Interaction between bone, the neuroendocrine system and metabolism
Limonard, E.J.

Citation for published version (APA):
Limonard, E. J. (2016). Interaction between bone, the neuroendocrine system and metabolism.

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

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
General introduction
Classically, the skeleton is defined by its mechanical properties; it is essential in the protection of internal organs and in locomotion. In addition, bone serves as a site for hematopoiesis and contributes to calcium and phosphorus homeostasis. In recent years, a further-reaching role has been suggested for the skeleton. In this thesis, we describe research investigating interactions between bone, the neuroendocrine system and metabolism.

Anatomy of bone

Bone consists of a collagen matrix strengthened by multiple crosslinks, on which calcium and phosphate are deposited in the form of hydroxyapatite. The matrix also contains noncollagenous proteins like osteocalcin (1). Two types of bone exist: cortical and trabecular bone. Cortical bone is composed of densely packed lamellae of mineralized collagen which provide mechanical strength. Trabecular bone is less dense and has a higher surface area. Trabecular bone is found inside long bones, the vertebrae and pelvis and provides elasticity and is metabolically more active than cortical bone. The cavity inside bones also houses bone marrow, which surrounds the trabecular bone elements.

Bone (re)modeling

Bone is a dynamic tissue that continuously regenerates. During development and growth, bone size and shape is achieved by bone modeling. Before puberty, growth hormone and insulin-like growth factor 1 (IGF-1) maintain slow, but continuous bone growth (2). Sex differences in bone proportion are established during the peripubertal years. Both androgens and estrogens have substantial effects on growing bone, although estrogen appears to play a more dominant role in both girls and boys (3-6). Estrogen initiates the pubertal growth spurt and ends it by inducing epiphyseal closure (7). Bone tissue gained during puberty is the main contributor to the final amount of bone that is achieved at skeletal maturity, also known as peak bone mass. Individual variances in peak bone mass exist, the majority of which are attributable to heredity factors (8).

After the skeleton has reached maturity, old bone is periodically replaced by new bone at the same location, in a process called bone remodeling (9, 10). This most likely serves to repair microdamage. With advancing age, in both men and women, bone is lost because the balance between the amount of old bone removed and new bone deposited in each remodeling process becomes negative (11). This age-associated loss of bone begins immediately after peak bone mass is achieved and results from compromised osteoblast numbers and/or function (10, 12). At menopause, the abrupt loss of estrogen increases the bone remodeling rate and the lifespan of osteoclasts and decreases that of osteoblasts (10, 11, 13). Within ten years following menopause, the rate of bone loss in women slows to that of aged-matched eugonadal men (12).
Bone cells

Osteoclasts are giant multinucleated cells responsible for bone resorption. They are formed by fusion of osteoclast-precursor cells of the hematopoietic stem cell lineage (14). Mature osteoclasts bind to the bone surface via integrins and create a confined acidified microenvironment into which proteinases like cathepsin K are released to break down the adjacent mineral and matrix (15). Osteoblasts and osteocytes express receptor activator of NFκB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF), which are essential for inducing commitment to the osteoclast lineage (16, 17). This process is inhibited by osteoprotegerin (OPG), a protein that prevents interaction of RANKL with RANK (18, 19). The progenitor cell of the osteoblast is the mesenchymal stem cell (MSC) which also give rise to bone marrow stroma cells, chondrocytes, myocytes and adipocytes (20). Differentiated osteoblasts synthesize and secrete products that constitute the bone matrix (21) and are also responsible for the subsequent mineralization of the matrix with hydroxyapatite (22, 23). Critical in the differentiation and proliferation of osteoblasts are runt-related transcription factor 2 (Runx2) and the Wnt signaling pathway (24, 25). At the end of their life-span the majority of osteoblasts undergo apoptosis. The remaining osteoblasts become linings cells, that cover bone surfaces or are entombed in the mineralized matrix as osteocytes (26, 27).

Osteocytes are the most abundant cell type in bone and are characterized by multiple cytoplasmatic processes that reach throughout bone tissue (28-30). Recently, many advances have been made to clarify the role of osteocytes in bone metabolism. Osteocytes sense mechanical stimuli with their dendrites and are able to communicate directly (gap junctions) and indirectly (paracrine signaling) with neighbouring cells and sites of bone resorption and formation (31). An example of osteocyte-osteoblast communication is decreased production of sclerostin, an antagonist of the Wnt signaling pathway, by mechanical loading (32-36). Osteocytes are also a source of pro- and anti-osteoclastogenic cytokines, like RANKL, OPG and M-CSF (37-39).

