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Influencing the Effect of Treatment of Diseases Related to Bone Remodelling by Dynamic Loading

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1. Introduction

1.1 Physiology of bone

Morphogenesis, growth and modelling of the skeletal system are dynamic processes, and the skeleton, once formed, is managed dynamically through remodelling. *Morphogenesis* begets growth. Morphogenesis is a consummate series of events during embryogenesis, bringing cells together to permit inductive opportunities – the outcome is a three-dimensional structure, such as a bone. The term *growth* embraces processes in endochondrally derived, tubular bones that increase length and girth prior to epiphyseal plate closure. In the cranium, the physis analog is the fontanelle. The process that permits bone growth is *modelling*, an active pageantry of cells embraced in mysterious partnership. Modelling produces functionally purposeful sizes and shapes of bones. Modelling drifts mainly determine outside bone diameter, cortical thickness, and the upper limit of bone strength. The final product of growth and modeling is a skeletal complex of 206 adult bones demanding continuous maintenance, which is accomplished by remodelling. Remodelling sustains structure and patches blemishes in the adult skeleton, while to homeostatic demands to ensure calcium and phosphate balance: "remodelling. . . is replacement of older by newer tissue in a way that need not alter its gross architecture or size"(Lieberman & Friedlaender, 2005).

Remodelling is a fundamental property of bone that permits adaptation to a changing mechanical environment. The skeleton's tissue-level functions and biomechanical influences on them were unknown before 1964 (Frost, 1964). The remodelling of bone tissue and orientation of osteons depends on very complex states of external loading caused by various positions and activities of human body which involve alternating extensions and shortenings of individual regions of bone tissue. Osteon orientation of the diaphysis of the long bones in man was confirmed on archaeological femurs and exactly biomechanically explained by Heřt et al. (Heřt et al., 1994). Rubin and Lanyon (Rubin & Lanyon, 1984; 1985a) proved in their experiments that bone reacts to intermittent strains only in defined range

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1000 – 2000-2500 microstrains. Longitudinal strain 1000 microstrain in compression is one that would shorten a bone by 0.1 %. One microstrain is defined as 10^{-6} original lengths at shortening and by $0.5\ 10^{-6}$ of original length in tension.

H.M. Frost has defined the minimum effective strain (Frost, 1987c). The alternating strains above that threshold level 2000 – 2500 microstrains (overuse) affect modelling and remodeling activities in ways that change the size and configuration of growing bones (bone formation) to their new mechanical usage and return their strains to the threshold level (i.e. feedback). Vice versa, the alternating strains below 1000 microstrains (disuse) causes bone resorption.

The newer Utah paradigm of bone physiology by H.M. Frost (Frost, 2000; 2004) includes in part the skeleton's tissue-level "nephron equivalents" (the tissue-level multicellular units that provide special skeletal activities and functions, e.g. modelling drifts, remodelling), precursor cells (osteoblasts and osteoclasts in bone), mechanical effects, microdamage physiology, a marrow mediator mechanism, creep physiology (Frost, 1987c), mechanostats, maintenance activities that tend to preserve the mechanical competence of skeletal organs and the related feedback. Nowadays, the most part of authors distinguishes the modelling of bones as a form of sculpting which determines the shape, size and proportions of long bones by locally modifying their directions and speed of growth, from remodelling, signifying a quantised turnover of bone in remodelling packets called "basic multicellular units (BMU)" which couple an initial resorption process to formation processes in the same place of the bone surface (periosteal, Haversian, cortical-endosteal and trabecular). The bone remodelling begins with a resting surface, a resorption cavity (Howship's lacuna) is excavated by ostoeclasts, which osteoblasts then refill with new bone. In a simplified way, modelling of bones can be described like this: packets of bone are removed where the mechanical demand of the skeleton is low and new bone is formed at those sites where mechanical strains are repeatedly detected.

In summary, succinctly according to Frost, "Growth determines size. Modelling molds the growing shape. Remodelling then maintains functional competence (replacement, maintenance and homeostasis)." The processes of macromodelling and minimodelling continue in the adult skeleton, where macromodelling increases the ability of bone to resist bending (by expanding periosteal and endosteal cortices) and minimodelling rearranges trabeculae to best adapt to functional challenges (Frost, 1987b; c; 2000; 2004; Kimmel, 1993).

In the Utah paradigm the biologic mechanisms that determine skeletal health and disorders still need "nonmechanical things" in order to work. "Nonmechanical things (agents)" include sex, age, diet, vitamins, hormones, other humoral agents, genes, cytokines, membrane receptors and ligands, biochemical reactions, apoptosis, pinocytosis, etc. Mechanostat is the combination of biologic mechanisms that adapts skeletal strength and architecture to the needs of voluntary physical activities (Frost, 1987b). In load-bearing skeletal organs mechanical factors guide those mechanisms and cells in time and anatomical space, including their effects on skeletal strength and architecture. Nonmechanical factors can help or modulate that guidance but cannot replace it. E.g., so they cannot normalise skeletal organs in paralysed limbs.

Because of the organic components such as collagen, proteoglycans, elastine and intercellular fluid, *the bone tissue has viscoelastic properties* which are manifested by long-term viscoelastic deformation changes occurring in contradiction of elastic behaviour even under constant loading and after unloading. These long-term strain changes continue much longer

than those nearly instantaneous ones and depending on the moment of loading and unloading. Starting from the foregoing facts and considerations, Sobotka and Mařík (Sobotka & Mařík, 1995) arrived at the deformational-rheological theory of remodelling of bone tissue according to which the stimulating mechanical effects depend not only on the amount but also on the duration of deformation changes. The elastic after-effect then involves a relatively long continuation of deformation changes under non-varying loading. In this manner, the existence of remodeling effects even at rest can be explained. These effects are used at ortotic treatment (Culik et al., 2008; Mařík et al., 2003) and physiotherapy for many years.

1.2 Bone metabolism—RANK/RANKL/OPG concept

Remodelling of skeleton is a complex process performed by the coordinated activities of osteoblasts and osteoclasts. Osteoblasts originate from pluripontent mesenchymal stem cells, which also give rise to chondrocytes, muscle cells, adipocytes and stromal bone marrow cells and are the cells responsible for the synthesis of the bone matrix. Osteoclasts are derived from hemopoietic stem cells of the monocyte-macrophage lineage and are the only cells capable of resorbing mineralised bone (Manolagas, 2000). It is generally concluded the osteoclasts resorb bone during growth, modelling and remodelling. The interactions between osteoblasts and osteoclasts, which guarantee a proper balance between bone gain and loss, is known as coupling (Rodan & Martin, 1981). The birth and death of osteoblasts and osteoclasts are controlled by local factors such as cytokines, growth factors and prostaglandins that are produced by skeletal and non-skeletal tissues. The effects of these factors can be mediated through autocrine, paracrine or even endocrine signal pathways, although factors produced by skeletal tissue and stored in bone may have more direct effects (Rucker et al., 2002). Many of these factors not only have redundant effects on bone cells, but can also modulate their own and each other's production in a cascade fashion (Manolagas, 2000). Thus even a small change of concentration of one factor can dramatically affect the concentrations of others.

Terms osteoblast and osteocyte were originally used to define the active and inactive stages, respectively, of the same cell type. Osteocytes play the active role e.g. in the sensing and transmission of mechanical strains. There are stromal cells (marrow-associated and bone associated) that include fibroblastic and reticular cells which constitute and secrete the collagen framework (i.e. the stroma) and bone lining cells that closely resemble osteocytes as regards their ultrastructure. Bone lining cells differ from osteocytes in that they retain their bone-forming potentiality and thus, under appropriate stimuli, can reconvert into osteoblasts (Miller et al., 1989). It should be said that all osteoblasts were found to be in contact with vascular dendrites of mature osteocytes, i.e. dendrites radiating from the osteocyte plasma membrane facing the bone vascular surface. While vascular dendrites continue to elongate, during bone deposition, in order to remain in contact with the osteoblastic lamina, mineral dendrites of the newly formed osteocytes do not seem to grow. Marotti (Marotti, 1996) with co-workers morphologically proved that the cells of the osteogenic lineage form a continuous cytoplasmatic network from the vascular endothelium to the osteocytes, passing through the stromal cells and the cells carpeting the bone surfaces, i.e. osteoblasts or bone lining cells. It appears that the overall system made up of the cells of the osteogenic lineage, including the vascular endothelium, constitutes a functional syncytium. It means that the transmission of signals throughout such a cellular system may occur by means of two mechanisms – wiring transmission (WT) and volume transmission

(VT) similarly like transmission of signals in the central nervous system. The concept of VT in bone simply corresponds to the well-known endocrine, paracrine and autocrine routes to the bone cells followed by hormones, cytokines and growth actors. VT should generally affect wider skeletal regions or even the whole skeleton, whereas WT would seem to participate in the local modulation of bone cells, particularly as far as mechanical stimuli are concerned. Cytoplasmic stress-strain and fluid movement (fluid flow in canalicular extracellular matrix) are possible operational mechanisms securing the osteocyte-osteoblast interaction and may function as a mechanism for the transduction of mechanical strain to osteocytes in bone (Lieberman & Friedlaender, 2005).

