*. (2011), LRP5 acts in an autonomous-osteoblast manner to control Wnt-mediated signaling. BDS is synthesized in the brain stem neurons by the TPH2 enzyme. It stimulates osteoblast activity indirectly through the hypothalamus. BDS decreases sympathetic tone and activity of the ADRβ2 receptor in an osteoblast plasma membrane. Production of BDS remains under control of leptin–adipocytes-derived hormones. Leptin indirectly inhibits BDS synthesis, causing a decreased osteoblast differentiation. TPH1 and TPH2, tryptophan hydroxylase 1 and 2; HTR1b/2c, 5-hydroxytryptamine (serotonin) receptor 1b/2c; LRP5, LDL-receptor-related protein 5; ADRβ2, beta 2-adrenergic receptor; Fzd, Frizzled receptor. Original artwork, data from Ducy & Karsenty (2010) and Cui *et al*. (2011). A full colour version of this figure is available at http://dx.doi.org/10.1530/JME-15-0067.

and simultaneous decrease in the total bone mass and length (D'Amelio *et al*. 2012). Moreover, leptin prevents excessive resorption of the bone, acting via a precursor protein of cocaine- and amphetamine-regulated transcript (CART) neuropeptide. Elefteriou *et al*. (2005) found that CART reduces the expression of RANKL, a key factor involved in osteoclast differentiation through non-adrenergic actions. Therefore, *Cart*$^{-/-}$mice lose more bone than WT littermates in response to intracerebroventricular leptin (Elefteriou 2005, Driessler & Baldock 2010).

A study of patients with depression treated with selective serotonin reuptake inhibitors showed that binding serotonin extracellularly (not only within a synaptic space but also in the gut) may exert a negative effect on bone mass (An *et al*. 2013). Data published by Gustafsson *et al*. (2006) point to the ability of osteoblasts and osteocytes to synthesize serotonin; this phenomenon was confirmed in an *in vitro* study of human and murine cells, analyzing the mRNA level for the Tph1-encoding gene transcript. This may point to another potential cellular regulatory mechanism of bone growth. As mentioned previously, contrary to its cerebral equivalent, the serotonin of intestinal origin inhibits bone formation. Recent studies documented tight association between the activity of intestinal enterochromaffin cells and osteoblasts in the regulation of bone growth; however, the exact nature of this interaction is still not completely understood and there are many unsolved controversies around this issue. According to one theory, osteoblasts may inhibit the synthesis of intestinal serotonin acting via the LRP5 protein present in the plasma membrane of the enterochromaffin cells (Fig. 3); this neutralizes the inhibitory effect of serotonin on bone growth and thus enables osteogenesis (Yadav *et al*. 2008). Deletion of the

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LRP5-encoding gene in enterochromaffin cells was reflected by the overexpression of *Tph1*, release of intestinal serotonin into circulation and inhibition of bone growth (Yadav *et al*. 2008). The postulated ability of osteoblasts to affect the synthesis of serotonin points to a significant cellular mechanism of bone growth control. However, Cui *et al*. (2011) obtained contradictory results, indicating that peripheral (i.e., gut-derived serotonin) does not affect bones and the role of LRP5 in the intestine is not related to Tph1 inhibition (Fig. 3). These authors rather suggest that osteoblasts activity is under control of LRP5, present in their plasma membrane, which is directly associated with the Wnt signaling pathway. It is the so-called autonomous-osteoblast control of the bone mass accrual. Thus, according to Cui *et al*. (2011), the mutations in osteoblasts, but not enterochromaffin cells-derived LRP5, lead to different impaired skeletal phenotypes, e.g., high bone mass or osteoporosis–pseudoglioma syndrome. Serotonin may also regulate the activity of osteoclasts. Chabbi-Achengli *et al*. (2012) revealed that serotonin is vital not only for osteoblasts but also for osteoclast differentiation and showed that the synthesis of serotonin by these cells may be additionally enhanced by RANKL, generated by osteoblasts. All of this evidence points to the crucial role of serotonin in the regulation of both bone formation and bone resorption.