Sympathetic control of bone remodeling

Sympathetic nervous system

The autonomic nervous system is a, largely unconscious, central mediator in maintaining whole-body homeostasis. The autonomic nervous system is divided in the parasympathetic and sympathetic nervous system, which have opposite actions in most cases. The sympathetic nervous system acts via catecholamines that bind to adrenergic receptors. There are three subfamilies of adrenoceptors: alpha-1 adrenerceptor (subdivided into alpha-1A, alpha-1B and alpha-1D), alpha-2 adrenerceptor (subdivided into alpha-2A, alpha-2B and alpha-2C) and beta-adrenerceptor (subdivided into beta-1, beta-2 and beta-3) (40, 41). All three adrenergic receptor subfamilies are expressed widely throughout the body. In addition, alpha-2 adrenergic receptors are also found in the brainstem. These receptors are activated by released neurotransmitters and serve as autoreceptors regulating catecholamine release. Therefore, activation of these presynaptic inhibitory receptors leads to a reduction in sympathetic tone (42, 43). Like many other cell types in the body, adrenergic receptors are also expressed in bone cells (44-50).
Central control of bone mass

Bone metabolism, like most other homeostatic functions, is also under control of the sympathetic nervous system. The first evidence supporting this concept was the finding that leptin-deficient mice (ob/ob mice) have a high bone mass in spite of hypogonadism and hypercortisolism (51). This high bone mass phenotype can be rescued by intracerebroventricular (ICV) infusion of leptin, advocating the existence of a central control for the regulation of bone metabolism (52). In mice with an osteoblast-specific knockout for the beta-2 adrenergic receptor ICV leptin infusion does not alter the high bone mass phenotype, suggesting that the sympathetic nervous system, via the beta-2 adrenergic receptor on the osteoblast, is necessary for the central action of leptin on bone. (53).

In agreement with these experimental studies, pharmacological inhibition and stimulation of beta-adrenergic receptors in rodent studies increased and decreased bone mass, respectively (54, 55). The role of the sympathetic nervous system in bone turnover was further supported by the high bone mass phenotype in mice with low sympathetic activity, due to dopamine β-hydroxylase deficiency (56). Recently, the role of the sympathetic nervous system in bone metabolism was extended by investigating a mouse model of chronic sympathetic hyperactivity due to a double knockout of alpha-2A and alpha-2C adrenoceptor genes, inducing increased norepinephrine release (50). Unexpectedly, these mice were characterized by high sympathetic tone and a phenotype of high bone mass with increased bone formation and decreased bone resorption, rather than the expected low bone mass. This finding is in contrast with previous studies and suggests that the mechanisms whereby the sympathetic nervous system regulates skeletal homeostasis are far more complicated than previously thought.

It is uncertain whether human bone metabolism is also under control of the sympathetic nervous system. Activation of the sympathetic nervous system is generally considered to contribute to bone loss. This concept is supported by a case-control study comparing bone turnover in pheochromocytoma patients and controls (57). This study showed that pheochromocytoma patients have increased bone resorption, which normalizes after adrenalectomy. In line with these results, one would expect that pharmacological beta-adrenergic blockade by beta-adrenergic receptor antagonists (beta-blockers) is beneficial to the skeleton. Many epidemiological studies on the association between beta-blocker use and fracture risk have been conducted and showed inconclusive results, with beta-blockers having positive, negative or no effects on bone mass (58-70). Two recent meta-analyses indicate a small but significant risk reduction (15% and 17%, respectively) of any fracture in patients treated with beta-blockers (71, 72). This risk reduction was however associated with the use of beta-1 selective blockers, rather than non-selective beta-blockers.

In rodents, signaling through the beta-2 adrenergic receptor on the osteoblast influences bone remodeling. Three polymorphisms of the beta-2 adrenergic receptor are known to influence receptor function in vitro and in vivo. A recent case-control study therefore examined whether this altered receptor function has an effect on bone metabolism (73). In four large cohorts, polymorphisms in the beta-2 adrenergic receptor were not associated with fracture risk or bone mineral density. To date, only one study investigated the association of two single nucleotide polymorphisms (SNPs) located in the alpha-2A adrenergic gene with bone
remodeling markers and bone mineral density (49). Significant associations were observed between alpha-2A adrenergic receptor gene locus and bone mineral density at the lumbar spine and cathepsin K, C-terminal crosslinking telopeptides of collagen type I (CTx), osteocalcin and bone-specific alkaline phosphatase levels. In sum, the role, if any, of the sympathetic nervous system in human bone remodeling remains unclear.

The abovementioned data suggest that the other branch of the autonomic nervous system, the parasympathetic nervous system (PSNS), may also affect bone remodeling. Genetic studies have shown that the PSNS, acting through the muscarinic 3 receptor, located in the locus coeruleus, is a positive regulator of bone mass by increasing bone formation and decreasing bone resorption (74).

