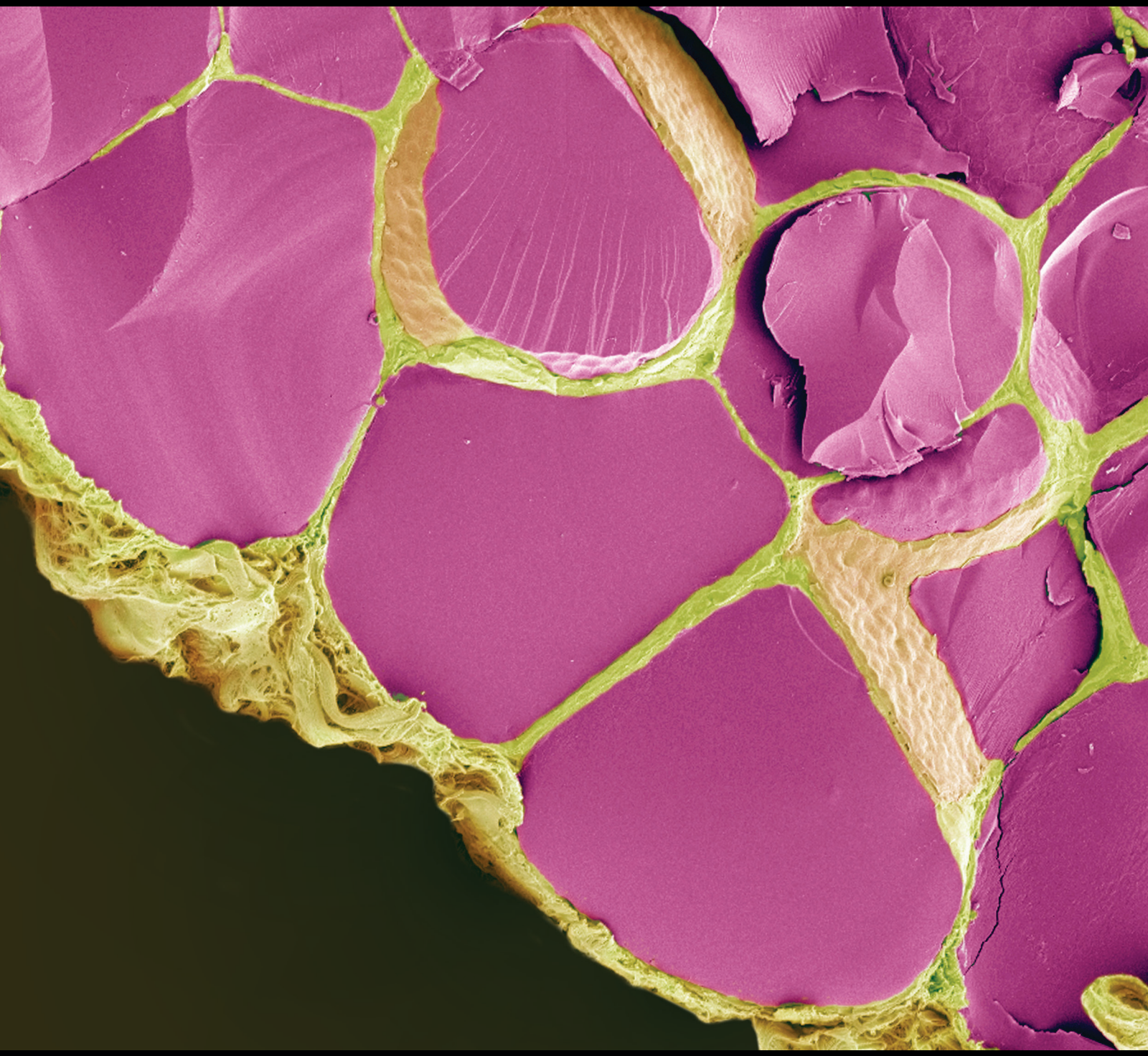
International Journal of Endocrinology

The Endocrine Role of the Skeleton

Guest Editors: Andrea Del Fattore, Cristina Sobacchi, Martina Rauner, and Amélie Coudert



The Endocrine Role of the Skeleton

International Journal of Endocrinology

The Endocrine Role of the Skeleton

Guest Editors: Andrea Del Fattore, Cristina Sobacchi, Martina Rauner, and Amélie Coudert

Editorial Board

Contents

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2015, Article ID 265151, 2 pages
http://dx.doi.org/10.1155/2015/265151

Editorial

The Endocrine Role of the Skeleton

Andrea Del Fattore,[1] Cristina Sobacchi,[2,3] Martina Rauner,[4] and Amélie Coudert[5]

[1]*Bambino Gesù Children's Hospital, 00146 Rome, Italy*
[2]*UOS/IRGB, Milan Unit, National Research Council (CNR), 20138 Milan, Italy*
[3]*Humanitas Clinical and Research Center, 20089 Rozzano, Italy*
[4]*Medical Faculty of the Technische Universität Dresden, 01309 Dresden, Germany*
[5]*Institut National de la Santé et de la Recherche Médicale U1138, Centre de Recherche des Cordeliers, 75006 Paris, France*

Correspondence should be addressed to Andrea Del Fattore; andrea.delfattore@opbg.net

Received 28 December 2014; Accepted 28 December 2014

In recent years, many advances have been made in the understanding of the role of the skeleton in whole body pathophysiology.

The original vision of the bone as a static tissue with limited functions is now completely abolished. Indeed it is now well established that bone is a dynamic connective tissue subjected to a continuous process of resorption and formation. This activity is crucial, since correct bone homeostasis, skeleton integrity, and mechanical properties rely on a perfect coupling between osteoclasts and osteoblasts functions. Indeed recent studies demonstrated that the bone remodelling process is an important regulator of many functions not only limited to the bone, but also correlated with the whole body physiology.

In this special issue, experts in the bone field addressed the interplay between bone and energy metabolism, immune system, male fertility, and kidney and documented how bone has emerged as an endocrine "gland."

In particular, osteocalcin is indicated as a key factor mediating the endocrine functions of bone, with special focus on glucose metabolism and male fertility. J. Shao et al. described how osteocalcin targets β-cells in the pancreas and adipocytes to regulate insulin production and sensitivity and Leydig cells to control testicular function. Moreover the authors reviewed the mechanisms regulating osteocalcin production and activation, with particular attention to 1α,25-dihydroxyvitamin D3 and vitamin K, and reported studies in humans supporting the relevance of osteocalcin in energy metabolism and highlighting differences between men and mice as well as aspects requiring further investigation. M. F. Faienza et al. analyzed the bone-pancreas loop, focusing the attention not only on osteocalcin, but also on other proteins, such as osteoprotegerin, vitamin D, gastric inhibitory polypeptide (GIP), and adiponectin, involved in the regulation of insulin function and glucose metabolism. Antiresorptive drugs might impact the bone-pancreas interplay; however conflicting results exist on this aspect and deserve further efforts from the scientific community. In particular, the development of new drugs simultaneously targeting the skeleton, glucose metabolism, and the adipose tissue can be envisaged. Besides the effects of bone osteocalcin on male fertility, the endocrine role of oestrogens on human male skeleton was also discussed in this special issue in the paper by V. Rochira et al. The authors described the effects exerted by oestrogens in the physiological events occurring in male bone throughout life such as longitudinal skeletal growth, skeletal proportion, achievement of peak bone mass, and maintenance of bone mineral density. Moreover the authors discussed clinical aspects of oestrogen deficiency.

In separate chapters, the interplay between bone and kidney is presented. Y. Takei et al. described how bone homeostasis is finely regulated by a complex mechanism including FGFs (*fibroblast growth factors*)/FGF receptors signalling. In particular they emphasized how FGF23, expressed by osteocytes/osteoblasts, reduces the levels of serum phosphate and $1,25(OH)_2D_3$ and the relevance of its coreceptor αKlotho, produced by kidney distal tubular cells, in the physiologic regulation of mineral metabolism. In a clinical study

S. Rotondi et al. investigated whether a soluble form of Klotho could represent a marker of renal damage of CKD- (chronic kidney disease-) MBD (mineral bone disorder), indicative of the cross-talk between bone and kidney, and concluded that soluble Klotho could be considered an early marker of mineral metabolism impairment in renal disease.

Finally, bone diseases, such as osteopetrosis and osteoporosis, are revisited with the special focus on their relevance for the endocrine role of the skeleton and on the relationship with other diseases, respectively. Indeed, A. E. Coudert et al. described how osteoclast dysfunctions lead to osteopetrosis and have allowed shedding some lights on several aspects of the bone biology that were not well known, discovering the interaction between bone and stomach, insulin metabolism, male fertility, immune system, bone marrow, and fat.

Regarding osteoporosis studies, P. Jackuliak and J. Payer analyzed the osteoporosis risk in diabetic patients. Moreover the authors made clinical considerations and discussed the use of bone mineral density and the trabecular bone score to study different bone properties, quantity and quality, respectively. M. Bolanowski et al. investigated how many hormonal disorders such as Cushing's syndrome, hyperprolactinemia, acromegaly, hypogonadism, and hypopituitarism influence bone metabolism and can result in secondary osteoporosis.

This special issue should be of interest for basic and clinical researchers since it covers a wide range of topics regarding bone research. Original studies and reviews have been published with the aim to report and summarize the latest findings regarding the relevance of the skeleton in the whole body physiology.

Acknowledgment

We are very grateful to all the authors, who submitted papers for this issue and contributed with their excellent work to publish this successful special issue.

Andrea Del Fattore
Cristina Sobacchi
Martina Rauner
Amélie Coudert

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2015, Article ID 967673, 9 pages
http://dx.doi.org/10.1155/2015/967673

Review Article

Bone Regulates Glucose Metabolism as an Endocrine Organ through Osteocalcin

Jin Shao, Zhi Wang, Tieyi Yang, Hui Ying, Yan Zhang, and Shuyi Liu

Department of Orthopaedics, Shanghai Pudong New Area Gongli Hospital/Clinical School, The Second Military Medical University, Shanghai 200135, China

Correspondence should be addressed to Tieyi Yang; yangtieyi@163.com

Received 9 June 2014; Revised 21 August 2014; Accepted 18 September 2014

Academic Editor: Andrea Del Fattore

Skeleton was considered as a dynamic connective tissue, which was essential for mobility, calcium homeostasis, and hematopoietic niche. However more and more evidences indicate that skeleton works not only as a structural scaffold but also as an endocrine organ, which regulates several metabolic processes. Besides osteoprotegerin (OPG), sclerostin (SOST), and Dickopf (DKK) which play essential roles in bone formation, modelling, remodelling, and homeostasis, bone can also secret hormones, such as osteocalcin (OCN), which promotes proliferation of β cells, insulin secretion, and insulin sensitivity. Additionally OCN can also regulate the fat cells and male gonad endocrine activity and be regulated by insulin and the neural system. In summary, skeleton has endocrine function via OCN and plays an important role in energy metabolism, especially in glucose metabolism.

1. Introduction

Bone has been only considered as a structure organ for centuries. Classical understanding of skeleton is that bone functions as the structure scaffold in animals and contains three types of cells: osteoblast, osteocyte, and osteoclast. Osteoblasts come from mesenchymal stem cells and can differentiate to osteocytes, which stay in the bone matrix. Osteoblasts are responsible for bone formation, and its activity can be regulated by several pathways such as WNT signaling pathway. Osteoclasts are a type of bone cells, which resorb bone and derived from the fusion of mononuclear cells belonging to the monocyte/macrophage lineage. Osteocytes and osteoblasts can secrete some signaling factors, such as WNT, OPG, and SOST, in order to regulate the coupling function of osteoblast and osteoclast; in this way bone obtains the ability to model and remodel itself. Recent reports indicate that SOST, a WNT antagonist, downregulates the WNT signaling activity by crosstalk with parathyroid hormone (PTH) and bone morphogenetic protein (BMP) in order to regulate bone cell differentiation, proliferation, bone formation, and bone resorption activities [1–4]. Even though there are plenty of evidences indicating that bone can secrete protein factors in the circulation and regulate itself, all these secreted factors cannot be called hormones.

However, in the past decades with the development of technology we found that skeleton can also be an endocrine organ, which regulates not only itself but also other organs. The most well known and studied two hormones secreted by skeleton are OCN and fibroblast growth factor 23 (FGF23) [5] (Figure 1). In this review we would focus on OCN, especially on its function as a hormone in metabolism homeostasis.

2. Endocrine Functions of Bone through OCN

2.1. Production of OCN. OCN is specially synthesized and secreted by cells of osteoblast lineage, such as osteoblast and osteocyte [6, 7]. In humans OCN is also known as bone γ-carboxyglutamic acid-containing protein (BGP) and it is one of the richest noncollagenous proteins in bone matrix. The most of OCN is found in bone matrix and only a small amount in blood, because it has strong affinity to bone matrix.

OCN has proosteoblastic or bone-building function. Osteoblasts can secret OCN to stimulate osteoblastic differentiation and osteocytic maturation. The *OCN* gene has

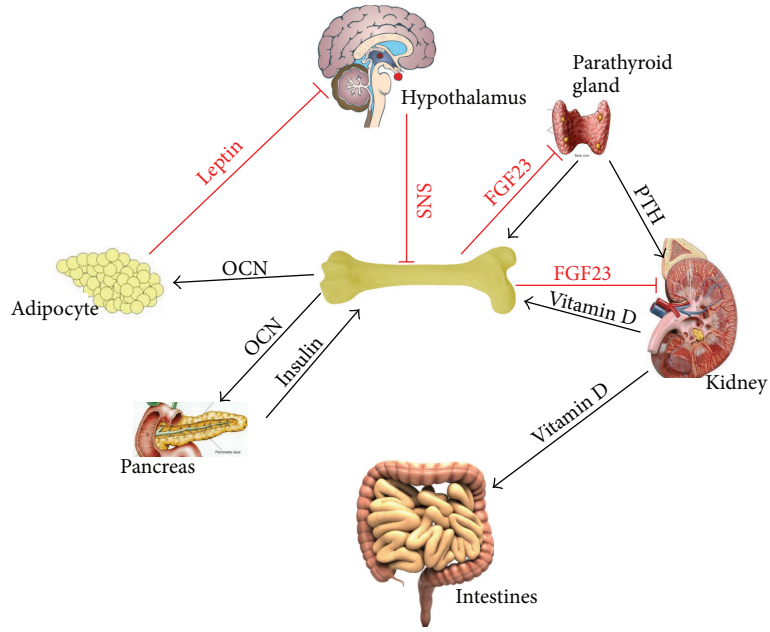


Figure 1: Skeleton regulates mineral and energy homeostasis. In mineral homeostasis, low level of circulating calcium stimulates the parathyroid gland to release PTH, which later upregulates blood calcium levels by stimulating osteoclastic bone resorption, renal calcium reabsorption, and renal production of Vitamin D to increase intestinal calcium absorption. Increased serum phosphate and Vitamin D stimulate FGF23 synthesis and releasing in bone, which subsequently inhibits PTH production from the parathyroid gland, inhibits Vitamin D production in the kidney, and promotes renal phosphate excretion. Leptin inhibits bone formation and the homeostatic function of the skeleton indirectly through SNS. However, SNS signalling also increases the production of OCN from bone, which feeds into the positive loop. OCN affects pancreatic β cells and increases insulin level, which has feedback effect on bone, driving further production of OCN. OCN also acts on fat to increase the production of adiponectin and upregulates insulin sensitivity. FGF23, fibroblast growth factor 23; PTH, parathyroid hormone; SNS, sympathetic nervous system.

4 exons and 3 introns, and more than 70% of the exon sequences are conserved both in human and in mice. The OCN protein contains 46–50 amino acid residues (varying from different species), the synthesis process of which is a little complicated (Figure 2). Firstly, the transcription is regulated by 1α,25-dihydroxy-Vitamin D3. After the translation it is only preproosteocalcin, which contains 98 amino acid residues composed of three parts: a 23-residue signal protein, a 26-residue target propeptide, and a 49-residue mature protein. Proteolysis of the prepropeptide will form the mature OCN [8]. At position 17, 21 and 24 of the 49-residue peptide second carboxyl groups (–COOH) are added to form γ-carboxyglutamyl residues, which are essential for its activity regulation.

According to the carboxylation level, OCN can be divided into two groups; one is called undercarboxylated osteocalcin (ucOCN), and the other is carboxylated osteocalcin (cOCN). The decarboxylated form ucOCN is the active form of OCN as a hormone, which has little affinity to bone so that most of ucOCN will circulate with the blood, while most of cOCN will be stored in the bone matrix because of its strong affinity to bone matrix. Usually the concentration of ucOCN is not controlled by the protein synthesis, but by the decarboxylation of OCN and its releasing from the bone matrix. This issue will be discussed in Section 2.3 later.

2.2. OCN Targeting Organs

2.2.1. OCN Targets β Cells and Adipocytes. There are evidences indicating that in *Ocn* knocked out mice the total amount of insulin in serum is downregulated, and the *Ocn* knocked out mice have so-called impaired glucose-stimulated insulin secretion (GSIS) and poor glucose tolerance phenotype [9]. At the same time the serum adiponectin, a protein hormone that modulates several metabolic processes such as glucose regulation and fatty acid oxidation, was also reduced [10]. All these results suggest that OCN may target β cells and insulin targeting tissue such as muscle, liver, and adipocyte in order to regulate both insulin secretion and sensitivity [11, 12]. Later studies show that the change of insulin sensitivity is probably mediated by adiponectin instead of direct interaction with OCN [13]. This result indicates that adipocytes are probably one target of ucOCN, and ucOCN functions as the activity form of OCN in circulation.

There are also many evidences indicating that ucOCN can regulate β cells in pancreatic islets. Not only insulin 1 and insulin 2 can be upregulated by ucOCN but also the proliferation of β cells. Further research indicates that CyclinD1, CyclinD2, and Cdk4 in β cells can also be regulated by ucOCN, and in this way it is not difficult to understand why ucOCN can stimulate β cell proliferation [9, 12, 14].

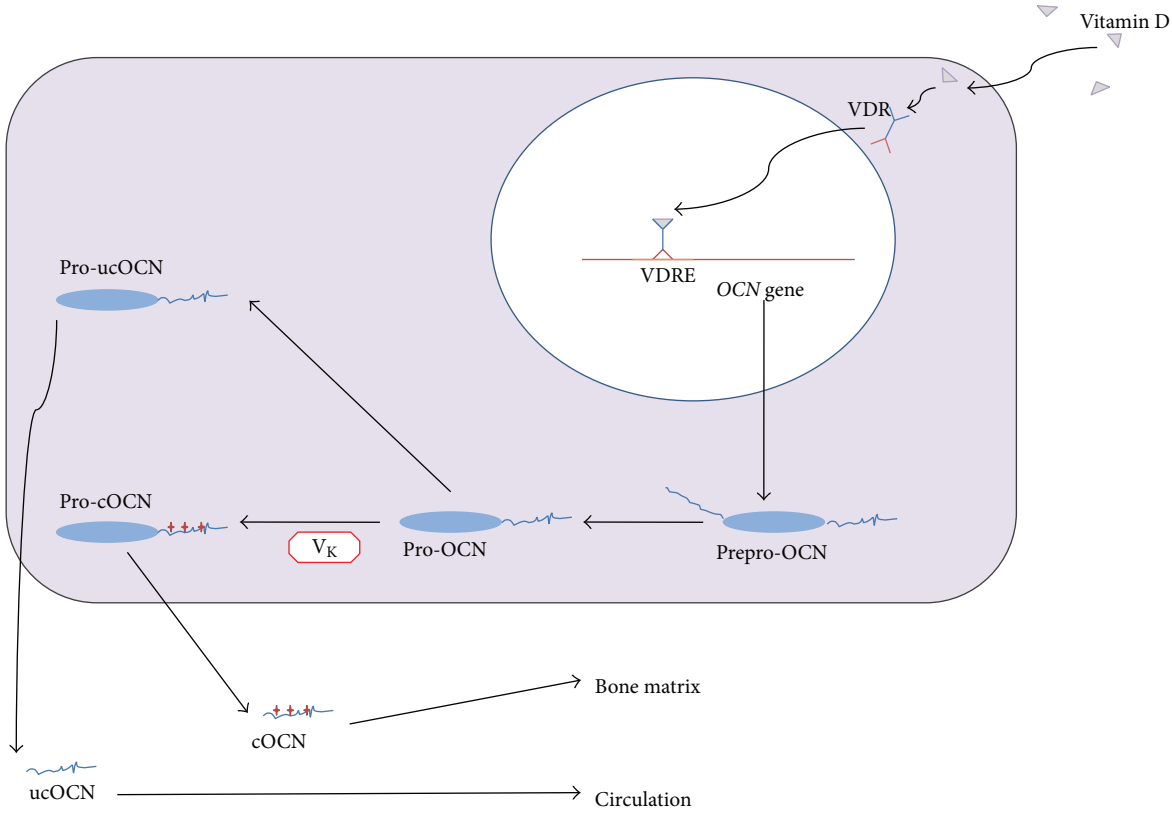


Figure 2: OCN is synthesized in osteoblasts. *OCN* is mainly expressed in osteoblasts. After transcription, which is stimulated by Vitamin D, the prepro-OCN peptide will be proteolysed and forms a prepeptide (23 aa) and a pro-OCN peptide (75 aa). The pro-OCN peptide can be carboxylated at Glu residues 17, 21, and 24, resulting in formation of Gla residues in a Vitamin K dependent process. Generally, this process only occurs in a proportion of newly synthesized proosteocalcin. Then Gla and Glu pro-OCN peptides are subjected to a final proteolytic process that produces cOCN and ucOCN. Both forms are released from osteoblasts in a process which is calcium dependent. While the carboxylated Gla residues are involved in calcium and hydroxyapatite binding, allowing OCN deposition on mineralized bone matrix, ucOCN has a low affinity for hydroxyapatite and is more easily released into the circulation. OCN, osteocalcin; ucOCN, uncarboxylated osteocalcin; VDR, Vitamin D receptor; VDRE, Vitamin D receptor element.

β cells proliferation and insulin secretion can be significantly affected at low concentrations of ucOCN (6–60 pM) [15]. The mice lacking osteoblasts have not only problems on bone density and strength but also impaired glucose metabolism, such as high blood glucose, low insulin secretion, and insulin resistance. All these phenotypes mimic the defects in *Ocn* knocked out mice. However, administration of OCN can restore glucose and insulin level in circulation but only partially insulin sensitivity [16]. This indicates that osteoblasts may have some OCN independent ways to regulate insulin sensitivity.

Although more and more evidences show that OCN can regulate β cells and increase the insulin sensitivity of insulin targeting cells, the receptor of OCN or more correctly ucOCN has not been identified yet. What is more, we do not even know how OCN regulates the insulin sensitivity. There are only evidences indicating adiponectin mediates the insulin sensitivity regulation of OCN [13]. Whether some other mechanisms involved in this progress is still not clear. We should also not ignore cOCN, which usually stays in the bone matrix. The biological function of cOCN is still unclear. Is it only storage of ucOCN? Does it have some unknown functions on metabolism of bone formation? Some *in vitro* studies indicated that cOCN could regulate bone formation and resorption; however, in the *Ocn* knocked out mice no negative bone effects were observed [17–19]. Anyway, a lot of questions still need to be answered.

2.2.2. OCN Regulates Testicular Function. OCN regulates male reproduction activity [20]. These were observed from the experimental studies on *Esp* and *Ocn* knocked out mice. With or without OCN, male mice showed different reproductive activity. Mice with high OCN activity (the *Esp* knocked out mice) had increased testicular volume and sperm count. On the contrary, the OCN absent mice (the *Ocn* knocked out mice) showed shriveled testes, epididymis, and seminal vesicles. However, the female in both *Esp* and *Ocn* knocked out groups did not show any defects in reproduction. *Ocn* specific deletion in leydig cells showed no such effects as described above [20]. This indicates that OCN secreted by skeleton regulates male reproduction activity as a hormone.

And fortunately the receptor in the leydig cell of ucOCN was identified. It is a G protein-coupled receptor (GPCR6A)

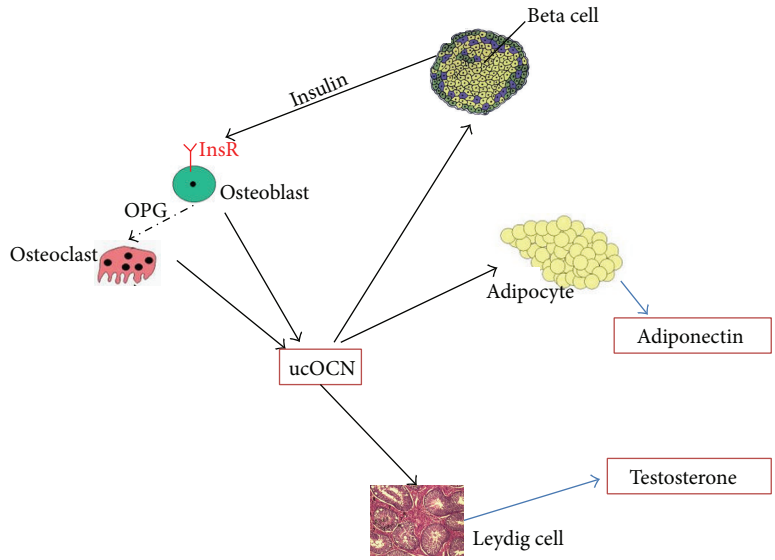


Figure 3: Endocrine actions of osteocalcin. Circulating OCN and particularly ucOCN (released during bone resorption activity) have direct effects on β cells, stimulating insulin synthesis. Meanwhile it affects adipocytes and stimulates adiponectin secretion. Adiponectin upregulates insulin sensitivity. In turn, insulin binds to insulin receptor of osteoblasts and affects osteoblasts; then OPG expression will be upregulated and in turn suppress the activity of osteoclasts. Osteoclast stimulates bone resorption with subsequent release of ucOCN in blood circulation. OCN also functions on leydig cells. It can increase their activity and testosterone production. OCN, osteocalcin; ucOCN, uncarboxylated osteocalcin; OPG, osteoprotegerin; InsR, insulin receptor.

and localized on the surface of the leydig cell. This 7-transmembrane protein was found because the *GPCR6A* knocked out mice copy the metabolic syndrome of the *Ocn* knocked out mice [21, 22]. In the past decades it was demonstrated that gonadal hormone (estrogen) can regulate bone formation [23, 24], and now we know that bone can also regulate testicle and testosterone secretion. This discovery suggested that there may be a novel mechanism regulating bone formation in some special stage, such as teenage time, in which rapid bone formation is associated with androgen secretion changing. In general, OCN, particularly ucOCN (released during bone resorption) targets directly β cells and stimulates insulin synthesis and secretion. At the same time it affects adipocytes and enhances adiponectin secretion. Adiponectin itself upregulates the insulin sensitivity of the insulin targeting cells [13]. Interestingly, OCN also targets leydig cells and regulates testosterone production and male reproductive activity (Figure 3).

2.3. Regulation of OCN Production and Activation

2.3.1. OCN Is Partly Regulated by 1α,25-Dihydroxyvitamin D3 and Vitamin K. Vitamin D is a kind of fat-soluble secosteroids which plays an essential role in absorption of calcium, phosphorus, iron, zinc, and their metabolism. In humans and mice the most active form of Vitamin D is 1α,25-dihydroxyvitamin D3 (Vitamin D3). Recent studies showed that the *Ocn* expression in osteoblasts can be partly stimulated by Vitamin D3 [25]. It has been reported that OCN usually functions to inhibit mineralization. More interestingly fibroblast growth factor 23 (FGF23), another bone secreted hormone, can affect kidney and inhibit Vitamin D synthesis. This loop suggested a feedback loop, in which bone can partly regulate its own hormone secretion [26].

Table 1: Metabolic phenotypes of *ESP* and *Ocn* knocked out mice.

	Δ*ESP* mice	Δ*Ocn* mice
Blood glucose	Decreased	Increased
Glucose tolerant	Increased	Decreased
Sensitive to insulin	Increased	Decreased
Beta cells proliferation	Increased	Decreased
ucOCN	Decreased	Absent

Studies showed that accumulation of osteocalcin in the ECM of human osteoblastic cultures stimulated by 1α,25-dihydroxyvitamin D3 is inhibited by warfarin (antagonist of Vitamin K), while Vitamin K2 enhanced the 1α,25-dihydroxyvitamin D3 effect [27], and 1α,25-dihydroxyvitamin D3 stimulated mineralization was significantly augmented by warfarin [28].