The aspects of RANK/RANKL/OPG biology were delineated during the past 13 years that are ushered in a totally new era of understanding of bone resorption. A number of labs using different methods and biological systems uncovered the new molecules (essential cytokines, receptors and ligands) in the Tumour Necrosis Factor family members and their biologic activities involved in the regulation of bone resorption (Martin, 2004).

Several factors have been associated with osteoclast formation, including PTH, 1,25-dihydroxy vitamin D_3, interleukins-1, -6, and -11, tumour necrosis factor (TNF), leukemia inhibitory factor, ciliary neurotropic factor, prostaglandins, macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor, and RANK (Teitelbaum, 2000).

In response to homeostatic demands, systemic humoral cues for cells of the BMU can include 1,25-dihydroxy vitamin D_3, androgen, calcitonin, estrogen, glucocorticoids, growth hormone (GH), parathormone (PTH) and thyroid hormone. PTH and 1,25-dihydroxy vitamin D_3 stimulate resorption, they are countered by calcitonin, which inhibits resorption. Mechanisms for interactions are still not well known. The key systemic signal for bone is estrogen (Pacifici, 1998): a decrease of this hormone can cause resorption to outstrip formation, bone mass falls, and the diagnosis for this disease is osteoporosis. Advancing age is associated with an increased serum level to PTH and a decrease in estrogen, which may evoke increased cytokine levels of IL-1, IL-6, TNF-α, and probably RANK-L (Eghbali-Fatourechi et al., 2003). Estrogen depletion provokes osteocyte apoptosis, and could cause bone loss (Tomkinson et al., 1997).

Local humoral cues can include BMPs (bone morphogenic proteins), FGF (fibroblast growth factor), IGF (insulin-like growth factor), TGF-β (tumour growth factor beta), PDGF (platelet-derived growth factor), PTHrP for formation and GM-CSF (granulocyte macrophage colony stimulating factor), ILs (interleukins 1,4,6,11,13,18), and M-CSF (macrophage colony-stimulating factor), leading to resorption (Raisz, 1999). TGF-β can promote both resorption and formation.

In addition to local factors, adhesion molecules (proteins expressed on the surface of bone cells and progenitors) also have important regulatory roles by mediating cell-cell and cell-matrix interaction that enable the migration of osteoprogenitors to the remodeling sites; anchor mature osteoblasts unto bone surface; and communicating local, hormonal and mechanical signals (Raisz, 1999). Circulating hormones and mechanical signals exert potent effects on skeletal metabolism by modulating the production and action of these local factors. The molecular and physiological mechanisms of control of osteoclast formation and activity have been explained with the discovery of three protein members of the TNF superfamily which have been proposed as final effectors for many of the local factors and hormones. Receptor activator of NF-κ B ligand (RANKL, also called Tumour Necrosis Factor-Related Activation-Induced Cytokine - TRANCE, osteoprotegerin ligand, or

osteoclast differentiating factor) is the type II membrane protein (cytokine) in cells of the osteoblastic lineage (committed preosteoblastic cells) which interacts with its receptor, receptor activator of NF-κB (RANK), on hematopoietic precursors (osteoclast progenitors) to promote osteoclast formation and maintain their viability and activity. RANKL binds to RANK with high affinity and, with the permissive effect of macrophage stimulating factor (M-CSF), this interaction is essential and sufficient for osteoclastogenesis. The process is further negatively regulated by the decoy receptor, the third non-membrane bound protein, osteoprotegerin (OPG), osteoclast inhibitory factor (OCIF) respectively, that is also produced by stromal/osteoblastic cells, and which binds to RANKL to prevent RANKL stimulation of osteoclast formation binding to RANK (Bekker et al., 2001; Simonet et al., 1997; Yasuda et al., 1998). Osteoprotegerin, has been shown to be a potent osteoclast inhibitor in vitro and in vivo studies (Simonet et al., 1997).

The RANK-RANKL-OPG pathway is coupled to the dual action of tumour growth factor beta (TGF-β) on osteoblasts. TGF-β, as well as other growth factors and specific components embedded in the bone matrix, are released by osteoclasts during bone resorption (Bonewald & Dallas, 1994). On one hand, TGF-β has the potential to stimulate osteoblast recruitment, migration and proliferation of osteoblast precursors (responding osteoblasts). On the other, TGF-β inhibits terminal osteoblastic differentiation into active osteoblasts (Alliston & Choy, 2001). TGF-β is also known to induce osteoclast apoptosis.

The evidence of all came from the validation studies in genetically manipulated mice or other rodent models that uncovered physiologic roles for these molecules. Overexpression of OPG resulted in mice with osteopetrosis because of failure to form osteoclasts (Simonet et al., 1997) whereas genetic ablation of OPG led to severe osteoporosis (Bucay et al., 1998; Mizuno et al., 1998). Genetic ablation of RANKL resulted in osteopetrosis because RANKL is necessary for normal osteoclast formation (Kong et al., 1999). Genetic ablation of RANK led to osteopetrosis also because it is the receptor for RANKL (Dougall et al., 1999).

1.3 Human bone diseases related to bone remodelling

Aging. In the healthy young adult skeleton, resorption and formation are balanced so that bone mass is maintained. Starting around the fourth or fifth decade of life, however, bone loss with age happens at all skeletal sites in both sexes and is characterized by a remodeling imbalance, in which resorption exceeds formation. With menopause (or male hypogonadism) the rate of bone loss increases dramatically, a change attributed to cellular mechanisms (Manolagas, 2000). The result is clinical disease osteoporosis. Both estrogen and androgens (perhaps through conversion to estrogen) normally suppress the production of IL-6, TNF and M-CSF, which stimulate the formation of osteoclasts and osteoblasts from the marrow. In addition, estrogen promotes osteoclast apoptosis (probably mediated through TGF-β), while exerting anti-apoptotic effects on osteoblast and osteocytes (Manolagas, 2000). As a result, loss of estrogen increases not only the number of active BMUs, but also the lifespan of osteoclasts while reducing the lifespan of osteoblasts and osteocytes. The increased lifespan of the osteoclasts, in particular, is thought responsible for the deepening of resorption cavities (Eriksen et al., 1999) and trabecular perforation leads to micro-structural weakness of bone and increased fracture risk in women in the early postmenopausal period (Rucker et al., 2002). In contrast to postmenopausal bone loss resulting from osteoclast hyperactivity, the inexorable bone loss seen with senescence in both sexes is thought to be osteoblast mediated. A decrease in osteoblast number decreases

bone formation (Manolagas, 2000). Although it is difficult to separate sex-steroid deficiency from aging effects, bone marrow osteoblastogenesis also decreases with age. The decrease in osteoblastogenesis is attributed to an over-expression of genes that redirect mesenchymal stem cells to differentiate into adipocytes rather than osteoblasts, as well as age-related decreases in the pulsatile excretion of growth hormone that result in decreases insulin-like growth factors (IGFs) and their binding proteins (Weinstein & Manolagas, 2000).

There are several other important endocrine factors implicated in age-related bone loss. With increasing age, the ability to absorb calcium from the gut decreases because of decreased levels of the active vitamin D hormone, 1,25-dihydroxy vitamin D, ($1,25(OH)_2D$). Although $1,25(OH)_2D$ itself has potent stimulatory effects on local factors that stimulate osteoclasts and osteoblasts, the major physiologic function of this hormone is to stimulate intestinal calcium absorption. Insufficient $1,25(OH)_2D$ reduces serum calcium that in turn increases synthesis and secretion of parathyroid hormone (PTH). PTH then increases bone remodeling to mobilize calcium from the skeleton. PTH has potent stimulatory effects on the development and activity of osteoblasts and interferes with bone formation at the transcriptional level (Rucker et al., 2002). Pharmacological doses of glucocorticoids also have various harmful effects bone remodeling. Glucocorticoid excess inhibits osteoblastogenesis, increases osteoblast and osteocyte apoptosis, suppresses circulating gonadal steroid production, and decreases calcium absorption (Manolagas, 1998).

The genetic basis of the several human *extremely rare heritable disorders of the RANK/RANKL/OPG pathway* was uncovered following the elucidation of the biological activity and significance of the pathway members (Whyte & Mumm, 2004). These remarkable skeletal disorders were found to reflect gene defects leading to constitutive activation of RANK or to deficiency of OPG. Hughes et al. (Hughes et al., 2000) investigated familial expansile osteolysis (FEO) and identified an activating 18-bp tandem in the gene encoding RANK (TNFRSF11A) in three affected kindred, and similar 27-bp duplication in an unusual, familial form of early-onset Paget disease of bone (PDB) in Japan. Whyte and Hughes (Whyte & Hughes, 2002) reported that a seemingly unique disorder designated expansile skeletal hyperphosphatasia (ESH) was allelic to FEO and involved 15-bp tandem duplication in RANK. Whyte et al. (Whyte et al., 2002) documented homozygous complete deletion of the gene encoding OPG (TNFRSF11B) as the first molecular explanation for idiopathic hyperphosphatasia, called juvenile Paget disease (JPD).

