Title: Bone Metabolism: A Note on the Significance of Mouse Models

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Short title: Bone metabolism and mouse models

Summary

This minireview briefly surveys the complexity of regulations governing the bone metabolism. The impact of clinical studies devoted to osteoporosis is briefly summarized and the emphasis is put on the significance of experimental mouse models based on an extensive use of genetically modified animals. Despite possible arising drawbacks, the studies in mice are of prime importance for expanding our knowledge on bone metabolism. With respect to human physiology and medicine, however, one should be always aware of possible limitations as the experimental results may not be, or may be only to some extent, transposed to humans. If applicable to humans, results obtained in mice provide new clues for assessing unforeseen treatment strategies for patients. A recent publication representing in our opinion the important breakthrough in the field of bone metabolism in mice is commented in detail. It provides an evidence that skeleton is endocrine organ that affects energy metabolism and osteocalcin, a protein specifically synthesized and secreted by osteoblasts, is a hormone

involved. If confirmed by other groups and applicable to humans, this study provides the awaited connection of long duration between bone disorders on one hand and obesity and diabetes on the other.

Key words: bone remodeling - osteoblast and osteoclast - osteoporosis - osteocalcin - obesity and diabetes

Introduction

Osteoporosis became a pandemic disease of the mankind with extensive health care and social demands (Raska and Broulik 2005, Teitelbaum 2007, Hofbauer *et al.* 2007). Based on the extensive statistics, particularly from countries in which convenient surveillance programme is being implemented, a very high percentage of elderly people, particularly women after menopause, experience an osteoporotic fracture frequently accompanied by the debilitating fate of the patient (Stepan *et al.* 2003, Teitelbaum 2007). Despite an enormous progress of our knowledge, due to both clinical and experimental results achieved in the last 10 years (e.g. Teitelbaum 2007, Harada and Rodan 2003, Boyle *et al.* 2003, Teitelbaum and Ross 2003, Karsenty 2006, Zaidi 2007), a synthetic view on the molecular mechanisms responsible for the etiopathology of the disease is still missing. This lack of knowledge should be attributed to a very complex, systemic regulation of the bone homeostasis that is reflected through the bone remodeling. The remodeling is a dynamic metabolic process that serves not only for the adjustment of the bone architecture to meet changing mechanical needs. It is important in maintaining plasma calcium homeostasis including reversible changes accompanying lactation period, it serves to adjust the biological parameters of the bone cellular elements and it helps to repair (micro)damage in the bone matrix (Stepan *et al.* 2003, Woodrow *et al.* 2006).

Skeleton is a dynamic organ. It comprises two types of bone: cortical and trabecular. Although both types are being continuously remodeled during human life-time, there is a much higher turnover in the trabecular one. It is generally claimed that two different cell lineages have emerged to serve distinct skeletal functions in the bone remodeling. Osteoblasts are derived from bone marrow mesenchymal stem cells, and construct and shape the skeleton for optimal performance. The osteoclasts, which are derived from the hematopoietic stem cells, resorb the bone and contribute to the maintenance of mineral homeostasis. The highly regulated balance, in time and space, between bone deposition and resorption (a relationship known as coupling) is crucial for the proper development and maintenance of bone size, shape and integrity. It is affected through the differentiation and functions of osteoclasts and osteoblasts that are determined not only by a high number of cytokines acting locally as well as by a direct communication between the two types of cells, but also by a multitude of hormones acting in a systemic way (Zaidi 2007, Cirmanova *et al.* 2008).

This being said, we should mention here obesity and Diabetes mellitus as these also represent pandemic dangers that similarly to osteoporosis arose only in the 20th century. Obesity represents a disorder of energy metabolism that predisposes to diabetes, hypertension, and hyperlipidemia, diseases characterized by insulin resistance and complicated by atherosclerosis (Semenkovich and Teitelbaum 2007, Adams *et al.* 2006, Semenkovich 2006). Diabetic patients suffer from various bone disorders, and osteoporosis is the most frequent metabolic bone disorder (Raska and Broulik 2005, Hofbauer *et al.* 2007). Both type 1 and type 2 diabetes are apparently associated with increased risk of bone fractures, although only type 1 diabetes is associated with lower bone density (Schwartz and Sellmeyer 2007). It has been generally believed that diabetes, through increased levels of blood glucose and decreased levels or effectiveness of insulin and insulin-like growth factor (IGF)-1, affects negatively osteoblast activity, and thus may play a role in osteoporosis (Inaba 2004).