The biological active form of OCN is ucOCN, while the cOCN has a strong affinity to bone matrix and binds tightly to hydroxyapatite, as a result little cOCN could be found in the serum [29]. Vitamin K is a cofactor for the glutamate carboxylase, which is required for carboxylation. In the absence of Vitamin K the serum level of ucOCN would be increased. On the contrary, high level of Vitamin K can reduce ucOCN level, which had been proved by Vitamin K administration in daily diet [30].

2.3.2. OST-PTP, the Product of Receptor-Type Tyrosine-Protein Phosphatase V (Esp) Controls OCN Activity. Generally speaking, there are two ways controlling OCN signaling, one is

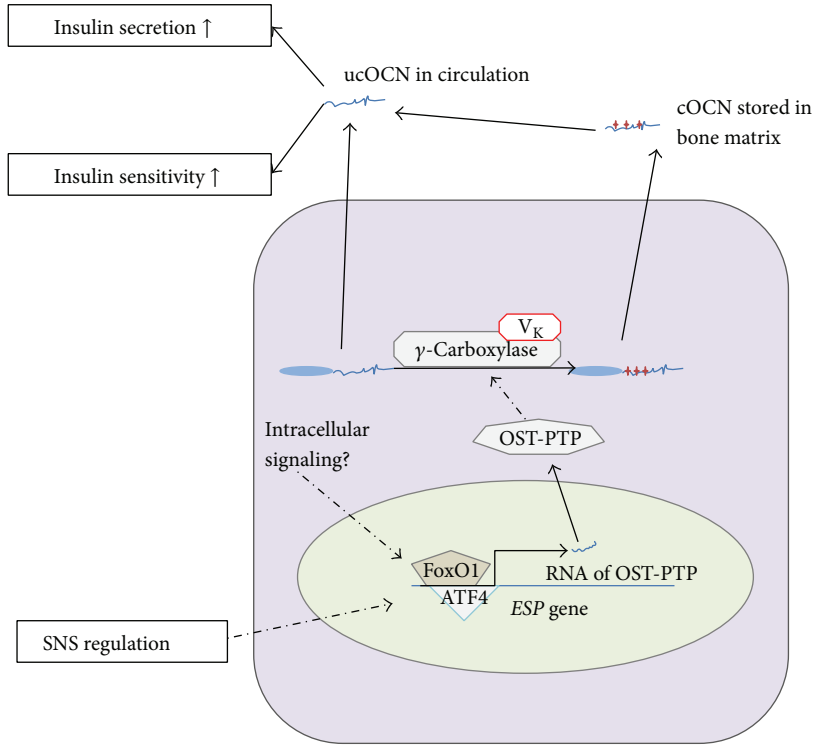


Figure 4: Proposed regulation of *Esp* expression and OCN carboxylation in mice. FoxO1 and ATF4 could bind to the promoter site to stimulate the transcription of *Esp*. The product of *Esp* is OST-PTP, which can suppress the carboxylation of prepro-OCN and in turn suppress the activity of OCN. V_K, Vitamin K; SNS, sympathetic nervous system; OCN, osteocalcin; ucOCN, uncarboxylated osteocalcin.

on the transcription level and the other is on the post-translational level. The product of *Esp* is an osteotesticular protein tyrosine phosphatase (OST-PTP), which is found in embryonic cells, Sertoli cells, and osteoblast [9]. Karsenty et al. found *Esp* knocked out mice have the opposite phenotype compared with the *Ocn* knocked out group (Table 1). The phenotypes of the *Esp* knocked out mice are as follows: hypoglycemia, high glucose-stimulated insulin secretion, glucose tolerance, pancreatic insulin content, and β cell proliferation [9]. *Esp* deletion (either totally knocked out or selectively knocked out in osteoblasts) resulted in the exact opposite phenotype as found in the *Ocn* knocked out mice. And *Esp* can rescue the phenotype of the high fat diet and gold thioglucose-induced obesity and diabetes, which mimics the phenotype of ucOCN treated mice [9, 12]. All these results let Lee et al. believe that OST-PTP may mediate the inactivation of OCN via γ-carboxylation. Indeed lacking one allele of *Ocn* in the *Esp* knocked out mice rescued the metabolic abnormality of the *Esp* absent mice. This result indicates that OST-PTP downregulates the activity of ucOCN [9]. As the total *Ocn* gene expression did not change in *Esp* knocked out mice, Ferron et al. suggested that OST-PTP was involved in the osteoclast-dependent ucOCN formation and releasing [31].

In the recent years the molecular mechanism of *Esp* regulation was revealed (Figure 4). Two transcription factors, forkhead box protein O1 (FOXO1) and cyclic AMP-dependent transcription factor (ATF-4), are involved in the regulation of the total OCN [32]. FOXO1 is a conservative modulator of glucose metabolism in many organs, as well as insulin synthesis in β cells, and ATF4 regulates expression of many genes which mostly are important for adult bone formation and development [33, 34]. Osteoblast specific *FoxO1* knocked out mice have low blood glucose level and high glucose tolerance [35], which is similar to the mice with high ucOCN level. This indicated that FoxO1 may be a modulator of *Ocn* transcription or posttranslational modification. Further studies on knocked out mice showed that FoxO1 could upregulate OST-PTP. Molecular experimental studies showed that FoxO1 could bind to a cognate FoxO1 binding site in the *Esp* promoter area and stimulate the expression of the downstream *Esp*; then OST-PTP would activate the carboxylation of OCN or ucOCN. The new formed cOCN would bind to hydroxyapatite and stay in bone matrix, and as a result the ucOCN would be downregulated [35]. These studies also found the binding site for ATF4 in the *Esp* promoter. Now it is not surprising that ATF4 can suppress obesity in mice and involve in the glucose homeostasis in human [36–38]. In fact even though ATF4 is mainly expressed in osteoblast, it functions quite similar to FoxO1. Via stimulating OST-PTP transcription ATF4 suppress the activity of OCN, in this way suppressing insulin secretion and insulin sensitivity [39, 40]. More details of OCN regulation

are still unknown, and the endocrine regulation of OCN will be discussed in the later section of this review.

3. Bone Regulates Glucose Metabolism via OCN

3.1. OCN in Glucose Metabolism. As mentioned in the sections above, in the *Ocn* knocked out mice the blood glucose concentration was higher than that of the wild type. Serum insulin in the knocked out mice would be downregulated and the mice tended to be obesity [12]. All these indicate that OCN may be essential in energy metabolism, especially in glucose metabolism. With administration of OCN or deletion of *Esp* (OST-PTP increases ucOCN, the active form of OCN), the mice achieved higher activity of insulin, lower blood glucose, higher glucose tolerance, and higher insulin sensitivity with normal glucagon levels [9]. This abnormality is mainly caused by the increased number or mass of pancreatic islets and β cells. This means that OCN can increase insulin secretion and insulin sensitivity in some tissues such as muscles and adipose tissue. The increasing sensitivity of insulin is probably mediated by adiponectin, which can be upregulated by ucOCN [9, 12, 41]. cOCN would be stored in the bone matrix and released during bone resorption [10, 42]. Osteoclasts would secret protons and decrease the pH value during resorption; this would facilitate the decarboxylation of cOCN and by the way the active form ucOCN would enter the circulation to stimulate β cells proliferation and insulin secretion [31, 43]. However, more details of the mechanism need to be revealed.

3.2. Feed-Forward Effect of Insulin. Insulin, synthesized and modified in β cells in pancreas, is an essential protein hormone in almost all vertebrates. Through clinical observation we know that insulin signaling can also affect the bones. Insulin receptor (InsR) is required for osteoblast survival, proliferation, and differentiation [44, 45]. Runx2 is a transcription factor which is essential for osteoblastic differentiation and skeletal morphogenesis. Studies showed that the expression of Runx2 was decreased in osteoblasts lacking InsR [45, 46]. However, insulin dose does not directly regulate Runx2 activity. Instead, insulin downregulates Twist2, an inhibitor of Runx2 activity [46], to suppress the activity of Runx2 [43].

Insulin can also promote bone resorption. However, it does not directly work on osteoclasts but on osteoblasts. Insulin signaling can decrease osteoprotegerin (OPG) expression in osteoblasts via binding to InsR [31]. OPG, a key regulator of osteoclasts, together with receptor activator of nuclear factor-κ ligand (RANKL) via receptor activator of nuclear factor-κ (RNAK) regulates the differentiation and activity of osteoclasts [2, 47, 48]. During bone resorption the osteoclasts secrete proton and the pH value will be low. This acidic environment facilitates decarboxylation and the concentration of ucOCN will be upregulated. In turn ucOCN increases β cells proliferation, insulin secretion, and insulin sensitivity [16, 49]. Thus, insulin signaling and OCN form a feed-forward loop, in which insulin affects osteoblasts and in turn increases its own secretion and sensitivity via ucOCN. Insulin signaling can decrease OPG expression in osteoblast via binding to InsR [31]. And InsR is downstream molecule of OST-PTP, which inhibits phosphorylation of InsR and stops insulin signaling in osteoblasts [50, 51]. This feed-forward model explains why the osteoblastic InsR knocked out mice have low serum insulin level and high insulin resistance.

3.3. Endocrine Regulation of OCN. As mentioned above, the relationship between OCN and insulin is not a feedback relationship, so there must be some other signaling pathways to regulate the OST-PTP activity. If not, the serum insulin concentration cannot stay in a certain level with only the feed-forward loop between bone and pancreatic islets. Some studies suggested that sympathetic nervous system (SNS) may control the expression of *Esp* via leptin, a hormone secreted by adipocytes [41]. The main function of leptin is regulating the amount of fat stored in the body. It can adjust both the sensation of hunger and energy expenditures. The target of leptin signaling is the brain. It inactivates the 5-hydroxylase 2 (Tph2), which is known as the rate-limiting enzyme of serotonin synthesis [52]. *Tph2* knocked out mice have osteoporotic and anorectic phenotype, indicating this source of serotonin can regulate osteoblasts. Yadav et al. have proved that leptin-dependent regulation of OCN carboxylation appeared to be through the hypothalamus [53, 54]. The SNS acts through β2-adrenergic receptors (Adrb2) in osteoblasts to upregulate the *Esp* expression [41], and in so doing the activity of ucOCN would be suppressed.

3.4. OCN in Human

3.4.1. ESP Does Not Play the Same Role in Human. The *ESP* gene in human is a so-called pseudogene which does not have a functional product. Its function may be replaced by another or some other proteins [55]. Years ago a study showed that in human dephosphorylation of InsR can be achieved by protein-tyrosine phosphatase 1B (PTP1B) instead of OST-PTP [31]. So it seems that the mechanism of OCN regulation in human is different from that in mice.

3.4.2. The Circadian Rhythm of OCN in Human Is Different from That in Mice. In human the concentration of OCN will arrive at its peak in the early morning and its lowest point in the afternoon, while the mice will have a high level of OCN in the day and the level of OCN reaches its lowest point at night during sleep [56]. In the older studies the researchers could only test the concentration of total OCN, now there are several ways to test the level of ucOCN, such as ucOCN specific ELISA and hydroxyapatite (HAP) binding assay [19, 57]. With these technological development, recent studies showed that the ratio of ucOCN/OCN usually did not change, while the total amount of ucOCN in circulation had the similar changes as the circadian rhythm of OCN [8, 58–62].

3.4.3. Clinical Studies of OCN. In clinical studies the data were mostly focused on the effects of OCN on bone formation

and metabolism. Children and adolescents, who have the need to increase bone formation, usually have higher levels of OCN and ucOCN and this may facilitate the utilization of glucose in bone [63]. Clinical studies also showed that bone loss was associated with high level of ucOCN in human, and in older people high OCN level predicted increased fracture risk or lower bone density [64, 65]. The phenomenon can be explained by studies in mice that bone resorption facilitates decarboxylation of OCN and releases OCN from bone matrix to the blood [10]. However, whether OCN has effects on skeleton or not is still not clear; the OCN knocked out mice seem quite normal. The minor differences between the knocked out and wild type mice suggest that OCN may play some roles in regulation of bone mineralization [17].

Only in recent years many studies are focused on the hormone functions of ucOCN and OCN in energy metabolism. Patients who suffer from type 1 or type 2 diabetes mellitus have lower level of OCN than healthy people [66–69]. Patients with obesity also show lower level of OCN [70, 71]. We have already discussed that adiponectin mediates the insulin sensitivity. However, studies showed in male patients with type 2 diabetes adiponectin was associated with ucOCN/OCN ratio instead of the concentration of ucOCN, while in female patients it was correlated with OCN, but not ucOCN [72]. It is now very clear that OCN regulates testicular function and does not have such function on female gonad [20]. Whether it has something to do with the differences between male and female diabetic patients is still unknown. Anyway, more studies need to be done to reveal the mechanism of this phenomenon.

4. Conclusion

Studies in animal models showed that ucOCN targets β cells in the pancreas to directly regulate insulin synthesis and regulates insulin sensitivity through adiponectin [13]. And experiments in mice showed that ucOCN may be a potential therapy for diabetic patients [66]. One of the regulators of OCN is OST-PTP, which promotes the carboxylation of OCN and reduces the concentration of ucOCN or the ratio of ucOCN/OCN [8].

There are also many clinical evidences indicating that OCN or probably ucOCN is associated with fasting glucose and insulin sensitivity [63]. However, OST-PTP is not a functional protein in human, and the exact role which OCN plays in human still needs to be studied.

Anyway all these studies suggest us skeleton can behave not only as a structure scaffold but also as an endocrine organ, which regulates energy metabolism, even though all the studies are mostly done in mice and only partially confirmed in human [55]. Therefore, more investigations are needed to reveal the total functions of OCN as a hormone in energy metabolism.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Jin Shao and Zhi Wang contributed equally to this work.

Acknowledgments

This work was supported by National Natural Science Foundation of China (81201367), Key Discipline Construction Project of Pudong Health Bureau of Shanghai (PWZx2014-09), Academic Leaders Training Program of Pudong Health Bureau of Shanghai (PWRd2012-16), and Shanghai Municipal Natural Science Foundation (10ZR1427700).

References

[1] R. Baron and M. Kneissel, "WNT signaling in bone homeostasis and disease: from human mutations to treatments," *Nature Medicine*, vol. 19, no. 2, pp. 179–192, 2013.

[2] S. Khosla, "Minireview: the OPG/RANKL/RANK system," *Endocrinology*, vol. 142, no. 12, pp. 5050–5055, 2001.

[3] M. E. Brunkow, J. C. Gardner, J. van Ness et al., "Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein," *The American Journal of Human Genetics*, vol. 68, no. 3, pp. 577–589, 2001.

[4] M. Katoh, "Networking of WNT, FGF, Notch, BMP, and Hedgehog signaling pathways during carcinogenesis," *Stem Cell Reviews*, vol. 3, no. 1, pp. 30–38, 2007.

[5] S. Fukumoto and T. J. Martin, "Bone as an endocrine organ," *Trends in Endocrinology and Metabolism*, vol. 20, no. 5, pp. 230–236, 2009.

[6] P. V. Hauschka, J. B. Lian, D. E. Cole, and C. M. Gundberg, "Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone," *Physiological Reviews*, vol. 69, no. 3, pp. 990–1047, 1989.

[7] P. A. Price, "Gla-containing proteins of bone," *Connective Tissue Research*, vol. 21, no. 1–4, pp. 51–57, 1989.

[8] A. J. Lee, S. Hodges, and R. Eastell, "Measurement of osteocalcin," *Annals of Clinical Biochemistry*, vol. 37, part 4, pp. 432–446, 2000.

[9] N. K. Lee, H. Sowa, E. Hinoi et al., "Endocrine regulation of energy metabolism by the skeleton," *Cell*, vol. 130, no. 3, pp. 456–469, 2007.

[10] K. K. Ivaska, T. A. Hentunen, J. Vääräniemi, H. Ylipahkala, K. Pettersson, and H. K. Väänänen, "Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption *in vitro*," *The Journal of Biological Chemistry*, vol. 279, no. 18, pp. 18361–18369, 2004.

[11] I. Kanazawa and T. Sugimoto, "The relationship between bone and glucose/lipid metabolism," *Clinical Calcium*, vol. 23, no. 2, pp. 181–188, 2013.

[12] M. Ferron, E. Hinoi, G. Karsenty, and P. Ducy, "Osteocalcin differentially regulates β cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 13, pp. 5266–5270, 2008.

[13] A. S. Lihn, S. B. Pedersen, and B. Richelsen, "Adiponectin: action, regulation and association to insulin sensitivity," *Obesity Reviews*, vol. 6, no. 1, pp. 13–21, 2005.

[14] G. L. Klein, "Insulin and bone: recent developments," *World Journal of Diabetes*, vol. 5, no. 1, pp. 14–16, 2014.

[15] S. L. Booth, A. Centi, S. R. Smith, and C. Gundberg, "The role of osteocalcin in human glucose metabolism: marker or mediator?" *Nature Reviews Endocrinology*, vol. 9, no. 1, pp. 43–55, 2013.

[16] Y. Yoshikawa, A. Kode, L. Xu et al., "Genetic evidence points to an osteocalcin-independent influence of osteoblasts on energy metabolism," *Journal of Bone and Mineral Research*, vol. 26, no. 9, pp. 2012–2025, 2011.

[17] A. L. Boskey, S. Gadaleta, C. Gundberg, S. B. Doty, P. Ducy, and G. Karsenty, "Fourier transform infrared microspectroscopic analysis of bones of osteocalcin-deficient mice provides insight into the function of osteocalcin," *Bone*, vol. 23, no. 3, pp. 187–196, 1998.

[18] T. Reinehr and C. L. Roth, "A new link between skeleton, obesity and insulin resistance: relationships between osteocalcin, leptin and insulin resistance in obese children before and after weight loss," *International Journal of Obesity*, vol. 34, no. 5, pp. 852–858, 2010.

[19] P. Vergnaud, P. Garnero, P. J. Meunier, G. Bréart, K. Kamihagi, and P. D. Delmas, "Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS study," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 3, pp. 719–724, 1997.

[20] F. Oury, G. Sumara, O. Sumara et al., "Endocrine regulation of male fertility by the skeleton," *Cell*, vol. 144, no. 5, pp. 796–809, 2011.

[21] M. Pi, L. Chen, M.-Z. Huang et al., "GPRC6A null mice exhibit osteopenia, feminization and metabolic syndrome," *PLoS ONE*, vol. 3, no. 12, Article ID e3858, 2008.

[22] M. Pi and L. D. Quarles, "Multiligand specificity and wide tissue expression of GPRC6A reveals new endocrine networks," *Endocrinology*, vol. 153, no. 5, pp. 2062–2069, 2012.

[23] Y. Miyauchi, Y. Sato, T. Kobayashi et al., "HIF1alpha is required for osteoclast activation by estrogen deficiency in postmenopausal osteoporosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 41, pp. 16568–16573, 2013.

[24] X. Dai, C. Wang, J. Dai et al., "Association of single nucleotide polymorphisms in estrogen receptor alpha gene with susceptibility to knee osteoarthritis: a case-control study in a Chinese Han population," *BioMed Research International*, vol. 2014, Article ID 151457, 12 pages, 2014.

[25] J. van de Peppel and J. P. T. M. van Leeuwen, "Vitamin D and gene networks in human osteoblasts," *Frontiers in Physiology*, vol. 5, article 137, Article ID Article 137, 2014.

[26] D. J. DiGirolamo, T. L. Clemens, and S. Kousteni, "The skeleton as an endocrine organ," *Nature Reviews Rheumatology*, vol. 8, no. 11, pp. 674–683, 2012.

[27] Y. Koshihara and K. Hoshi, "Vitamin K_2 enhances osteocalcin accumulation in the extracellular matrix of human osteoblasts in vitro," *Journal of Bone and Mineral Research*, vol. 12, no. 3, pp. 431–438, 1997.

[28] V. J. Woeckel, C. Bruedigam, M. Koedam, H. Chiba, B. C. J. van der Eerden, and J. P. T. M. van Leeuwen, "1α,25-Dihydroxyvitamin D3 and rosiglitazone synergistically enhance osteoblast-mediated mineralization," *Gene*, vol. 512, no. 2, pp. 438–443, 2013.

[29] J. R. Cairns and P. A. Price, "Direct demonstration that the vitamin K-dependent bone Gla protein is incompletely γ-carboxylated in humans," *Journal of Bone and Mineral Research*, vol. 9, no. 12, pp. 1989–1997, 1994.

[30] L. J. Sokoll and J. A. Sadowski, "Comparison of biochemical indexes for assessing vitamin K nutritional status in a healthy adult population," *American Journal of Clinical Nutrition*, vol. 63, no. 4, pp. 566–573, 1996.

[31] M. Ferron, J. Wei, T. Yoshizawa et al., "Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism," *Cell*, vol. 142, no. 2, pp. 296–308, 2010.

[32] S. Kousteni, "FoxO1, the transcriptional chief of staff of energy metabolism," *Bone*, vol. 50, no. 2, pp. 437–443, 2012.

[33] R. T. Franceschi, C. Ge, G. Xiao, H. Roca, and D. Jiang, "Transcriptional regulation of osteoblasts," *Cells Tissues Organs*, vol. 189, no. 1–4, pp. 144–152, 2008.

[34] W. Wang, N. Lian, L. Li et al., "Atf4 regulates chondrocyte proliferation and differentiation during endochondral ossification by activating Ihh transcription," *Development*, vol. 136, no. 24, pp. 4143–4153, 2009.

[35] M.-T. Rached, A. Kode, B. C. Silva et al., "FoxO1 expression in osteoblasts regulates glucose homeostasis through regulation of osteocalcin in mice," *Journal of Clinical Investigation*, vol. 120, no. 1, pp. 357–368, 2010.

[36] M. Delépine, M. Nicolino, T. Barrett, M. Golamaully, G. Mark Lathrop, and C. Julier, "EIF2AK3, encoding translation initiation factor 2-α kinase 3, is mutated in patients with Wolcott-Rallison syndrome," *Nature Genetics*, vol. 25, no. 4, pp. 406–409, 2000.

[37] H. P. Harding, H. Zeng, Y. Zhang et al., "Diabetes mellitus and exocrine pancreatic dysfunction in *Perk*−/− mice reveals a role for translational control in secretory cell survival," *Molecular Cell*, vol. 7, no. 6, pp. 1153–1163, 2001.

[38] D. Ron and P. Walter, "Signal integration in the endoplasmic reticulum unfolded protein response," *Nature Reviews Molecular Cell Biology*, vol. 8, no. 7, pp. 519–529, 2007.

[39] T. Yoshizawa, E. Hinoi, Y. J. Dae et al., "The transcription factor ATF4 regulates glucose metabolism in mice through its expression in osteoblasts," *Journal of Clinical Investigation*, vol. 119, no. 9, pp. 2807–2817, 2009.

[40] A. Kode, I. Mosialou, B. C. Silva et al., "FoxO1 protein cooperates with ATF4 protein in osteoblasts to control glucose homeostasis," *Journal of Biological Chemistry*, vol. 287, no. 12, pp. 8757–8768, 2012.

[41] E. Hinoi, N. Gao, D. Y. Jung et al., "The sympathetic tone mediates leptin's inhibition of insulin secretion by modulating osteocalcin bioactivity," *Journal of Cell Biology*, vol. 183, no. 7, pp. 1235–1242, 2008.

[42] C. M. Gundberg and R. S. Weinstein, "Multiple immunoreactive forms of osteocalcin in uremic serum," *Journal of Clinical Investigation*, vol. 77, no. 6, pp. 1762–1767, 1986.

[43] K. Fulzele, R. C. Riddle, D. J. DiGirolamo et al., "Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition," *Cell*, vol. 142, no. 2, pp. 309–319, 2010.

[44] J. C. Brüning, M. D. Michael, J. N. Winnay et al., "A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance," *Molecular Cell*, vol. 2, no. 5, pp. 559–569, 1998.

[45] K. Fulzele, D. J. DiGirolamo, Z. Liu, J. Xu, J. L. Messina, and T. L. Clemens, "Disruption of the insulin-like growth factor type 1 receptor in osteoblasts enhances insulin signaling and action," *The Journal of Biological Chemistry*, vol. 282, no. 35, pp. 25649–25658, 2007.

[46] P. Bialek, B. Kern, X. Yang et al., "A twist code determines the onset of osteoblast differentiation," *Developmental Cell*, vol. 6, no. 3, pp. 423–435, 2004.

[47] B. F. Boyce and L. Xing, "Functions of RANKL/RANK/OPG in bone modeling and remodeling," *Archives of Biochemistry and Biophysics*, vol. 473, no. 2, pp. 139–146, 2008.

[48] M. Pérez-Sayáns, J. M. Somoza-Martín, F. Barros-Angueira, J. M. G. Rey, and A. García-García, "RANK/RANKL/OPG role in distraction osteogenesis," *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology*, vol. 109, no. 5, pp. 679–686, 2010.

[49] T. Yoshizawa, "Diabetes mellitus and osteoporosis. Insulin signaling and bone/glucose/energy metabolism," *Clinical Calcium*, vol. 22, no. 9, pp. 1367–1373, 2012.

[50] S. Avnet, F. Perut, M. Salerno, L. Sciacca, and N. Baldini, "Insulin receptor isoforms are differently expressed during human osteoblastogenesis," *Differentiation*, vol. 83, no. 5, pp. 242–248, 2012.

[51] T. L. Clemens and G. Karsenty, "The osteoblast: an insulin target cell controlling glucose homeostasis," *Journal of Bone and Mineral Research*, vol. 26, no. 4, pp. 677–680, 2011.

[52] D. J. Walther, J.-U. Peter, S. Bashammakh et al., "Synthesis of serotonin by a second tryptophan hydroxylase isoform," *Science*, vol. 299, no. 5603, p. 76, 2003.

[53] V. K. Yadav, F. Oury, N. Suda et al., "A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure," *Cell*, vol. 138, no. 5, pp. 976–989, 2009.

[54] C. J. Rosen, "Leptin's RIGHT turn to the brain stem," *Cell Metabolism*, vol. 10, no. 4, pp. 243–244, 2009.

[55] W. Cousin, A. Courseaux, A. Ladoux, C. Dani, and P. Peraldi, "Cloning of hOST-PTP: the only example of a protein-tyrosine-phosphatase the function of which has been lost between rodent and human," *Biochemical and Biophysical Research Communications*, vol. 321, no. 1, pp. 259–265, 2004.

[56] P. Szulc and P. D. Delmas, "Biochemical markers of bone turnover: potential use in the investigation and management of postmenopausal osteoporosis," *Osteoporosis International*, vol. 19, no. 12, pp. 1683–1704, 2008.

[57] B. Merle and P. D. Delmas, "Normal carboxylation of circulating osteocalcin (bone Gla-protein) in Paget's disease of bone," *Bone and Mineral*, vol. 11, no. 2, pp. 237–245, 1990.

[58] L. J. Sokoll, S. L. Booth, K. W. Davidson, G. E. Dallal, and J. A. Sadowski, "Diurnal variation in total and undercarboxylated osteocalcin: Influence of increased dietary phylloquinone," *Calcified Tissue International*, vol. 62, no. 5, pp. 447–452, 1998.

[59] C. Rosenquist, P. Qvist, N. Bjarnason, and C. Christiansen, "Measurement of a more stable region of osteocalcin in serum by ELISA with two monoclonal antibodies," *Clinical Chemistry*, vol. 41, no. 10, pp. 1439–1445, 1995.

[60] P. Garnero, M. Grimaux, B. Demiaux, C. Preaudat, P. Seguin, and P. D. Delmas, "Measurement of serum osteocalcin with a human-specific two-site immunoradiometric assay," *Journal of Bone and Mineral Research*, vol. 7, no. 12, pp. 1389–1398, 1992.

[61] S. C. Corlett, M. Couch, A. D. Care, and A. R. Sykes, "Measurement of plasma osteocalcin in sheep: assessment of circadian variation, the effects of age and nutritional status and the response to perturbation of the adrenocortical axis," *Experimental Physiology*, vol. 75, no. 4, pp. 515–527, 1990.