The majority of *human metabolic bone diseases* are caused by excessive extent of bone resorption that exceeds the rate of bone formation, resulting in loss of bone mass. With accumulating evidence of the role of the OPG/RANKL/RANK cytokine system for normal osteoclast biology, it became clear that many clinically relevant metabolic disease in humans, including inflammatory bone diseases (e.g. rheumatoid arthritis), malignant bone tumours (e.g. myeloma or osteolytic metastases) and different forms of osteoporosis are caused by alterations of the OPG/RANKL/RANK system (Teitelbaum, 2000). Skeletal estrogen agonists (including 17 β-estradiol, raloxifene and genistein) induce osteoblastic OPG production through estrogen receptor-α activation in vitro, while immune cells appear to over-express RANKL in estrogen deficiency in vivo. OPG administration can prevent bone loss associated with estrogen deficiency as observed in both animal models and a small clinical study (Bekker et al., 2001). Glucocorticoids and immunosuppressants concurrently up-regulate RANKL and suppress OPG in osteoblastic cells in vitro, and glucocorticoids are among the most powerful drugs to suppress OPG serum levels in vivo. As for hyperparathyroidism, chronic PTH exposure concurrently enhances RANKL production

and suppresses OPG secretion through activation of osteoblastic protein kinase A in vitro which would favour increased osteoclastic activity. PTH receptors are largely expressed on the osteoblast surface. While continuous PTH exposure (binding these receptors) stimulates the production of RANKL and inhibits the production of OPG by osteoblasts. This mechanism enhanced the RANKL-to-OPG ratio by up to 25-fold and stimulated osteoclastogenesis. Later was proved that intermittent (pulsatile) PTH administration stimulated IGF-1 mRNA, an anabolic skeletal growth factor. PTH is currently involved in numerous clinical trials as an anabolic agent for the treatment of low bone mass in osteoporosis (Locking et al., 2003; Neer et al., 2001). In sum, RANKL/OPG imbalances is the likely etiology of metabolic bone diseases (Hofbauer et al., 2004).

These data point to the promise that targeted RANKL antagonist therapy could bring to the many clinical settings where excessive bone loss leads directly to increased morbidity and mortality. There is a few years experience with bisphosphonates, raloxifene, teriparatide (parathormone 1-84) and stroncium ranelate in treatment of different forms of osteoporosis (idiopathic postmenopausal and secondary osteoporosis) and heritable disorders of the RANK/RANKL/OPG pathway, too. *In published Czech case of Familial expansile osteolysis (Marik et al., 2006b) the treatment with bisphosphonates was successful and allowed surgical correction of severe shank deformity after normalisation of bone turnover (a note of the author). There are other rare heritable disorders with high bone turnover, e.g., Hajdu-Cheney syndrome (Marik et al., 2006a) and Pachydermoperiostitis (Latos-Bielenska et al., 2007), where treatment with bisphosphonates has a positive influence.*

Safety and efficacy of above mentioned drugs is still studied in clinical trials. At present, the basis for osteoporosis prevention and therapy is supplementation of vitamin D and calcium together with appropriate physical activities with respect to age. At present, clinical trials of osteoporosis with recombinant OPG and anti-RANKL provide additional support for innovative treatment strategy.

1.4 Physical activity and mechanical loading

It is well known that bone adapts to its environment; Galileo was among the first to recognize that body weight and activity were related to bone size (Galileo, 1638). This structure/ function relation was formally described in the late 19th century in what has been designated as Wolff's law (Wolff, 1892). Over time, Wolff's law promulgated into a teleological paradigm that bone is a well-designed engineering structure, adding bone and changing its architecture to minimize strain on the skeleton (McLeod et al., 1998). Frost and others (Frost, 1987a; Lanyon et al., 1982) described the mechanical regulation of bone as a "mechanostat", whereby bone increases its mass with the mechanical loading and, conversely, loses bone mass when there is no little or no mechanical stimulus. Supporting this structural efficiency paradigm is a wealth of observational and experimental evidence, such as loss of bone mass during disuse (Nishimura et al., 1994) or space flight (Morey & Baylink, 1978), and local bone hypertrophy related to mechanical loading (Haapasalo et al., 1994; Kravitz et al., 1985; Rubin & Lanyon, 1985a; Turner et al., 1994).

While the concept that the mechanical environment affects bone is well accepted, it remains unknown exactly what aspects of the mechanical milieu are paramount for osteogenesis. Much of what we do know about functional adaptations at the tissue level comes from well-controlled animal models to assess physical influences on bone formation. The intensity, duration and manner of the loading environment is translated and expressed as mechanical strain (relative deformation of a material) or other related parameters of the strain

environment, such as strain frequency, rate and gradients (Zernicke & Judex, 1999). These studies show that only dynamic loads increase bone formation. Furthermore, if the magnitude is high enough, increasing the number of strain cycles beyond a certain point does not increase bone mass (Rubin & Lanyon, 1984). On the other hand, strains need not be large in magnitude if strains are unusual in their distribution (Lanyon, 1996), high in frequency or rate (Turner et al., 1994), or have gradients (Gross et al., 1997; Judex et al., 1997).

It has been shown that exercise-induced bone formation is site-specific (Loitz & Zernicke, 1992) although few of the animal studies have taken this into account. Animal studies that relate the mechanical parameters to morphological changes in bone have demonstrated that the osteogenic stimulus varies with skeletal maturity. Central to elucidating precisely how bone adapts to mechanical stimulus is to know how bone interprets mechanical stimuli at the cellular level. Mechanotransduction is the process of converting mechanical stimuli into a cellular response and occurs in a wide variety of physiologic functions. In bone, mechanotransduction involves the transduction of a mechanical signal into a local signal perceived by cells, and followed by the transduction of this local signal into a biochemical signal to stimulate osteoblasts or osteoclasts to form or remove bone. In theory, all eukaryotic cells are sensitive to their mechanical environments (Ingber, 1997). In bone, osteoclasts, osteoblasts, osteocytes and bone lining cells are sensitive to mechanical stimulation in vitro and in vivo. Osteoblasts, however, make up only 5% of cells in adult bone, and osteoclasts comprise under 1%. Thus, even if all active osteoblasts were directly stimulated, the effect would not significantly increase bone mass (Duncan & Turner, 1995). To facilitate an adaptive modeling/remodeling response, osteoprogenitors must be recruited to the bone surface. Rather than the mechanical signal directly stimulating osteoblasts or osteoclasts directly, it is hypothesized that osteocytes or bone lining cells, which make up approximately 95% of all bone cells (Parfitt, 1994), act as the sensor cells. That hypothesis is a function of the connectivity of these cells: osteocytes are connected to neighboring osteocytes and lining cells on the bone surface by a network of slender long processes linked via gap junctions (Shapiro, 1997). Thus communication is enabled through the bone matrix. Since neither osteocytes nor bone lining cells resorb or form new bone, they signal to “effector” cells (osteoclasts and osteoblasts) to produce bone adaptations (Duncan & Turner, 1995). Mechanical loading can activate osteocytic production of autocrine or paracrine factors, such as prostaglandins, nitric oxide (NO), and IGF (Zaman et al., 1997). Experimental evidence implicates fluid flow as a local signal for stimulating osteocytes (Weinbaum et al., 1994). When bone is loaded, interstitial fluid flows from the medullary canal into the vascular system and lacunar spaces of bone tissue. Fluid flow stimulates osteocytes directly through shear stresses or indirectly by electric fields (streaming potentials) (Otter et al., 1985).

The stimulus for remodeling can come from internal factors (e.g., hormones, cytokines-growth factors) and external factors (e.g., physical activity and mechanical loading). It is widely accepted that physical activity benefits the musculoskeletal system but the mechanisms affecting bone mass and density that are set off by physical activity in general and mechanical loading in particular are still poorly understood. It appears that mechanical strain inhibits RANKL production and up-regulates OPG production in vitro. Hence, lack of mechanical strain during immobilisation (disuse) may favour an enhanced RANKL-to-OPG ratio leading to increase bone loss. Nowadays, it is believed that the static loading is not osteogenic. Instead, the dynamic loading plays the essential role of stimulating the bone

remodelling process, which is supported by many experimental and clinical studies. Increasing age, declining levels of sex hormones, or calcium deficiencies produce an imbalance between resorption and formation resulting in bone loss. Physical activity through its mechanical effects on bone can mitigate this bone loss. Optimal mechanical stimuli differ between growing and mature bone, and mature bone is influenced by aging or other systemic factors such as nutrition and hormones. Recently so-called Whole Body Wibration (WBW) has been introduced to improve impaired biomechanical function of the musculoskeletal system in adults. The therapeutic principle is based on the activation of proprioceptive spinal circuits. These reflexes can be induced by upright standing on a vibrating platform. The application of vibrations increased bone formation and the metabolism in skeletal muscles and skin. WBW is characterised to prevent the loss of bone and muscle mass in immobilised adults. WBW improves inter- and intramuscular co-ordination over induction of agonists and antagonists in the neuromuscular system. At present, some clinical trials confirm therapeutic effects of the Cologne Standing-and-Walking-Trainer powered by Galileo on the mobility of children and adolescents affected with diseases characterised by a disease-related sarcopenia due to physical immobilisation such as patients with osteogenesis imperfecta (OI), infantile cerebral palsy and Meningomyelocele (Schönau, 2008). The effect of WBW is also studied in muscular dystrophy patients and children with juvenile idiopathic arthritis with the aim to improve muscular force and motor function.
Greater understanding of how mechanical stimuli interact with systemic factors is central for the development of more effective exercise programs in the prevention of bone loss, as well as enhancing complementary of exercise and pharmacological therapies.