Humoral control of bone remodeling

Bone marrow adipose tissue
Bone marrow adipose tissue, an unique component of the bone marrow cavity, is functionally distinct from and not subject to the same regulation as the other fat depots in the body (75). This is best exemplified by states of caloric restriction, which increases bone marrow adipose tissue while subcutaneous fat is lost (76). Previous observations indicate that young men have higher bone marrow fat than young women, and a rather constant accumulation of bone marrow fat with aging (77). Whereas in women bone marrow fat remains relatively stable before menopause and rapidly increases after menopause (78-80). As a result bone marrow fat is lower in young women compared to young men and higher in older women than in older men (80). Conditions associated with reduced bone mass, like osteoporosis (81), starvation (82), alcoholism (83), spinal cord injury (84) and bed rest (85), are also characterized by marrow fat accumulation. And in subjects using medications that have a detrimental effect on bone, including glucocorticosteroids and thiazolidinediones, higher levels of marrow fat are found (86, 87). The inverse relationship between bone marrow fat and bone mineral density has long been recognized, and can be explained by a common precursor cell shared by osteoblasts and adipocytes (20). Consequently, the number of mature osteoblasts and bone marrow adipocytes in the marrow cavity is influenced by differentiation of the mesenchymal stem cell towards one phenotype and away from the other (88). The rapid increase in bone marrow fat after menopause, resulted in a growing interest in the role of estrogen in determining mesenchymal stem cell faith. In vitro studies have shown that MSCs and cells of the osteoblast lineage express estrogen receptors (89, 90) and that estrogen stimulates differentiation of human and murine MSCs to osteoblasts at the expense of adipocytes (91, 92).

For a long time, marrow adipocytes were regarded as passive fillers of space not occupied by other tissue (93). More recently it has been recognized that bone marrow adipocytes play an active role in the bone marrow microenvironment by secreting adipokines and fatty acids suspected to have a negative effect on osteoblast proliferation and function (94, 95), a process also known as lipotoxicity. Lipotoxicity can be one of the reasons that a negative relationship
between high bone marrow fat and fracture risk has been reported (96, 97). Recent evidence suggests that bone marrow also functions as an endocrine organ with systemic effects, by the secretion of adiponectin, a hormone with beneficial metabolic effects (98-101).

**Dietary content and bone**

Because adipocytes and osteoblasts originate from the same precursor cell, a correlation between obesity and bone metabolism is strongly assumed. Whether obesity protects bone or leads to loss of bone mass remains controversial. The traditional view is that overweight is beneficial to bone (102-106). Recently there are an increasing amount of reports describing detrimental effects of excessive body fat on bone (107-109). As a result of the worldwide increasing prevalence of obesity, diets have become increasingly popular. While treatment of obesity is associated with improved health outcome, some evidence suggests that diet composition, in particular fat content, acts negatively on bone. Children with epilepsy treated with a low-carbohydrate, high-fat ketogenic (LCHF) diet show reduced growth, poor mineral status and lower bone mineral density (110-113). And animals studies have shown adverse effects on bone quantity and quality in rats fed LCHF diets (114-134).

Several mechanisms for the adverse effects of some dietary contents on bone have been proposed. Low-carbohydrate diets can generate acidosis, which promotes calcium mobilization from bone to maintain a neutral pH, finally leading to an increase of urinary calcium (135-137). Furthermore, dietary fat can reduce the absorption of calcium, as a result of the formation of calcium soaps (water-insoluble calcium salts of fatty acids) (138-142). In addition, products of lipid or lipoprotein oxidation may contribute to preferential differentiation of bone marrow progenitor cells towards an adipogenic lineage as compared with osteogenic and inducing osteoclastic differentiation (121, 123, 124, 143, 144).

**Bone as an endocrine organ**

Clinical observations have linked the regulation of bone mass and energy metabolism; the absence of food intake causes an arrest of growth and low bone mass (145). A link between bone mass and fertility has also been suggested, best exemplified by the fact that sex steroids are essential for skeletal growth and maturation and that gonadal failure leads to bone loss (7). This raised the hypothesis that bone, energy metabolism and reproduction are connected, suggesting that the skeleton is not only a recipient of hormonal input, but acts as an endocrine organ itself.