[62] J. Nishimura, N. Arai, and J.-I. Tohmatsu, "Measurement of serum undercarboxylated osteocalcin by ECLIA with the "Picolumi ucOC" kit," *Clinical calcium*, vol. 17, no. 11, pp. 1702–1708, 2007.

[63] C. M. Gundberg, S. D. Nieman, S. Abrams, and H. Rosen, "Vitamin K status and bone health: an analysis of methods for determination of undercarboxylated osteocalcin," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 9, pp. 3258–3266, 1998.

[64] M. K. Shea, E. J. Benjamin, J. Dupuis et al., "Genetic and non-genetic correlates of vitamins K and D," *European Journal of Clinical Nutrition*, vol. 63, no. 4, pp. 458–464, 2009.

[65] P. Garnero, "Markers of bone turnover for the prediction of fracture risk," *Osteoporosis International*, vol. 11, no. 6, pp. S55–S65, 2000.

[66] L. R. McCabe, "Understanding the pathology and mechanisms of type I diabetic bone loss," *Journal of Cellular Biochemistry*, vol. 102, no. 6, pp. 1343–1357, 2007.

[67] M. Inaba, Y. Nishizawa, K. Mita et al., "Poor glycemic control impairs the response of biochemical parameters of bone formation and resorption to exogenous 1,25-dihydroxyvitamin D3 in patients with type 2 diabetes," *Osteoporosis International*, vol. 9, no. 6, pp. 525–531, 1999.

[68] P. Gerdhem, A. Isaksson, K. Åkesson, and K. J. Obrant, "Increased bone density and decreased bone turnover, but no evident alteration of fracture susceptibility in elderly women with diabetes mellitus," *Osteoporosis International*, vol. 16, no. 12, pp. 1506–1512, 2005.

[69] J. M. Kindblom, C. Ohlsson, O. Ljunggren et al., "Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men," *Journal of Bone and Mineral Research*, vol. 24, no. 5, pp. 785–791, 2009.

[70] E. F. Papakitsou, A. N. Margioris, K. E. Dretakis et al., "Body mass index (BMI) and parameters of bone formation and resorption in postmenopausal women," *Maturitas*, vol. 47, no. 3, pp. 185–193, 2004.

[71] P. Ravn, G. Cizza, N. H. Bjarnason et al., "Low body mass index is an important risk factor for low bone mass and increased bone loss in early postmenopausal women," *Journal of Bone and Mineral Research*, vol. 14, no. 9, pp. 1622–1627, 1999.

[72] I. Kanazawa, T. Yamaguchi, M. Yamauchi et al., "Serum undercarboxylated osteocalcin was inversely associated with plasma glucose level and fat mass in type 2 diabetes mellitus," *Osteoporosis International*, vol. 22, no. 1, pp. 187–194, 2011.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2015, Article ID 729352, 12 pages
http://dx.doi.org/10.1155/2015/729352

Review Article

Functional Diversity of Fibroblast Growth Factors in Bone Formation

Yuichiro Takei, Tomoko Minamizaki, and Yuji Yoshiko

Department of Calcified Tissue Biology, Hiroshima University Institute of Biomedical & Health Sciences, 1-2-3 Kasumi Minami-ku, Hiroshima 734-8553, Japan

Correspondence should be addressed to Yuji Yoshiko; yyuji@hiroshima-u.ac.jp

Received 20 June 2014; Revised 23 August 2014; Accepted 31 August 2014

Academic Editor: Martina Rauner

The functional significance of fibroblast growth factor (FGF) signaling in bone formation has been demonstrated through genetic loss-of-function and gain-of-function approaches. FGFs, comprising 22 family members, are classified into three subfamilies: canonical, hormone-like, and intracellular. The former two subfamilies activate their signaling pathways through FGF receptors (FGFRs). Currently, intracellular FGFs appear to be primarily involved in the nervous system. Canonical FGFs such as FGF2 play significant roles in bone formation, and precise spatiotemporal control of FGFs and FGFRs at the transcriptional and posttranscriptional levels may allow for the functional diversity of FGFs during bone formation. Recently, several research groups, including ours, have shown that FGF23, a member of the hormone-like FGF subfamily, is primarily expressed in osteocytes/osteoblasts. This polypeptide decreases serum phosphate levels by inhibiting renal phosphate reabsorption and vitamin D_3 activation, resulting in mineralization defects in the bone. Thus, FGFs are involved in the positive and negative regulation of bone formation. In this review, we focus on the reciprocal roles of FGFs in bone formation in relation to their local versus systemic effects.

1. Introduction

Bone is a connective tissue with a mineralized extracellular matrix that provides support to the body and affects calcium (Ca)/phosphate (inorganic phosphate; Pi) metabolism. Osteoblasts are involved in bone formation via secretion of the organic matrix "osteoid" and the subsequent facilitation of hydroxyapatite crystal formation. Large multinucleated osteoclasts play an active role in bone resorption. Bone formation and resorption, that is, bone metabolism, are regulated by local versus systemic factors. The former includes growth factors and receptor activator of nuclear factor κ-β ligand (RANKL) and its receptor RANK. Representatives of the latter include parathyroid hormone (PTH), 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$), and calcitonin [1]. Growing evidence suggests that additional interactions between bone and extraskeletal organs affect, during development, aging and pathogenesis. For example, undercarboxylated osteocalcin secreted by osteoblasts acts on pancreatic β-cells to promote insulin production, which is involved in the regulation of energy metabolism [2]. Osteoblast lineage cells compose hematopoietic [3, 4] and cancer stem cell niches [5], thereby affecting the fates of their stem cells. The adipocyte-derived hormone leptin acts on its specific receptors in the hypothalamus, increases sympathetic activity in bone, and exerts antiosteogenic effects [6]. Serotonin (5-HT) secreted by enterochromaffin cells binds to its receptor 5-HT_2BR in preosteoblasts and inhibits their proliferation [7]. Further studies in this field are of significance with regard to understanding the precise functions of bone.

Fibroblast growth factors (FGFs) are pleiotropic growth factors that regulate cell proliferation, migration, and differentiation in many organs including bone. Twenty-two family members of FGFs (FGF1–23, wherein FGF15 is the mouse ortholog of human FGF19) have been identified in mammals so far. FGFs can be divided into three subfamilies: canonical, hormone-like, and intracellular [8]. Numerous studies have shown that canonical FGFs, such as FGF2, act in bone. Hormone-like FGF family members are the most recently identified FGFs, and the discovery of these, especially

the clinical and experimental studies of FGF23, led us to explore the additional roles of FGFs in bone. Not only FGF23 but also FGF2 is exclusively expressed in osteoblast lineage cells and shares specific receptors (FGF receptors, FGFRs) to transduce intracellular signals, although the effects of these FGFs are variable. The intracellular FGFs, FGF11–14, have been well studied in neurons but not in bone and, therefore, are not discussed here. This review, therefore, provides new insights into the roles of FGFs during bone formation and compares canonical versus hormone-like FGFs.

2. The FGF and FGFR Family Members and Their Signaling Pathways

Canonical FGFs, including FGF2, comprise the most common subfamily that transduces signals through FGFR tyrosine kinases. A heparin-binding domain is conserved among most FGFs, and heparan sulfate (HS) is an integral component for the acquisition of the binding affinity of FGFs to FGFRs. Therefore, these polypeptides can be retained in the extracellular matrix in the vicinity of their secreting cells. Thus, canonical FGFs act as autocrine and/or paracrine factors [10, 11]. The hormone-like subfamily members, FGF15/19, FGF21, and FGF23, contain extra structural features at the C-terminus and require the membrane proteins αKlotho/βKlotho as cofactors rather than HS to bind to FGFRs [8, 12]. This hallmark difference may pertain to the dynamic properties of the two subfamilies. Both canonical and hormone-like FGFs show their biological activities by activating four distinct FGFRs (also known as the existence of splicing variants "b" and "c" of FGFR1–3) with different binding affinities. For information on the binding affinity of individual FGFs to FGFRs, refer to other reviews and papers (see, e.g., [13]). Many studies have found that tyrosine phosphorylation of the intracellular domain of FGFRs activates the Ras-mitogen-activated protein kinase (MAPK) pathways, including extracellular signal-regulated kinase (ERK)1/2, p38, and c-Jun N-terminal kinase (JNK), the phosphatidylinositol 3-kinase- (PI3 K-) Akt pathway, and the phospholipase C (PLC)γ-protein kinase C (PKC) pathway (Figure 1) (see, e.g., [14]). Overall, the spatiotemporal dynamics of FGFs and FGFRs may determine how the FGF family members exert their proper activities in particular cells and tissues.

It is also worth noting that negative and positive modulators expressed in a wide range of cells and tissues play precise roles in FGF signaling, and this may further complicate the functional profiles of FGFs. The sprouty (SPRY) family is a highly conserved group of negative feedback loop modulators of growth factor-mediated MAPK activation that was originally described in *Drosophila* [15]; thereafter, four mammalian orthologs (SPRY1–4) have been identified. Either FGF3 or FGF8 upregulates both mRNA and protein levels of Spry4, while increased Spry4 inhibits both FGF3 and FGF8 signaling by interfering with the downstream activation of FGFR1 in zebrafish blastomeres [16]. Similar expression of *Fgf* genes (*Sef*) encodes a conserved putative transmembrane protein that has sequence similarity with the intracellular domain of the interleukin-17 receptor. This modulator acts as a feedback-induced antagonist of FGF8/Ras/Raf/MAPK signaling in the development of zebrafish embryos [17]. In contrast, Canopy1 (CNPY1) was identified as a positive feedback regulator for FGF-induced signaling [18]. This positive feedback loop between the polypeptide and FGF8/FGFR1 is involved in the cluster formation of dorsal forerunner cells during gastrulation in zebrafish [19]; however, its underlying mechanism in mammals remains to be elucidated.

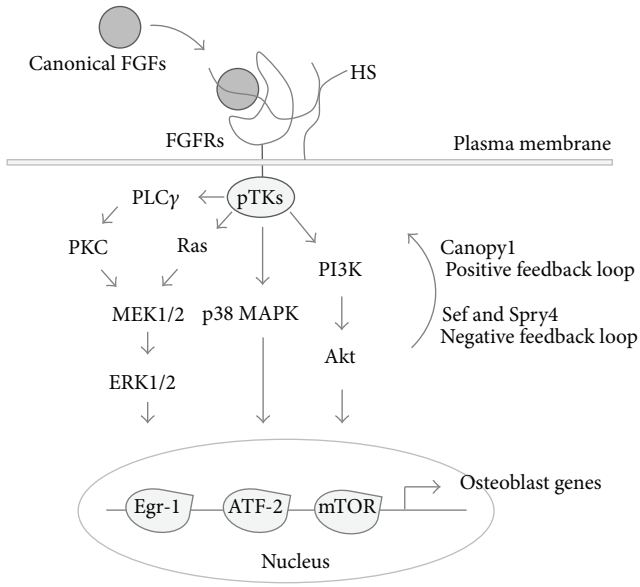


Figure 1: FGF/FGFR signaling and its feedback loops. Ligand-dependent activation of FGFR tyrosine kinases induces ERK1/2, p38 MAPK, and Akt phosphorylation and subsequent upregulation of their downstream transcriptional factors such as early growth response protein-1 (Egr-1), activating transcriptional factor- (ATF-) 2, and mammalian target of rapamycin (mTOR). These transcription factors regulate the expression of genes involved in osteoblastogenesis. Canopy1 acts as positive feedback factor for FGF/FGFR signaling. Sef and Spry4 silence FGF/FGFR signaling. pTKs: phosphorylated tyrosine kinases; PLCγ: phospholipase C γ; PKC: protein kinase C; MEK: mitogen-activated protein kinase; PI3 K: phosphoinositide 3-kinase.

3. Roles of Canonical FGFs on Bone Formation

In addition to our previous data on FGFRs [9], here we show the expression profile of *Fgfs* in a well-established fetal rat calvaria cell model (Figure 2). Among these, *Fgf9* and hormone-like *Fgf23* are abundant and vary in expression levels during osteoblast development. Table 1 summarizes the primary roles of FGFs in bone formation in multiple models. Human calvaria cell cultures describe, in detail, the roles of FGF2 in osteoblastogenesis [20]. When treated at early developmental stages, FGF2 inhibits alkaline phosphatase (ALP) activity, collagen synthesis, and matrix mineralization and increases cell proliferation; however, when treated at late developmental stages, it has no obvious effects. Because the *in vivo* effects of FGF2 on bone formation are apparent, its potential therapeutic benefit in pediatric surgery and

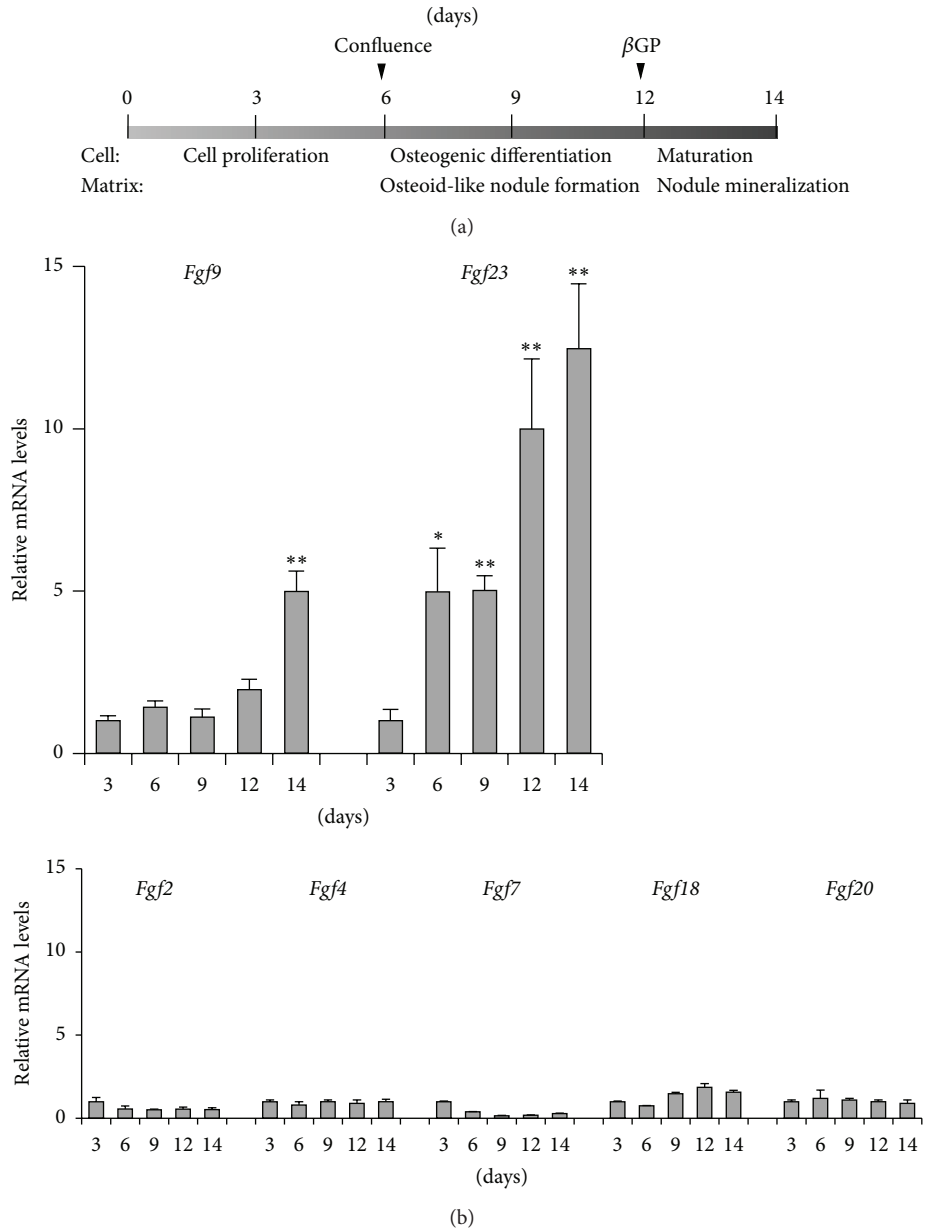


Figure 2: Expression profiling of *Fgf* genes in rat calvaria cell cultures. (a) Outline of osteoblast development. Rat calvaria cells from 21-day-old fetal rats [9] were plated at 3,000 cells per cm^2 and grown in αMEM supplemented with 10% fetal calf serum plus 50 μg/mL ascorbic acid. Cells proliferate, reach confluence at day 6, and subsequently initiate osteoid-like nodule formation. To determine matrix mineralization, 10 mM β-glycerophosphate (βGP) is added to cultures for 2 days before culture termination. (b) Distinct gene expression patterns of *Fgfs* during osteoblast development. Total RNA was routinely prepared as indicated time points, and cDNA synthesis and quantitative real-time RT PCR (qPCR) were performed using standard protocols. Ribosomal protein L32 was used as internal control. Data represent means ± S.D. $n = 3$. Statistical significance of differences was analyzed with one-way or two-way analysis of variance (ANOVA) with repeated measures, followed by Tukey's multiple comparison test. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus day 3.

periodontal disease is under consideration [21, 22]. The significant anabolic actions of FGF2 in bone have been widely demonstrated in several animal models; see, for example, growth plate and trabecular bone in growing rats that received daily intravenous injections of FGF2 [23]. Local injections of FGF2 over the calvaria increase new bone formation in mice [24], and those into osteotomized sites of the tibia accelerate surgical fracture repair in rabbits [21]. FGF2 also has an ability to prevent trabecular bone loss in the vertebrae of ovariectomized rats possibly by increasing osteoadipogenic cell proliferation [25]. *Fgf2*-null ($Fgf2^{-/-}$) mice exhibit a significant decrease of femoral trabecular bone volume and bone formation rate [26]. This can be explained by a downregulation of BMP-2 in $Fgf2^{-/-}$

osteoblasts, resulting in a decrease in ALP activity and nuclear accumulation of the master transcription factor of osteoblastogenesis Runx2 [27]. Furthermore, an inverse correlation between adipogenesis and osteogenesis is observed in *Fgf* $^{-/-}$ mice, and FGF2 blocks adipocyte formation and increases ALP-positive colony formation in bone marrow cell cultures independent of FGF2 [28]. In FGF2, most attention has been dedicated to the smallest 18-kDa variant (LMW). In addition, genetic manipulation of LMW FGF2 in skeletal tissues contributes to bone phenotypes *in vivo* [29]. However, there are several higher molecular weight (HMW) variants of the polypeptide. Additional information on the representative roles of the HMW variants in bone is shown below.

Compared with FGF2, other canonical FGFs have not been studied in detail (Table 1). Although *Fgf1* expression was not obvious in our model, its transcript appears to act in the same manner as FGF2 [30]. Intravenous administration of FGF1 increases bone formation of femoral diaphysis in normal rats [30] and tibial metaphysis in ovariectomized rats [24]. However, *Fgf1*$^{-/-}$ mice do not display any gross phenotypic defects [31]. Because deficiency of FGF1 in mice exacerbated high-fat diet-induced diabetic phenotypes, such as insulin resistance and defects in adipose remodeling in gonadal white adipose tissue, FGF1, may directly and/or indirectly act on bone. FGF4 is more specific to mesenchymal cells, but its subcutaneous injections increase trabecular bone mineral density in the mouse femur [32]. Much less is known about the roles of FGF6 [33], FGF7 [34], and FGF8 [35] in bone; the expression of *Fgf7* but not of *Fgf6* and *Fgf8* is detected in our calvaria cell model, and FGF6 shows catabolic effects on osteoblastic cells, but others have anabolic function *in vitro*. Histological evidence for chondrogenesis with the upregulation of the *Sox9* and *Col2a1* genes is seen in cranial mesenchymal cells of transgenic mice overexpressing FGF9, suggesting that FGF9 converts intramembranous ossification to endochondral ossification [36]. FGF9 also shows supportive effects on FGF2-dependent trabecular bone formation [37]. Among *Fgfs* expressed in our model, *Fgf9* is abundant during the late developmental stages, along with *Fgf23* levels (Figure 2). Notably, both mRNA levels are upregulated by 1,25$(OH)_2D_3$, while only *Fgf9* levels are suppressed by pretreatment of cycloheximide, a protein synthesis inhibitor, as well as the transcriptional inhibitor actinomycin D (Figure 3). Thus, 1,25$(OH)_2D_3$-dependent expression of *Fgf9* but not *Fgf23* may result from *de novo* protein synthesis. Additional role(s) and the precise regulatory mechanism of FGF9 in osteoblast functions remain to be elucidated. Functional anomalies in FGF10 signals may be involved in craniosynostosis [38], but there are no obvious effects of FGF10 in our rat (unpublished data) and mouse calvaria cells [39]. Treatment of mouse calvaria cells with FGF18 promotes proliferation and suppresses differentiation and matrix mineralization [39]. In *Fgf18*$^{-/-}$ mouse embryos, calvaria cell proliferation and bone mineralization and kyphosis are observed in the cervical and upper thoracic spine [40]. Together with the observation that treatment of mouse calvaria cells with FGF18 increases proliferation and decreases matrix mineralization [39], the effects of this polypeptide on bone formation appear to be similar to those of FGF2.

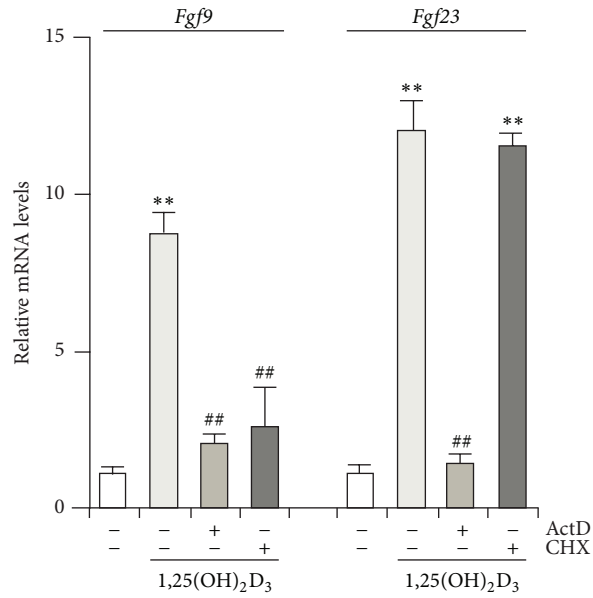


FIGURE 3: 1,25$(OH)_2D_3$ increases *Fgf9* and *Fgf23* gene expression at late development stages in rat calvaria cell cultures. Rat calvaria cells were obtained as shown in Figure 2. At day 11, nodule-forming cells were stripped by collagenase and replated (subcultures). Four days later, osteoblast subcultures were pretreated with or without actinomycin D (ActD) or cycloheximide (CHX), followed by incubation with 1 nM 1,25$(OH)_2D_3$ for 6 h. See the above mentioned for qPCR. Data represent means ± S.D. $n = 3$. Statistical significance of differences was analyzed with one-way or two-way analysis of variance (ANOVA) with repeated measures, followed by Tukey's multiple comparison test. $^{**}P < 0.01$ versus vehicle alone; $^{\#\#}P < 0.01$ versus 1,25$(OH)_2D_3$ alone.

4. Physiological and Pathological Importance of FGFRs in Bone

The dynamics of FGFRs are also an important determinant of FGF-mediated bone formation. Indeed, mutations in FGFR1 and FGFR2 account for the craniosynostosis and chondrodysplasia syndromes in humans [41–44], suggesting that both FGFRs are important for endochondral and intramembranous bone formation. Because *Fgfr1*$^{-/-}$ mice are embryonic lethal shortly after gastrulation [45], osteochondrocyte lineage- and osteoblast-specific FGFR1 knockout mice were generated under the control of the *proα1(II) collagen (Col2)* and *proα1(I) collagen (Col1)* promoters, respectively. *Col2*-mediated FGFR1 inactivation delays chondrocyte and osteoblast maturation, while *Col1*-dependent FGFR1 deficiency accelerates osteoblast differentiation with stimulated mineral deposition and reduces osteoclast activity [46]. Gain-of-function missense mutations in *Fgfr2* (S252W and P253R) cause craniosynostosis syndromes, including Crouzon and Apert syndromes [47, 48]. Indeed, heterozygous *Fgfr2* (S252W) mutant mice show midline sutural

Table 1: Roles of FGFs in bone.

FGFs		Models						
		In vivo		*Ex vivo*	*In vitro*	Outcomes	Animals	Ref.
Types	Members	Genetic manipulations	Recombinant proteins		Recombinant proteins and so forth			
Canonical	FGF1	Systemic deletion				No obvious effects	Mice	[31]
			Injections over the calvaria			Osteoblastic cell proliferation and new bone formation ↑	Mice	[24]
			Intravenous injections			Prevention of the ovariectomized (OVX)-related bone loss	OVX Rats	
					Osteoblasts	Cell proliferation ↑, but nodule formation and mineralization ↓	Rats	[30]
	FGF2	Systemic deletion		Calvaria cells		Cell proliferation ↓	Mice	[26]
				Bone marrow cells		ALP-positive colonies and mineralized nodules ↓	Mice	[26, 28]
						Trabecular bone ↓		
		Osteoblast-specific expression of human HMW FGF2				Dwarfism, osteomalacia, none mineral density ↓, serum phosphate levels, and FGF23 expression ↑	Mice	[95]
				Bone marrow cells		ALP-positive colonies and mineralized nodules ↑		
		Osteoblast-specific expression of human LMW FGF2				Increased bone formation ↑, sFRP-1 expression ↓	Mice	[29]
		Systemic deletion of human LMW FGF2		Bone marrow cells		Reverse effects as above		
			Intravenous injections			Growth plate width and trabecular bone ↑ and periosteal bone ↓	Rats	[23]
			Subcutaneously injections			Osteoid volume in lumbar vertebra ↑	OVX Rats	[25]
			Injections over the calvaria			Osteoblastic cell proliferation and new bone formation ↑	Mice	[24]
			Single local injection into the distracted callus			Bone formation in the callotasis model ↑	Rabbits	[21]
			Subcutaneous transplantations of human bone marrow cells treated with FGF2			New bone formation in trabecular bone ↑	Nude mice	[37]

Table 1: Continued.

FGFs		Models						
		In vivo		*Ex vivo*	*In vitro*	Outcomes	Animals	Ref.
Types	Members	Genetic manipulations	Recombinant proteins		Recombinant proteins and so forth			
					Bone marrow cells	Cell proliferation and matrix mineralization ↑	Humans	
						Cell proliferation ↑, matrix mineralization ↓	Mice	[39]
					Calvaria cells	Differentiation stage-specific effects; cell proliferation ↑, Osteogenic differentiation ↓ in less mature cells Matrix mineralization ↑ in more mature cells	Humans	[20]
					Osteoblasts from trabecular bone	Cell proliferation ↑, ALP activity, and matrix mineralization ↓	Humans	[33]
	FGF4		Subcutaneous injections			Bone formation ↑	Mice	[32]
	FGF6				Osteoblasts from trabecular bone	Cell proliferation ↑, ALP activity, and matrix mineralization ↓	Humans	[33]
	FGF7				Embryonic stem cells	Mineralized nodules and osteoblast marker gene expression ↑	Mice	[34]
	FGF8				Osteogenic ROB-26 cells	ALP activity and *Runx2* expression ↑	Rats	[35]
	FGF9		Subcutaneous transplantations of human bone marrow cells treated with FGF2 plus FGF9			Effect of FGF2 on new bone formation in trabecular bone ↑	Nude mice	[37]
					Bone marrow cells	Effect of FGF2 on cell proliferation and mineralization ↑	Humans	
	FGF10	Systemic deletion in *Fgfr2* mutant mice				Rescue of craniosynostosis and skeletal defects	Mice	[38]
					Calvaria cells	No obvious effects	Mice	[39]

Table 1: Continued.