1.5 Available models of bone remodelling

With the development of computer-aided strategies and based on the knowledge of bone geometry, applied forces, and elastic properties of the tissue, it may be possible to calculate the mechanical stress transfer inside the bone (Finite Elements analysis or FE analysis). The change of stresses is followed by a change in internal bone density distribution. This allows to formulate mathematical models that can be used to study functional adaptation quantitatively and furthermore, to create the bone density distribution patterns (Beaupré et al., 1990; Carter, 1987; Weinans et al., 1992). Such mathematical models have been built in the past. Since they calculate just mechanical transmission inside the bone and not considering cell-biologic factors of bone physiology, they just partially correspond to the reality seen in living organisms. Basically, there are essentially two groups of models for bone remodelling. One assumes that the mechanical loading is the dominant effect, almost to the exclusion of other factors, and treatment of biochemical effects are included in parameter with no physical interpretation (Beaupré et al., 1990; Carter, 1987; Doblaré & García, 2002; Huiskes et al., 1987; Ruimerman et al., 2005; Turner et al., 1997). The results or predictions of these models yield the correct density distribution patterns in physiological cases. However, they have a limited ability to simulate disease. The second group, the biochemical models, consider control mechanisms of bone adaptation in great detail, but with limited possibilities for including mechanical effects that are known to be essential (Komarova et al., 2003; Lemaire et al., 2004; Müller, 2005).
We realize that biochemical reactions are initiated and influenced primarily by genetic effects and then by external biomechanical effects (stress changes). Our thermodynamic model enables to combine biological and biomechanical factors (Klika & Maršík, 2009b).

Such a model may also reflect changes in remodelling behaviour resulting from pathological changes to the bone metabolism or from hip joint replacement. However, it is a model and thus it is a great simplification of the complex process of bone remodelling. In this paper, a more detailed description of biochemical control mechanisms will be added to the mentioned model (Klika & Maršík, 2009b) which in turn leads to possibility to study several concrete bone related diseases using this model.

2. Simulation of diseases and their treatment

In our previous work, the influence of mechanical stimulation on (chemical) interactions in general was studied and it was shown how to comprise this effect into a model of studied biochemical processes (Klika & Maršík, 2009a). These findings were used to describe the bone remodelling phenomenon (Klika et al., 2008; Maršík et al., 2009; Maršík et al., 2005). Most actual version of this model with identified parameters which has captured the main features of bone remodelling is currently under revision in Biomechanics and Modelling in Mechanobiology (Klika & Maršík, 2009b)[1]. In this chapter, an extension of the mentioned bone remodelling model (influences of concrete biochemical factors) will be presented where the essential significance of dynamic loading will still be apparent. The approach cannot be so straightforward, actually, bounds of applicability will be searched.

Firstly, fundamental control factors will be mentioned. As was mentioned in the introduction, the RANKL-RANK-OPG pathway is essential in the bone remodelling control. Osteoprotegerin (OPG) inhibits binding of ligand RANKL to receptor RANK and thus prevents osteoclastogenesis. Since osteoclasts are the only resorbing agents in bone, osteoprotegerin "protects bone" (osteo-protege). Further, one of the major problems connected to bone remodeling is a rapid bone loss after menopause that affects a significant portion of women after 50 years of age. Menopause is linked to a rapid decrease in estrogen levels. And because estrogen significantly affects bone density, it would be beneficial to be able to simulate the influence of estrogen levels on the bone remodelling process. Similarly, the parathyriod hormone PTH, tumour growth factor TGF-β_1, and nitric oxide NO play a significant role during the bone adaptation process.

PTH causes a release of calcium from the bone matrix and induces MNOC differentiation from precursor cells, estrogen has complex effects with final outcome in decreasing bone resorption by MNOC, calcitonin decreases levels of blood calcium by inhibiting MNOC function, and osteocalcin inhibits mineralisation (Sikavitsas et al., 2001). The discovery of the RANKL-RANK-OPG pathway enabled a more detailed study of the control mechanisms of bone remodelling. Robling et al. states that all PTH, PGE (prostaglandin), IL (interleukin), and vitamin D are "translated" by corresponding cells (osteoblasts) into RANKL levels (Robling et al., 2006). Further, nitric oxide NO is known to be a strong inhibitor of bone resorption and recently it has been known that it works in part by suppressing the expression of RANKL and, moreover, by promoting the expression of OPG (Robling et al., 2006). Both these effects eventually lead to a decrease of numbers of active osteoclasts MNOC, which in turn causes decrease of bone resorption. Kong et al. mentions that the OPG expression is induced by estrogen (Kong & Penninger, 2000). Boyle et al. add that OPG

[1] Please, contact the authors for update about this paper

production by osteoblasts is based on anabolic stimulation from TGF-β or estrogen (Boyle et al., 2003). Martin also deals with the question how hormones and cytokines influence contact-dependent regulation of MNOC by osteoblasts. He summaries results from the carried out experiments (mainly in vitro) that PTH, IL-11, and vitamin D ($1.25(OH)_2D_3$ more precisely) promotes RANKL formation, which in turn increases osteoclastogenesis (Martin, 2004).

RANKL-RANK-OPG pathway mediates many of these above mention biochemical factors. Moreover, RANKL levels also reflect microcrack density. Hence, it is essential to incorporate this pathway into our model. The connection will be enabled through the amount RANKL-RANK bonds that are one of the components of developed model, noted as RR, see (Klika & Maršík, 2009b).

2.1 Incorporation of RANKL-RANK-OPG pathway into the bone remodelling model

A new model for RANKL-RANK-OPG chain kinetics will be formulated and added to the mentioned model of bone remodelling (fundamental ideas can be found in (Maršík et al., 2009) and its most recent version is under review in Biomechanics and Modelling in Mechanobiology (Klika & Maršík, 2009b)). RANKL is a ligand molecule and binds to RANK forming a bond, here noted as RR and its molar concentration as [RR], between osteoblasts and precursors of osteoclasts. Osteoblasts also secrete a decoy receptor osteoprotegerin OPG[2] that binds with high affinity to RANKL and thus prevents the needed connection between osteoblasts and osteoclastic precursors.

The reaction scheme of interaction of the mentioned molecules can be described as follows:

$$\begin{aligned} RANKL + RANK &\overset{k_{\pm 1}}{\rightleftarrows} RR, \\ RANKL + OPG &\overset{k_{\pm 2}}{\rightleftarrows} RO_{\text{inactive}}, \end{aligned} \tag{1}$$

where RO_{inactive} represents the bond between the decoy OPG and ligand RANKL. Using the law of mass action (Klika & Maršík, 2009a) we may infer kinetics of the above mentioned interactions. Only the simplification, when assuming a relation between forward and backward reaction rates $k_{+i} \gg k_{-i}$, is not applicable here. We get

$$\begin{aligned} \frac{\mathrm{d}n_{RANKL}}{\mathrm{d}\tau} &= -n_{RANKL}(\beta_{\mathrm{RK}}^{\mathrm{RRO}} + n_{RANKL} - n_{OPG}) + \\ &\quad + \delta_{-1}^{\mathrm{RRO}}(\beta_{\mathrm{RR}}^{\mathrm{RRO}} - n_{RANKL} + n_{OPG}) - \\ &\quad - \delta_{+2}^{\mathrm{RRO}} n_{RANKL} n_{OPG} + \delta_{-2}^{\mathrm{RRO}}(\beta_{\mathrm{RO}}^{\mathrm{RRO}} - n_{OPG}), \\ \frac{\mathrm{d}n_{OPG}}{\mathrm{d}\tau} &= -\delta_{+2}^{\mathrm{RRO}} n_{RANKL} n_{OPG} + \delta_{-2}^{\mathrm{RRO}}(\beta_{\mathrm{RO}}^{\mathrm{RRO}} - n_{OPG}), \end{aligned} \tag{2}$$

where

[2] Osteoblasts are not the only producers of OPG - in fact, around 60 % is produced by cells in heart, kidney, and liver (Boyce & Xing, 2008).

$$\delta_{-1}^{\mathrm{RRO}} = \frac{k_{-1}}{k_{+1}[\mathrm{RANKL}_{\mathrm{stand}}]},$$
$$\delta_{-2}^{\mathrm{RRO}} = \frac{k_{-2}}{k_{+1}[\mathrm{RANKL}_{\mathrm{stand}}]},$$
$$\delta_{+2}^{\mathrm{RRO}} = \frac{k_{+2}}{k_{+1}}, \tag{3}$$
$$\beta_{\mathrm{RO}}^{\mathrm{RRO}} = \frac{C_{\mathrm{RO}}}{[\mathrm{RANKL}_{\mathrm{stand}}]} = \frac{[\mathrm{RO}_0]+[\mathrm{OPG}_0]}{[\mathrm{RANKL}_{\mathrm{stand}}]},$$
$$\beta_{\mathrm{RR}}^{\mathrm{RRO}} = \frac{C_{\mathrm{RR}}}{\mathrm{RANKL}_{\mathrm{stand}}} = \frac{[\mathrm{RR}_0]+[\mathrm{RANKL}_0]-[\mathrm{OPG}_0]}{[\mathrm{RANKL}_{\mathrm{stand}}]}.$$

Again $k_{\pm i}$ are reaction rate coefficients, δ_i are interaction rates, and β_j^{RRO} represents the normalized initial molar concentrations of corresponding substances, denoted with index 0 and finally [$\mathrm{RANKL}_{\mathrm{stand}}$] represents standard serum level of RANKL used for normalisation of molar concentrations of substance i, n_i. All the parameters have evidently a physical interpretation and are measurable. However, hardly any such in vivo data for humans is available. Fortunately, the recent progress in the understanding of bone remodelling control enabled in vitro studies of individual factors.