In this minireview, we shall first shortly survey the possibilities of clinical studies dealing with osteoporosis, the significance of the experimental mouse model and the complexity of the bone metabolism. We shall then highlight a recent contribution describing endocrine function of skeleton and coupling regulation of bone and energy metabolism (Lee *et al.* 2007) the results of which, if confirmed by other groups, represent the breakthrough in the field of bone metabolism. In addition, if applicable to humans, this study provides a link between bone misfunctioning on one hand and obesity and diabetes on the other.

Clinical studies and the significance of experimental mouse models

Clinical studies dealing with osteoporosis can be in the first approximation divided in two categories, one with the aim to prevent catabolic resorptive processes in the bone, and the other in which anabolic processes are potentiated in order to enhance the bone formation (Stepan *et al.* 2003, Canalis *et al.* 2007). Even though such a division seems to be, due to an extensive cross-talk of various metabolic pathways as well as systems feedback loops involved, artificial, in practical terms it represents a convenient scheme for the treatment. We should also mention here the importance of clinical studies involving patients with established polymorphisms (respectively mutations) in genes involved directly or indirectly in the bone metabolism (Liu *et al.* 2006, Krizova *et al.* 2007).

Osteoporosis is a multigenic and multifaceted metabolic disease, with tens of identified genes being involved, and results from the interplay between genetic and environmental factors that also involve daily habits of an individual (Canalis *et al.* 2007, Stepan *et al.* 2003, Liu *et al.* 2006, Teitelbaum 2007). Imbalances of bone remodeling can result in smaller or larger perturbations in skeletal structure and function, and potentially in diseases like osteoporosis. Therefore, already the prevention is of utmost importance.

Each patient suffering from osteoporosis frequently exhibits pleiotropic symptoms. An individual patient, representing a very specific entity, can respond to a given treatment differently with regard to another patient exhibiting the same manifestations of the disease. This difficulty is to a large extent surmounted via double blind long term studies involving a large group of persons. Such clinical studies of irreplaceable importance provide treatment schemes and define also respective dangers ensuing from the use of individual tested drugs (Stepan *et al.* 2003, Canalis *et al.* 2007). On the other hand, such studies may also exhibit a drawback. In practical terms, the most proper control group does not exist since it is ethically difficult or even impossible not to medicate the patients from the control group in long term studies.

The limitations of clinical studies are to be identified with the nature of the results obtained. In human medicine, we are usually limited to the investigation of the bone mineral density (BMD) and a measure of relevant biological markers in serum and urine (Zikan and Stepan 2002). Consequently, these phenomenological studies cannot provide information about the molecular processes taking place in various tissues or organs, including the bone, that are to be identified as genuine players responsible for the etiopathogenesis of the disease. Moreover, the results obtained by different research groups are not always completely comparable. For this purpose, for instance, the same choice of the site in a given bone for BMD evaluation should be standardly used by all research groups. But BMD data alone may not necessarily provide the proper information about bone structure or strength (Zaidi 2007).

In contrast to clinical studies, mouse models allow for the investigation of molecular processes within the frame of bone remodeling and their use potentially permits to experimentally prove/disproof proposed specific hypotheses addressing the bone metabolism. Importantly in this respect, skeleton is a late acquisition during development (Karsenty 2006). This explains why a high degree of conservation of relevant factors (regulatory molecules

such as transcription factors, kinases, receptors, secreted molecules, or structural proteins) frequently exists between mice and humans. Due to the endeavour of tens of experimental laboratories exploring transgenic rodents, particularly mice, we witness today an enormous progress in the knowledge of bone metabolism and its (experimentally induced) disorders. At the same time, possible drawbacks of the results obtained have to be emphasized. Physiology of genetically modified animals may be biased due to adaptive and other changes ensuing from a partial or complete lack of the relevant gene, or from overexpressed genes in mice in which genes were knocked in. And importantly, the experimental results obtained may not be transposed, or transposed only to a limited extent, to humans.