FGFs		Models						
		In vivo		*Ex vivo*	*In vitro*	Outcomes	Animals	Ref.
Types	Members	Genetic manipulations	Recombinant proteins		Recombinant proteins and so forth			
	FGF18	Systemic deletion				Skeletal defects, proliferation of osteogenic cells, and maturation of osteoblasts ↓	Mice	[40]
					Calvaria cells	Cell proliferation ↑, matrix mineralization ↓	Mice	[39]
Hormone-like	FGF21	Overexpression				Trabecular bone ↓	Mice	[92]
		Systemic deletion				Reverse effects as above		
	FGF23	Systemic deletion				Bone mineralization ↓ with hyperphosphatemia	Mice	[81]
		Overexpression				Bone abnormality with hypophosphatemia and serum PTH levels ↑	Mice	[57]
		Osteoblast-specific overexpression of FGF23					Mice	[58]
					Calvaria cells with adenoviral FGF23 overexpression	Osteogenic differentiation and matrix mineralization ↓	Rats	[9]
					Osteoblastic MC3T-E1 cells	Cell proliferation ↑, matrix mineralization ↓	Mice	[87]

↑: increase; ↓: decrease. Ref.: References.

bone defects and craniosynostosis with abnormal osteoblastic proliferation and differentiation [49]. An *in vitro* study shows that constitutively active FGFR2 (S252W) induces the ERK1/2 and PKC pathways causing osteoblastic differentiation in the murine mesenchymal cell line C3H10T1/2 [50]. Three of the *Fgfr3* gain-of-function mutations have been reported to cause chondrodysplasia and craniosynostosis. Achondroplasia, the most common form of human dwarfism, is associated with the G380R mutation [51]. The P250R mutation causes Muenke syndrome, a common syndrome of craniosynostosis [52]. Crouzon syndrome and acanthosis nigricans, a skin pigmentation disorder, result from the A391E mutation [53]. Unlike FGFR1 and FGFR2 deficient mice, systemic *Fgfr3* null mice are viable and show progressive osteodysplasia with expanded growth plate cartilage [54]. Taken together, because FGF9, a preferred ligand for FGFR3, upregulates *osteopontin* (*Opn*) in chicken chondrocytes [55], FGFR3 signaling may affect chondrocytes rather than osteoblasts [54]. In contrast to these three FGFRs, there are quite a few reports about the relationship between FGFR4 and bone formation. Cool et al. indicated that FGFR4 is expressed in preosteoblasts and osteoblasts in neonatal mouse calvaria, suggesting that FGFR4 is involved in osteogenesis [56], but its role in bone remains unclear.

5. FGF23 and FGF19 Subfamily Members as Hormone-Like Factors

FGF23 is the last member of the FGF family, and its significant roles in Pi and vitamin D metabolism are obvious in genetically engineered mice [57–59] (also see review [60]). FGF23 was originally discovered as the gene responsible for autosomal dominant hypophosphatemic rickets [61] and thereafter as a phosphaturic factor produced by mesenchymal tumors in tumor-induced osteomalacia [62]. FGF23 is predominately expressed in osteoblasts/osteocytes [63–66]. Type I transmembrane protein αKlotho acts as a coreceptor for FGF23 to convert canonical FGFRs (FGFR1c, FGFR3c, and FGFR4) into a specific receptor for FGF23 [67, 68]. Therefore, organs expressing αKlotho, such as the kidney, parathyroid glands, and choroid plexus, appear to be targets of FGF23 [69]. FGF23 decreases the expression of renal type II sodium-phosphate cotransporters (*Slc34a1* and *Slc34a3*) and 25-hydroxyvitamin D_3 ($25(OH)D_3$) 1α-hydroxylase, resulting in a decrease in serum Pi and $1,25(OH)_2D_3$ levels, respectively, in mice and rats [70, 71]. Meanwhile, $1,25(OH)_2D_3$ induces *Fgf23* expression in rat osteosarcoma ROS17/2.8 cells [72] as well as our rat calvaria cells [73]. Together with the result that intraperitoneal injections of $1,25(OH)_2D_3$ into mice increase serum FGF23 levels, there seems to be a feedback loop between FGF23 and $1,25(OH)_2D_3$ [72]. FGF23 also decreases the expression of PTH [74], although this is not simply regulated by the FGF23-αKlotho axis [75]. Transgenic mice expressing constitutively active PTHR1 in osteocytes exhibit increased serum FGF23 levels independently of serum Ca and Pi levels and *Fgf23* expression in osteoblasts and osteocytes [76]. Comparison of *Fgfr1/3/4* single and double knockout mice indicates that FGFR1 and FGFR3/4 may be involved in renal Pi reabsorption [70] and vitamin D metabolism [77], respectively. Additional factors, for example, Pi [78], sympathetic activation [79], and circulating αKlotho [80], may be involved in FGF23 expression/production; however, the regulation of FGF23 expression is still under investigation.

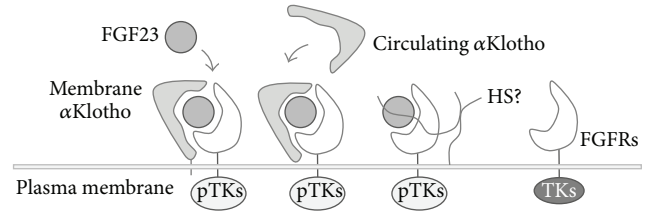


Figure 4: Possible klotho-dependent and klotho-independent mechanisms of FGF23 actions. FGF23 may activate FGFR tyrosine kinases with or without membrane and circulating αKlotho. TKs: nonphosphorylated tyrosine kinases.

Both of ectopic (hepatic) overexpression and osteoblast/osteocyte-specific overexpression of the *Fgf23* transgene result in lower bone mineral density of the femur with hypophosphatemia and high serum levels of PTH [57, 58]. The lack of either FGF23 or αKlotho causes aberrant Ca/Pi and vitamin D metabolism, thus ensuring skeletal anomalies and ectopic calcification [59, 81, 82]. $Fgf23^{-/-}/Opn^{-/-}$ double-knockout (DKO) mice mimic hyperphosphatemia in $Fgf23^{-/-}$ mice, but the severe osteoidosis in $Fgf23^{-/-}$ is markedly reduced [83]. $Fgf23^{-/-}/Slc34a1^{-/-}$ DKO mice reverse hyper- to hypophosphatemia in keeping with hypomineralization in bone [84]. These observations suggest that skeletal anomalies that involve FGF23 may result not only from serum Pi levels but also from intrinsic anomalies in bone. FGF23 may act independently of the membrane protein αKlotho (Figure 4). For example, overexpression of FGF23 in cultured rat calvaria cells impairs osteoblast differentiation and mineralized matrix formation but not mineralization, via activation of FGFR1 [9]. One plausible explanation is that the existence of the soluble form (circulating αKlotho) shedding from the extracellular domain of αKlotho [85, 86] may act as a cofactor for FGF23. In fact, effects of FGF23 in MC3T3-E1 cells (a mouse osteoblastic cell line) cultured with circulating αKlotho [87] mimic the results observed in rat calvaria cells [9]. In mouse chondrocytes, FGF23 activates FRS2α, FGFR substrate 2α, and ERK1/2, resulting in a decrease in chondrocyte proliferation in the presence of circulating αKlotho [88]. In contrast, αKlotho is not required for FGF23 action in some cells. For instance, FGF23 can induce the hypertrophy of neonatal rat ventricular cardiomyocytes, in which αKlotho is not detected [89]. In addition, FGF23 decreases PTH secretion in thyroparathyroid organ cultures from parathyroid-specific αKlotho-deficient mice [75]. It is still unknown why FGF23 targets the kidney and parathyroid glands, even in the presence of circulating αKlotho and/or the ubiquitous expression of FGFRs.

The roles of two other members of the hormone-like FGF19 subfamily, FGF19 and FGF21, in bone formation remain to be elucidated. *Fgf19* transcripts are predominantly expressed in the ileum, while *Fgf21* mRNA is expressed in the liver, pancreas, and white adipose tissue [90]. In skeletal tissue under normal conditions, FGF19, but not FGF21, is also detectable at the protein level in human fetal growth plate cartilage [91]. Interestingly, the treatment of mouse bone marrow cells with FGF21 increases *βKlotho* and *Fgf21* mRNA expression, especially in the presence of rosiglitazone [92], an agonist of the master regulator for adipogenesis, PPARγ, possibly affecting bone formation. Thus, genetic FGF21 loss and gain of function in mice increase and decrease bone mass [92], respectively, suggesting that FGF21/βKlotho may act as an inhibitor of bone formation.

6. Local and Systemic Effects of FGFs during Bone Formation, Focusing on FGF2, FGF21, and FGF23

As above, FGF2 and FGF23 may exhibit distinct activities during different stages of osteoblast differentiation, such as cell proliferation versus matrix (osteoid) mineralization. In contrast to osteogenic cell proliferation, differentiation, and associated matrix formation, the molecular mechanism(s) underlying matrix mineralization remains to be fully elucidated. Human FGF2 has multiple isoforms via an alternative initiation of translation at CUG codons from a single *FGF2* gene: LMW and high (HMW FGF2, 22-kDa, 22.5-kDa, 24-kDa, and 34-kDa) molecular forms [93]. LMW FGF2—exactly the same FGF2 as described above—is predominantly expressed in osteoblast precursors and activates intracellular signaling via FGFR in an autocrine/paracrine manner. While recent evidence indicates that extracellular LMW FGF2 can translocate to the nucleus after internalization [94], there is little evidence for this process in bone to date. The HMW FGF2 isoforms are not released from the cells and localized to the nucleus and regulate gene expression to exert specific effects. Transgenic mice overexpressing human HMW FGF2 (22-kDa, 23-kDa, and 24-kDa) under the *Col1* promoter (*Col3.6*) exhibit lower bone mineral density with decreased bone formation and increased bone resorption [95]. Interestingly, upregulation of *Fgf23* expression and hypophosphatemia are observed in these mice [95]. These observations may lead to the development of an additional framework for understanding the effects of the HMW FGF2 and FGF23 on bone mineralization.

It is well known that elevated serum FGF23 levels are the most common predictor in patients with chronic kidney disease [96]. Serum FGF23 levels are positively correlated to aortic arterial calcification in hemodialysis patients [97]. Recent studies demonstrate that FGF23 exacerbates left ventricle hypertrophy where αKlotho might not be expressed [89] and elevated plasma FGF23 levels are associated with low body mass index and dyslipidemia in dialysis patients [98]. Thus, systemic actions of FGF23 may reach organs dependently and independently of αKlotho. Although skeletal tissues do not express *Fgf21* under normal conditions, circulating FGF21 seems to suppress osteoblastogenesis and induce adipogenesis [92]. Also, FGF21 itself enhances *Fgf21* and *βKlotho* expression in bone marrow-derived adipocytes, and increases in FGF21 and βKlotho have a synergetic effect on its signaling in local area [92]. Comprehensive analyses are needed to determine the local versus systemic effects of FGF21 on bone. Taken all together, FGFs expressed in bone are involved in bone formation directly and indirectly, which indicates that FGFs mediate the interrelationships between bone and other organs under normal and/or clinical situations. The clinical importance of FGF23/21 is now becoming clearer owing to the recent findings in FGF research. However, precise elucidation of FGF mechanisms is still required.

7. Conclusion

The skeleton is a multipotent organ that is fundamental for the survival of vertebrates. Bone and mineral homeostasis are strictly controlled by multiple mechanisms including FGF/FGFR signaling. Canonical and hormone-like FGFs regulate bone formation at different developmental stages in different ways, and these members may compensate for one another in bone and/or extraskeletal tissues. In order to understand these mechanisms, the balance between local and systemic regulation needs to be considered.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] X. Feng and J. M. McDonald, "Disorders of bone remodeling," *Annual Review of Pathology*, vol. 6, pp. 121–145, 2011.

[2] M. Ferron, M. D. McKee, R. L. Levine, P. Ducy, and G. Karsenty, "Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice," *Bone*, vol. 50, no. 2, pp. 568–575, 2012.

[3] L. M. Calvi, G. B. Adams, K. W. Weibrecht et al., "Osteoblastic cells regulate the haematopoietic stem cell niche," *Nature*, vol. 425, no. 6960, pp. 841–846, 2003.

[4] J. Zhang, C. Niu, L. Ye et al., "Identification of the haematopoietic stem cell niche and control of the niche size," *Nature*, vol. 425, no. 6960, pp. 836–841, 2003.

[5] H. Iwasaki and T. Suda, "Cancer stem cells and their niche," *Cancer Science*, vol. 100, no. 7, pp. 1166–1172, 2009.

[6] S. Takeda, F. Elefteriou, R. Levasseur et al., "Leptin regulates bone formation via the sympathetic nervous system," *Cell*, vol. 111, no. 3, pp. 305–317, 2002.

[7] C. Collet, C. Schiltz, V. Geoffroy, L. Maroteaux, J.-M. Launay, and M.-C. de Vernejoul, "The serotonin 5-HT_{2B} receptor controls bone mass via osteoblast recruitment and proliferation," *The FASEB Journal*, vol. 22, no. 2, pp. 418–427, 2008.

[8] M. Kuro-o, "Endocrine FGFs and Klothos: emerging concepts," *Trends in Endocrinology & Metabolism*, vol. 19, no. 7, pp. 239–245, 2008.

[9] H. Wang, Y. Yoshiko, R. Yamamoto et al., "Overexpression of fibroblast growth factor 23 suppresses osteoblast differentiation

and matrix mineralization in vitro," *Journal of Bone and Mineral Research*, vol. 23, no. 6, pp. 939–948, 2008.

[10] R. T. Böttcher and C. Niehrs, "Fibroblast growth factor signaling during early vertebrate development," *Endocrine Reviews*, vol. 26, no. 1, pp. 63–77, 2005.

[11] B. Thisse and C. Thisse, "Functions and regulations of fibroblast growth factor signaling during embryonic development," *Developmental Biology*, vol. 287, no. 2, pp. 390–402, 2005.

[12] H. Kurosu and M. Kuro-O, "Endocrine fibroblast growth factors as regulators of metabolic homeostasis," *BioFactors*, vol. 35, no. 1, pp. 52–60, 2009.

[13] M. Mohammadi, S. K. Olsen, and O. A. Ibrahimi, "Structural basis for fibroblast growth factor receptor activation," *Cytokine and Growth Factor Reviews*, vol. 16, no. 2, pp. 107–137, 2005.

[14] L. Dailey, D. Ambrosetti, A. Mansukhani, and C. Basilico, "Mechanisms underlying differential responses to FGF signaling," *Cytokine and Growth Factor Reviews*, vol. 16, no. 2, pp. 233–247, 2005.

[15] N. Hacohen, S. Kramer, D. Sutherland, Y. Hiromi, and M. A. Krasnow, "*Sprouty* encodes a novel antagonist of FGF signaling that patterns apical branching of the *Drosophila* airways," *Cell*, vol. 92, no. 2, pp. 253–263, 1998.

[16] M. Fürthauer, F. Reifers, M. Brand, B. Thisse, and C. Thisse, "*Sprouty4* acts in vivo as a feedback-induced antagonist of FGF signaling in zebrafish," *Development*, vol. 128, no. 12, pp. 2175–2186, 2001.

[17] M. Fürthauer, W. Lin, S.-L. Ang, B. Thisse, and C. Thisse, "Sef is a feedback-induced antagonist of RAs/MAPK-mediated FGF signalling," *Nature Cell Biology*, vol. 4, no. 2, pp. 170–174, 2002.

[18] Y. Hirate and H. Okamoto, "Canopy1, a novel regulator of FGF signaling around the midbrain-hindbrain boundary in zebrafish," *Current Biology*, vol. 16, no. 4, pp. 421–427, 2006.

[19] T. Matsui, S. Thitamadee, T. Murata et al., "Canopy1, a positive feedback regulator of FGF signaling, controls progenitor cell clustering during Kupffer's vesicle organogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 24, pp. 9881–9886, 2011.

[20] F. Debiais, M. Hott, A. M. Graulet, and P. J. Marie, "The effects of fibroblast growth factor-2 on human neonatal calvaria osteoblastic cells are differentiation stage specific," *Journal of Bone and Mineral Research*, vol. 13, no. 4, pp. 645–654, 1998.

[21] H. Okazaki, T. Kurokawa, K. Nakamura, T. Matsushita, K. Mamada, and H. Kawaguchi, "Stimulation of bone formation by recombinant fibroblast growth factor-2 in callotasis bone lengthening of rabbits," *Calcified Tissue International*, vol. 64, no. 6, pp. 542–546, 1999.

[22] M. Kitamura, M. Akamatsu, M. MacHigashira et al., "FGF-2 stimulates periodontal regeneration: results of a multi-center randomized clinical trial," *Journal of Dental Research*, vol. 90, no. 1, pp. 35–40, 2011.

[23] H. Nagai, R. Tsukuda, and H. Mayahara, "Effects of basic fibroblast growth factor (bFGF) on bone formation in growing rats," *Bone*, vol. 16, no. 3, pp. 367–373, 1995.

[24] C. R. Dunstan, R. Boyce, B. F. Boyce et al., "Systemic administration of acidic fibroblast growth factor (FGF-1) prevents bone loss and increases new bone formation in ovariectomized rats," *Journal of Bone and Mineral Research*, vol. 14, no. 6, pp. 953–959, 1999.

[25] J. I. Aguirre, M. E. Leal, M. F. Rivera, S. M. Vanegas, M. Jorgensen, and T. J. Wronski, "Effects of basic fibroblast growth factor and a prostaglandin E2 receptor subtype 4 agonist on osteoblastogenesis and adipogenesis in aged ovariectomized rats," *Journal of Bone and Mineral Research*, vol. 22, no. 6, pp. 877–888, 2007.

[26] A. Montero, Y. Okada, M. Tomita et al., "Disruption of the fibroblast growth factor-2 gene results in decreased bone mass and bone formation," *Journal of Clinical Investigation*, vol. 105, no. 8, pp. 1085–1093, 2000.

[27] T. Naganawa, L. Xiao, J. D. Coffin et al., "Reduced expression and function of bone morphogenetic protein-2 in bones of *Fgf2* null mice," *Journal of Cellular Biochemistry*, vol. 103, no. 6, pp. 1975–1988, 2008.

[28] L. Xiao, T. Sobue, A. Esliger et al., "Disruption of the *Fgf2* gene activates the adipogenic and suppresses the osteogenic program in mesenchymal marrow stromal stem cells," *Bone*, vol. 47, no. 2, pp. 360–370, 2010.

[29] L. Xiao, P. Liu, X. Li et al., "Exported 18-kDa isoform of fibroblast growth factor-2 is a critical determinant of bone mass in mice," *Journal of Biological Chemistry*, vol. 284, no. 5, pp. 3170–3182, 2009.

[30] K. T. Tang, C. Capparelli, J. L. Stein et al., "Acidic fibroblast growth factor inhibits osteoblast differentiation in vitro: altered expression of collagenase, cell growth-related, and mineralization-associated genes," *Journal of Cellular Biochemistry*, vol. 61, no. 1, pp. 152–166, 1996.

[31] D. L. Miller, S. Ortega, O. Bashayan, R. Basch, and C. Basilico, "Compensation by fibroblast growth factor 1 (FGF1) does not account for the mild phenotypic defects observed in FGF2 null mice," *Molecular and Cellular Biology*, vol. 20, no. 6, pp. 2260–2268, 2000.

[32] S. Kuroda, S. Kasugai, S. Oida, T. Iimura, K. Ohya, and T. Ohyama, "Anabolic effect of aminoterminally truncated fibroblast growth factor 4 (FGF4) on bone," *Bone*, vol. 25, no. 4, pp. 431–437, 1999.

[33] M. Bosetti, M. Leigheb, R. A. Brooks, F. Boccafoschi, and M. F. Cannas, "Regulation of osteoblast and osteoclast functions by FGF-6," *Journal of Cellular Physiology*, vol. 225, no. 2, pp. 466–471, 2010.

[34] Y.-M. Jeon, S.-H. Kook, S.-J. Rho et al., "Fibroblast growth factor-7 facilitates osteogenic differentiation of embryonic stem cells through the activation of ERK/Runx2 signaling," *Molecular and Cellular Biochemistry*, vol. 382, no. 1-2, pp. 37–45, 2013.

[35] K. Omoteyama and M. Takagi, "FGF8 regulates myogenesis and induces Runx2 expression and osteoblast differentiation in cultured cells," *Journal of Cellular Biochemistry*, vol. 106, no. 4, pp. 546–552, 2009.

[36] V. Govindarajan and P. A. Overbeek, "FGF9 can induce endochondral ossification in cranial mesenchyme," *BMC Developmental Biology*, vol. 6, article 7, 2006.

[37] T. Kizhner, D. Ben-David, E. Rom, A. Yayon, and E. Livne, "Effects of FGF2 and FGF9 on osteogenic differentiation of bone marrow-derived progenitors," *In Vitro Cellular and Developmental Biology—Animal*, vol. 47, no. 4, pp. 294–301, 2011.

[38] M. K. Hajihosseini, R. Duarte, J. Pegrum et al., "Evidence that Fgf10 contributes to the skeletal and visceral defects of an apert syndrome mouse model," *Developmental Dynamics*, vol. 238, no. 2, pp. 376–385, 2009.

[39] T. Shimoaka, T. Ogasawara, A. Yonamine et al., "Regulation of osteoblast, chondrocyte, and osteoclast functions by fibroblast growth factor (FGF)-18 in comparison with FGF-2 and FGF-10," *The Journal of Biological Chemistry*, vol. 277, no. 9, pp. 7493–7500, 2002.

[40] N. Ohbayashi, M. Shibayama, Y. Kurotaki et al., "FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis," *Genes and Development*, vol. 16, no. 7, pp. 870–879, 2002.

[41] L. Chen and C.-X. Deng, "Roles of FGF signaling in skeletal development and human genetic diseases," *Frontiers in Bioscience*, vol. 10, no. 2, pp. 1961–1976, 2005.

[42] G. M. Morriss-Kay and A. O. M. Wilkie, "Growth of the normal skull vault and its alteration in craniosynostosis: insights from human genetics and experimental studies," *Journal of Anatomy*, vol. 207, no. 5, pp. 637–653, 2005.

[43] D. M. Ornitz and P. J. Marie, "FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease," *Genes & Development*, vol. 16, no. 12, pp. 1446–1465, 2002.

[44] D. M. Ornitz, "FGF signaling in the developing endochondral skeleton," *Cytokine and Growth Factor Reviews*, vol. 16, no. 2, pp. 205–213, 2005.

[45] T. P. Yamaguchi, K. Harpal, M. Henkemeyer, and J. Rossant, "*fgfr*-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation," *Genes and Development*, vol. 8, no. 24, pp. 3032–3044, 1994.

[46] A. L. Jacob, C. Smith, J. Partanen, and D. M. Ornitz, "Fibroblast growth factor receptor 1 signaling in the osteo-chondrogenic cell lineage regulates sequential steps of osteoblast maturation," *Developmental Biology*, vol. 296, no. 2, pp. 315–328, 2006.

[47] E. W. Jabs, X. Li, A. F. Scott et al., "Jackson-Weiss and Crouzon syndromes are allelic with mutations in fibroblast growth factor receptor 2," *Nature Genetics*, vol. 8, no. 3, pp. 275–279, 1994.

[48] A. O. M. Wilkie, S. F. Slaney, M. Oldridge et al., "Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome," *Nature Genetics*, vol. 9, no. 2, pp. 165–172, 1995.

[49] Y. Wang, R. Xiao, F. Yang et al., "Abnormalities in cartilage and bone development in the Apert syndrome FGFR2$^{+/S252W}$ mouse," *Development*, vol. 132, no. 5, pp. 3537–3548, 2005.

[50] H. Miraoui, K. Oudina, H. Petite, Y. Tanimoto, K. Moriyama, and P. J. Marie, "Fibroblast growth factor receptor 2 promotes osteogenic differentiation in mesenchymal cells via ERK1/2 and protein kinase C signaling," *The Journal of Biological Chemistry*, vol. 284, no. 8, pp. 4897–4904, 2009.

[51] F. Rousseau, J. Bonaventure, L. Legeai-Mallet et al., "Mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia," *Nature*, vol. 371, no. 6494, pp. 252–254, 1994.

[52] M. Muenke, K. W. Gripp, D. M. McDonald-McGinn et al., "A unique point mutation in the fibroblast growth factor receptor 3 gene (FGFR3) defines a new craniosynostosis syndrome," *The American Journal of Human Genetics*, vol. 60, no. 3, pp. 555–564, 1997.

[53] G. A. Meyers, S. J. Orlow, I. R. Munro, K. A. Przylepa, and E. W. Jabs, "Fibroblast growth factor receptor 3 (FGFR3) transmembrane mutation in Crouzon syndrome with acanthosis nigricans," *Nature Genetics*, vol. 11, no. 4, pp. 462–464, 1995.

[54] C. Deng, A. Wynshaw-Boris, F. Zhou, A. Kuo, and P. Leder, "Fibroblast growth factor receptor 3 is a negative regulator of bone growth," *Cell*, vol. 84, no. 6, pp. 911–921, 1996.

[55] S. Weizmann, A. Tong, A. Reich, O. Genina, A. Yayon, and E. Monsonego-Ornan, "FGF upregulates osteopontin in epiphyseal growth plate chondrocytes: implications for endochondral ossification," *Matrix Biology*, vol. 24, no. 8, pp. 520–529, 2005.

[56] S. Cool, R. Jackson, P. Pincus, I. Dickinson, and V. Nurcombe, "Fibroblast growth factor receptor 4 (FGFR4) expression in newborn murine calvaria and primary osteoblast cultures," *International Journal of Developmental Biology*, vol. 46, no. 4, pp. 519–523, 2002.

[57] X. Bai, D. Miao, J. Li, D. Goltzman, and A. C. Karaplis, "Transgenic mice overexpressing human fibroblast growth factor 23 (R176Q) delineate a putative role for parathyroid hormone in renal phosphate wasting disorders," *Endocrinology*, vol. 145, no. 11, pp. 5269–5279, 2004.

[58] T. Larsson, R. Marsell, E. Schipani et al., "Transgenic mice expressing fibroblast growth factor 23 under the control of the α1(I) collagen promoter exhibit growth retardation, osteomalacia, and disturbed phosphate homeostasis," *Endocrinology*, vol. 145, no. 7, pp. 3087–3094, 2004.

[59] T. Shimada, M. Kakitani, Y. Yamazaki et al., "Targeted ablation of *Fgf23* demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism," *The Journal of Clinical Investigation*, vol. 113, no. 4, pp. 561–568, 2004.

[60] L. D. Quarles, "Skeletal secretion of FGF-23 regulates phosphate and vitamin D metabolism," *Nature Reviews Endocrinology*, vol. 8, no. 5, pp. 276–286, 2012.

[61] K. E. White, W. E. Evans, J. L. H. O'Riordan et al., "Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23," *Nature Genetics*, vol. 26, no. 3, pp. 345–348, 2000.

[62] T. Shimada, S. Mizutani, T. Muto et al., "Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 11, pp. 6500–6505, 2001.

[63] Y. Yoshiko, H. Wang, T. Minamizaki et al., "Mineralized tissue cells are a principal source of FGF23," *Bone*, vol. 40, no. 6, pp. 1565–1573, 2007.

[64] M. Mirams, B. G. Robinson, R. S. Mason, and A. E. Nelson, "Bone as a source of FGF23: regulation by phosphate?" *Bone*, vol. 35, no. 5, pp. 1192–1199, 2004.

[65] R. Masuyama, I. Stockmans, S. Torrekens et al., "Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts," *The Journal of Clinical Investigation*, vol. 116, no. 12, pp. 3150–3159, 2006.

[66] S. Liu, W. Tang, J. Zhou, L. Vierthaler, and L. D. Quarles, "Distinct roles for intrinsic osteocyte abnormalities and systemic factors in regulation of FGF23 and bone mineralization in Hyp mice," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 293, no. 6, pp. E1636–E1644, 2007.

[67] I. Urakawa, Y. Yamazaki, T. Shimada et al., "Klotho converts canonical FGF receptor into a specific receptor for FGF23," *Nature*, vol. 444, no. 7120, pp. 770–774, 2006.

[68] H. Kurosu, Y. Ogawa, M. Miyoshi et al., "Regulation of fibroblast growth factor-23 signaling by Klotho," *Journal of Biological Chemistry*, vol. 281, no. 10, pp. 6120–6123, 2006.

[69] A. Imura, Y. Tsuji, M. Murata et al., "α-klotho as a regulator of calcium homeostasis," *Science*, vol. 316, no. 5831, pp. 1615–1618, 2007.