Quinn et al. studied the influence of RANKL and OPG concentration on a number of osteoclasts (more precisely, TRAP positive multinucleated osteoclasts) in a dose-dependent way (Quinn et al., 2001). We would like to use this data to determine the above mentioned parameters of the RANKL-RANK-OPG model. Because the carried out experiments are studying effects of RANKL and OPG separately, the reaction scheme (1) may be splitted into two separate reactions for parameter setting. This is convenient because the kinetics of a single biochemical reaction can be described using a single differential equation (in this case non-linear). Moreover, both normalised differential equations corresponding to these two reactions can be written in the same form:

$$\dot{x} = -Ax^2 - Bx + C, \quad A > 0, C > 0, \tag{4}$$

where $A = 1$, $B = \beta_{\mathrm{RK}} + \delta_{-1}$, $C = \delta_{-1}\beta_{\mathrm{RR}}$ for the RANKL reaction and $A = \delta_{+2}$, $B = \delta_{+2}\beta_{\mathrm{RANKL}} + \delta_{-2}$, $C = \delta_{-2}\beta_{\mathrm{RO}}$ for OPG reaction. The normalised form is also useful because it decreases the number of unknown parameters. The differential equation (4) has the following solution for positive constants A, C and for initial value x_0:

$$x(\tau) = \left[\frac{2A}{\sqrt{B^2+4AC}}\left(1+\frac{1+\frac{2A}{\sqrt{B^2+4AC}}\left(x_0+\frac{B}{2A}\right)}{1-\frac{2A}{\sqrt{B^2+4AC}}\left(x_0+\frac{B}{2A}\right)}e^{\sqrt{B^2+4AC}\tau}\right)\right]^{-1} \cdot$$
$$\cdot\left[\left(1-\frac{B}{\sqrt{B^2+4AC}}\right)\frac{1+\frac{2A}{\sqrt{B^2+4AC}}\left(x_0+\frac{B}{2A}\right)}{1-\frac{2A}{\sqrt{B^2+4AC}}\left(x_0+\frac{B}{2A}\right)}e^{\sqrt{B^2+4AC}\tau}-\frac{B}{\sqrt{B^2+4AC}}-1\right]. \tag{5}$$

Because we know the analytic form of function describing the kinetics of RANKL (and OPG), we may use the least square method for determination of the unknown parameters according to the carried out experiments. Data from the Quinn et al. in vitro experiment relates RANKL (and OPG) concentration to MNOC concentration (the number of osteoclasts per well). The mentioned reaction scheme (1) of RANKL-RANK-OPG interaction has an output product denoted as RR. Thus, to be able to use the mentioned data from Quinn et al., we need to relate RANKL-RANK bonds ([RR]) to the number of osteoclasts ([MNOC]). To get a precise prediction of this relationship from the presented model we would also need to know the analytical solution of the system of ODEs that describe the bone remodelling process (Klika & Maršík, 2009b; Maršík et al., 2009), which is not possible. On the other hand, the interaction that describes the relation between RANKL-RANK bonds and MNOC concentration is the first one in our bone remodelling scheme (Klika & Maršík, 2009b; Maršík et al., 2009) and it will be assumed that the number of formed and active osteoclasts is proportional to the RR concentration. It means that it was assumed that in vitro, where no remodellation occurs, the formation of osteoclasts may be described by:

$$RR \rightleftarrows MNOC.$$

This assumption will be used just for purposes of parameter setting and from final results it will be possible to see if this simplification was too great or not.

The next issue we have to deal with is finding a possible relation between in vitro and in vivo data. In vivo ones are more or less unavailable, especially in such a detail that is needed for parameter setting. Further, determination of standard serum levels of OPG and RANKL is needed. The problem is that in most cases in vitro concentrations have to be much higher to reach a similar effect as in vivo. Moreover, no such relation may exist. It will be assumed that there is a correspondence among these two approaches and that it is linear, i.e. in vivo data can be gained from in vitro after appropriate scaling of concentrations.

The search for standard serum levels of osteoprotegerin and RANKL was not simple. Studies differ greatly in the presented values. Kawasaki states that the standard level of osteoprotegerin is 250 $\frac{pg}{\mu l}$ (Kawasaki et al., 2006) and Moschen et al. mention 800 $\frac{pg}{\mu l}$ (Moschen et al., 2005). Further, Eghbali-Fatourechi et al. determined OPG serum levels to be 2.05 $\frac{pmol}{l}$ (Eghbali- Fatourechi et al., 2003). The probable cause of these discrepancies lies in differently used techniques of gaining osteoprotegerin and measuring its concentration. Kawasaki et al. measured the amount of RANKL in gingival crevicular fluid, Moschen et al. performed collonic explant cultures from biopsies and consequently measured RANKL and OPG levels using an ELISA kit, and Eghbali-Fatourechi used a different cell preparation technique followed by measurement with an ELISA kit. One of the manufacturers of the ELISA kit for assessment OPG levels cites several studies on OPG levels in humans and also submits results from their own research (OPG ELISA kit, 2006). At least all these measurements are carried out by the same measurement technique and are comparable. Therefore, we set standard OPG and RANKL levels according to data that are there referred to - $[\mathrm{RANKL}_{\mathrm{stand}}] = 0.84\ \frac{pmol}{l} = 55 \cdot 0.84\ \frac{pg}{ml} = 46.2\ \frac{pg}{ml}$ and $[\mathrm{OPG}_{\mathrm{stand}}] = 1.8\ \frac{pmol}{l} = 20 \cdot 1.8\ \frac{pg}{ml} = 36\ \frac{pg}{ml}$ in serum (Kudlacek et al., 2003), where the knowledge of molecular weights $MW_{\mathrm{RANKL}} = 55\ 10^3$, $MW_{\mathrm{OPG}} = 20\ 10^3$ was used (OPG ELISA kit, 2006; RANKL product data sheet, 2008). Now it is needed to find a reasonable relation with in vitro data from Quinn that will be

used for the least squares method for parameter estimation. The following consideration will be used: the physiological range of levels of OPG and RANKL will be found and consequently related to studied effective in vitro range by Quinn. OPG serum levels found in human are 12–138 $\frac{pg}{ml}$ = 0.6–6.9 $\frac{pmol}{l}$ and RANKL serum levels are 0–250 $\frac{pg}{ml}$ = 0–4.55 $\frac{pmol}{l}$ with standard values of 0.84 $\frac{pmol}{l}$ for RANKL and 1.8 $\frac{pmol}{l}$ for OPG, respectively. When we relate these values to the in vitro ranges of RANKL 0–500 $\frac{ng}{ml}$ and of OPG 0–30 $\frac{ng}{ml}$, we get the in vitro equivalents for standard values: $[\mathrm{RANKL_{invitrostand}}]$ = 92.3 $\frac{ng}{ml}$, $[\mathrm{OPG_{invitrostand}}]$ = 7.83 $\frac{ng}{ml}$.

A list of parameters that will be determined by least squares from the RANKL experiment are the following:

$$\delta_{-1}^{\mathrm{RRO}}, \tau_{7\,\mathrm{days}}^{\mathrm{RRO}}, n_{RK_0}, n_{RR_0},$$

where $\tau_{7\,\mathrm{days}}^{\mathrm{RRO}}$ is the dimensionless time that corresponds to 7 days. Before the parameter setting by curve fitting (least square method) is carried out, it is reasonable to have at least some estimation of parameter values. Because the normalisation was done by division with term $k_{+1}[\mathrm{RANKL_{stand}}]^2$ and from (3), we get:

$$\tau_{7\,\mathrm{days}}^{\mathrm{RRO}} = tk_{+1}C_{\mathrm{RR}}\,|_{t=7\,days} = 6\,10^5\,10^7\,10^{-12} \doteq 10^0,$$

$$n_{RR_0} \doteq 10^0,$$

$$n_{RK_0} \doteq 10^0,$$

$$\delta_{-1^{\mathrm{RRO}}} = \frac{k_{-1}}{k_{+1}[\mathrm{RANKL_{stand}}]} \doteq k_{-1}10^5,$$

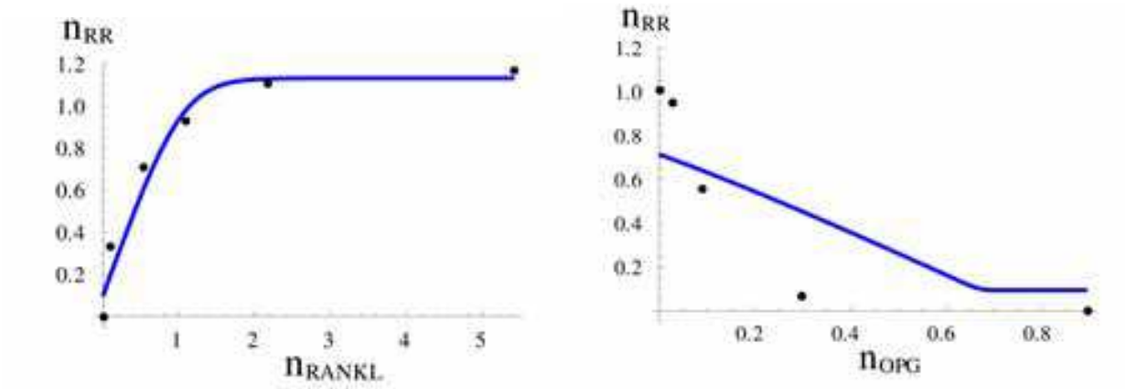
where the value of k_{+1} was estimated from the parameter setting in the bone remodeling model, standard value of RANKL $[\mathrm{RANKL_{stand}}] \doteq 1\ \frac{pmol}{l}$ was mentioned above, and the k_{-1} value may be anywhere in $(0, 10^7)$ but most probably lower than one.