The regulation of bone metabolism is complex (see e.g. Fig. 1 in Cirmanová *et al.* 2008 and Fig. 2 in Gallo *et al.* 2008). Frequent cross-talks in regulatory pathways, accompanied by signal divergence, do occur and feedback loops modulate the whole system. In this context, the experimental approaches with mice have been largely refined. Accordingly, genes to be investigated are either knocked in, knocked out, conditionally knocked out or their expression is modulated, but frequently only in the target cell types within a given organ, such as bone. Always bearing in mind the inherent drawbacks of the genetically modified animal model, direct involvement of specific molecular processes can thus be potentially pinned down with a limited interference from the various regulatory pathways or even the whole system.

Survey of the regulation of bone metabolism

The regulation of bone metabolism may be categorized from various points of view such as proosteogenic and antiosteogenic or local and systemic, including both neuronal and humoral subcategories; we shall stick here to the latter one. This chosen categorization of regulations represents a simplified view since some pathways exhibit both systemic and local

features (see Fig 1. in Cirmanová *et al.* 2008) such as leptin pathways (see below). We have to emphasize that our knowledge of regulation comes from two, not always reconcialable sources, human medicine and experiments with animal models. Accordingly, although leptin significance has not yet been proven in a convenient way in humans, leptin is without any doubt an important regulator of bone remodeling in mice (Karsenty 2006).

Contemporary literature usually divides systemic bone regulation into two main subcategories, the recently discovered neuronal and the already well known humoral regulations. The moment which led to such a new view on the systemic regulation of bone metabolism was the discovery that leptin, an important regulator of energy metabolism (Zhang *et al.* 1994), regulates also bone remodeling through a hypothalamic relay in mice and that action of leptin on bone formation through this hypothalamic relay is mainly inhibitory (Ducy *et al.* 2000). Subsequent studies revealed that sympathetic nervous system (SNS) is one of the major effectors of this relay, and that its action on bone is accomplished through β2-adrenergic receptors present on osteoblasts (Takeda *et al.* 2002). When these receptors are activated, they trigger within the osteoblast two different downstream molecular cascades, first cascade affects the clock genes and inhibits osteoblast proliferation, second cascade promotes receptor activator of nuclear factor-κB ligand (RANKL; see below) secretion and thereby increases osteoclastogenesis (Elefteriou *et al.* 2005, Fu *et al.* 2005). Therefore, in the first approximation, one may say that the increase of sympathetic activity through the central leptinonergic pathway leads to decreased bone formation and also increased bone resorption.

Furthermore, the egress of hematopoietic stem cell precursors of the osteoclast lineage from the hematopoietic stem cell niche was also found to be controlled by adrenergic signaling through the osteoblasts (Katayama *et al.* 2006, Zaidi 2007).

Importantly, leptin also centrally stimulates expression of anorexogenic hypothalamic neuropeptide called cocaine and amphetamine regulated transcript (CART) (Elefteriou *et al.*

2005). As result, RANKL expression is, via an unknown mechanism, downregulated in osteoblasts and bone resorption is thus decreased (Elefteriou *et al.* 2005, Karsenty 2006, Cirmanova *et al.* 2008). Therefore leptin seems to centrally regulate bone resorption in two opposite ways. First, leptin stimulates bone resorption through the leptin/SNS pathway. Second leptin inhibits bone resorption through CART expression (Karsenty 2006, Cirmanova *et al.* 2008). Moreover, it has been recently shown that CART apparently acts more as a circulating hormone than a neuropeptide (Singh *et al.* 2008).

Within the context of hypothalamic regulations, two other hypothalamic neuropeptides, neuropeptide Y (NPY) and neuromedin U (NMU), apparently regulate not only energy metabolism but also bone mass. Neuropeptide Y is expressed in the central nervous system as well as in peripheral tissues. There are several types of Y receptors present in all kinds of tissues and both bone and adipose tissues are known to be centrally regulated by hypothalamicY2 receptors and the mice with knocked out Y2 gene exhibit increased bone mass (Lundberg *et al.* 2007). Osteoblastic cells express Y1-receptors and the deletion of the relevant leads to increased bone and adipose mass (Baldock *et al.* 2007). Neuromedin U, an anorexogenic neuropeptide, has been recently identified as a potent regulator of bone formation and NMU deficient mice showed increased bone formation (Sato *et al.* 2007). Leptin intracerebroventricular infusions or β2-adrenergic agonist treatment in NMU-deficient mice did not show any decrease in bone mass and bone formation. Moreover, expression of molecular clock genes was downregulated in NMU-deficient mice. These data indicate that NMU could be a central mediator of the leptin-dependent regulation of bone mass (Sato *et al.* 2007).