The role of Wnt/β-catenin pathway in bone metabolism, and factors controlling this signaling pathway

The Wnt glycoproteins are involved in the control of many developmental processes, including the formation of mesoderm and organogenesis (Logan & Nusse 2004). The results of recent studies suggest that these proteins may also play a role in the development of some neoplasms (Sharma *et al*. 2014, Zhang *et al*. 2014). A total of 19 members of the Wnt family were described to date. Wnt-mediated signaling controls renewal and proliferation of stem cells and stimulates differentiation of osteoprogenitor cells (Baron & Kneissel 2013, Clevers 2013). In particular, Wnt7b and Wnt10b are postulated to play the significant important roles in the process of bone mass control (Kubota *et al*. 2010). Mice with mutations of the Wnt7b and Wnt10b genes presented with impaired osteogenesis and slower bone growth rate respectively (Bennett *et al*. 2007, Tu *et al*. 2007). However, it also is worth mentioning two other members of the Wnt superfamily, namely Wnt1 and Wnt16, which have recently been revealed to play an important role in either the bone formation or repression of osteoclastogenesis and the prevention of cortical bone fragility fractures respectively (Laine *et al*. 2013, Moverare-Skrtic *et al*. 2014).

The Wnt proteins can activate at least three independent signaling pathways that are crucial for bone remodeling. The most important of them is the so-called canonical Wnt pathway, involving a cytoplasmic protein, β-catenin. Another pathway, the so-called non-canonical Wnt pathway, involves two distinct mechanisms: planar cell polarity (important during embryogenesis) and the regulation of calcium ion concentration within the bone cells (Wang *et al*. 2014).

The canonical Wnt pathway (Fig. 4) may be interrupted due to the blockade of the Wnt signaling for the Frizzled (Fzd) receptors expressed on the osteoblast surface. This blockade, for example, associated with the activity of SOST, leads to degradation of β-catenin, resulting from phosphorylation of this protein. The latter reaction is catalyzed by glycogen synthase kinase 3 beta (GSK3β), acting in complex with other proteins, e.g., adenomatous polyposis coli (APC) or axin. If the Wnt signal reaches osteoblasts, their Fzd receptors bind to their co-receptors, LRP5 or LRP6, and deactivate the GSK3β complex; this enables the passing of the β-catenin signal to the cell nucleus and activation of genes that control the proliferation and differentiation of osteoblasts via T-cell factor/lymphoid-enhancer factor transcription factors (Kubota *et al*. 2010). Wnt signaling is currently emerging as an essential pathway targeted in bone regeneration (Leucht & Helms 2015). Minear *et al*. (2010) have shown that another member of the Wnt family (Wnt3a) stimulates the proliferation of skeletal progenitor cells and accelerates their differentiation into osteoblasts, when packaged into liposomal vesicles and delivered into mouse skeletal defects. The liposome-based delivery of Wnt3a enhances its activity and results in faster bone regeneration, i.e., increased mineralization and osteoid formation, when compared to the control (liposome-entrapped PBS; Minear *et al*. 2010). These data suggest that Wnt signaling can be amplified successfully at the injury site, being an alternative for bone morphogenetic proteins (BMPs)-based bone therapies. Recently, Leucht *et al*. (2013) also demonstrated that liposomal Wnt3a enhances cell survival and reestablishes the osteogenic capacity of bone grafts from aged rabbits. This may indicate a new strategy of bone therapy, e.g., in individuals suffering from diminished bone healing potential.

Apart from other genes, the Wnt signaling pathway controls the expressions of *RUNX-2* and *C-MYC*, i.e., the