The least square method with the used data from Quinn et al. (Quinn et al., 2001) and the analytic function as described above gives the following estimates:

$$\delta_{-1}^{\mathrm{RRO}} = 4.92\,10^{-6}, \tau_{7\,\mathrm{days}}^{\mathrm{RRO}} = 4.64, n_{RK_0} = 1.037, n_{RR_0} = 0.0947. \quad (6)$$

If we compare these values with their order estimation above, we see that the values are acceptable and the curve fit is as well, see figure 1a.

Now, we may proceed with OPG parameters. The difference is that if we use only the second reaction of RANKL-RANK-OPG reaction scheme (1), we do not know how initial OPG concentration influences the number of bonds between RANKL and RANK. However, this influence is mediated by a decrease in number of available ligands RANKL by binding with OPG. Because OPG binds with higher affinity to ligand RANKL than this ligand to its



(a) RANKL (b) OPG

Fig. 1. RANKL and OPG fitted solutions (blue curves) by least squares method to data measured (dots) by Quinn et al. (Quinn et al., 2001). Firstly, n_{RR} as a function of n_{RANKL_0} is determined and consequently n_{RR} as a function of n_{OPG_0}, created by embedding dependency of [RANKL] on [OPG] and of [RR] on [RANKL] concentration, was found.

receptor RANK (otherwise the decoy effects of OPG would be very limited), it will be assumed that OPG binds to RANKL more rapidly than the competiting reaction. The reason for this is again in the need of analytic solution of differential equations that govern the kinetics of mentioned processes (we was not able to solve the full system of two differential equations (2) so the mentioned simplification was needed; again, from the results to come it seems reasonable). Thus, the influence of levels of osteoprotegerin on the RR concentration may be mediated by an appropriate modification of initial concentration of RANKL which in turn affects the resulting RR concentration. Schematically:

$$2^{\text{nd}} \text{ reaction in (1)} \rightarrow [\text{OPG}](t)$$

and consequently $[\text{RANKL}_0] = [\text{OPG}](\tau_{\text{OPG}})$, which is used in

$$1^{\text{st}} \text{ reaction of (1)} \rightarrow [\text{RR}][t_{7\text{days}}]$$

where τ_{OPG} is a time to be determined.

The already determined parameters from the RANKL setting will be used and only the yet unknown will be determined, i.e.

$$\delta_{-2}^{\text{RRO}}, \delta_{+2}^{\text{RRO}}, \tau_{\text{OPG}}^{\text{RRO}}, n_{RO_0},$$

Again, the least squares in the case of OPG give the following estimates (based on data from Quinn and the fact that molecular weight of RANKL is 55 10^3 and of OPG 20 10^3):

$$\delta_{-2}^{\text{RRO}} = 5.86\,10^{-19}, \delta_{+2}^{\text{RRO}} = 12.96, \tau_{\text{OPG}}^{\text{RRO}} = 11.36, n_{RO_0} = 6.135. \quad (7)$$

Also, the values are admissible and the curve fit as well (the function here is much more complicated because OPG concentration is firstly used to determine an initial RANKL concentration for a consecutive reaction that finally gives [RR] outcome), see figure 1b.

If the mentioned results of parameter estimation are combined, all the needed values of parameters of RANKL-RANK-OPG model (3) may be inferred:

$$
\begin{aligned}
\delta_{-1}^{\mathrm{RRO}} &= \frac{k_{-1}}{k_{+1}[\mathrm{RANKL}_{\mathrm{stand}}]} = 4.92\,10^{-6},\\
\delta_{-2}^{\mathrm{RRO}} &= \frac{k_{-2}}{k_{+1}[\mathrm{RANKL}_{\mathrm{stand}}]} = 5.86\,10^{-19},\\
\delta_{+2}^{\mathrm{RRO}} &= \frac{k_{+2}}{k_{+1}} = 12.96,\\
\beta_{\mathrm{RO}}^{\mathrm{RRO}} &= \frac{C_{\mathrm{RO}}}{[\mathrm{RANKL}_{\mathrm{stand}}]} = \frac{[\mathrm{RO}_0]+[\mathrm{OPG}_0]}{[\mathrm{RANKL}_{\mathrm{stand}}]} = 6.135 + n_{OPG_0},\\
\beta_{\mathrm{RR}}^{\mathrm{RRO}} &= \frac{C_{\mathrm{RR}}}{[\mathrm{RANKL}_{\mathrm{stand}}]} = \frac{[\mathrm{RR}_0]+[\mathrm{RANKL}_0]-[\mathrm{OPG}_0]}{[\mathrm{RANKL}_{\mathrm{stand}}]} = 0.0947 + n_{RANKL_0} - [\mathrm{OPG}_0],\\
\beta_{\mathrm{RK}}^{\mathrm{RRO}} &= \frac{C_{\mathrm{RK}}}{[\mathrm{RANKL}_{\mathrm{stand}}]} = \frac{[\mathrm{RK}_0]-[\mathrm{RANKL}_0]+[\mathrm{OPG}_0]}{[\mathrm{RANKL}_{\mathrm{stand}}]} = 1.037 - n_{RANKL_0} + n_{OPG_0},\\
\tau_{7\mathrm{days}}^{\mathrm{RRO}} &= 4.64.
\end{aligned}
\tag{8}
$$

Interconnection between this RRO model and bone remodelling model is mediated by [RR]. The concentration of RR influences the value of parameter β_1 in the developed thermodynamic bone remodelling model, see (Klika & Maršík, 2009b). There are different normalizations used in these two mentioned models and we assume that in the case of standard values of RANKL and OPG, the parameter β_1 should have its standard value (corresponding to "healthy" state). Further, the typical normalised concentration of RR in bone remodeling model is $n_{RR} \in (1.35, 1.41)$ in standard state (see (Klika & Maršík, 2009b)). Thus:

$$\beta_1 = 1.41 / 0.79 n_{RR} - 0.81, \tag{9}$$

which gives the value $\beta_1 = 0.6$ for standard values of RANKL and OPG because n_{RR} under these condition equals 0.79 and n_{RR} is a result of the interaction in RANKL-RANK-OPG pathway at time $\tau_{7\mathrm{days}}^{\mathrm{RRO}}$. As can be seen, the value of n_{RR} influences only β_1, i.e. it acts only as a modification of initial conditions of the bone remodelling model. However, it will be seen in the results below that it sufficiently captures the influence of the whole pathway.

The increase in ligand concentration RANKL should lead to an increase in osteoclast formation, and consequently, the decrease of bone tissue density, and conversely, osteoprotegerin OPG prevents osteoclastogenesis. Modelling of this pathway is carried out through solving kinetic equations (2) with the above mentioned parameter values (8). Consequently, the output value of n_{RR} is used as an input variable in the bone adaptation model - (9). Tab. 1 gives an idea of how the added RANKL-RANK-OPG pathway may influence bone density (percentual changes of n_{RR} are more or less in accordance with data found in Quinn et al. (Quinn et al., 2001).