In additon to leptin, the humoral action of which is commented within the microinverinmental context (see below), humoral control of bone remodeling encompasses many well known hormones. Parathyroid hormone (PTH) is the most important regulator of

calcium metabolism and its continuous secretion is a potent stimulator of bone resorption (Bisello *et al.* 2004). PTH also increases renal reabsorption of calcium and excretion of phosphates and also stimulates renal calcitriol production (Mundy and Guise 1999). On the other hand, if PTH is secreted or administered intermittently it acts as a potent anabolic bone agent (Neer *et al.* 2001). The molecular basis of this effect is still not clearly understood, but the recent experiments show that the increase of osteoblast number is due to the ability of intermittent PTH to delay osteoblast apoptosis (Jilka *et al.* 1999, Martin *et al.* 2006). Calcitriol is essential in increasing the intestinal absorption of calcium and phosphates (Mundy and Guise 1999). The ablation experiments with vitamin D receptor show that the effects of calcitriol on the bone metabolism are indirect through changes of the calcium levels (Demay 2006). Growth hormone (GH) is anabolic, but its action is mainly indirect, through secreting IGF-1, this factor representing the active physiological stimulator of bone formation (Yakar *et al.* 2002, Zhang *et al.* 2002). Genetic deficiency of IGF-1 causes growth retardation and osteopenia, and this bone loss is not rescued by GH administration (Zaidi 2007). Glucocorticoids (GCs) have very complex effect on bone, but there is no doubt that an excess of GCs, which is encountered during long-term glucocorticoid therapy, has suppressive effect on bone formation (Weinstein 2001, Weinstein *et al.* 1998). More recently it has been shown, that osteoclasts are the intermediary cells, which are necessary for glucocorticoid-induced osteoblast inhibition (Kim *et al.* 2006). But still the glucocorticoid-induced osteoclast-to-osteoblast signal remains to be identified. Furthermore, GCs directly target mature osteoclasts and specifically disrupt their cytoskeleton (Kim *et al.* 2006). Such a disruption restricts bone resorption and therefore also retards bone remodeling, and thus decreases bone formation. The retarded remodeling, which is characteristic for long-term GC therapy, leads to brittleness of the bones. This raises an argument that for an effective prevention of skeletal complications in long term GC therapy, some restoration of osteoclast activity is required

(Teitelbaum 2007). In contrast, the reason why there is a transient increase in bone resorption during short-term GC treatment is unknown (Dovio *et al.* 2004). Simple explanation of the effect of short-term GC therapy could be through overriding pro-resorptive effect of inflammatory cytokines, which are abundant in GC treated diseases such as autoimmune ones (Teitelbaum 2007). As these inflammatory cytokines decrease during long-term GC therapy, the suppressive effect of GCs on osteoresorption becomes apparent (Teitelbaum 2007). Calcitonin is an important hormone during pregnancy and particularly lactation because it protects the bone against the demineralizating effects of estrogen deficiency and increased levels of mammary-secreted parathyroid hormone-related protein (PTHrp; see below) during this life period (Woodrow *et al.* 2006). Its exact role in the adult skeleton is still to be determined. Although there is a clear receptor-mediated inhibitory effect on osteoclasts, experimental ablations of calcitonin gene lead to a high bone mass, rather then to osteopenia (Hoff *et al.* 2002, Zaidi 2007). Thyroid hormones have anabolic effect on bone during growth. Their lack during growth results in delayed ossification and bone mineralization (Bassett *et al.* 2008, Bassett *et al.* 2007a). In contrast their excess in adults, which is seen during e.g. thyrotoxicosis, causes osteoporosis (Bassett *et al.* 2007b, Bassett *et al.* 2007a). Androgens act through androgen receptors (ARs), which are present in both osteoblasts and osteoclasts. Their action is essential for normal skeletal development and preservation of bone mass (Sato *et al.* 2002). The experiments with AR ablation result in an increased bone resorption in male mice. AR ablation in female mice does not notably affect the bone metabolism possibly because of the dominant role of estrogen receptors (Kawano *et al.* 2003, Nakamura *et al.* 2007). Estrogens (ES) play a key role in the control of bone mass (Manolagas *et al.* 2002). They stimulate osteoblast proliferation and decrease their apoptosis (Kousteni *et al.* 2002). They reduce the effect of RANKL in the osteoclast precursors and impair the osteoclast formation (Srivastava *et al.* 2001). They also induce apoptosis in bone-resorbing osteoclasts