**Endocrine functions of osteocalcin**

In search of a bone-specific component that could be involved in energy metabolism, osteocalcin knockout mice were investigated. Osteocalcin is secreted by osteoblasts (1) and is one of the main protein components of the bone matrix. Low concentrations of osteocalcin are detectable in the circulation and serve as a marker of bone formation. Circulating osteocalcin
exists in two forms, carboxylated on three glutamate residues or undercarboxylated (1). Osteocalcin knockout mice present with a metabolic phenotype of glucose intolerance, insulin resistance, increased fat mass and decreased energy expenditure (146). Osteocalcin administration to wild-type mice caused an increase in blood insulin levels, enhanced glucose tolerance and improved insulin sensitivity (147, 148). In vitro studies confirmed the beneficial metabolic actions of osteocalcin; in co-culture assays wild-type mouse osteoblasts enhanced insulin secretion by pancreatic islets, while osteocalcin -/- osteoblasts were not able to do so (149). In vitro treatment of islet cells with carboxylated and undercarboxylated osteocalcin further revealed that only undercarboxylated osteocalcin could increase expression of insulin and should be considered as the active form of osteocalcin (149).

The observation that male osteocalcin knockout mice bred poorly, suggested that osteocalcin might also play a role in male reproduction. Osteocalcin knockout mice are characterized by decreased testis and epididymal weights, a decreased sperm count and low circulating testosterone levels (150). The hypothesis was confirmed in co-culture assays; supernatant of osteocalcin -/- osteoblasts were unable to increase testosterone production in testis explants and primary Leydig cell cultures (150). Moreover, treating primary Leydig cells with increasing amounts of undercarboxylated osteocalcin, resulted in a dose-dependent increase in testosterone secretion, as does treating wild-type mice with osteocalcin injections (150).

The identification of the endocrine function of osteocalcin in mice resulted in a growing interest in the metabolic role of osteocalcin in humans. In two recent reviews of the literature the majority of the studies investigated observed a negative correlation of serum undercarboxylated or total osteocalcin levels with blood glucose, insulin resistance, diabetes, obesity and markers metabolic syndrome (151, 152). When interpreting these data, the correlative nature of these studies should be kept in mind, as well as the analytical aspects of different assays related to various forms of osteocalcin.

The endocrine role of osteocalcin in human testosterone production was tested in several cross-sectional studies. Only one large population-based study showed osteocalcin to be positively associated with total testosterone levels (153). A systematic genomic analysis of a cohort of 59 male subjects with an identical subfertile phenotype as osteocalcin knockout mice, identified a loss-of-function mutation in the osteocalcin receptor in two subjects, establishing the first genetic evidence that osteocalcin fulfils an endocrine function in humans (154).
Aim and outline of thesis

The general aims of this thesis were to study:
• The role of the sympathetic nervous system in human bone remodeling
• The humoral control of bone remodeling via bone marrow fat and dietary content
• The role of bone as an endocrine organ

Part I: Sympathetic control of bone remodeling
In chapter 2 we prospectively studied the effect of pharmacological modulation of the beta-adrenergic receptor on human bone metabolism. In chapter 3 we describe the effect of a reduced sympathetic tone, accomplished by pharmacological alpha-2 adrenergic receptor stimulation on human bone in vivo. In chapter 3 we also investigate the effect of direct stimulation of the alpha-2 adrenergic receptor in osteoclasts in vitro. To further elucidate the role of alpha-2 adrenergic receptor we investigated the association of polymorphisms in the alpha-2 adrenergic gene with fracture risk and bone mineral density in chapter 4.

Part II: Humoral control of bone remodeling
Although bone marrow adipose tissue is well established as a component of the bone marrow compartment, longitudinal data on changes in bone marrow fat in humans are missing. In chapter 5 we describe the variation in vertebral bone marrow fat fraction among ovulating premenopausal women. We also determined the short-term effect of 17-β estradiol administration on vertebral bone marrow fat fraction in postmenopausal women. With the rising prevalence of obesity, diets have become increasingly popular. While treatment of obesity is associated with improved health outcome, evidence suggests that diet composition can act negatively on bone. In chapter 6 we describe the effect of different amounts of dietary fat and carbohydrate content on bone turnover in healthy subjects.

Part III: Bone as an endocrine organ
In mice, osteocalcin has been identified as a hormone with important metabolic and reproductive actions. In humans, numerous correlative studies have investigated the role of osteocalcin in energy metabolism. Data on osteocalcin in human gonadal function are limited. In chapter 7 we describe the association of osteocalcin levels with the pituitary-gonadal axis in an unselected large cohort of older men. In chapter 8 we investigate the effect of changes in osteocalcin levels on glucose tolerance, insulin sensitivity and testosterone concentrations by means of a prospective intervention study.


107. Fain JN. Release of inflammatory mediators by human adipose tissue is enhanced in obesity and primarily by the nonfat cells: a review. Mediators of inflammation. 2010;2010:513948.


128. Lecka-Czernik B, Stechschulte LA, Czernik PJ, Dowling AR. High bone mass in adult mice with diet-induced obesity results from a combination of initial increase in bone mass followed by attenuation in bone formation; implications for high bone mass and decreased bone quality in obesity. Mol Cell Endocrinol. 2015.


General introduction