2.2 Incorporation of estradiol effects into the bone remodelling model

Estradiol is a major estrogen hormone in humans. Kong and Penninger mention that osteoprotegerin expression is promoted by estrogen (Kong & Penninger, 2000). Hofbauer et

The predicted effects of RANKL and OPG serum levels on bone density			
$\frac{[\text{RANKL}]}{pmol}$	$\frac{[\text{OPG}]}{pmol}$	n_{RR} [1]	normalised bone density [1]
0.84 (standard)	1.8 (standard)	0.790	100%(0.811)
4.55	1.8	1.132	76.9%(0.624)
0.1	1.8	0.13	**172.6% (1.40)
0.84	6.9	0.276	**152.9% (0.1.24)
0.84	0.6	0.892	92.5% (0.75)

Table 1. The predicted effects of the RANKL-RANK-OPG pathway on bone density. n_{RR} is a result from the RANKL-RANK-OPG pathway model, and consequently, bone density (the number in parentheses in the last column) is predicted from the presented thermodynamic bone remodelling model based on the calculated n_{RR}. The asterisk in the front of values notices that it may be necessary to intermit the treatment after a certain time:
* - after a longer time, ** - after a shorter period. Simulated or predicted data by model that are boxed are in accordance with data found in literature - (Kudlacek et al., 2003).

al. studied in vitro responses of osteoprotegerin production to estradiol levels (Hofbauer et al., 1999). They clearly showed that osteoprotegerin levels are dose-dependent on estradiol concentrations in vitro. We will take advantage of this observation and incorporate estradiol effects into the presented model.
As was mentioned, estradiol promotes osteoprotegerin expression. Thus, we may describe this fact using the following interaction:

$$Estradiol + OPG_{\text{producers}} + Substratum \overset{k_{\pm 1}}{\rightleftarrows} OPG + OPG_{\text{producers}}, \tag{10}$$

where $OPG_{\text{producers}}$ represents the group of cells that are expressing OPG and a mixture of substances needed for osteoprotegerin production is noted as *Substratum*. Similarly, as in case of RANKL-RANK-OPG pathway, a differential equation describing kinetics of estradiol concentration can be derived:

$$\frac{\mathrm{d}[\text{Estradiol}]}{\mathrm{d}\tau} = -[\text{Estradiol}](\beta^{\text{estr}}_{Substr} + [\text{Estradiol}]) + \delta^{\text{estr}}_{-1}(\beta^{\text{estr}}_{\text{OPG}} - [\text{Estradiol}]), \tag{11}$$

where

$$\begin{aligned} \delta^{\text{estr}}_{-1} &= \frac{k_{-1}}{k_{+1}[\text{RANKL}_{\text{stand}}]}, \\ \beta^{\text{estr}}_{\text{OPG}} &= \frac{C_{\text{OPG}}}{[\text{RANKL}_{\text{stand}}]} = \frac{[\text{OPG}_0] + [\text{Estradiol}_0]}{[\text{RANKL}_{\text{stand}}]}, \\ \beta^{\text{estr}}_{\text{Substr}} &= \frac{C_{\text{Substr}}}{[\text{RANKL}_{\text{stand}}]} = \frac{[\text{Substr}_0] - [\text{Estradiol}_0]}{[\text{RANKL}_{\text{stand}}]}. \end{aligned} \tag{12}$$

Again, this differential equation can be rewritten into (4) where $A = 1$, $B = \beta_{\text{Substr}} + \delta_{-1}$, $C = \delta_{-1}\beta_{\text{OPG}}$. Therefore, we know the analytical function that describes the evolution of estradiol concentration in time from its initial concentration. In vitro data from Hofbauer et al. will be used for estimation of these parameter values. Thus it is needed to know how the initial concentration of estradiol influences osteoprotegerin concentration after 24 hours. For this purpose we will use a relation between OPG and estradiol concentration following from (10):

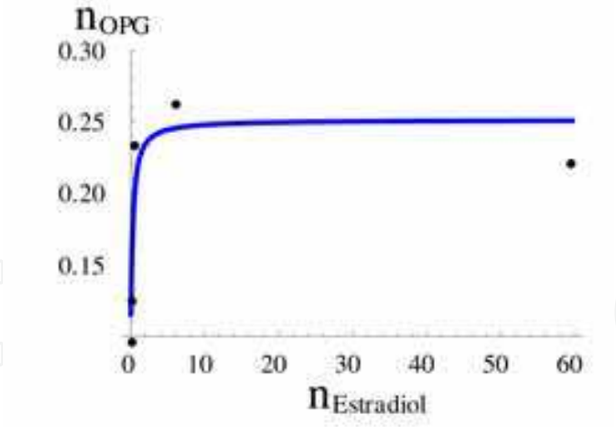


Fig. 2. Estradiol fitted solution (blue curve) by least squares method to data measured (dots) by Hofbauer et al. (Hofbauer et al., 1999).

$$[\text{OPG}] = \beta_{\text{OPG}}^{\text{estr}} - [\text{Estradiol}].$$

Now we may use the data from Hofbauer et al. to estimate all the parameters; a least square method will be used. Firstly, we need to normalise data from the experiment. Normalisation of concentrations and β_i parameters was carried out by [$\text{RANKL}_{\text{Standard}}$] concentration:

$$\frac{10^{-10}\ \text{M}}{[\text{RANKL}_{\text{Standard}}]} = \frac{10^{-10}\ \frac{mol}{l}}{[\text{RANKL}_{\text{invitrostand}}]} = 0.0596.$$

Similarly, the other concentrations may be normalised.
The least square method gives the following values of parameters and the data fit is depicted in figure 2:

$$\delta_{-1}^{\text{estr}} = 0.145,\ \tau_{24\text{h}}^{\text{estr}} = 26.17, n_{Substr_0}^{\text{estr}} = 0.018. \tag{13}$$

The studied in vitro concentrations of estradiol most probably differs from serum levels found in human. It is needed to find a relation between in vitro and in vivo data. In other words, the in vitro data is used for gaining a qualitative fit because in vitro experiments enable dose-dependent studies that are needed. Consequently, a suitable scaling is used to obtain in vivo concentration values while the qualitative fit (shape of curve) is kept.
Ettinger et al. describe standard values of estradiol in humans 40–60 $\frac{pg}{ml}$ (Ettinger et al., 1998). Further, from the data mentioned in this study we may observe that there is a significant correlation between estrogen serum levels and bone density. Concretely, the difference in bone density between a group of women with mean estradiol level 10–25 $\frac{pg}{ml}$ and a group with < 5 $\frac{pg}{ml}$ was +5.7% (higher bone density in the case with higher estradiol levels). From here it follows, that we may define standard estradiol serum level to be 50 $\frac{pg}{ml}$ = 184 $\frac{pmol}{l}$ ($\text{MW}_{\text{Estradiol}}$ = 272.38 (Estradiol analyzing method PV2001, 2001)) and further that a change from 35% of standard level (the average of the first group - 17.5 $\frac{pg}{ml}$) to 2.5% (the average of the second group - 2.5 $\frac{pg}{ml}$) causes a decrease in bone density by 5.7%.

Firstly, linkage of this simple model of estradiol influence on osteoprotegerin production with bone remodelling model is naturally mediated by RANKL-RANK-OPG pathway and thus by the already mentioned model of this control pathway. The predicted value of osteoprotegerin concentration based on estrogen level will be used as an input into RANKL-RANK-OPG model, and consequently, will be translated into appropriate change in number of active osteoclasts (see the previous subsection).

Now the aim is to determine the in vitro equivalent of the standard level of estradiol and to find a linear relation between the predicted normalised value of OPG from this model and of the RANKL-RANK-OPG model that would lead to behaviour as observed in vitro. If these considerations are used, one will find out that the in vitro equivalent of the standard level of estradiol is 10^{-8} M and the searched linear relation is:

$$[\mathrm{OPG}_0]^{\mathrm{RRO}} = k[\mathrm{OPG}]^{\mathrm{estr}}(\tau_{24\mathrm{h}}^{\mathrm{estr}}) + c,$$

where k = 2, constant c is opted so that normalised standard values of OPG coincide, $[\mathrm{OPG}_0]^{\mathrm{RRO}}$ represents the input value (initial concentration) of OPG for RANKL-RANK-OPG model, and $[\mathrm{OPG}]^{\mathrm{estr}}(\tau)$ represents the predicted normalised concentration of OPG at time τ based on estradiol level.

The normal range of estradiol serum levels is 40–60 $\frac{pg}{ml}$. It can be seen that predicted bone density is almost constant in this range (variation is 0.2%), see Tab. 2. After menopause, estradiol levels decrease to 10–25 $\frac{pg}{ml}$ in some women (Ettinger et al., 1998), which have almost normal bone density (1% decrease). However, in some women there is a more dramatic drop in estrogen (< 5 $\frac{pg}{ml}$) and bone density is approximately 5.7% lower than in the previously mentioned group (most probably this leads to osteoporosis). The same behaviour is observed here (more precisely the parameters were opted to capture this

The predicted effects of estradiol serum levels on bone density	
[Estradiol] $\frac{pg}{ml}$	normalised bone density [1]
60	100,1% (0.812)
50 (standard)	100%(0.811)
40	99.9%(0.810)
20	99.0% (0.803)
17.5	98.9%(0.802)
10	97.7% (0.792)
2.5	93.1%(0.755)

Table 2. The predicted effects of estradiol serum levels on bone density. Estradiol influences OPG expression, which in turn influences osteoclastogenesis. Consequently, bone density (the number in parentheses in the last column) is predicted from the presented bone remodeling model based on the calculated [RR]. Simulated or predicted data by model that are boxed are in accordance with data found in literature - (Ettinger et al., 1998) - here the observed effect in human is a decrease by 5.7% when the estradiol level is changed from 17.5 to 2.5 $\frac{pg}{ml}$.

effect): 0.755/0.802 = 94.1%. Simulation predicts that the more affected group of women experiences 6.9% decrease in bone density due to estrogen drop. Interestingly, these values and prediction may be valid for men as well, if they experience such changes in estrogen levels, because Hogervorst et al. states that estradiol levels in elderly men is 83.47 $\frac{pmol}{l}$= 22.8 $\frac{pg}{ml}$ which is in considered range of concentrations (Hogervorst et al., 2004). If these values in elderly men and women are compared, it can be seen that there is a considerable difference which may contribute to higher occurrence of osteoporosis in women than in men.

3. Examples of predictions of bone remodelling based on the presented model

We may now simulate the response of bone remodelling to changing environment, both mechanical and biochemical. Similarly, as was described in (Maršík et al., 2009), density distribution patterns may be obtained using FEM. The results from the previous section will be used.