(Kameda *et al.* 1997, Kousteni *et al.* 2002). Decrease of ES, which is seen after menopause, causes osteoporosis in a significant number of women. Until lately, bone's response to ES withdrawal has been understood as a complex reaction of inflammatory and osteoclastogenic cytokines in which osteoblasts were the primary estrogen targets (Clowes *et al.* 2005). Importantly, two recent publications using transgenic mice have shed new light on molecular mechanisms governing ES protection of bone (Nakamura *et al.* 2007, Krum *et al.* 2008). Apoptic Fas ligand (FasL) gene, being under control of estrogen receptor α (ERα, is the key player, but its activation is shown to take place either in osteoclasts (Nakamura *et al.* 2007) or in osteoblasts (Krum *et al.* 2008). In the first case, it is shown that ES induces in an autocrine manner the activation of the FasL gene. This activation then leads to suppression of bone resorption through apoptosis of differentiated osteoclasts (Nakamura *et al.* 2007). In contrast, Krum *et al.* (2008) describe a paracrine mechanism in which ES affects osteoclast survival through the upregulation of FasL in osteoblasts (and not osteoclasts). This leads to an induction of a paracrine signal originating in osteoblasts that results in apoptosis of pre-osteoclasts. The FasL is identified as the key gene in both cases but further work is needed in order to establish which mechanism really applies. The action of thyroid stimulating hormone (TSH) and follicle stimulating hormone (FSH) has been considered as indirect through the action of thyroid and gonadal hormones (Zaidi 2007). Recent reports suggest, however, that TSH and FSH have also direct effect on bone cells that overrides the effect of thyroid and gonadal hormones (Abe *et al.* 2003, Sun *et al.* 2006). Both hormones apparently interact reciprocally with MAP kinases, nuclear factor-κB and Akt kinases downstream of RANKL. In addition, both TSH and FSH probably also function in a reciprocal manner via tumor necrosis factor (TNF)-α as the downstream mediator (Iqbal *et al.* 2006, Hase *et al.* 2006). However, some of the recent results do not support this dominant effect of FSH and TSH

(Bassett *et al.* 2007b, Bassett *et al.* 2008, Nakamura *et al.* 2007) and thus further research needs to be performed to clarify this issue.

The second category of regulations involves local regulations within the bone microenvironment in which the molecular cross talk between bone cell types, bone and immune cell types, but in the first row the cross talk between osteoclasts and osteoblasts, is the primary mechanism regulating bone remodeling. The central role of the cross talk is played by the RANKL/RANK/OPG system, where RANK stands for receptor activator of nuclear factor-κB and OPG for osteoprotegerin (Khosla 2001, Boyle *et al.* 2003). Osteoblasts, stromal stem cells and activated immune cells express RANKL, which is a cytokine from the TNF superfamily and its action is to activate RANK receptors on osteoclasts or their precursors. After the RANK receptors are activated, they promote activation and survival of osteoclasts or differentiation of osteoclast precursors, and thus lead to osteoresorption. This action of RANKL is inhibited by OPG which is a decoy receptor and is also released from osteoblasts and osteogenic stromal cells, and RANKL/OPG ratio controls the degree of bone resorption (Khosla 2001, Hofbauer *et al.* 2000, Boyle *et al.* 2003). Although some cytokines and hormones can control bone resorption and formation directly, many of them control it by modulating the RANKL/RANK/OPG system (Boyle *et al.* 2003). Furthermore, e.g. TNF-α, a potent osteoclastogenic cytokine, stimulates osteoblast production of RANKL and also acts directly on osteoclasts and their precursors in an osteoclastogenic manner. The osteoclastogenic action of interleukin (IL)-7 is now explained by promoting proliferation of T-cells and their secretion of TNF-α and RANKL (Clowes *et al.* 2005). Macrophage colony stimulating factor is indispensable during normal differentiation of osteoclasts and its deficiency impairs osteoclastogenesis. Interferon (IFN)-γ in inflammatory conditions blocks RANKL-mediated osteoclastogenesis. In summary, TNF-α, IL-7 (as well as IL-6) and RANKL seem to be pro-osteoclastogenic, IFN-γ and OPG (as well as IL-4, IL-10 and

transforming growth factor-β) seem to inhibit osteoclastogenesis (Clowes *et al.* 2005, Zaidi 2007).