[70] J. Gattineni, C. Bates, K. Twombley et al., "FGF23 decreases renal NaPi-2a and NaPi-2c expression and induces hypophosphatemia in vivo predominantly via FGF receptor 1," *The American Journal of Physiology—Renal Physiology*, vol. 297, no. 2, pp. F282–F291, 2009.

[71] H. Saito, K. Kusano, M. Kinosaki et al., "Human fibroblast growth factor-23 mutants suppress Na^+-dependent phosphate

co-transport activity and 1α,25-dihydroxyvitamin D_3 production," *The Journal of Biological Chemistry*, vol. 278, no. 4, pp. 2206–2211, 2003.

[72] S. Liu, W. Tang, J. Zhou et al., "Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D," *Journal of the American Society of Nephrology*, vol. 17, no. 5, pp. 1305–1315, 2006.

[73] R. Yamamoto, T. Minamizaki, Y. Yoshiko et al., "1α,25-dihydroxyvitamin D_3 acts predominately in mature osteoblasts under conditions of high extracellular phosphate to increase fibroblast growth factor 23 production *in vitro*," *Journal of Endocrinology*, vol. 206, no. 3, pp. 279–286, 2010.

[74] I. Z. Ben-Dov, H. Galitzer, V. Lavi-Moshayoff et al., "The parathyroid is a target organ for FGF23 in rats," *The Journal of Clinical Investigation*, vol. 117, no. 12, pp. 4003–4008, 2007.

[75] H. Olauson, K. Lindberg, R. Amin et al., "Parathyroid-specific deletion of Klotho unravels a novel calcineurin-dependent FGF23 signaling pathway that regulates PTH secretion," *PLoS Genetics*, vol. 9, no. 12, Article ID e1003975, 2013.

[76] Y. Rhee, N. Bivi, E. Farrow et al., "Parathyroid hormone receptor signaling in osteocytes increases the expression of fibroblast growth factor-23 in vitro and in vivo," *Bone*, vol. 49, no. 4, pp. 636–643, 2011.

[77] J. Gattineni, K. Twombley, R. Goetz, M. Mohammadi, and M. Baum, "Regulation of serum 1,25$(OH)_2$ vitamin D_3 levels by fibroblast growth factor 23 is mediated by FGF receptors 3 and 4," *The American Journal of Physiology—Renal Physiology*, vol. 301, no. 2, pp. F371–F377, 2011.

[78] N. Arai-Nunota, M. Mizobuchi, H. Ogata et al., "Intravenous phosphate loading increases fibroblast growth factor 23 in uremic rats," *PLoS ONE*, vol. 9, no. 3, Article ID e91096, 2014.

[79] M. Kawai, S. Kinoshita, S. Shimba, K. Ozono, and T. Michigami, "Sympathetic activation induces skeletal *Fgf23* expression in a circadian rhythm-dependent manner," *The Journal of Biological Chemistry*, vol. 289, no. 3, pp. 1457–1466, 2014.

[80] R. C. Smith, L. M. O'Bryan, E. G. Farrow et al., "Circulating αKlotho influences phosphate handling by controlling FGF23 production," *Journal of Clinical Investigation*, vol. 122, no. 12, pp. 4710–4715, 2012.

[81] M. Kuro-o, Y. Matsumura, H. Aizawa et al., "Mutation of the mouse *klotho* gene leads to a syndrome resembling ageing," *Nature*, vol. 390, no. 6655, pp. 45–51, 1997.

[82] Y. Takei, H. Yamamoto, T. Sato et al., "Stanniocalcin 2 is associated with ectopic calcification in α-klotho mutant mice and inhibits hyperphosphatemia-induced calcification in aortic vascular smooth muscle cells," *Bone*, vol. 50, no. 4, pp. 998–1005, 2012.

[83] Q. Yuan, Y. Jiang, X. Zhao et al., "Increased osteopontin contributes to inhibition of bone mineralization in FGF23-deficient mice," *Journal of Bone and Mineral Research*, vol. 29, no. 3, pp. 693–704, 2014.

[84] D. Sitara, S. Kim, M. S. Razzaque et al., "Genetic evidence of serum phosphate-independent functions of FGF-23 on bone," *PLoS Genetics*, vol. 4, no. 8, Article ID e1000154, 2008.

[85] Y. Matsumura, H. Aizawa, T. Shiraki-Iida, R. Nagai, M. Kuro-O, and Y.-I. Nabeshima, "Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein," *Biochemical and Biophysical Research Communications*, vol. 242, no. 3, pp. 626–630, 1998.

[86] T. Shiraki-Iida, H. Aizawa, Y. Matsumura et al., "Structure of the mouse klotho gene and its two transcripts encoding membrane and secreted protein," *FEBS Letters*, vol. 424, no. 1-2, pp. 6–10, 1998.

[87] V. Shalhoub, S. C. Ward, B. Sun et al., "Fibroblast growth factor 23 (FGF23) and α-klotho stimulate osteoblastic MC3T3.E1 cell proliferation and inhibit mineralization," *Calcified Tissue International*, vol. 89, no. 2, pp. 140–150, 2011.

[88] M. Kawai, S. Kinoshita, A. Kimoto et al., "FGF23 suppresses chondrocyte proliferation in the presence of solubleα-klotho both in vitro and in vivo," *Journal of Biological Chemistry*, vol. 288, no. 4, pp. 2414–2427, 2013.

[89] C. Faul, A. P. Amaral, B. Oskouei et al., "FGF23 induces left ventricular hypertrophy," *Journal of Clinical Investigation*, vol. 121, no. 11, pp. 4393–4408, 2011.

[90] B. Angelin, T. E. Larsson, and M. Rudling, "Circulating fibroblast growth factors as metabolic regulators—a critical appraisal," *Cell Metabolism*, vol. 16, no. 6, pp. 693–705, 2012.

[91] P. Krejci, D. Krakow, P. B. Mekikian, and W. R. Wilcox, "Fibroblast growth factors 1, 2, 17, and 19 are the predominant FGF ligands expressed in human fetal growth plate cartilage," *Pediatric Research*, vol. 61, no. 3, pp. 267–272, 2007.

[92] W. Wei, P. A. Dutchak, X. Wang et al., "Fibroblast growth factor 21 promotes bone loss by potentiating the effects of peroxisome proliferator-activated receptor γ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 8, pp. 3143–3148, 2012.

[93] I. Delrieu, "The high molecular weight isoforms of basic fibroblast growth factor (FGF-2): an insight into an intracrine mechanism," *FEBS Letters*, vol. 468, no. 1, pp. 6–10, 2000.

[94] P. Claus, F. Döring, S. Gringel et al., "Differential intranuclear localization of fibroblast growth factor-2 isoforms and specific interaction with the survival of motoneuron protein," *Journal of Biological Chemistry*, vol. 278, no. 1, pp. 479–485, 2003.

[95] L. Xiao, T. Naganawa, J. Lorenzo, T. O. Carpenter, J. D. Coffin, and M. M. Hurley, "Nuclear isoforms of fibroblast growth factor 2 are novel inducers of hypophosphatemia via modulation of FGF23 and KLOTHO," *The Journal of Biological Chemistry*, vol. 285, no. 4, pp. 2834–2846, 2010.

[96] M. Wolf, "Update on fibroblast growth factor 23 in chronic kidney disease," *Kidney International*, vol. 82, no. 7, pp. 737–747, 2012.

[97] Z. Chen, X. Chen, J. Xie et al., "Fibroblast growth factor 23 is a predictor of aortic artery calcification in maintenance hemodialysis patients," *Renal Failure*, vol. 35, no. 5, pp. 660–666, 2013.

[98] J. R. Montford, M. Chonchol, A. K. Cheung et al., "Low body mass index and dyslipidemia in dialysis patients linked to elevated plasma fibroblast growth factor 23," *American Journal of Nephrology*, vol. 37, no. 3, pp. 183–190, 2013.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2015, Article ID 872193, 8 pages
http://dx.doi.org/10.1155/2015/872193

Research Article

Soluble α-Klotho Serum Levels in Chronic Kidney Disease

Silverio Rotondi,[1] Marzia Pasquali,[1] Lida Tartaglione,[1] Maria Luisa Muci,[1] Giusy Mandanici,[1] Cristiana Leonangeli,[1] Silvia Sales,[1] Alessio Farcomeni,[2] and Sandro Mazzaferro[1]

[1]*Department of Cardiovascular, Respiratory, Nephrology, Geriatric, and Anesthetic Sciences, "Sapienza" University, 5 Piazzale Aldo Moro, 00185 Rome, Italy*
[2]*Department of Public Health and Infectious Diseases, Section of Statistics, "Sapienza" University, 5 Piazzale Aldo Moro, 00185 Rome, Italy*

Correspondence should be addressed to Sandro Mazzaferro; sandro.mazzaferro@uniroma1.it

Received 8 August 2014; Accepted 17 November 2014

Academic Editor: Andrea Del Fattore

Transmembrane α-Klotho (TM-Klotho), expressed in renal tubules, is a cofactor for FGF23-receptor. Circulating soluble-α-Klotho (s-Klotho) results from TM-Klotho shedding and acts on Phosphate (P) and Calcium (Ca) tubular transport. Decreased TM-Klotho, described in experimental chronic kidney disease (CKD), prevents actions of FGF23 and lessens circulating s-Klotho. Thus, levels of s-Klotho could represent a marker of CKD-MBD. To evaluate the clinical significance of s-Klotho in CKD we assayed serum s-Klotho and serum FGF23 in 68 patients (age 58 ± 15; eGFR 45 ± 21 mL/min). s-Klotho was lower than normal (519 ± 183 versus 845 ± 330 pg/mL, $P < .0001$) in renal patients and its reduction was detectable since CKD stage 2 ($P < .01$). s-Klotho correlated positively with eGFR and serum calcium (Cas) and negatively with serum phosphate (Ps), PTH and FGF23. FGF23 was higher than normal (73 ± 51 versus 36 ± 11, $P < .0002$) with significantly increased levels since CKD stage 2 ($P < .001$). Our data indicate a negative effect of renal disease on circulating s-Klotho starting very early in CKD. Assuming that s-Klotho mirrors TM-Klotho synthesis, low circulating s-Klotho seems to reflect the ensuing of tubular resistance to FGF23, which, accordingly, is increased. We endorse s-Klotho as an early marker of CKD-MBD.

1. Introduction

Alpha-Klotho (Klotho) is a transmembrane (TM) protein primarily expressed in the kidney distal tubular cells [1] where it acts as an obligate coreceptor for the bone derived protein fibroblast growth factor-23 (FGF23) [2, 3]. In fact, TM-Klotho is required for FGF23 regulation of both renal handling of phosphate and renal synthesis of calcitriol [4]. At variance with TM-Klotho, soluble α-Klotho (s-Klotho) is the circulating protein resulting from the shedding of the extracellular domain of TM-Klotho operated by two metalloproteinases of the ADAM (a disintegrin and metalloproteinase domain-containing protein) family: ADAM 10 and ADAM 17 [5]. Importantly, s-Klotho also acts as a paracrine substance (with no receptor identified so far) with specific and FGF23 independent renal and extra-renal effects [6, 7]. In particular, s-Klotho inhibits the sodium-phosphate cotransporter NaPi2a expression in the proximal tubules thus generating a phosphaturic effect additive to and independent of FGF23 [7, 8] and activates the ion channel TRPV5 in the distal tubules, thus increasing tubular reabsorption of calcium [9]. In summary, α-Klotho, with its transmembrane and soluble forms, is deeply involved with the physiologic regulation of mineral metabolism [10].

Experimental models of CKD evidence early reduction of renal Klotho mRNA expression and of both TM-Klotho and s-Klotho [11, 12], and this is considered responsible for the development of kidney tubular cell resistance to FGF23. In agreement, in a model of selective Klotho deletion in the distal tubule, bone synthesis of FGF23 increased possibly aimed at overcoming tubular resistance; as a result fractional excretion of phosphate increased and calcitriol synthesis

declined. These animal models have been invoked to describe the early changes that in renal patients anticipate the development of the newly identified CKD-MBD syndrome [13] or, as pointed out in a recent paper, disorder [14].

Available data on serum levels of s-Klotho in CRF patients almost invariably describe reduced values, a finding referred to a primary proportional reduction of TM-Klotho, as described in animal studies [16–18]. Moreover, circulating serum levels of s-Klotho are not always correlated with estimated glomerular filtration rate (eGFR) [19–22]. Excessive variability of some of the commercial kits available for s-Klotho assay in humans, as described by Heijboer et al. [23] could contribute, at least partially, to these conflicting results. Actually, Klotho assays have not been largely validated and various forms of circulating alpha-klotho could be responsible for this variability. Nonetheless, a very recent paper based on renal biopsies of patients with glomerulonephritis and variable renal damage, for the first time showed reduced tubular TM-Klotho expression associated with reduced serum levels of s-Klotho. Importantly, these patients had almost undetectable changes in the standard markers of mineral metabolism [24]. As a whole, the diagnostic role of the assay of s-Klotho as a useful biomarker of early mineral metabolism derangements in CKD patients may be relevant but warrants further research [19–22].

In the present study, we evaluated the diagnostic performance of s-Klotho in our CKD population of patients by using the most accredited commercial s-Klotho assay [23]. We aimed at confirming the reduction of circulating levels of s-Klotho and at verifying the links with FGF23 and with other markers of mineral metabolism derangements.

2. Methods

2.1. Subjects. We enrolled in the study, from our outpatient unit, eligible patients who gave informed consent. Inclusion criteria were white race, age 18–80 years, on conservative therapy with no evidence of acute underlying illness and naïve to treatment with any active or precursor metabolite of vitamin D.

Fasting blood samples were drawn from all participants to measure creatinine (Crs), albumin, calcium (Ca), phosphate (P), parathyroid hormone (PTH), 25(OH)-vitamin D (25D), 1,25$(OH)_2$-vitaminD (1,25D), fibroblast growth factor-23 (FGF23), and soluble-α-Klotho (s-Klotho). We also collected fasting spot urine samples from all participants at the time of blood sampling to measure creatinine, phosphate, and calcium. In each patient, we recorded clinical parameters and prescribed therapies.

We classified CKD stage according to the National Kidney Foundation Disease Outcomes Quality Initiative clinical practice guidelines (KDOQI) [15].

Thirty normal subjects, recruited among the employees and fellows attending our Unit, served as control to obtain our reference values in particular for s-Klotho and FGF23. They were 14 males and 16 females, 35.0 ± 12.4 years of age with normal renal function (eGFR: 105.8 ± 15.4 mL/min/1,73 m^2) negative urinary dipstick and no evidence of acute or chronic underlying illness.

2.2. Assays. Serum creatinine (kinetic alkaline picrate method), albumin (bromcresol purple method), Ca (cresolphthalein-complexone method), and P (ammonium molybdate method) were assayed by routine, standard colorimetric techniques with a Technicon RA-500 analyzer (Bayer Corporation Inc, Tarrytown, NY).

Serum PTH was assayed by an immunoradiometric technique (DiaSorin, Stillwater, MN, USA) based on a double antibody against the intact molecule; our normal values are within 10–55 pg/mL, with intra- and interassay variations of 6.5% and 9.8%, respectively.

Serum 25D determination was done with a commercial kit (Dia-Sorin, Stillwater, MN, USA) that included sample purification with acetonitrile followed by a ^{125}I-based radioimmunoassay. Intra- and interassay coefficients of variation were 10.8% and 9.4%, respectively.

Serum levels of 1,25D were measured with a radioimmunoassay according to the manufacturer's protocol (IDS Ltd, Boldon, UK) including a monoclonal immunoextraction, followed by quantitation with a standard ^{125}I-based radioimmunoassay. Intra- and interassay coefficients of variation were <12% and <14%, respectively. The normal range observed in our laboratory was between 19.5 and 67.0 pg/mL.

Serum levels of FGF23 were assayed with a commercially available kit (Kainos Lab Inc., Tokyo, Japan) that utilizes a 2-site ELISA for the full-length molecule. Two specific murine monoclonal antibodies recognized the biologically active FGF23, with a lower limit of detection of 3 pg/mL, and inter- and intraassay coefficients of variation of <5%. This assay has been demonstrated to be the most sensitive among the 3 different methods available [25]. In 30 normal subjects, we obtained a mean value of 29.8 ± 10.9 pg/mL (range 18–52 pg/mL), in line with reported data [25].

Serum levels of soluble alpha-Klotho were assayed with a novel enzyme-linked immunosorbent assay (ELISA) method detecting human s-Klotho developed first by establishing a monoclonal antibody with strong affinity for human Klotho protein, recognizing with high selectivity the tertiary protein structure of its extracellular domain (Immuno-Biological Laboratories Co., Ltd.). This ELISA system can specifically detect and measure the circulating serum s-Klotho levels in humans [26]. It was recently tested by Heijboer et al. [23] and the within- and between-run variation of the α-Klotho IBL was <5 and <8%, respectively. Measurements in serum and EDTA plasma samples were in agreement ($R^2 = 0.99$; $n = 20$) and linearity was tested by dilution in two samples with a concentration of 1929 and 2864 pg/mL. In one sample, 2-, 4-, and 8-time dilutions gave results as expected (100–117% of expected values). In our experience, in 30 normal subjects we obtained a mean value of 845 ± 330 pg/mL (range 2048–481 pg/mL).

We estimated glomerular filtration rate (eGFR) according to the abbreviated modification of diet in renal disease (MDRD) equation (eGFR = 186 × serum creatinine$^{-1.154}$ × age$^{-0.203}$ × 0.742 if female) [27].

Table 1: Clinical characteristics of the CKD patients.

	CKD
Number of patients	68
M/F	37/31
Mean age, years	58 ± 15
eGFR, mL/min	45 ± 21
Mean body mass index	27 ± 4
Diabetes, number (%)	7 (10%)
Renal diagnosis	
Glomerulonephritis/vasculitis	16 (24%)
Interstitial nephritis	15 (22%)
Hypertensive/large vessel disease	18 (26%)
Hereditary nephropathy	4 (6%)
Unknown or missing data	15 (22%)
Therapies	
ACE inhibitor	28 (41%)
Angiotensin II receptor blockade	13 (19%)
Beta blocker	15 (22%)
Diuretic	10 (15%)
Insulin or oral hypoglycemic agent	6 (9%)

Values are mean ± standard deviation.
M/F: men/female; eGFR estimated glomerular filtration rate; ACE: angiotensin converting enzyme.

2.3. Statistical Analysis. Data are expressed as means ± SD for Gaussian variables or median and IQR when normality was not tenable.

We used Kolmogorov-Smirnov test to evaluate normality of continuous measurements.

Spearman correlation was used to assess monotonic covariation of measurements. Tests on Spearman correlation were Bonferroni adjusted for multiplicity. Nonparametric ANOVA (Kruskall-Wallis test) was used to compare measurements among groups and post-hoc comparisons in pairs were conducted by Bonferroni adjusted Mann-Whitney tests.

Log-measurements were confirmed to be normally distributed and were used as outcomes in multivariate regression models. The final multivariate model was obtained by minimizing the Akaike information criterion via a forward stepwise regression. All tests are two tailed and (adjusted) P-values <0.05 were considered as statistically significant.

Analyses were performed using the open source software package R version 3.0.2.

3. Results

For this study we recruited sixty-eight CKD patients, and their clinical and biochemical parameters are reported in Tables 1 and 2. Renal function, as reflected by eGFR, averaged 45 ± 21 mL/min (median ± SD) and included CKD stages from 2 to 4 (stage 2, $n = 22$ (32%); stage 3, $n = 28$ (41%); stage 4, $n = 18$ (27%)).

Table 2: Biochemistries in CKD.

Number of patients	68
Cas mg/dL	9,4 ± ,6
Ps mg/dL	3,5 ± ,7
PTH pg/mL	60,0 ± 31,7
25D ng/mL	23,7 ± 11,1
1,25D pg/mL	24,8 ± 13,2

Values are mean ± standard deviation.
Cas: serum calcium; Ps: serum phosphate; PTH: parathyroid hormone; 25D: 25(OH)-vitaminD; 1,25D: $1,25(OH)_2$-vitaminD.

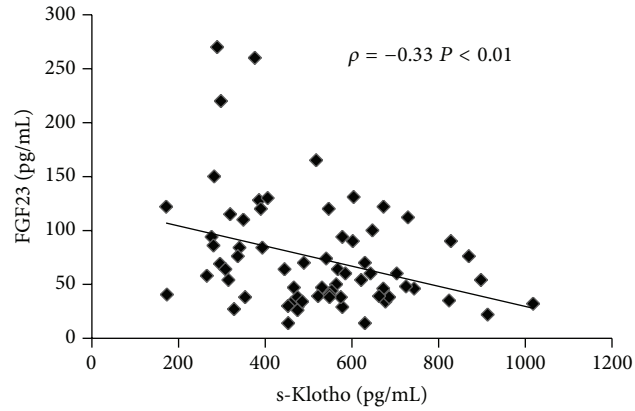


Figure 1: Correlation test of s-Klotho levels with FGF23 levels in CKD.

Table 3: s-Klotho and FGF23 in CKD versus reference value.

	CKD (68)	Reference values (30)	P=
s-Klotho, pg/mL	519 ± 183	845 ± 330	.0001
FGF23, pg/mL	73 ± 51	36 ± 11	.0002

Mann-Whitney test; values are mean ± standard deviation.
s-Klotho: soluble-Klotho; FGF23: fibroblast growth factor-23.

3.1. s-Klotho, FGF23, and eGFR. s-Klotho levels in our CKD patients were significantly lower (519 ± 183 versus 845 ± 330 pg/mL, $P < .0001$, Table 3) and FGF23 levels were significantly higher (73 ± 51 versus 36 ± 11 pg/mL, $P < 0002$, Table 3) than our reference values.

In patients s-Klotho and FGF23 were negatively correlated ($\rho = -0.33$, $P < .01$, Figure 1). In addition, there was a positive correlation between eGFR and s-Klotho ($\rho = 0.43$, $P < .001$, Figure 2(a)) and a negative correlation between eGFR and FGF23 ($\rho = -0.66$, $P < .0001$, Figure 2(b)).

When we evaluated s-Klotho in the different CKD stages, we found reduced levels since CKD stage 2, with a more significant reduction in CKD stages 3 and 4 (reference values = 845 ± 330, pg/mL; stage 2 = 611 ± 191, pg/mL, $P < .01$; stage 3 = 529 ± 160, pg/mL, $P < .001$; stage 4 = 393 ± 142, pg/mL; $P < .001$. Figure 3(a)). Levels of FGF23 were significantly higher since CKD stage 2 (reference values = 36 ± 11, pg/mL; stage 2 = 46 ± 18, pg/mL, $P < .001$; stage 3 = 57 ± 28, pg/mL, $P < .002$; stage 4 = 136 ± 58, pg/mL, $P < .001$. Figure 3(b)) with no further increment in later CKD stage. Multivariate analysis performed to identify the best

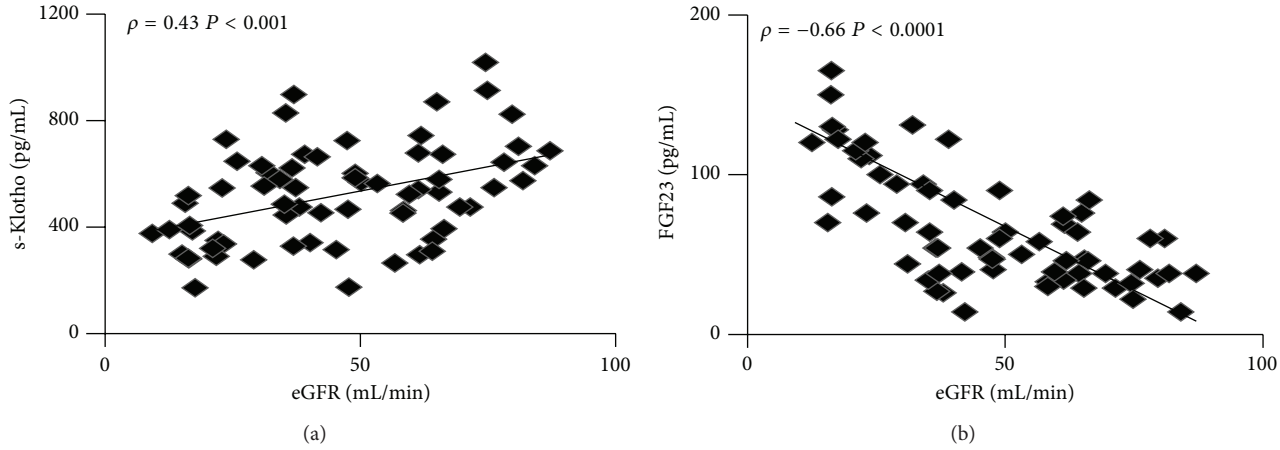


Figure 2: Correlation tests of eGFR with s-Klotho levels (a) and FGF23 (b).

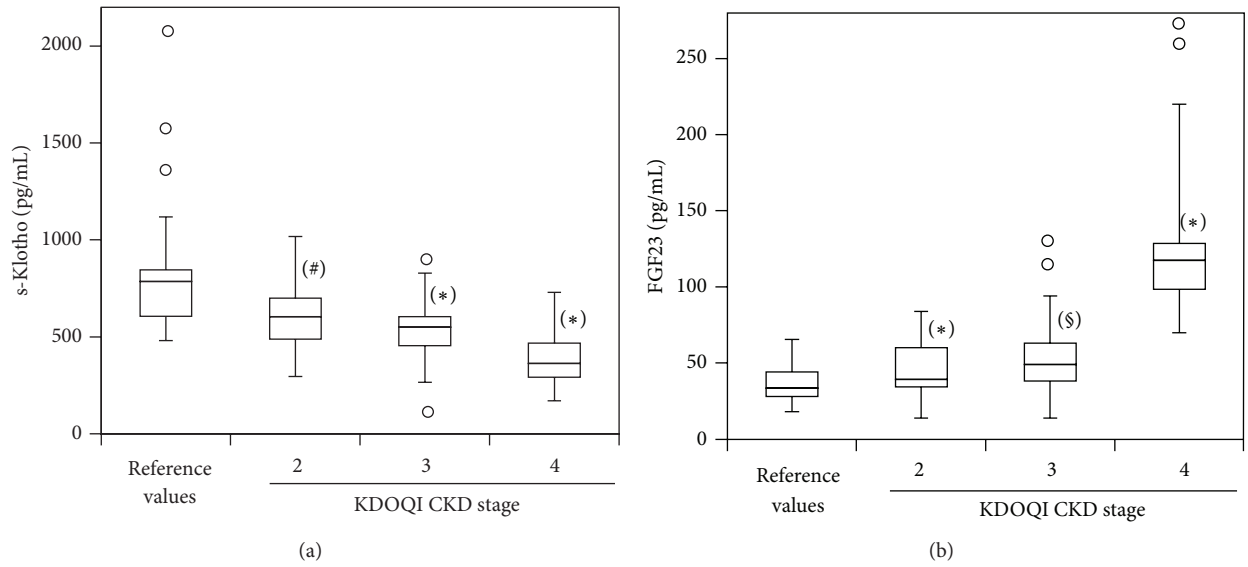


Figure 3: s-Klotho (soluble Klotho) (a) and FGF23 (fibroblast growth factor 23) and (b) serum levels stratified by CKD stage and compared with reference values. Indicated are medians, first and third quartiles, minimal and maximal values. Values between 1.5 and 3 times IQR above the third quartile or below the first are represented by circles. Wilcoxon rank-sum tests. KDOQI: National Kidney Foundation Disease Outcomes Quality Initiative clinical practice guidelines [15]. $^{\#}P < .01$ versus reference value; $^{*}P < .001$ versus reference value; $^{\S}P < .002$ versus reference value.

Table 4: Multivariate analysis performed in CKD to identify predictors of s-Klotho.

VAR	Coef.	CI	$P=$
eGFR, mL/min	.006	.002, .010	.004
Cas, mg/dL	.193	.034, .035	.020

VAR: variable; coef: coefficient of linear regression; CI: confidence interval.

Table 5: Multivariate analysis performed in CKD to identify predictors of FGF23.