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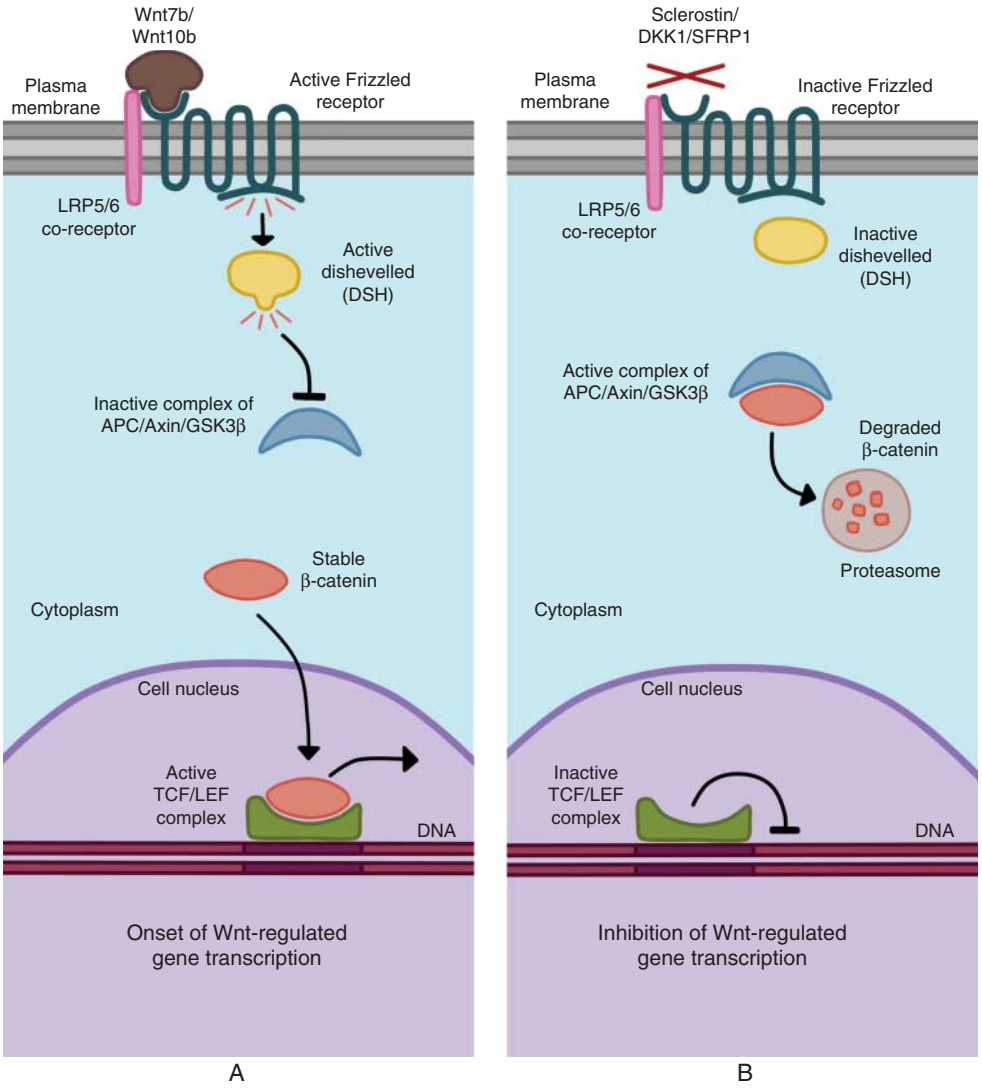

Figure 4

(A) Activation of canonical Wnt/β-catenin pathway in osteoblast via, e.g., Wnt7b and Wnt10b proteins. Fzd receptor interacts with Wnt7b or Wnt10b causing the activation of Dsh protein, which inactivates the complex of APC/Axin/GSK3β. This enables β-catenin transduction of the signal triggering transcription of Wnt-dependent genes in the osteoblast nucleus. (B) Inhibition of osteoblast activity by Wnt/β-catenin pathway inhibitors, e.g., sclerostin (SOST), sFRP1, and DKK1. SOST binding to LRP5/6 co-receptors in the osteoblast plasma membrane disables the signal transduction from Fzd to Dsh. This triggers a phosphorylation of β-catenin by APC/Axin/GSK3β and its further proteosomal degradation. Degraded β-catenin is unable to activate a Wnt-dependent gene transcription in the nucleus of osteoblast. Fzd, Frizzled receptor; Dsh, dishevelled; APC/Axin/GSK3β, the complex of adenomatous polyposis coli (APC), axin, and glycogen synthase kinase 3 beta (GSK3β); sFRP1, soluble Frizzled-related protein 1; DKK1, Dickkopf-1; LRP5 or LRP6, LDL-receptor-related protein 5 or 6; TCF, T-cell factor; LEF, lymphoid-enhancer factor. Original artwork, data from Bennett *et al.* (2007). A full colour version of this figure is available at http://dx.doi.org/10.1530/JME-15-0067.