Recent studies define local intermittent production of PTHrp by early osteoblast progenitors and osteocytes as a crucial endogenous anabolic factor, which supports osteoblast differentiation and survival, and thus bone formation (Miao *et al.* 2005, Kronenberg 2006, Bisello *et al.* 2004). Moreover, intermittent administration of PTHrp to patients is also anabolic (Horwitz *et al.* 2003). In contrast, although apparently not within the microenvironmental context, prolongated and elevated levels of PTHrp seen under specific physiological conditions (lactation) or malignancies, has been shown to support osteoclastogenesis and bone resorption (Woodrow *et al.* 2006, Broadus *et al.* 1988, Bisello *et al.* 2004, Kronenberg 2006, Jilka 2007).

Concerning non-central action of leptin, it should be noted that both humoral and local effects take apparently place as adipocytes in bone marrow also secrete leptin (Laharrague *et al.* 1998). Leptin receptors have been shown to be present in a large number of peripheral tissues, including cell elements in bone(Cirmanova *et al.* 2008, Zhang *et al.* 2005). With respect to its action, several studies support a concept that the effect of leptin within the bone microenvironment is likely proosteogenic (Thomas *et al.* 1999, Holloway *et al.* 2002, Burguera *et al.* 2001, Cornish *et al.* 2002, Khosla 2002). In contrast to central effect of leptin, its local effect seems to be realized via upregulation of OPG pathway (Holloway *et al.* 2002, Burguera *et al.* 2001), although the involvement of other pathways cannot be excluded. It should be noted, however, that several reports questioned the importance of the direct leptin effect on bone (Ducy *et al.* 2000, Takeda *et al.* 2002, Patel and Elefteriou 2007, Astudillo *et al.* 2008).

Finally, we have to mention the omnipresent effect of mechanical stimuli which is essential for the maintenance of skeletal mass. Quintessence of this effect is the transduction

of mechanical signals into the cell, termed mechanotransduction, which then regulates expression of various genes (Liedert *et al.* 2006, Zaidi 2007).

We conclude this short survey on regulations with a prologue to the next subchapter. It appears that bone metabolism and metabolism of nutrients have many common regulatory mechanisms involving e.g. GH, corticoids, leptin, NPY and NMU, TNF-α, CART. As obesity protects mammals from osteoporosis (Tremollieres *et al.* 1993), Karsenty and collaborators (Ducy *et al.* 2000, Karsenty 2006) proposed that bone remodeling and energy metabolism could be regulated by the same hormone(s). They indeed showed that leptin affects the bone in mice (Karsenty 2006). As most hormonal regulations are controlled by feedback loops such that a cell type affected by a hormone sends signals influencing the hormone producing cell, and if indeed bone cells influcence the level of activity of hormone-producing cells, then osteoblasts should strike back and affect energy metabolism.

Bone is endocrine organ regulating energy metabolism

With the aim of identifying osteoblast-enriched genes affecting energy metabolism, Karsenty and collaborators (Lee *et al.* 2007) generated mutant mouse strains lacking genes encoding signaling molecules expressed only or preferentially in osteoblasts. Through this effort they inactivated in an osteoblast-specific manner Esp, a gene expressed just in osteoblasts and Sertoli cells, that encodes a receptor-like protein tyrosine phosphatase termed osteotesticular protein tyrosine phosphatase (OST-PTP) (Morrison and Mauro 2000, Schiller and Mauro 2005). Mice lacking OST-PTP (Esp mice) were shown to be hypoglycemic and to have increased β-cell proliferation and insulin secretion, increased insulin sensitivity, higher adiponection production, higher energy expenditure, lower visceral fat, and were protected from obesity and glucose intolerance (Lee *et al.* 2007). *Ex vivo* co-culture experiments showed that osteoblasts from Esp mice increased insulin expression in islets of Langerhans