VAR	Coef.	CI	$P=$
eGFR, mL/min	−.019	−.023, −.014	.0001
1,25D, pg/mL	−.016	−.024, −.008	.0001

VAR: variable; coef.: coefficient of linear regression; CI: confidence interval.

predictors of s-Klotho (Table 4) and FGF23 (Table 5) included eGFR and serum calcium for s-Klotho and eGFR and 1,25D serum level for FGF23.

3.2. s-Klotho, FGF23, and Mineral Metabolism. s-Klotho correlated negatively with PTH ($\rho = -0.28$, $P < .05$, Figure 4(a)) and Ps ($\rho = -0.28$, $P < .05$, Figure 4(b)) and positively with Ca ($\rho = 0.30$, $P < .01$, Figure 4(c)) while no correlation was found between s-Klotho and 1,25D, FE_{PO_4}, and FE_{Ca}.

FGF23 levels correlated positively with PTH ($\rho = 0.43$, $P < .001$, Figure 5(a)), Ps ($\rho = 0.51$, $P < .001$, Figure 5(b))

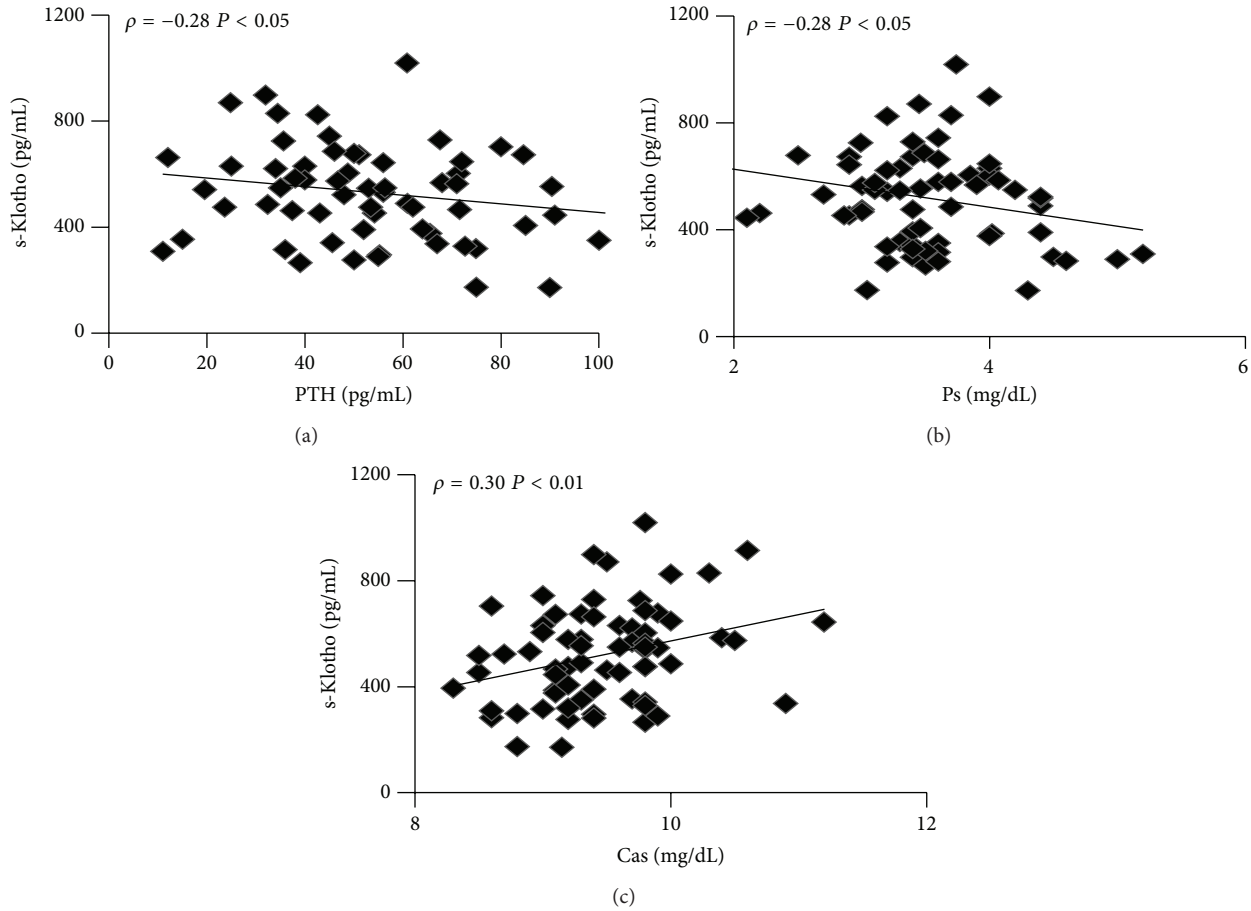


Figure 4: Correlation tests of s-Klotho levels with: (a) PTH, (b) Ps, (c) Cas.

and FE_{PO_4} ($\rho = 0.47$, $P < .001$, Figure 5(d)) and negatively with 1,25D ($\rho = -0.39$, $P < .001$, Figure 5(c)). No correlation existed with Ca and FE_{Ca}.

4. Discussion

In agreement with experimental models of CKD [11, 12] and with papers in the literature [19, 21] our patients showed markedly reduced s-Klotho serum levels as compared to reference control. Moreover, our mean values in CKD and in normal controls are comparable to those available in papers the literature that employ our same method of assay [19, 28–30]. It is therefore suggested that although s-Klotho proteins are not homogeneous, consistent data can be obtained by different groups.

Renal function negatively affected s-Klotho levels with detectable reduction starting from CKD stage 2. Since s-Klotho is normally excreted in the urine [7–9], this reduction in serum levels along with progressive renal damage can be most probably explained by reduced renal synthesis. In fact, in case of stable renal Klotho production, a reduced excretion due to renal damage would increase serum levels. Alternatively, if renal excretion persisted to be normal, serum levels would not predictably decrease. On the contrary, an increased renal excretion seems highly improbable since this would contradict the available clinical [31] and experimental [32] data of reduced urinary s-Klotho in CKD. In our experience, the best predictor of s-Klotho was eGFR and this is confirmatory of the hypothesis we have just considered. The other positive predictor of s-Klotho in our study was serum Ca as if a regulatory role of s-Klotho would be present even in case of renal failure. This seems possible if we recall the direct effect of s-Klotho on TRPV5. However, the pathomechanism may not be straightforward since we did not find correlation between s-Klotho and FE_{Ca}. Therefore, we should guess a more complex interplay with other changing parameters like eGFR, PTH, and 1,25D.

The negative relationship we found between s-Klotho and serum P is interesting due to the reported direct effect of circulating s-Klotho on the renal expression of NaPi2a. However, no correlation was evident with renal FE_{PO_4}. Since s-Klotho and FGF23 correlate negatively in CKD but exert a similar positive effect on renal expression of NaPi2a, it is possible that the action of s-Klotho is shadowed by FGF23. Finally, the negative relationship with PTH should be regarded as secondary to reduced renal function.

Serum levels of FGF23 were higher than normal in our CKD population with increments detectable since CKD stage 2. Correlations of FGF23 were negative with eGFR and 1,25D, and positive with PTH, Ps and FE_{PO_4}. Multivariate analysis

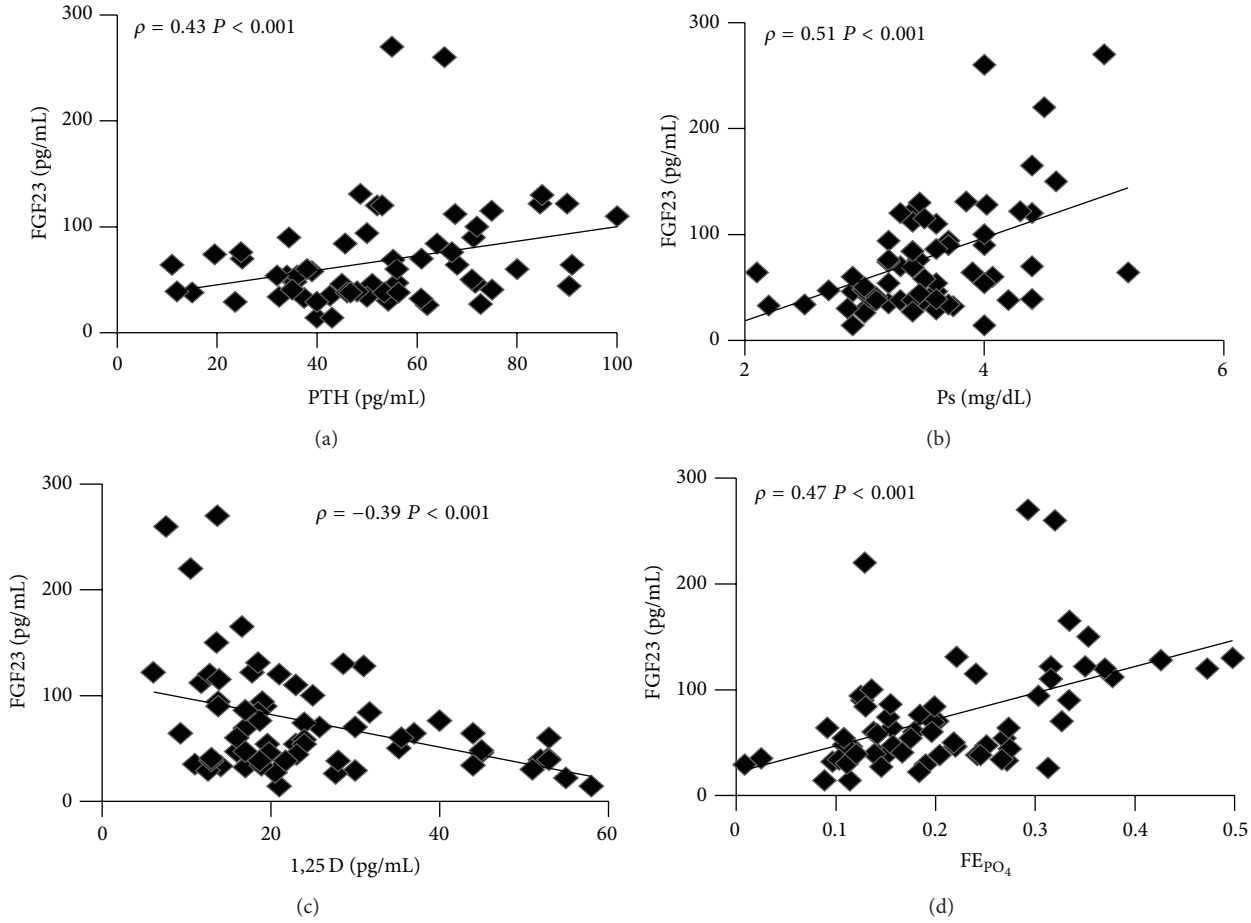


Figure 5: Correlation tests of FGF23 with: (a) PTH, (b) Ps, (c) 1,25D, (d) FE_{PO_4}.

evidenced eGFR and 1,25D as best predictors. These data suggest that bone cells somehow sense the reduction of eGFR very early and increase FGF23 synthesis mainly aiming at reducing 1,25D synthesis. In agreement, in a mice model of CKD administration of FGF23 antibodies resulted in a significant and dose dependent increase of 1,25D [33]. In our CKD population, the consensual increments of serum P and PTH along with that of FGF23 can be regarded, at variance, as mainly secondary to the reduction of renal function.

Interestingly in our study, a negative relationship emerged between s-Klotho and FGF23. This favors the hypothesis that s-Klotho, whose circulating levels are strongly related to renal function and are most probably secondary to reduced expression of TM-Klotho, can be regarded as a sensitive biomarker of TM-Klotho expression, useful to appreciate early development of tubular resistance to FGF23. Consequently, bone synthesis of FGF23 is increased. A recent paper with histologic data from patients with glomerulonephritis showed parallel reduction of renal Klotho and of s-Klotho together with increments in FGF23, which is in agreement with our data [24]. Transgenic animals selectively null for Klotho throughout the nephron, as reported recently by Lindberg et al., have negligible shedding of Klotho from renal explants and circulating levels reduced by 80%, thus revealing the kidney as a major contributor of circulating Klotho [34].

Significantly in our study, levels of s-Klotho and of FGF23 are roughly halved and, respectively, doubled in a population with an average eGFR of 45 mL/min/1,73 but with substantially normal values of Ca and P and with mean values of 1,25D and of PTH, respectively, at the lower and higher upper limits of normality. This confirms that changes in serum Ca and P are not reliable to detect early CKD-MBD, while s-Klotho seems more sensitive than PTH and 1,25D.

Limitations of our study are several. First, the number of evaluated patients is rather low. In fact, an increased number of cases would have reinforced the reliability of our results that, however, are in line with published data in the literature. Second, we did not include CKD stage 1 patients and this does not allow us to verify if s-Klotho diminish even in case of "normal" renal function. Certainly, this important issue warrants further investigations. Third, we did not measure urinary s-Klotho. Effectively, evidence of reduced urinary Klotho in our experience would have reinforced the hypothesis of reduced renal synthesis. However, scanty, available papers report reduced urinary Klotho in patients with renal failure [31] and in animals selectively null for nephron Klotho [34], both results confirming our hypothesis.

In conclusion, our results favor the hypothesis that renal Klotho synthesis diminishes early in renal disease and that s-Klotho proportionally lessens; bone detects these changes

somehow and increases FGF23 production. Accordingly, s-Klotho represents an early marker of renal damage and of ensuing CKD-MBD, indicative of the cross-talk between bone and kidney.

Conflict of Interests

The authors declare no conflict of interests for this paper.

References

[1] I. S. Mian, "Sequence, structural, functional, and phylogenetic analyses of three glycosidase families," *Blood Cells, Molecules and Diseases*, vol. 24, no. 2, pp. 83–100, 1998.

[2] H. Kurosu, Y. Ogawa, M. Miyoshi et al., "Regulation of fibroblast growth factor-23 signaling by Klotho," *The Journal of Biological Chemistry*, vol. 281, no. 10, pp. 6120–6123, 2006.

[3] I. Urakawa, Y. Yamazaki, T. Shimada et al., "Klotho converts canonical FGF receptor into a specific receptor for FGF23," *Nature*, vol. 444, no. 7120, pp. 770–774, 2006.

[4] S. Liu, W. Tang, J. Zhou et al., "Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D," *Journal of the American Society of Nephrology*, vol. 17, no. 5, pp. 1305–1315, 2006.

[5] C.-D. Chen, S. Podvin, E. Gillespie, S. E. Leeman, and C. R. Abraham, "Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 50, pp. 19796–19801, 2007.

[6] M. C. Hu, M. Kuro-O, and O. W. Moe, "Klotho and chronic kidney disease," *Contributions to Nephrology*, vol. 180, pp. 47–63, 2013.

[7] M. C. Hu, M. Kuro-o, and O. W. Moe, "Renal and extrarenal actions of Klotho," *Seminars in Nephrology*, vol. 33, no. 2, pp. 118–129, 2013.

[8] M. C. Hu, M. Shi, J. Zhang et al., "Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule," *The FASEB Journal*, vol. 24, no. 9, pp. 3438–3450, 2010.

[9] S.-K. Cha, B. Ortega, H. Kurosu, K. P. Rosenblatt, M. Kuro, and C.-L. Huang, "Removal of sialic acid involving Klotho causes cell-surface retention of TRPV5 channel via binding to galectin-1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 28, pp. 9805–9810, 2008.

[10] M. Kuro-O, "Phosphate and klotho," *Kidney International*, vol. 79, no. 121, pp. S20–S23, 2011.

[11] H. Aizawa, Y. Saito, T. Nakamura et al., "Downregulation of the klotho gene in the kidney under sustained circulatory stress in rats," *Biochemical and Biophysical Research Communications*, vol. 249, no. 3, pp. 865–871, 1998.

[12] J. Yu, M. Deng, J. Zhao, and L. Huang, "Decreased expression of klotho gene in uremic atherosclerosis in apolipoprotein E-deficient mice," *Biochemical and Biophysical Research Communications*, vol. 391, no. 1, pp. 261–266, 2010.

[13] H. Olauson, K. Lindberg, R. Amin et al., "Targeted deletion of klotho in kidney distal tubule disrupts mineral metabolism," *Journal of the American Society of Nephrology*, vol. 23, no. 10, pp. 1641–1651, 2012.

[14] M. Cozzolino, P. Ureña-Torres, M. G. Vervloet et al., "Is chronic kidney disease-mineral bone disorder (CKD-MBD) really a syndrome?" *Nephrology Dialysis Transplantation*, vol. 29, no. 10, pp. 1815–1820, 2014.

[15] National Kidney Foundation, "K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification," *American Journal of Kidney Diseases*, vol. 39, pp. S1–S266, 2002.

[16] M. C. Hu, M. Shi, J. Zhang et al., "Klotho deficiency causes vascular calcification in chronic kidney disease," *Journal of the American Society of Nephrology*, vol. 22, no. 1, pp. 124–136, 2011.

[17] N. Koh, T. Fujimori, S. Nishiguchi et al., "Severely reduced production of klotho in human chronic renal failure kidney," *Biochemical and Biophysical Research Communications*, vol. 280, no. 4, pp. 1015–1020, 2001.

[18] T. Akimoto, T. Kimura, Y. Watanabe et al., "The impact of nephrectomy and renal transplantation on serum levels of soluble Klotho protein," *Transplantation Proceedings*, vol. 45, no. 1, pp. 134–136, 2013.

[19] I. Pavik, P. Jaeger, L. Ebner et al., "Secreted Klotho and FGF23 in chronic kidney disease stage 1 to 5: a sequence suggested from a cross-sectional study," *Nephrology Dialysis Transplantation*, vol. 28, no. 2, pp. 352–359, 2013.

[20] S. Seiler, M. Wen, H. J. Roth et al., "Plasma Klotho is not related to kidney function and does not predict adverse outcome in patients with chronic kidney disease," *Kidney International*, vol. 83, no. 1, pp. 121–128, 2013.

[21] M. Wan, C. Smith, V. Shah et al., "Fibroblast growth factor 23 and soluble klotho in children with chronic kidney disease," *Nephrology Dialysis Transplantation*, vol. 28, no. 1, pp. 153–161, 2013.

[22] S. Devaraj, B. Syed, A. Chien, and I. Jialal, "Validation of an immunoassay for soluble Klotho protein: decreased levels in diabetes and increased levels in chronic kidney disease," *American Journal of Clinical Pathology*, vol. 137, no. 3, pp. 479–485, 2012.

[23] A. C. Heijboer, M. A. Blankenstein, J. Hoenderop, M. H. De Borst, and M. G. Vervloet, "Laboratory aspects of circulating α-Klotho," *Nephrology Dialysis Transplantation*, vol. 28, no. 9, pp. 2283–2287, 2013.

[24] H. Sakan, K. Nakatani, O. Asai et al., "Reduced renal α-Klotho expression in CKD patients and its effect on renal phosphate handling and vitamin D metabolism," *PLoS ONE*, vol. 9, no. 1, Article ID e86301, 2014.

[25] E. A. Imel, M. Peacock, P. Pitukcheewanont et al., "Sensitivity of fibroblast growth factor 23 measurements in tumor-induced osteomalacia," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 6, pp. 2055–2061, 2006.

[26] Y. Yamazaki, A. Imura, I. Urakawa et al., "Establishment of sandwich ELISA for soluble alpha-Klotho measurement: age-dependent change of soluble alpha-Klotho levels in healthy subjects," *Biochemical and Biophysical Research Communications*, vol. 398, no. 3, pp. 513–518, 2010.

[27] A. S. Levey, J. P. Bosch, J. B. Lewis, T. Greene, N. Rogers, and D. Roth, "A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation," *Annals of Internal Medicine*, vol. 130, no. 6, pp. 461–470, 1999.

[28] K. Yokoyama, A. Imura, I. Ohkido et al., "Serum soluble α-klotho in hemodialysis patients," *Clinical Nephrology*, vol. 77, no. 5, pp. 347–351, 2012.

[29] M. Kitagawa, H. Sugiyama, H. Morinaga et al., "A decreased level of serum soluble Klotho is an independent biomarker associated with arterial stiffness in patients with chronic kidney disease," *PLoS ONE*, vol. 8, no. 2, Article ID e56695, 2013.

[30] A. Nowak, B. Friedrich, F. Artunc et al., "Prognostic value and link to atrial fibrillation of soluble Klotho and FGF23 in

hemodialysis patients," *PLoS ONE*, vol. 9, no. 7, Article ID e100688, 2014.

[31] T. Akimoto, H. Yoshizawa, Y. Watanabe et al., "Characteristics of urinary and serum soluble Klotho protein in patients with different degrees of chronic kidney disease," *BMC Nephrology*, vol. 13, article 155, 2012.

[32] M.-C. Hu, M. Shi, J. Zhang, H. Quīones, M. Kuro-O, and O. W. Moe, "Klotho deficiency is an early biomarker of renal ischemia-reperfusion injury and its replacement is protective," *Kidney International*, vol. 78, no. 12, pp. 1240–1251, 2010.

[33] H. Hasegawa, N. Nagano, I. Urakawa et al., "Direct evidence for a causative role of FGF23 in the abnormal renal phosphate handling and vitamin D metabolism in rats with early-stage chronic kidney disease," *Kidney International*, vol. 78, no. 10, pp. 975–980, 2010.

[34] K. Lindberg, R. Amin, O. W. Moe et al., "The kidney is the principal organ mediating klotho effects," *Journal of the American Society of Nephrology*, vol. 25, pp. 2169–2175, 2014.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2015, Article ID 165215, 15 pages
http://dx.doi.org/10.1155/2015/165215

Review Article

The Endocrine Role of Estrogens on Human Male Skeleton

Vincenzo Rochira,[1,2] Elda Kara,[1] and Cesare Carani[1]

[1] *Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Via P. Giardini 1355, 41126 Modena, Italy*
[2] *Azienda USL di Modena, Nuovo Ospedale Civile Sant'Agostino Estense (NOCSAE), Via P. Giardini 1355, 41126 Modena, Italy*

Correspondence should be addressed to Vincenzo Rochira; vincenzo.rochira@unimore.it

Received 23 August 2014; Accepted 14 November 2014

Academic Editor: Martina Rauner

Before the characterization of human and animal models of estrogen deficiency, estrogen action was confined in the context of the female bone. These interesting models uncovered a wide spectrum of unexpected estrogen actions on bone in males, allowing the formulation of an estrogen-centric theory useful to explain how sex steroids act on bone in men. Most of the principal physiological events that take place in the developing and mature male bone are now considered to be under the control of estrogen. Estrogen determines the acceleration of bone elongation at puberty, epiphyseal closure, harmonic skeletal proportions, the achievement of peak bone mass, and the maintenance of bone mass. Furthermore, it seems to crosstalk with androgen even in the determination of bone size, a more androgen-dependent phenomenon. At puberty, epiphyseal closure and growth arrest occur when a critical number of estrogens is reached. The same mechanism based on a critical threshold of serum estradiol seems to operate in men during adulthood for bone mass maintenance via the modulation of bone formation and resorption in men. This threshold should be better identified in-between the ranges of 15 and 25 pg/mL. Future basic and clinical research will optimize strategies for the management of bone diseases related to estrogen deficiency in men.

1. Introduction

In the human male, testosterone (T) and estradiol (E_2) are the main circulating sex steroids acting on bone tissue. The first is produced from the Leydig cells in the testis, while the latter derives from the aromatization of the androgens by means of the enzymatic complex of aromatase [1]. Aromatase is a cytochrome P450 enzyme encoded by the *CYP19A1* gene that plays a key role in estrogen biosynthesis: it catalyzes the conversion of Δ^4-androstenedione into estrone and that of T into E_2 [2, 3]. Aromatase is widely expressed in a large number of tissues such as testis (Sertoli and Leydig cells), ovary (granulosa cells and luteal corpus), brain (including hypothalamus), hair follicles, and fibroblasts [2, 3]. Adipose tissue also expresses aromatase and it constitutes an important source of estrogens, especially in men [1–4]. In men, in fact, E_2 is mainly produced by the testis and secondarily by adipose tissue [2–4]. Biological actions of estrogens are mediated by their receptor (ER) that belongs to the nuclear receptors family, and, to date, two different ERs have been identified: ER-α and ER-β [5]. A further nongenomic pathway of estrogen action has been described probably involving a plasma membrane interaction of the ER [6, 7].

Animal [8, 9] and human [10, 11] models of male congenital estrogen deficiency offered a new scenario useful for better understanding estrogen effects on male bone as well as several organs and tissues in men [12–14]. All these physiological actions of estrogens in men remained overlooked for a long time (see [13] for review). In the past, estrogen was also erroneously considered indispensable for blastocyst implantation [15] and congenital estrogen defects are supposed to be incompatible with life [10, 11]. The discovery of the first cases of congenital estrogen defects in humans allowed understanding that aromatase deficiency is due to mutations of the gene coding for the aromatase enzyme complex, which leads to lack of both estrogen synthesis and action, while estrogen resistance is due to mutations of the genes coding for estrogen receptors and leads to resistance to estrogen actions even in presence of circulating estrogens [10, 11].

This review will focus on the role of estrogens on human male bone according to all skeletal physiological events that occur *in vivo* in different life stages in men. The rise of T and E_2 in men at puberty progressively exposes bone to sex steroids, thus allowing them to act on the growing skeleton. Sex steroids modify the way through which immature bone develops in terms of size, structure, bone mineral density (BMD), and proportions till the achievement of final skeletal maturation. After the achievement of peak bone mass, estrogens continue to influence bone remodeling in adulthood, the decline of circulating E_2 being directly correlated with bone loss from adult to aging life.

2. Estrogen Effects on Bone from Early to Late Puberty

Very low levels of estrogens circulate in the blood even in male children during infancy, but their real physiological significance is not known [16]. In prepubertal boys with a genital Tanner stage 1, serum E_2 measured with the gold standard liquid chromatography tandem mass spectrometry (LC/MS/MS) starts to increase ranging from 0.5–1.0 to about 1.9 pg/mL in healthy controls and obese boys, respectively [17, 18]. Considering the developing skeleton to not be under the effects of estrogens before puberty in male fetuses and children is a good simplification, even though this is a poorly investigated field of research (Table 1).

The initial activation of the hypothalamic-pituitary-gonadal axis in male children resulting in a progressive, slow increase of sex steroids, including estrogens, characterizes the peripubertal period [17–19]. Bone exposure to low concentrations of estrogens leads to well-known estrogen-dependent bone changes in males [20, 21].

2.1. Effects of Estrogens on Longitudinal Skeletal Growth. The growth of long bones occurs at the growth plate, a thin layer of cartilage that separates the epiphysis from the metaphysis [22]. The growth plate consists of three distinct layers of resting (stem cell-like), proliferative and hypertrophic chondrocytes [22, 23]. The concept that sex steroids promote epiphyseal growth and maturation during puberty in both sexes was a well-known issue in endocrinology since the beginning of the last century [24–26]. This classical endocrinological theory was based on a well-distinct action of estrogen from androgen on bone. In fact, it was believed that the former leads to growth plate maturation only in women [27], while the latter leads to growth plate maturation only in men [28, 29]. As a matter of fact, the failure of epiphyseal closure and osteoporosis observed in adult men with congenital hypogonadotropic hypogonadism or with childhood onset severe T deficiency were both traditionally ascribed to insufficient bone exposure to androgen at puberty [30, 31]. Notwithstanding evidence on estrogen actions on bone maturation in men had become available since the 1980s [32–34]; this viewpoint lasted since the beginning of the 1990s when the idea that estrogen is the main sex steroid involved in male bone maturation started to advance [35] thanks to the description of the first cases of congenital defects of estrogen synthesis or action [36–40].

Table 1: Role of estrogens on male bone throughout lifespan.

Life stages	Effects of estrogens on bone
Fetal life	Poorly investigated/unknown
Infancy	Poorly investigated/unknown
Puberty	
Early puberty (detectable but low E_2)	**Growth plate lengthening** Rapid advancement of bone age Accelerated linear bone growth Increase in height velocity (growth spurt) Assurance of adequate target height attainment
Late puberty (high E_2)	**Thinning and progressive growth plate disappearance before epiphyseal closure** Bone maturation Advancement of skeletal maturation Progression of bone age in late puberty Epiphyseal fusion and growth arrest Achievement of final height Allowance of skeletal body proportions Achievement of adequate peak bone mass Bone size Bone length Periosteal bone apposition (crosstalk with androgens)
Adulthood	Maintenance of bone mass
Ageing	Prevention of bone loss

E_2: estradiol.