genes responsible for differentiation and proliferation of osteoblasts respectively (Zhu *et al.* 2008). Aside from SOST, osteocytes can release many other antagonists of the osteoblast Wnt/β-catenin pathway, e.g., DKK1 or soluble Frizzled-related protein 1 (sFRP1). Wnt-mediated signaling can also be modulated by glucocorticoids, which increase the expression of Wnt signaling antagonists (SOST and DKK1) in experimental studies in rodents and cell cultures (Almeida *et al.* 2011, Thiele *et al.* 2012). Because Wnt signaling is essential for a proper differentiation of osteoblasts as mentioned previously, glucocorticoids can decrease bone formation, leading to osteoporosis. Thus, inhibition of SOST, DKK1, or sFRP1 may potentially constitute a therapeutic target in patients with glucocorticoid-induced osteoporosis (Gifre *et al.* 2013, Guanabens *et al.* 2014).

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The role of SOST in the regulation of bone growth and remodeling

Several physiological mechanisms prevent both insufficient and excessive bone formation, equally unfavorable as the excessive (e.g., osteoporosis-associated) resorption. One of them is paracrine signaling, i.e., communication between osteocytes and osteoblasts. Recent studies unambiguously confirmed that the process of bone formation (i.e., proliferation and differentiation of osteoblasts) is modulated by SOST, an osteocyte-synthesized protein (Neve *et al.* 2012, Dallas *et al.* 2013). SOST is encoded by the sclerosis–bone dysplasia, sclerosteosis (*SOST*) gene, located on the long (q) arm of chromosome 17 at position 11.2. Owing to the presence of a cysteine knotlike domain in its secondary structure, SOST is classified as the differential-screening-selected gene aberrant in neuroblastoma glycoprotein (Balemans *et al.* 2001, Brunkow *et al.* 2001, van Bezooijen *et al.* 2005). Although the expression of mRNA for the *SOST* transcript is observed during embryogenesis of many tissues (McNulty *et al.* 2012), the product of this gene, SOST, is biosynthesized solely during the postnatal period and is specific for differentiated cells: osteocytes, hypertrophic mineralized chondrocytes and cementocytes (Winkler *et al.* 2003, van Bezooijen *et al.* 2004, 2009, Poole *et al.* 2005) and articular chondrocytes (Chan *et al.* 2011, Roudier *et al.* 2013). SOST is a down regulator of bone formation. However, besides inhibiting bone formation, it can also induce osteoclast formation (Wijenayaka *et al.* 2011). Binding to co-receptors of the LRP family, LRP5 or LRP6, SOST acts as an antagonist of Wnt/β-catenin signaling pathway (Fig. 4; Li *et al.* 2005) and thus inhibits survival, proliferation, and differentiation of osteoblasts and promotes survival of osteocytes. Both *in vitro* and *in vivo* studies showed that the intracellular Wnt/β-catenin signaling pathway can be activated due to mechanical stimulation of bone cells (Hens *et al.* 2005, Robinson *et al.* 2006, Lin *et al.* 2009). Very recently, Lara-Castillo *et al.* (2015) have postulated that mechanical load initially activates β-catenin signaling in osteocytes via a PG and Akt-mediated mechanism. According to these authors, initial activation of β-catenin signaling in the subset of osteocytes activates a subsequent propagation of the 'load signal' to the adjacent osteocytes, which further reaches the cells on the bone surfaces. This signal propagation and activation of β-catenin signaling in the osteocytes is connected to the decreased expression of the LRP5/6 and Wnt ligand inhibitors, e.g., SOST and DKK1. As mentioned previously, the inhibitory effect of SOST on bone formation is potentiated by glucocorticoids (Gifre *et al.* 2013) and can be counterbalanced by PTH, administered in the form of teriparatide (PTH 1–34); this evidence constitutes the basis of currently tested experimental therapeutic approaches, e.g., treatment of glucocorticoids-induced osteoporosis (Lau & Adachi 2010). Apart from NO, synthesis of SOST is also inhibited by PGs, especially PGE2 (Genetos *et al.* 2011). Furthermore, Papanicolaou *et al.* (2009) demonstrated the effects of BMPs on SOST expression at the mRNA/protein levels in different bone cell lines of the mouse origin (MLO-Y4, MLO-A5, and UMR 106.01). The authors have shown that in mature MLO-Y4 osteocytes, treatment with BMP2, BMP4, or BMP6 was without effect on Sost but induced a robust increase in Sost expression in immature MLO-A5 osteocytes. Oscillatory fluid flow applied to mature UMR 106.01 osteoblasts transiently decreased expression of SOST at both the mRNA and protein levels. The results obtained by Papanicolaou *et al.* (2009) indicate that BMP treatment and *in vitro* mechanical loading demonstrate opposite effects on SOST expression.