and an insulin-sensitizing adipokine, adiponectin (Otabe *et al.* 2007, Yamauchi *et al.* 2001), expression in adipocytes. In contrast, in transgenic mice overexpressing Esp selectively in osteoblasts, the metabolic and proliferation parameters seen in Esp mice were reversed. OST-PTP thus controlled down-stream the bioactivity of an osteoblast-derived secreted molecule regulating adiponectin as well as insulin expression, and glucose homeostasis.

In the search for such an osteoblast secreted molecule, Lee *et al.* (2007) focused on osteocalcin since mice lacking gene Ocn for osteocalcin (Ocn mice) are obese. Osteocalcin has several features of a hormone as it is cell-specific molecule, synthesized as a pre-promolecule and secreted (Hauschka *et al.* 1989, Price 1989). Osteocalcin may also undergo, via the action of OST-PTP, a posttranslational modification whereby its glutamic acid residues are carboxylated to form γ-carboxyglutamic acid (Gla) residues (Hauschka *et al.* 1989).

The phenotype of Ocn mice, with decreased β-cell proliferation and energy expenditure and increased insulin resistance, was exactly the opposite of Esp mice. However, in experiments in which Ocn mice were administred glucose together with recombinant osteocalcin, i.e. non-Gla modified protein, they were rescued as decreased glucose levels and increased secretion of insulin were seen. Moreover, *ex vivo*, non-Gla modified osteocalcin stimulated cyclin D1, a molecular marker of cell proliferation (Kushner *et al.* 2005), and insulin expression, in islet β-cells, and adiponectin in adipocytes. Thus, although the authors did not exclude the participation of other secreted osteoblast-specific molecules (Lee *et al.* 2007), they provided evidence that osteocalcin regulated the glucose level (Fig. 1).

The production and clearance of osteocalcin were normal in Esp mice, so that OST-PTP did not regulate Ocn gene expression (Lee *et al.* 2007). The results obtained thus suggested that in Esp mice, there was a gain of osteocalcin metabolic activity. Most importantly, this was demonstrated as Esp mice being heterozygous for Ocn exhibited a

remarkable reversal of all their metabolic characteristics, and β-cell proliferation was reduced in these mice. In addition, if normal osteoblasts were co-cultured with adipocytes treated with warfarin, an inhibitor of γ-carboxylation (Berkner 2005), a substantial increase of adiponectin expression was observed. All together, these results provided an evidence that Esp and Ocn lied in the same regulatory pathway, showed that OSP-PTP, via the γ-carboxylation, downregulated the metabolic bioactivity of osteocalcin, and established a link between bone and energy metabolism, both in wild type and transgenic mice (Fig. 1; Lee *et al.* 2007, Ferron *et al.* 2008). Furthermore, Ferron *et al.* (2008) provided evidence that osteocalcin differentially regulates β-cell and adipocyte gene expression and that continuous administration of osteocalcin acts in wild type mice to improve glucose handling and reduce fat mass, and that, as a result, it can reduce the severity of obesity and experimental type 2 diabetes.

Concluding remarks

We provided here a short survey on the impact of clinical studies devoted to osteoporosis and the complexity of regulations governing the bone metabolism. We emphasized the significance of the experimental mouse model. Despite possible drawbacks ensuing from the use of knocked out and transgenic animals, the mouse model is of prime importance for expanding our knowledge on bone metabolism.

We commented here in detail the recent study (Lee *et al.* 2007) that, if confirmed by other groups, represents the real breakthrough in the field of bone metabolism. The results obtained demonstrated a new regulatory pathway and for the first time "closed" the circle - adipocytes and β-cells had been known to talk to bone, and Lee *et al.* (2007) have shown that bone was talking back. However, many important facets of this conversation are still to be elucidated. For instance, osteocalcin interacting partners in target cells/tissues have to be

characterized and hiearchy of ensuing regulations to be established. In fact, the exact interrelationship between bone regulating energy metabolism via osteocalcin and feedback hormonal loop (loops) is not known. It is not known through which regulation pathway leptin strikes back onto the regulation by osteocalcin, how osteoblasts "sense" the necessity to regulate energy metabolism and what are mechanisms leading to Gla-modification of osteocalcin and secretion of non-Gla modified osteocalcin.