Example - menopause

During menopause, a decline in estradiol levels occur. In some women, the decrease is very dramatic (a drop bellow 5 $\frac{pg}{ml}$ is observed, whereas a standard serum level is 40–60 $\frac{pg}{ml}$) while in some not (serum level remains above 20 $\frac{pg}{ml}$), see section 2.2. Further it was observed that, together with estradiol, there is a decline in nitric oxide levels (van't Hof and Ralston, 2001). An example of a woman who is physically active (correct mechanical stimuli on regular daily basis, i.e. approximately 20000 steps per day) but in a consequence of menopause has decreased serum levels of estradiol is depicted in figure 3. The presented model predicts a decrease of 8% in bone tissue density, which does not seem to be osteoporosis yet. This may be because menopause is accompanied by more effects than these two mentioned (as the mentioned decrease in NO) and also most probably because they are less physically active (may be caused by pain). If we combine the 8% decrease (figure 3) caused by menopause alone with another 9% decline (not yet published results) caused by improper loading, we get a significant drop by almost 20% in the overall bone density of the femur, which can be considered as osteoporotic state. One possible treatment of bone loss connected with menopause is treated with hormone therapy (HRT). Simulation of such a treatment that increased estradiol serum levels to 20 $\frac{pg}{ml}$ is given in figure 3. Again, the importance of mechanical stimulation shown when increased physical activity (running 30 minutes every other day) increases bone density in similar fashion as HRT treatment (the same figure). And best results are reached when both effects are combined and even the original bone tissue density can be restored - figure 3.

4. Conclusion

A natural goal of the modelling of a process in the human body is to help in understanding its mechanisms and ideally to help in the treatment of diseases related to this phenomenon. For this reason, more detailed influences of various biochemical factors were added.

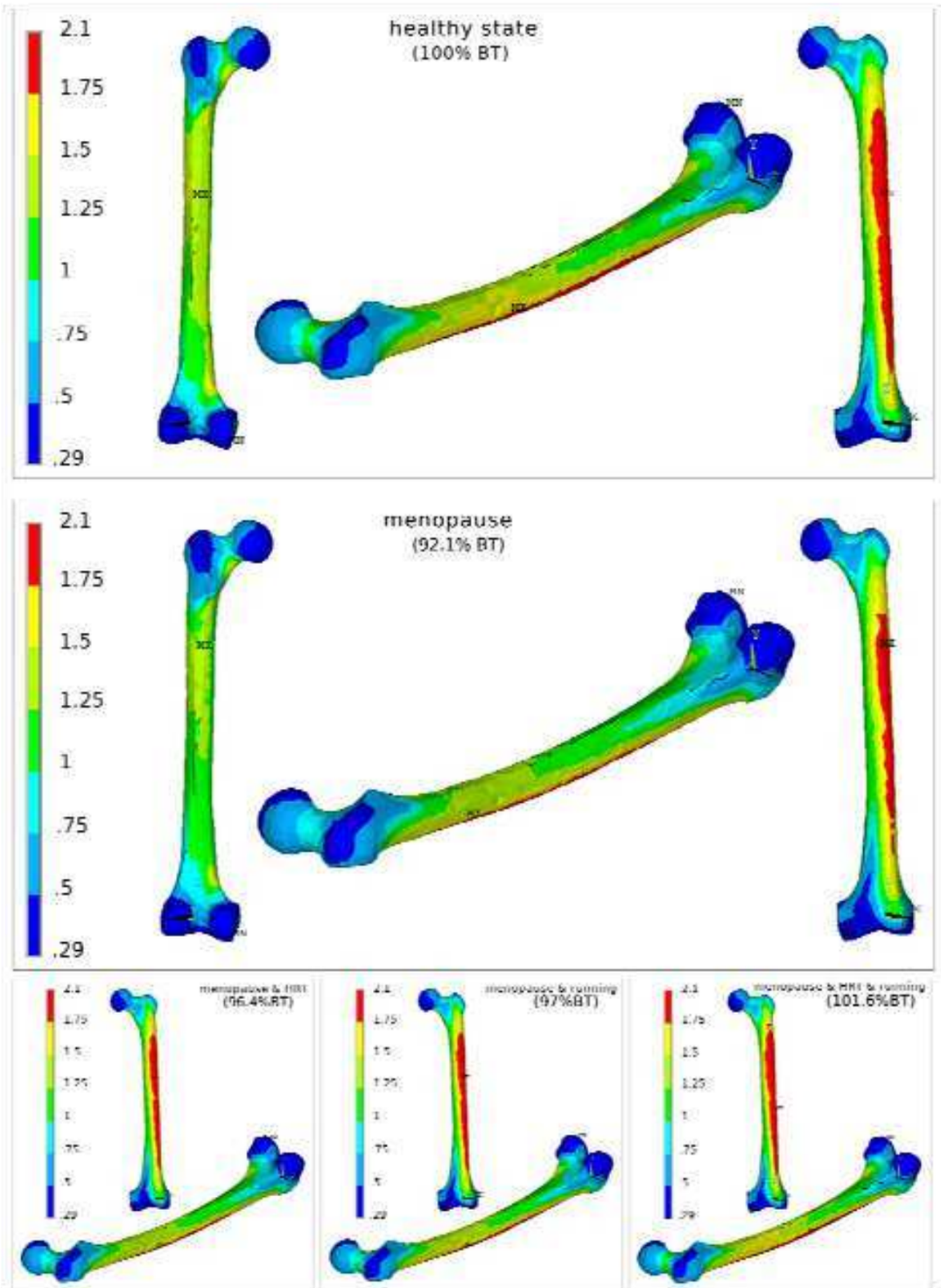


Fig. 3. Prediction of the menopause effect on bone quality (estradiol levels decreased to 2.5 $\frac{pg}{ml}$ l), treatment proposal, and its simulation - hormonal treatment (HRT), running (30 minutes every other day). Notice the change of bone mass (BM) of the whole femur.

Nowadays, the RANKL-RANK-OPG chain is deemed to be one of the most important biochemical controls of the bone remodelling process. The direct cellular contact of osteoclast precursor with stromal cells is needed for osteoclastogenesis. This contact is mediated by the receptor on osteoclasts and their precursor, RANK, and ligand RANKL on osteoblasts. Osteoprotegerin binds with higher affinity to RANK which inhibits the receptor-ligand interaction and as a result, it reduces osteoclastogenesis. Thus, the raise in OPG concentration results in a smaller number of resorbing osteoclasts, which leads to higher bone tissue density. The results discussed in the presented work have exactly the same behaviour. Similarly, the effects of RANKL, RANK, and estradiol were added to the mentioned model. Consequently, a disease, menopause, and its possible treatment were simulated. These results were partially validated by clinical studies found in literature.

However, the impression that the presented model is able to simulate the bone remodeling process in the whole complexity is not correct. It has limitations, as mentioned below, in the spatial precision of the results (i.e. actual structure of bone tissue) and also some control mechanisms cannot be included (e.g. TGF-β effects). But still, the model can be at least considered as a summary of known important factors, comprising much of the currently known knowledge of the bone remodelling phenomenon, with some predictive capabilities and encouraging predictive simulations.

Since the presented model is a concentration model, it cannot be used arbitrarily. The limitation is, of course, in the spatial precision of results. The minimal volume unit (finite element) should be sufficiently large to contain enough of all the substances entering the reaction schemes, namely osteoclasts and osteoblasts. It surely cannot be used on the length scales of BMU where it is no longer guaranteed that any osteoclast is present. There are approximately 10^7 BMU in a human skeleton present at any moment (Klika & Maršík, 2009b) and, because bones have a total volume of 1.75l, there is 1 BMU per 0.175 mm^3 on average at any moment. In other words, the presented model cannot be used for length scales smaller than $\sqrt[3]{0.175\,mm^3}$ and we recommend that it is not used at length scales below $\sqrt[3]{0.5\,mm^3} \doteq 0.8\,mm$.

Ongoing applications of the model include simulations of the 3D geometries of the femur and vertebrae (FE models) under various conditions (both biochemical and mechanical). The preliminary results are encouraging and show the correct density distribution. Currently, we are working on bone modelling (change of shape of bone) model that would add the possibility to adapt bone shape to its mechanical environment as it is observed in vivo. Further, we would like to have a more detailed description of the inner structure of bone as an outcome of the model. Most probably, a homogenisation technique will be used for addressing this goal.

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Dynamic Modelling

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When talking about modelling it is natural to talk about simulation. Simulation is the imitation of the operation of a real-world process or systems over time. The objective is to generate a history of the model and the observation of that history helps us understand how the real-world system works, not necessarily involving the real-world into this process. A system (or process) model takes the form of a set of assumptions concerning its operation. In a model mathematical and logical assumptions are considered, and entities and their relationship are delimited. The objective of a model – and its respective simulation – is to answer a vast number of “what-if” questions. Some questions answered in this book are: What if the power distribution system does not work as expected? What if the produced ships were not able to transport all the demanded containers through the Yangtze River in China? And, what if an installed wind farm does not produce the expected amount of energyt? Answering these questions without a dynamic simulation model could be extremely expensive or even impossible in some cases and this book aims to present possible solutions to these problems.

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