Five mutations of the *SOST* gene were described to date, including three associated with a the premature termination of translation and another two resulting in impaired splicing of the *SOST* transcript during post-transcriptional processing. Furthermore, impaired bone formation may result from the deletion of the DNA segment regulating the expression of the *SOST* gene (Loots *et al.* 2005). As a result of this mutation, SOST no longer exerts its inhibitory effect on bone formation, which leads to hyperplasia, a typical manifestation of sclerosteosis and van Buchem disease (Balemans *et al.* 2001, 2005, Brunkow *et al.* 2001, Loots *et al.* 2005, Kim *et al.* 2008). Understanding the molecular background of these two conditions provided novel therapeutic options for patients with excessive bone formation and individuals with osteoporosis (McClung *et al.* 2014). One example of such novel therapy is a MAB that selectively binds SOST (trade name: romosozumab); the results of ongoing clinical trials (currently phase 3) point to high efficacy of this agent in the management of osteoporosis (van Dinther *et al.* 2013, Pinkerton *et al.* 2013). This raises hopes for successful treatment of this condition frequently diagnosed in postmenopausal women. Excessive production of SOST may also potentially be inhibited with blosozumab, a specific monoclonal anti-SOST antibody already tested in a phase 1 clinical trial on healthy, postmenopausal women (McColm *et al.* 2014).

Conclusion

The skeleton is not a static structure. Throughout the lifetime, it undergoes a continuous remodeling, i.e., a

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dynamic reorganization of the bone. This phenomenon is observed either under physiological conditions, as an effect of dynamic forces generated during usual everyday activities or during regeneration of the bone after trauma. Bone remodeling occurs in response to various factors, e.g., hormones, cytokines, chemokines and biomechanical, external stimuli, and is vital not only for the maintenance of a normal bone mass and strength but also for mineral homeostasis. Bone remodeling is specifically regulated by a crosstalk between bone cells. The process of bone remodeling involves resorption, controlled by osteoclasts, and bone formation, associated with the activity of osteoblasts. However, osteocytes located within the mineralized bone matrix seem to play a superior role in the regulation of bone remodeling at a cellular and molecular level. Recently osteocytes, previously considered metabolically inactive cells, have raised interest as key regulatory components of the bone and one of the most important endocrine cells of the body. Impaired synthesis and secretion of various osteocyte-derived molecules, especially SOST, may lead to many skeletal pathologies, resulting from either too low (e.g., in van Buchem disease) or too high (e.g., in postmenopausal osteoporosis) synthesis of this protein, which may promote excessive or cause decreased bone formation (and thus regeneration) respectively (Zhou *et al*. 2013). Bone is a richly innervated and vascularized tissue. Therefore, the CNS with its neurotransmitters and neuropeptides, growth factors, and hormones along with other systems play vital roles in the process of bone turnover.

Apart from skeletal innervation, the need for a tight control of bone remodeling is also reflected by the activity of extra-skeletal regulators of the process, e.g., cerebral and hypothetically intestinal serotonin. As a source of serotonin, the brain stem plays a pivotal role in the control of new bone formation.

Constant remodeling and adaptation of bone tissue to mechanical loads is not the only principal role of the skeletal system. Bones are increasingly referred to as the central hormonal organs of the human body, not only regulating metabolism of the skeletal system but also strongly affecting the function of other organs and tissues. This is of vital importance as many pathologies of the skeleton may give rise to systemic disorders. Therefore, further identification of other molecular mechanisms related to bone remodeling and metabolism will not only provide better insight into these processes but also define novel strategies for the treatment of skeletal as well as systemic diseases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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