The clinical phenotype presented by the unique male patient described to date [36] with estrogen resistance was very close to that of men with aromatase deficiency [37, 40]. It is characterized by tall stature, a history of continuous linear growth into adulthood, unfused epiphyses, progressive genu valgum, eunuchoid proportion of the skeleton, delayed bone age, and osteoporosis. In 1997, Carani et al. [38] demonstrated that transdermal E_2 replacement is effective in obtaining complete epiphyseal closure, final skeletal maturation, the arrest of growth in height, the increase in BMD, and peak bone mass [10, 38]. This result was subsequently replicated by many other authors in all aromatase-deficient men described so far [40–46]. On the other hand, six months of treatment with high doses of T, given before the diagnosis of aromatase deficiency in an attempt to arrest continuous linear growth, had no effects on bone age in this patient [10, 38]. Besides, E_2 treatment was not effective in the estrogen-resistant man, as expected [36]. All these findings suggest that epiphyseal closure is an estrogen-dependent phenomenon even in males and that androgens by themselves are not effective to ensure a normal skeletal development during late pubertal stages [10, 11, 21–23] (Table 1).

Later on, these findings opened the way to studies investigating the role of estrogens on bone growth and maturation not only in the context of congenital estrogen defects but also in normal boys. Serum E_2 was found to increase simultaneously with T levels during puberty in boys and to correlate directly with chronological and skeletal age,

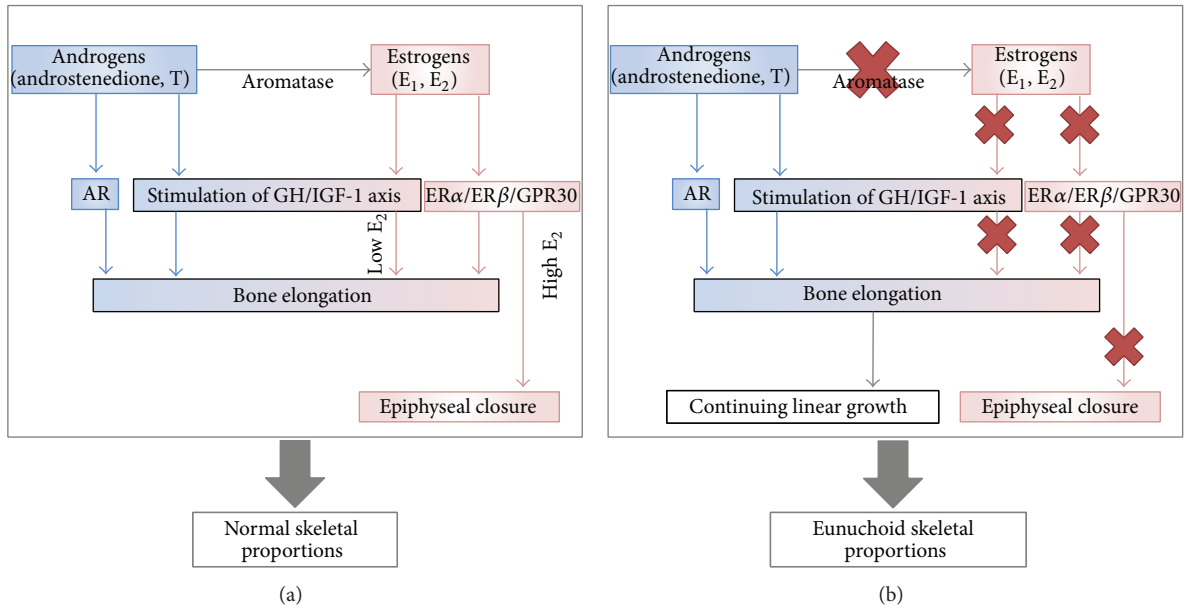


Figure 1: Direct and indirect effects of increasing circulating estrogens and their depletion on the growth plate (a), and effects of estrogen deprivation on bone elongation and epiphyseal status (b). AR: androgen receptor; E1: estrone; E_2: estradiol; T: testosterone; ER-α: estrogen receptor alpha; ER-β: estrogen receptor beta; GPR30: membranous-G-protein-coupled estrogen receptor.

height, weight, and pubertal stages, thus confirming the crucial role of estrogens on bone physiology at puberty even in the male [19, 21] (Table 1). In particular, estrogens seem to have a dose-dependent effect on growth plates [21, 47]: actually low doses of E_2 stimulate ulnar growth in boys [33], while higher doses lead to an inhibition of this process of growth [20]. Recently, circulating serum E_2 measured by LC/MS/MS resulted directly related to both the genital Tanner stage and the skeletal maturation in pubertal boys [18]. In addition, serum E_2 was significantly higher and bone age more advanced in obese boys compared with healthy boys at the same pubertal stage [48]. The excess of adipose tissue in obese boys probably accounts for increased aromatization of androgens into estrogens and for the advancement of bone age due to the higher amounts of circulating estrogens [18, 48]. This result is in line with the well-known gender difference in the progression of skeletal maturation, which is more rapid in women than in men and parallels gender differences in the way serum E_2 increases throughout puberty [19, 20, 33, 35]. At the beginning of puberty, when circulating E_2 is low, the prevailing effect of E_2 consists in the promotion of chondrocytes proliferation within the growth plate, resulting in growth plate lengthening and accelerated bone elongation (Figure 1) (Table 1). This corresponds to the increase of height velocity occurring during pubertal growth spurt that is postulated to be under estrogen control (Table 1) [21, 40, 47]. As puberty goes on, the rise in serum T ensures high E_2 circulating levels, typical of late puberty; E_2 inhibits chondrocyte proliferation and stimulate chondrocyte differentiation, thus inducing the progressive ossification of the growth plate and its final disappearance (Figure 1). At present, the amount of E_2 required for shifting from the increase in length of the growth plate to its growth deceleration and final closure of the growth plate line is not known in detail. Data available in literature clearly show that no difference in serum T is present between men with idiopathic hypogonadotropic hypogonadism with fused epiphyses compared to those with unfused epiphyses [30], but no data are available in literature on serum estrogens in these rare conditions. This implies that androgen is not involved in the process of shifting from growth plate elongation to progressive growth plate thinning and final disappearance (Table 1).

Based on the poor compliance of an aromatase-deficient man, we tried to develop a dose-response relationship between serum E_2 and radiological changes of the long bones in terms of bone age [45]. Due to patient's poor compliance, serum E_2 remained below 20 pg/mL for a long time without any change of bone age and growth plate appearance at X-ray [45]. The closure of the epiphyses was obtained only several months later when E_2 rose above 20 pg/mL and the patient was taking the right dose of transdermal E_2 [45]. This suggests that serum E_2 above 20 pg/mL is necessary for epiphyseal cartilage fusion [45] and that only in the case of severe estrogen deficiency the epiphyses remain still open despite the advancement of the chronological age (Figure 2). The same results can be deduced from a recent study comparing sex steroids, pubertal stage, and skeletal maturation between obese and lean boys [17]. If boys at the end of puberty (with a genital Tanner stage 5) are considered, bone age (of about 18 years on average) was greatly advanced and consistent with fused epiphyses in obese boys with a mean serum E_2 clearly above 20 pg/mL (median 34.8 pg/mL, min–max: 25.6–41.1 pg/mL), while bone age (of about 16 years on average) was less advanced and consistent with still unfused epiphyses in lean boys with a mean serum E_2 below 20 pg/mL (median 15.7 pg/mL, min–max: 13.2–21.0 pg/mL) [17].

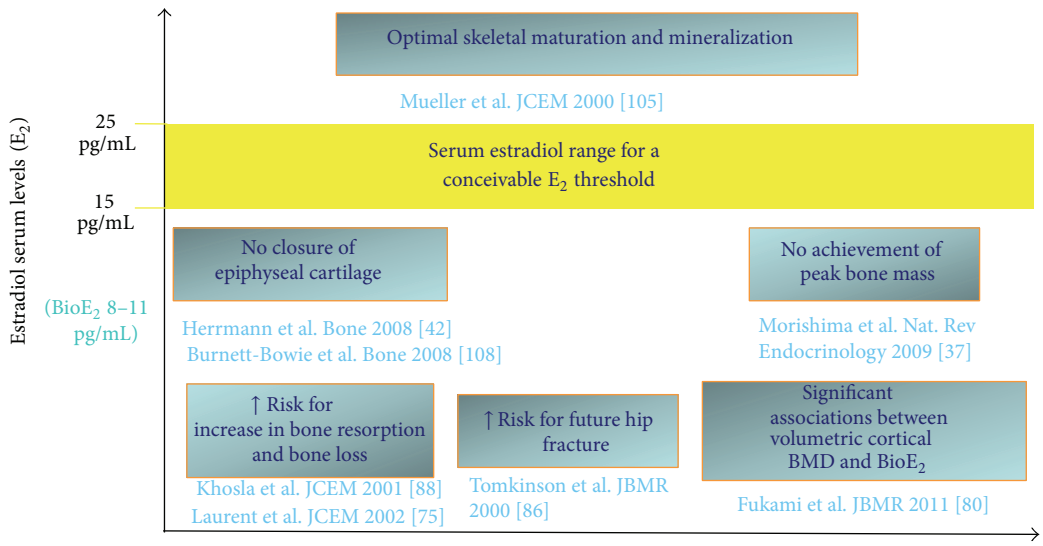


Figure 2: Proposed range for a critical serum estradiol threshold above which both skeletal maturation and mineralization can proceed in an optimal way. E_2: estradiol; $BioE_2$: Bioavailable estradiol.

The molecular mechanism through which estrogens act on the growth plate *in vivo* is still not known in detail, but recently advance on this issue has been reached [22, 23]. Several convincing evidence suggests that the growth plate width progressively decreases as a consequence of a process of senescence involving the chondrocytes, mainly in the resting zone [49]. How this senescence occurs and progresses is not known, but several mechanisms such as apoptosis, autophagy, chondrocytes differentiation into osteoblasts, and hypoxia have been proposed and are currently object of undergoing investigation by basic scientists [23]. Certainly, estrogens exert a strong effect on one or more of these pathways finally resulting in the promotion of chondrocytes involution and in the assurance of final epiphyseal closure followed by growth arrest [21–23]. What is evident is that the number of both chondrocytes and progenitor cells progressively decreases in the resting zone of the growth plate and that estrogens accelerate this process, especially when they reach a critical level [50]. Both ER-α and ER-β are expressed by human epiphyseal chondrocytes [51, 52]; moreover, the membranous-G-protein-coupled estrogen receptor, namely, GPR30 [7], is also expressed in the hypertrophic zone of human growth plate [53]. Furthermore, the fact that aromatase is also expressed by chondrocytes which are able to produce estrogens [54–56] implies that both circulating and locally produced estrogens are able to exert their actions within the growth plate via the activation of all the available estrogen transduction signaling pathways [22, 23]. Low levels of estrogens, similar to those locally produced *in vivo*, are able to promote chondrocytes proliferation and to protect them from cell death *in vitro* [57]. This mechanism might explain why low circulating and/or locally produced estrogens enhance longitudinal growth during early puberty. Conversely, the expression of the estrogen receptor GPR30 decreases dramatically during pubertal progression in humans when circulating estrogens reach the highest values typical of late puberty and longitudinal growth decelerates up to cessation [53]. GPR30 is a good candidate for explaining estrogen actions on growth plate since knockout mice in which this receptor is disrupted do not respond to estrogen in terms of longitudinal growth deceleration and cessation [58]. ER-β, rather than ER-α [59], seems to be mainly involved in the induction of growth plate fusion in response to supraphysiological E_2 exposure [60]. However, the complex interaction between estrogens and their receptors within the growth plate remains to be elucidated in detail. In the unique man with estrogen resistance, epiphyseal fusion did not occur at the expected time, despite high circulating E_2 and normally functioning ER-β [36]. Besides, cultured cells obtained from the bone biopsy of this patient did not respond to estrogen exposure, differently from wild type cells [61, 62]. This issue is further complicated by the possible crosstalk between ER-α and ER-β. Theoretically, in fact, it is possible that the residual truncated N-terminal fragment of the disrupted ER-α may have acted as a negative inhibitor of ER-β in this patient [62, 63], thus accounting for the very delayed epiphyseal closure in this estrogen-resistant man reached at the age of 35.5 years [61].

Other hormones are good candidates for explaining growth plate proliferation, longitudinal bone growth, and growth plate involution [23]. Among them, growth hormone (GH) and insulin-like growth factor-1 (IGF-1) exert an anabolic effect on bone and are necessary for longitudinal bone growth and growth acceleration during infancy and at the time of the growth spurt, respectively [23]. The role of GH and IGF-1 on longitudinal bone growth and growth spurt could be even indirect through the well-known ability of estrogens to enhance GH and IGF-1 secretion, an event that occurs during late puberty and that concurs to accelerate growth during the pubertal spurt [10, 11, 35, 64] (Figure 1). However, longitudinal bone growth might occur and progress also independently from the GH/IGF-1 status but at a lower rate since men with aromatase deficiency continue to slowly

increase their stature during adulthood, despite severe GH deficiency [65]. Accordingly, GH response to GHRH plus Arginine in four patients with aromatase deficiency was significantly lower than that in normal subjects, both before and after transdermal E_2 replacement therapy with E_2 [65]. In particular, E_2 replacement did not restore normal GH secretion and IGF-1 that remained significantly lower than normal age-matched controls [65]. The fact that estrogen replacement treatment was effective on epiphyseal closure and growth arrest in all patients with aromatase deficiency [40], despite insufficient GH and IGF-1 production [65], implies that GH and IGF-1 do not play a major role in the process of growth plate closure in humans. These data suggest that a tall stature (higher than the genetic target) may be reached despite the coexistence of GH deficiency in these patients. Even though we have no data about the GH-IGF1 axis in these patients during their childhood and puberty, they were able to increase their stature during the first period of E_2 treatment, soon before epiphyseal closure [40]. Besides, when aromatase inhibitors are administered at puberty in order to increase final height, usually a reduction of GH and IGF1 levels is observed but a benefit on final height is even obtained thanks to the epiphyseal fusion blockade and a longer time available for growth [66, 67] (Figure 2). All these data suggest that both longitudinal bone growth and a slow progressive increase in height during adulthood are possible even in presence of circulating GH and IGF-1 lower than normal on condition that epiphyseal growth plates remains open. This condition might lead to the development of tall stature [65]. Outside the context of these rare clinical conditions that help to better know how sex steroids act on the growing skeleton in healthy children and boys, GH and IGF-1 remain very important physiological determinants of growth during infancy and puberty since they ensure bone elongation and a normal height velocity [64]. Accordingly, GH deficiency represents one of the most important causes of growth retardation and (if untreated) of final short stature. Indeed, r-hGH replacement treatment is effective in restoring a normal height velocity in children and boys with GH deficiency [64].

2.2. Effects of Estrogens on Skeletal Proportions. In men, estrogen at puberty modulates both growth and the increase in stature in a fascinating way that allows accelerating the growth of the appendicular skeleton for a brief period only—characterized by low but detectable serum E_2 (early puberty)—while preserving, at the same time, harmonic skeletal proportions. Accordingly, the further increase of serum estrogens triggers both epiphyseal closure and cessation of growth during mid-to-late puberty, thus avoiding the development of an altered ratio between the appendicular and the axial skeleton. Even though androgens alone are not able to induce bone maturation, they exert direct and indirect anabolic action on bone before, during, and after puberty (Figure 1). In particular, during puberty, androgens probably promote the continuous linear growth, especially at the level of long bones as substantiated by the expression of androgen receptors within the human growth plate [23, 68] and the promotion of growth sustained by androgens through the elongation of the growth plate, at least in rats [69] (Figure 1). Disproportional growth of long bones leads to eunuchoid proportions of the skeleton characterized by the prevailing length of arms and legs over the spine. Usually the eunuchoid skeleton is defined by the finding of an abnormal upper to lower segment ratio (<0.88) and by the predominance of the arm span over the patient's height (ratio > 1) [40, 47, 70]. Men with estrogen deficiency have a prolonged time available for linear growth thanks to a still open growth plate and they exhibit eunuchoid body proportions (Figure 1) [40, 47], which worsen if they are not treated with exogenous estrogens [43]. Conversely, if the onset of E_2 treatment starts at the proper time, during early puberty, body proportions are unaffected in males with aromatase [41]. This evidence highlights the concept that estrogen rather than androgen is necessary also for a harmonic skeletal growth (Figure 1) (Table 2). As a matter of fact, normal skeletal proportions are found in patients affected by complete androgens insensitivity syndrome (CAIS) where normal-to-high E_2 serum levels allow epiphyseal closure at the right timing, despite the absence of androgen action [71, 72]. The adult height of patients with CAIS usually corresponds, in fact, to both the calculated target and mean height of men rather than females standard [72]. All clinical conditions that lead to severe T deficiency before the completion of puberty and that are characterized by eunuchoid proportions of the skeleton [70] share the same mechanism of severe estrogen deficiency secondary to hypogonadism [73] (Figure 1). Thus, relative severe estrogen deficiency secondary to insufficient androgen production leads to eunuchoid body proportions in prepubertal male hypogonadism [70, 73], 17,20-lyase deficiency and combined 17α-hydroxylase and 17,20-lyase deficiency [40, 47].

2.3. Effects of Estrogens on Bone Mass Accrual and Attainment of Peak Bone Mass. The peak of bone mass (bone growth and BMD increase during childhood and young adulthood) determines the total amount of bone mineralized tissue available during adulthood till aging [74, 75]. Once the peak of bone mass is achieved during puberty and early adulthood (before the age of about 30 years), the bone becomes unable to reach further significant bone mass accrual. Thus, the bone mass remains stable or decreases on the basis of the balance between bone formation and resorption [74, 75]. The achievement of peak bone mass is prompted by the rise in sex steroids at puberty. Even this process was previously supposed to be under the control of estrogens in females and of androgens in males [10, 11, 76]. All the events that can negatively interfere with the achievement of peak bone mass predispose to the development of osteoporosis later in life in both sexes [74, 75]. Among them, physiological or pathological conditions that determine sex steroid deficiency result in impaired peak of bone mass, such as in the case of prepubertal hypogonadism or delayed puberty in boys and anorexia nervosa in girls [30, 31, 75].

Probably androgens alone are not sufficient for the achievement of a normal BMD and an optimal peak of bone mass since severe osteoporosis was reported in all

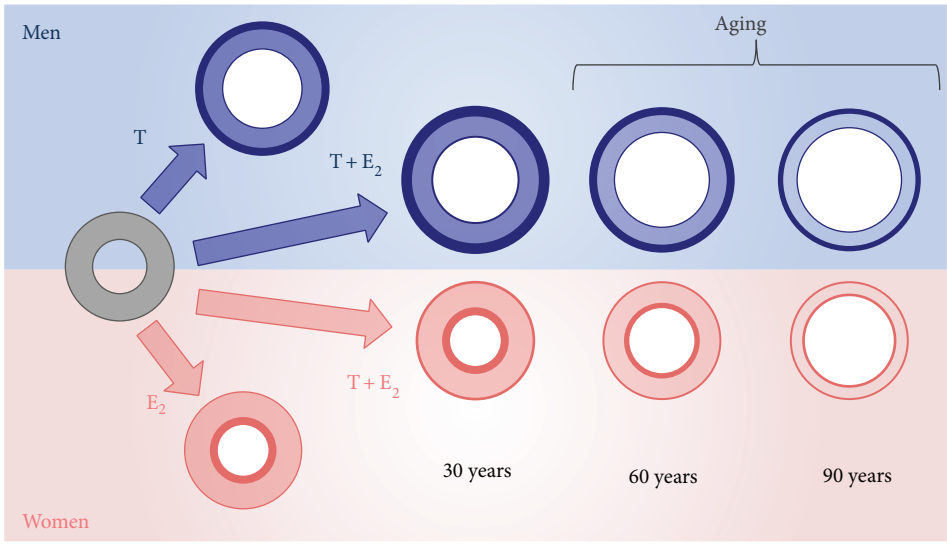


Figure 3: Schematic representation of the role of estrogen and androgen bone size according to gender. The effects of sex steroids on cortical and trabecular bone are represented. Bone size is reached in late puberty and early adulthood as depicted at the left of the panel where the effect of each sex steroid and their sum are shown according to gender. In men, the combined action of both T and E_2 led to greater bone size and cortical thickness than in women. The prevailing effect of E_2 is consistent with higher endosteal bone formation in women. Bone loss during aging occurs in a different fashion between man and women and is subordinate to the baseline conditions. Women lose more bone on the endosteal surface and in the trabecular portion of bone, while men lose mainly bone mass in the cortical bone (right side of the panel). E_2: estradiol, T: testosterone; modified in part from figures published in [75, 77].

young adults with estrogen resistance [36] or aromatase deficiency [38–40]. The importance of estrogens for the acquisition of peak bone mass during puberty is evident by the results obtained from both human [47] and mice models [8] of estrogen deficiency (Table 1). Several mice models of estrogen deficiency have been generated and all confirmed that even in rodent estrogens mediate most of the actions exerted on bone by androgens [8, 77, 78]. In particular, the knock-out of the ER-α in male mice leads to the increase of trabecular bone, the reduction of cortical bone, and the decrease of longitudinal bone growth, while the knock-out of the ER-β does not impact cortical and trabecular bone [78]. All these data reinforce the importance of ER-α in male bone homeostasis [78, 79]. Studies performed on animal models, however, do not allow transposing all the results to human male bone physiology due to substantial differences in sex steroid and bone physiology among species [77]. In rodent, in fact, circulating estrogens are very low and often undetectable so that the intracrine role of estrogens prevails over that of serum E_2 [77]. Furthermore, in rodent, sex steroids do not bind sex hormone binding globulin, and finally the process of bone maturation is different in rodent due to the absence of epiphyseal cartilage [77, 78]. The idea that estrogen is the main sex steroid involved in the acquisition of peak bone mass has been confirmed by other several data available in literature from rare models of sex steroids deficiency and from studies on pubertal boys. In men with CAIS, the peak of bone mass is only in part reduced, with intermediate values in-between those of male and female subjects [72], while men with aromatase excess syndrome display an increased BMD at the end of puberty due to high circulating estrogens throughout puberty [80]. In addition, outside the context of rare syndromes of sex steroids deficiency, several other studies involving pubertal boys clearly demonstrate that peak bone mass is under the control of estrogen even in men (Table 1) [17, 18, 48, 81–83]. During puberty, E_2 leads to the increase of bone mass mainly by increasing BMD, especially at the level of cortical bone whereas T contributes to increase the bone size, a phenomenon that is mainly mediated by the mechanical load exerted on bone by the increasing muscle mass [18]. As far as bone geometry is concerned, E_2 seems to be negatively associated to endosteal circumference [18] and seems to positively influence the increase of cortical thickness both at the level of radius and tibia [18]. Thus, both estrogens and androgens seem to be necessary for normal bone mass accrual during puberty. Androgens limit endosteal expansion and estrogens ensure adequate periosteal bone expansion [18] (Figure 3). The final result is a bone size greater than that of the female counterpart. Compared to females, male bone has, in fact, a larger cortical portion due to greater periosteal apposition and a larger endosteal circumference due to reduced endosteal apposition [18, 75, 77] (Figure 3). As a result of all these events, final peak bone mass is determined by the increase of BMD during puberty and early adulthood plus the remaining more slow bone accrual that continues till the 3rd decade of life and accounts for about 20% of peak bone mass [83].

3. Estrogen Effects on Bone during Adulthood

3.1. Estrogen and the Maintenance of BMD. In order to maintain a biomechanically efficient bone, the skeleton needs

to continuously remodel and repair the microcracks that develop both in the trabecular and cortical bone during lifetime [75, 83]. This process of remodeling occurs in basic multicellular units (BMUs) which include osteoclasts, osteoblasts, and osteocytes [84] that act altogether by coupling bone resorption and bone formation. The balance between bone formation and bone resorption determines the maintenance (if the two processes balance out the amount of bone mass) or the loss (in the case of resorption higher than formation) of bone mass in men [74, 84]. Sex steroids exert a direct action on the BMUs and can regulate, at least in part, bone remodeling [83–85]. While this effect was traditionally ascribed to estrogens in females, in the last 20 years, several studies progressively have disclosed the same outcome in men [62, 74, 75, 83–85].

Evidence from basic research had already demonstrated a direct estrogen action on bone cells and more data, especially on biomolecular mechanisms of action, have been progressively obtained till now [84, 85]. In particular, estrogens inhibit the apoptosis of osteocytes both in trabecular and cortical bone [84, 86]. They reduce bone resorption by means of both direct and indirect effects on osteoclasts [84] and act on osteoblasts, by inhibiting their apoptosis [84, 87]. In general, estrogen regulates bone remodeling by (i) inhibiting the activation of bone remodeling and the initiation of new BMUs; (ii) reducing the number and activity of osteoclasts (i.e., inhibition of their differentiation and promotion of apoptosis) and bone resorption; and (iii) increasing the number and the activity of osteoblasts (i.e., promotion of their commitment and differentiation and inhibition of apoptosis) and bone formation [77, 79, 84, 88]. For all these reasons, bone loss in man is mainly related to relative estrogen deficiency [74, 77, 84, 88, 89], while androgens have a minor role [74, 75, 83, 84]. Accordingly, the net effect of androgens *per se* on bone mass *in vivo* is quite poor. DHT, for example, is not able to increase BMD in patients with benign prostate hyperplasia [90]. In these patients, lumbar BMD decreases of about 1.5% after 24 months of treatment as a result of the net compensation between the poor anabolic effects of high circulating DHT and its prevalent negative effect on BMD. The latter is due to the inhibition of gonadotropin secretion and the reduction of sera T and E_2, with a reduced estrogen action on bone [90].

Several studies investigated the relationship between estrogen and BMD in men through different types of study design and all unequivocally demonstrate that estrogen action on the male bone is more determinant than androgen action [74, 75, 77, 83, 84, 89]. A normal BMD is observed in male patients with CAIS [72]. Serum E_2 and relative estrogen deficiency resulted associated with altered bone turnover markers [91], BMD [92–98], and fracture risk [99–102]. Most of these findings were confirmed by large epidemiological longitudinal studies [92, 94, 96–101]. Altogether, these studies provide evidence that E_2 is a better predictor of male bone health than T.

In addition, E_2 administered to male to female transsexuals [103–105] or to men with prostate cancer [106] significantly increases BMD, despite endogenous serum T suppression. Otherwise, aromatase inhibitors lead to alterations of bone turnover markers [107] and impairment of BMD [108]. Finally, genetic studies revealed an association between ERs [109, 110] or aromatase enzyme [111, 112] polymorphisms with decreased BMD in men.

Exogenous E_2 acts in a dose-dependent fashion on male bone since it restores a normal BMD in aromatase-deficient men given at a dose that ensures stable serum E_2 levels within the normal male range [40, 113, 114]. A daily dose of E_2 lower than 20 μg is usually unable to keep serum E_2 within the normal range in these patients. The result is BMD worsening or failure in restoring a normal BMD in men previously treated with higher doses [113] and in naive patients [45], respectively. Similarly, in adult men without genetic diseases involving estrogen pathways, E_2 seems to be protective for bone but only when serum levels are above a critical threshold [102]. In cohort studies, this threshold has been settled in-between 15 and 20 pg/mL [115]. By studying the effects of estrogen treatment in an aromatase-deficient man [45], this threshold has been more precisely determined as being around 16 pg/mL (Figure 2). This value has been also confirmed by studies on fracture risk in older men (Figure 2) [102]. Recently Khosla et al. stated that a serum E_2 level at least above 25 pg/mL is certainly protective for bone in men (Figure 2) [88]. All these data suggest that serum E_2 levels of 20 pg/mL or above are needed for optimal skeletal maturation and achievement of optimal peak bone mass [116]. This threshold is very close to that required also for the epiphyseal closure (Figure 2) [45, 116].

The concept that estrogens act on bone only when a specific amount is reached is well established, whereas the precise threshold value remains to be settled. Differences in study design and overall in methods used for estrogen assays might explain the discrepancy between values obtained from different studies. In the future, the wide use of the gold standard methods (LC/MS/MS) for the measurement of estrogen in serum will provide more reliable information on the exact threshold value: the latter will probably fall within the suggested range of 15–25 pg/mL (Figure 2). Furthermore, future studies will be of help in disclosing if individual, genetic differences in estrogen sensitivity might influence the amount of estrogens needed for a full estrogen action on bone.