Most importantly, until the experimental results (Lee *et al.* 2007) are proven to apply in humans, they cannot be directly applied to human physiology and medicine. Interestingly, non Gla-modified osteocalcin was shown to reduce the severity diabetes 2 in mice (Ferron *et al.* 2008) and it should be noted that diabetic patients exhibit lowered osteocalcin levels (Rosato *et al.* 1998, Gerdhem *et al.* 2005). However, a protein tyrosine phosphatase, performing the modification of osteocalcin, remains to be identified in humans (Cousin *et al.* 2004, Schiller and Mauro 2005).

If the results of Lee *et al.* (2007) are applicable to humans, the link between bone metabolism and osteoporosis on one hand and obesity and diabetes on the other becomes apparent. And consequenly, the pathogenesis of some degenerative diseases of energy metabolism may then be more complex than anticipated. Accordingly, new schemes for treating obesity, diabetes, and their complications may apply (Semenkovich and Teitelbaum 2007). OSP-PTP is, for the time being, known to be produced just in osteoblasts and Sertoli cells, the pharmacological downmodulation of relevant human tyrosine phosphatase levels could be the straightforward approach. The use of warfarine (Berkner 2005) is also to be considered as it was up to now used to reduce the risk of thrombosis, but a systematic assessment of glucose metabolism and adipocity in these patients was not performed. Next, biphosphonate treatment affects osteoclasts, but decreased osteocalcin levels ensue from such therapy (Greenspan *et al.* 2005, Semenkovich and Teitelbaum 2007). Also, glucocorticoid

therapy is common and predisposes patients to both diabetes and osteoporosis. Chronic administration of steroids affects osteoclasts and osteoblasts, suppressing bone formation and thus osteocalcin expression (Kim *et al.* 2006, Semenkovich and Teitelbaum 2007).

Witnessing today´s exponential expansion of our knowledge of bone metabolism arising from the use of mice models, we are confident that many persisting questions will be answered in the near future. And we hope that this will apply, even though in a longer run, with respect to questions relevant to human physiology and medicine.

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Abbreviations

AR - androgen receptor; Akt kinase - serine/threonine protein kinase; BMD - bone mass density; CART - cocaine and amphetamine regulated transcript; ER - estrogen receptor; ES - estrogens; Esp - gene coding OSP-PTP; Esp mice - mice with knocked out OSP-PTP gene; FasL - fibroblast-associated surface antigen ligand; FSH - follicle stimulating hormone; GC - glucocorticoid; GH - growth hormone; Gla - γ-carboxyglutamic acid modification; IGF - insulin-like growth factor; IL - interleukin; MAP kinase - mitogen activated protein kinase; NMU - neuromedin; NPY - neuropeptide Y; Ocn - osteocalcin gene; Ocn mice - mice with knocked out osteocalcin gene; OPG - osteoprotegerin; OST-PTP - osteotesticular protein tyrosine phosphatase; PTH - parathyroid hormone; PTHrp - parathyroid hormone-related protein; RANK - receptor activator of nuclear factor-κB; RANKL - RANK ligand; SNS -

sympathetic nervous system; TNF - tumor necrosis factor; TSH - thyroid stimulating hormone.

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Legend to Figure

Fig. 1. Osteoblasts affect energy metabolism via uncarboxylated osteocalcin.

Osteoblastic Esp gene encodes OST-PTP protein that favours, through an unknown mechanism, a modification of osteocalcin, an osteoblast-specific protein. This modification consists of the γ-carboxylation of osteocalcin. Uncarboxylated osteocalcin (○), but not γ-carboxylated osteocalcin (●), affects energy metabolism. Down-modulation of Esp gene leads to an increased level of secreted uncarboxylated osteocalcin by osteoblasts. Secreted uncarboxylated osteocalcin affects pancreatic β-cells and adipocytes. As the result, β-cell proliferation as well as insulin (⬟) and adiponection (✦) secretion are increased. This leads to increased peripheral insulin sensitivity and improved glucose handling.

Fig. 1. Osteoblasts affect energy metabolism via uncarboxylated osteocalcin.