In conclusion, clinical and basic research demonstrate that E_2 is the main sex steroid required for bone homeostasis in men [74, 77, 83, 84, 88, 89].

3.2. Estrogens and Bone Size. Bone size exhibits evident gender differences that are ascribed mainly to sex steroids actions on bone [83, 117, 118]. Estrogen controls the final length of long bones by acting on epiphyseal closure (see the paragraph above for details). The length of long bones is greater in men than in women. This could be once again the effect of the more rapid increase of estrogens at puberty in women which is responsible for an anticipated epiphyseal closure and growth cessation [17, 18, 48]. Actually, bone mass and strength are greater in men than in women; probably these differences are due to different length and bone structure among the two sexes (Figure 3) [76]. Bone

size, in fact, is larger in men than in women mostly as a consequence of a wider width of bone, especially of its cortical portion (Figure 3) [75, 77, 115, 118, 119]. The enlargement of the periosteum involves the appendicular skeleton, and it mainly occurs from puberty to the 3rd decade of life [75]. In men, this process leads to continuous increase of the cortical thickness (Figure 3) [119–121]. Conversely, in females, this process ceases earlier and does not continue during young adulthood [119–121]. In addition, the very high amount of estrogen in women is responsible mainly for endosteal bone formation. The final result is that adult females have smaller cortical bone portion and a shorter endosteal circumference than males (Figure 3) [75, 77, 115–121].

It has been suggested that the different levels of circulating androgens between the two sexes can explain this sexual dimorphism in bone structure. As the final pathways of sex steroids actions in bone are the same in the two sexes, this sexual dimorphism in bone size probably comes from indirect actions of sex steroids on tissues different from bone. With this in view, a possible role of T in establishing bone size can be explained by sex differences in muscle mass [122] which start to appear at the time of puberty [48]. Muscle mass is androgen-dependent [122] and greater muscle mass can exert greater mechanical action on bone [123], thus resulting in increased bone size and bone mass [75]. Obese boys, for example, have larger muscle size and larger bones in the legs if compared with lean boys at the same pubertal stage [48]. Furthermore, when T is administered to ovariectomized female-to-male transsexuals, both muscle and skeletal mass change and the final musculoskeletal system resembles that of the male counterpart [124].

The old view postulated that androgen stimulates periosteal expansion in men, whereas estrogen inhibits periosteal apposition in women. The finding of significant periosteal expansion prompted by estrogen treatment in a boy with aromatase deficiency [41] suggests a more complex cross-talk between these two hormones within the bone periosteal surface [125]. Estrogens might exert a permissive action on androgens, thus facilitating and promoting their anabolic effect on the periosteum; this event does not take place or is minimized when estrogens are lacking or are below the normal range (Figure 3) [125]. Lesson from rare models of estrogen or androgen deficiency seems to confirm this hypothesis. In fact, bone size has a female appearance in XY patients with CAIS [126, 127]. Accordingly, Vandenput and colleagues [128] studied the role of the androgen receptor in the skeletal homeostasis of androgen-resistant, testicular feminized, male mice and observed that bone size and bone formation at the periosteal surface depend also on a functional androgen receptor. The mechanism consists in a permissive estrogen role on androgens and seems to operate also on trabecular bone homeostasis [114]. This permissive estrogen action, however, needs to be confirmed and better clarified on a molecular point of view.

In conclusion, the role of each sex steroid on bone size and on gender difference is a complex phenomenon that involves also other endocrine systems like the GH/IGF-1 and that needs still to be better clarified.

4. Estrogen Effects on Bone While Aging

The progressive decline of T that usually occurs with advancing age might result in a corresponding decrease of circulating estrogens in men [74, 98, 102, 129]. Relative estrogen deficiency in elderly men with low T is common and has been clearly demonstrated in most of the longitudinal studies on sex steroids in older men [98, 101, 102, 129]. There is, however, the possibility that both androgens and estrogens do not decline with advancing age, especially in older men in a good health status [130]. A mild-to-severe reduction of circulating T in older men who develop age-related hypogonadism is directly responsible for bone loss, but the concomitant decrease of circulating E_2 has a main impact on bone health [14, 129]. In older men, the 70–85% of the decrease in BMD related to sex steroids decline is imputable to estrogen deficiency while only the remaining 15–30% is imputable to androgens [74, 84, 91]. Relative estrogen deficiency causes a decrease of both trabecular [83, 88, 89, 98–101] and cortical [131] bone in older men, leading to a bone structure that strongly resembles that of young aromatase-deficient men [74, 132]. Bone size, in fact, decreases at least in part as an effect of contraction of the cortical portion of the bone [75, 77, 116, 117]. However, the main changes related to aging that occur in male bone regard the bone structure. In particular, cortical porosity increases with age [75, 77, 131] while BMD decreases [75, 77, 83, 84]. BMD changes are due to the reduction of trabecular bone volume that is sustained by the thinning of the trabeculae rather than a reduction of their number, the latter being a mechanism involved in female bone aging [75, 77, 83, 84]. Another mechanism involved in male bone aging is endosteal resorption. In men, endocortical resorption is less pronounced than in women but substantially contributes to decreasing cortical cross-sectional area and consequently bone strength (Figure 3) [74, 75, 77, 83, 84, 88, 89, 98–101, 131].

To what extent the decrease in BMD due to relative estrogen deficiency contributes to the incidence of fractures in aging men with late-onset hypogonadism is still not completely clear. Men with congenital estrogen deficiency were classically considered at risk to develop fracture, but only recently a history of pathological fractures of the forearm after minimal trauma has been observed in a man with aromatase deficiency [46]. Long-term outcome concerning fractures in those rare male patients remains still not available [40]. Data obtained from older men demonstrate that E_2 is inversely associated with BMD [92–98] and seems to predict fractures better than T [99–102]. However, not all the older men with low serum T develop relative estrogen deficiency [98, 102] and probably only men with concomitant low serum E_2 are at high risk of fracture. Individual differences in both aromatase activity and expression probably might explain why in the presence of low serum T only a subgroup of men with late-onset hypogonadism has relative estrogen deficiency (Table 2) [74]. However, well designed studies aimed at exploring this hypothesis are still lacking. Among men with low serum T, those with concomitant estrogen deficiency should be considered to be at high risk of osteoporotic fractures (Table 2), especially when serum E_2 is below 25 pg/mL

Table 2: Risk of osteoporosis and fractures along with clinical manifestations according to estrogen status in elderly men.

	Elderly men with late onset hypogonadism	
Estrogenic status	**Relative estrogen deficiency** (less functioning aromatase)	**Normal circulating estrogens** (normal functioning aromatase)
Clinical phenotype	Low serum E_2	Normal serum E_2
	Normal E_2/T ratio	Impaired E_2/T ratio
	Highest gonadotropins	Less increased gonadotropins
	Severely impaired BMD	Normal to moderate BMD decrease
	Osteoporosis	Osteopenia
		Risk of developing gynecomastia
Fracture risk	**High**	**Low**

E_2: estradiol; T: testosterone; BMD: bone mineral density.

(Figure 2). T deficiency further contributes to increasing the risk of fractures in these patients. Accordingly, muscle mass reduction that is related to androgen depletion [122] worsens BMD [123] and increases the risk of falling in elderly men [115, 133].

In conclusion, elderly male might present relative estrogen deficiency that may be implied in several age-related conditions [14] that can affect and worsen quality of life, including bone loss.

5. Areas of Uncertainties

Even though now the crucial role of estrogen on bone homeostasis is well established many uncertainties still remain to be clarified both in the field of basic and clinical research as well as in the field of translational endocrinology.

5.1. The Research Corner. Estrogen effect on male skeleton during fetal life and childhood has not yet been investigated while preliminary data during pubertal development are becoming available [16–18, 48] (Table 1). Furthermore, data on the effects of estrogen deprivation on the growing skeleton in men with congenital estrogen deficiency are scanty [40].

The exact molecular mechanism of estrogen action on the process of skeletal maturation remains to be established in details. We still do not know whether estrogens are directly involved in epiphyseal closure or whether their effect is mediated by a more complex hormonal network (endocrine and/or paracrine) including cytokines and growth factors. The lack of animal models useful to study the pathophysiology of the growth plate complicates the advancements on this issue. The models available present several limitations. The physiology of growth plate and the process of ossification of long bones in rodents are very different from those of humans [22, 23, 60]. The rabbit is closer to human but presents several significant differences [22, 23, 49–51].

While the role of circulating estrogens on bone maturation and accrual has been clarified, the contribution of intracrine estrogen production is unknown. Furthermore, the other endocrine systems and growth factors recruited by estrogen and having an important role within the bone remains to be identified.

The effects on bone of selective estrogen receptor modulators (SERMs) remain doubtful due to conflicting results available in the literature. Raloxifene, a SERM with a proven estrogen agonist action on bone with estrogen antagonist actions on other tissues, was not effective in inducing epiphyseal closure in an aromatase-deficient man after 24 months of treatment [134]. Conversely, tamoxifen, another SERM with agonist action on bone, induces permanent growth arrest through the apoptosis of chondrocytes in rats [135]. Raloxifene, however, is able to increase BMD both in aromatase-deficient men [134] and in men with prostate cancer [136, 137].

5.2. The Clinical Corner. About 20 years of research in the field of estrogen on bone did not lead to significant changes in clinical practice. Therapeutic strategies aiming to target estrogens for inducing changes in the male bone include aromatase inhibitors for the treatment of short stature in children and adolescents and estrogen-like compound for osteoporosis in adult and elderly men. None of these strategies, however, reached the expected results and nowadays they remain confined to the clinical research area without any real impact and extensive use in clinical practice.

Aromatase inhibitors are effective in increasing final height [67, 138] but the results in terms of centimeters reached are less than those expected if the epiphyseal closure is completely blocked. Aromatase inhibitors decelerate the advancement of bone age [67, 138], but the latter is not completely blocked as it happens in aromatase-deficient men [67, 138]. Probably even the most potent 3rd generation aromatase inhibitors are not able to completely block the enzyme [139] or, alternatively, after a first period of successful blockade, a phenomenon of escape might occur since serum E_2 tends to progressively increase even though at a lower rate than in the placebo group [138]. This probably also accounts for the lack of reported undesired effects, such as osteoporosis, which would be expected in the case of complete blockade of the enzyme. Recently, however, Hero et al. reported vertebral deformities in boys treated with letrozole [140]. It is not clear whether severe prepubertal and pubertal estrogen deficiency might lead to skeletal deformities. Sporadic skeletal deformities (i.e., kyphosis and bilateral femoral osteonecrosis) have been observed in men with estrogen deficiency, but the cause-effect relationship with estrogen deprivation needs to be confirmed [40]. Finally, further data from clinical trials are required in order to obtain final data on safety for the use of aromatase inhibitors in boys since theoretically more adverse events are expected to occur [141, 142].

The most critical issue in clinical practice is the poor reliability of the commercially available assays. Their accuracy and reproducibility are insufficient especially in the low range of serum E_2 typical of men [40, 74, 143]. The development of new techniques that are considered to be the gold standard, such as LC/MS/MS, is providing precise standard methodologies useful for serum estrogen measurement [143].

In the last years, these new methodologies are becoming even more widespread among clinical laboratories since they could be cost and time saving, especially in laboratories that perform a great number of assays per day. At present, serum E_2 is not currently part of the work-up used for the clinical diagnosis and management of male osteoporosis. Several authors, however, are going to introduce this biochemical test in the clinic when the lab outcomes are accurate. Thus, we will be able to improve our knowledge about the real critical amount of circulating estrogens required to ensure bone health.

Osteoporosis still remains an overlooked and undermanaged disease in older men [74, 144], while all the advancements in the field of estrogen deficiency and bone did not result in practical, significant changes in the approach to male osteoporosis. All these uncertainties reflect the wide differences among physicians approaching estrogen deficiency in the context of osteoporosis in older men [74, 144] as well as all the difficulties in identifying a way to physiologically increase serum E_2 without concomitantly impairing androgen production. The only practicable way remains T therapy that is able to restore both normal sera T and E_2 with consequent beneficial effects on bone in the presence of a normal functioning enzyme [144]. However, T replacement treatment should be considered only after careful evaluation of potential benefits and disadvantages since it could be harmful especially in older men who are not in a good health status [145–147]. It should be remarked that T treatment is currently not a treatment of choice for male osteoporosis [148].

6. Conclusions

Several evidence support the view that estrogens are the main sex steroids involved in processes such as bone maturation, bone mass accrual, and epiphyseal closure in men. Estrogen actions on bone, especially on bone maturation, remained quite unaltered among both gender and species during evolution, thus suggesting a high degree of conservative functions for estrogen that are also confirmed by the high degree of homologies of the aromatase enzyme. Conversely, estrogen actions on other tissues and organs are determinant in ensuring gender differences (e.g., primary and secondary sexual characteristics) and in promoting sexual divergence between the two sexes during evolution. The existence of a threshold level for serum E_2 that is necessary for ensuring skeletal maturation and adequate bone size and BMD confirms how complex the way estrogen acts on bone in men is. All these evidence contribute to make the issue of estrogen action on bone a fascinating one in the field of both basic and experimental research and encourage researches in order to find new strategies for the management and treatment of bone diseases related to estrogen deficiency.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] P. C. MacDonald, J. D. Madden, P. F. Brenner, J. D. Wilson, and P. K. Siiteri, "Origin of estrogen in normal men and in women with testicular feminization," *The Journal of Clinical Endocrinology & Metabolism*, vol. 49, no. 6, pp. 905–916, 1979.

[2] E. R. Simpson, M. S. Mahendroo, G. D. Means et al., "Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis," *Endocrine Reviews*, vol. 15, no. 3, pp. 342–355, 1994.

[3] W. L. Miller and R. J. Auchus, "The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders," *Endocrine Reviews*, vol. 32, no. 1, pp. 81–151, 2011.

[4] C. Stocco, "Tissue physiology and pathology of aromatase," *Steroids*, vol. 77, no. 1-2, pp. 27–35, 2012.

[5] E. Enmark and J.-Å. Gustafsson, "Oestrogen receptors—an overview," *Journal of Internal Medicine*, vol. 246, no. 2, pp. 133–138, 1999.

[6] C. J. Gruber, W. Tschugguel, C. Schneeberger, and J. C. Huber, "Mechanisms of disease: production and actions of estrogens," *The New England Journal of Medicine*, vol. 346, no. 5, pp. 340–352, 2002.

[7] C. M. Revankar, D. F. Cimino, L. A. Sklar, J. B. Arterburn, and E. R. Prossnitz, "A transmembrane intracellular estrogen receptor mediates rapid cell signaling," *Science*, vol. 307, no. 5715, pp. 1625–1630, 2005.

[8] J. F. Couse and K. S. Korach, "Estrogen receptor null mice: what have we learned and where will they lead us?" *Endocrine Reviews*, vol. 20, no. 3, pp. 358–417, 1999.

[9] Y. Murata, K. M. Robertson, M. E. E. Jones, and E. R. Simpson, "Effect of estrogen deficiency in the male: the ArKO mouse model," *Molecular and Cellular Endocrinology*, vol. 193, no. 1-2, pp. 7–12, 2002.

[10] M. Faustini-Fustini, V. Rochira, and C. Carani, "Oestrogen deficiency in men: where are we today?" *European Journal of Endocrinology*, vol. 140, no. 2, pp. 111–129, 1999.

[11] M. M. Grumbach and R. J. Auchus, "Estrogen: consequences and implications of human mutations in synthesis and action," *The Journal of Clinical Endocrinology & Metabolism*, vol. 84, no. 12, pp. 4677–4694, 1999.

[12] B. J. Deroo and K. S. Korach, "Estrogen receptors and human disease," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 561–570, 2006.

[13] V. Rochira, A. R. M. Granata, B. Madeo, L. Zirilli, G. Rossi, and C. Carani, "Estrogens in males: what have we learned in the last 10 years?" *Asian Journal of Andrology*, vol. 7, no. 1, pp. 3–20, 2005.

[14] J. S. Finkelstein, H. Lee, S.-A. M. Burnett-Bowie et al., "Gonadal steroids and body composition, strength, and sexual function in men," *The New England Journal of Medicine*, vol. 369, no. 11, pp. 1011–1022, 2013.

[15] F. W. George and J. D. Wilson, "Sex determination and differentiation," in *The Physiology of Reproduction*, E. Knobil and I. J. Neill, Eds., pp. 2–27, Raven Press, New York, NY, USA, 1988.

[16] K. Bay, A. M. Andersson, and N. E. Skakkebaek, "Estradiol levels in prepubertal boys and girls—analytical challenges," *International Journal of Andrology*, vol. 27, no. 5, pp. 266–273, 2004.

[17] S. Vandewalle, Y. Taes, T. Fiers et al., "Sex steroids in relation to sexual and skeletal maturation in obese male adolescents," *The Journal of Clinical Endocrinology and Metabolism*, vol. 99, no. 8, pp. 2977–2985, 2014.

[18] S. Vandewalle, Y. Taes, T. Fiers et al., "Associations of sex steroids with bone maturation, bone mineral density, bone geometry, and body composition: a cross-sectional study in healthy male adolescents," *The Journal of Clinical Endocrinology and Metabolism*, vol. 99, no. 7, pp. E1272–E1282, 2014.

[19] K. O. Klein, P. M. Martha Jr., R. M. Blizzard, T. Herbst, and A. D. Rogol, "A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. II. Estrogen levels as determined by an ultrasensitive bioassay," *The Journal of Clinical Endocrinology & Metabolism*, vol. 81, no. 9, pp. 3203–3207, 1996.

[20] G. B. Cutler Jr., "The role of estrogen in bone growth and a maturation during childhood and adolescence," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 61, no. 3–6, pp. 141–144, 1997.

[21] V. Rochira, A. Balestrieri, M. Faustini-Fustini, and C. Carani, "Role of estrogen on bone in the human male: insights from the natural models of congenital estrogen deficiency," *Molecular and Cellular Endocrinology*, vol. 178, no. 1-2, pp. 215–220, 2001.

[22] A. S. Chagin and L. Sävendahl, "Estrogens and growth: review," *Pediatric Endocrinology Reviews*, vol. 4, no. 4, pp. 329–334, 2007.

[23] J. Emons, A. S. Chagin, L. Sävendahl, M. Karperien, and J. M. Wit, "Mechanisms of growth plate maturation and epiphyseal fusion," *Hormone Research in Paediatrics*, vol. 75, no. 6, pp. 383–391, 2011.

[24] L. Wilkins, "Endocrine relationships and their influences upon growth and development," in *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence*, chapter 2, pp. 10–24, C. C. Thomas, Springfield, Ill, USA, 1950.

[25] H. S. Kuppermann, "Male endocrinology: hypogonadism in adolescent male and cryptorchidism," in *Human Endocrinology*, H. S. Kuppermann, Ed., Chapter 16, pp. 504–563, Davis Company Publishers, Philadelphia, Pa, USA, 1963.

[26] R. M. Blizzard, R. G. Thompson, A. Baghdassarian, A. Kowarski, C. J. Migeon, and A. Rodriguez, "The interrelationship of steroids, growth hormone and other hormones on pubertal growth," in *The Control of the Onset of Puberty*, M. M. Grumbach, G. D. Grave, and F. E. Mayer, Eds., p. 342, John Wiley & Sons, New York, NY, USA, 1974.

[27] W. A. Marshall, "Interrelationships of skeletal maturation, sexual development and somatic growth in man," *Annals of Human Biology*, vol. 1, no. 1, pp. 29–40, 1974.

[28] J. J. Trueta, "The growth and development of bones and joints: orthopedic aspects," in *Scientific Foundation of Pediatrics*, J. Davis and J. Dobbing, Eds., pp. 399–419, W.B. Saunders, Philadelphia, Pa, USA, 1974.

[29] J. J. van der Werff ten Bosch, "Testosterone as growth stimulant in man," *Pharmacology and Therapeutics*, vol. 2, no. 1, pp. 17–32, 1977.

[30] J. S. Finkelstein, A. Klibanski, R. M. Neer, S. L. Greenspan, D. I. Rosenthal, and W. F. Crowley Jr., "Osteoporosis in men with idiopathic hypogonadotropic hypogonadism," *Annals of Internal Medicine*, vol. 106, no. 3, pp. 354–361, 1987.

[31] J. S. Finkelstein, R. M. Neer, B. M. K. Biller, J. D. Crawford, and A. Klibanski, "Osteopenia in men with a history of delayed puberty," *The New England Journal of Medicine*, vol. 326, no. 9, pp. 600–604, 1992.

[32] J. S. van den Bosch, A. G. H. Smals, G. F. F. M. Pieters, I. M. Valk, and P. W. Kloppenborg, "Instant growth inhibition by low dose oestrogens in excessively tall boys," *Acta Endocrinologica*, vol. 100, no. 3, pp. 327–332, 1982.

[33] M. Caruso-Nicoletti, F. Cassorla, M. Skerda, J. L. Ross, D. L. Loriaux, and G. B. Cutler Jr., "Short term, low dose estradiol accelerates ulnar growth in boys," *The Journal of Clinical Endocrinology and Metabolism*, vol. 61, no. 5, pp. 896–898, 1985.

[34] G. B. Cutler Jr., F. G. Cassorla, J. L. Ross et al., "Pubertal growth: physiology and pathophysiology," *Recent Progress in Hormone Research*, vol. 42, pp. 443–470, 1986.

[35] P. A. Lee and S. F. Witchel, "The influence of estrogen on growth," *Current Opinion in Pediatrics*, vol. 9, no. 4, pp. 431–436, 1997.

[36] E. P. Smith, J. Boyd, G. R. Frank et al., "Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man," *The New England Journal of Medicine*, vol. 331, no. 16, pp. 1056–1061, 1994.

[37] A. Morishima, M. M. Grumbach, E. R. Simpson, C. Fisher, and K. Qin, "Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens," *Journal of Clinical Endocrinology and Metabolism*, vol. 80, no. 12, pp. 3689–3698, 1995.

[38] C. Carani, K. Qin, M. Simoni et al., "Effect of testosterone and estradiol in a man with aromatase deficiency," *The New England Journal of Medicine*, vol. 337, no. 2, pp. 91–95, 1997.

[39] J. P. Bilezikian, A. Morishima, J. Bell, and M. M. Grumbach, "Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency," *The New England Journal of Medicine*, vol. 339, no. 9, pp. 599–603, 1998.

[40] V. Rochira and C. Carani, "Aromatase deficiency in men: a clinical perspective," *Nature Reviews Endocrinology*, vol. 5, no. 10, pp. 559–568, 2009.

[41] R. Bouillon, M. Bex, D. Vanderschueren, and S. Boonen, "Estrogens are essential for male pubertal periosteal bone expansion," *The Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 12, pp. 6025–6029, 2004.

[42] B. L. Herrmann, B. Saller, O. E. Janssen et al., "Impact of estrogen replacement therapy in a male with congenital aromatase deficiency caused by a novel mutation in the CYP19 gene," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 12, pp. 5476–5484, 2002.

[43] L. Maffei, Y. Murata, V. Rochira et al., "Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment," *The Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 1, pp. 61–70, 2004.

[44] L. Maffei, V. Rochira, L. Zirilli et al., "A novel compound heterozygous mutation of the aromatase gene in an adult man: reinforced evidence on the relationship between congenital oestrogen deficiency, adiposity and the metabolic syndrome," *Clinical Endocrinology*, vol. 67, no. 2, pp. 218–224, 2007.

[45] F. Lanfranco, L. Zirilli, M. Baldi et al., "A novel mutation in the human aromatase gene: Insights on the relationship among serum estradiol, longitudinal growth and bone mineral density in an adult man under estrogen replacement treatment," *Bone*, vol. 43, no. 3, pp. 628–635, 2008.

[46] E. Pignatti, K. Unluhizarci, E. Kartal et al., "Complete aromatase deficiency in four adult men: detection of a novel mutation and two known mutations in the CYP19A1 gene," *Endocrine Abstracts*, vol. 32, p. 640, 2013.

[47] L. Zirilli, V. Rochira, C. Diazzi, G. Caffagni, and C. Carani, "Human models of aromatase deficiency," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 109, no. 3–5, pp. 212–218, 2008.

[48] S. Vandewalle, Y. Taes, M. van Helvoirt et al., "Bone size and bone strength are increased in obese male adolescents,"

The Journal of Clinical Endocrinology and Metabolism, vol. 98, no. 7, pp. 3019–3028, 2013.

[49] M. Weise, S. De-Levi, K. M. Barnes, R. I. Gafni, V. Abad, and J. Baron, "Effects of estrogen on growth plate senescence and epiphyseal fusion," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 12, pp. 6871–6876, 2001.

[50] O. Nilsson, M. Weise, E. B. Landman, I. L. Meyers, K. M. Barnes, and J. Baron, "Evidence that estrogen hastens epiphyseal fusion and cessation of longitudinal bone growth by irreversibly depleting the number of resting zone progenitor cells in female rabbits," *Endocrinology*, vol. 155, no. 8, pp. 2892–2899, 2014.

[51] V. Kusec, A. S. Virdi, R. Prince, and J. T. Triffitt, "Localization of estrogen receptor-α in human and rabbit skeletal tissues," *The Journal of Clinical Endocrinology & Metabolism*, vol. 83, no. 7, pp. 2421–2428, 1998.

[52] L. O. Nilsson, A. Boman, L. Sävendahl et al., "Demonstration of estrogen receptor-β immunoreactivity in human growth plate cartilage," *The Journal of Clinical Endocrinology & Metabolism*, vol. 84, no. 1, pp. 370–373, 1999.

[53] A. S. Chagin and L. Sävendahl, "Brief report: GPR30 estrogen receptor expression in the growth plate declines as puberty progresses," *The Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 12, pp. 4873–4877, 2007.

[54] O. K. Oz, R. Millsaps, R. Welch, J. Birch, and J. E. Zerwekh, "Expression of aromatase in the human growth plate," *Journal of Molecular Endocrinology*, vol. 27, no. 2, pp. 249–253, 2001.

[55] V. L. Sylvia, I. Gay, R. Hardin, D. D. Dean, B. D. Boyan, and Z. Schwartz, "Rat costochondral chondrocytes produce 17β-estradiol and regulate its production by 1α,25(OH)2D3," *Bone*, vol. 30, no. 1, pp. 57–63, 2002.

[56] B. C. J. van der Eerden, J. D. E. van Ven, C. W. G. M. Lowik, J. M. Wit, and M. Karperien, "Sex steroid metabolism in the tibial growth plate of the rat," *Endocrinology*, vol. 143, no. 10, pp. 4048–4055, 2002.

[57] A. S. Chagin, D. Chrysis, M. Takigawa, E. M. Ritzen, and L. Sävendahl, "Locally produced estrogen promotes fetal rat metatarsal bone growth; an effect mediated through increased chondrocyte proliferation and decreased apoptosis," *Journal of Endocrinology*, vol. 188, no. 2, pp. 193–203, 2006.

[58] S. H. Windahl, N. Andersson, A. S. Chagin et al., "The role of the G protein-coupled receptor GPR30 in the effects of estrogen in ovariectomized mice," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 296, no. 3, pp. E490–E496, 2009.

[59] A. E. Börjesson, M. K. Lagerquist, S. H. Windahl, and C. Ohlsson, "The role of estrogen receptor α in the regulation of bone and growth plate cartilage," *Cellular and Molecular Life Sciences*, vol. 70, no. 21, pp. 4023–4037, 2013.

[60] A. S. Chagin, M. K. Lindberg, N. Andersson et al., "Estrogen receptor-β inhibits skeletal growth and has the capacity to mediate growth plate fusion in female mice," *Journal of Bone and Mineral Research*, vol. 19, no. 1, pp. 72–77, 2004.

[61] E. P. Smith, B. Specker, B. E. Bachrach et al., "Impact on bone of an estrogen receptor-α gene loss of function mutation," *The Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 8, pp. 3088–3096, 2008.

[62] E. P. Smith, B. Specker, and K. S. Korach, "Recent experimental and clinical findings in the skeleton associated with loss of estrogen hormone or estrogen receptor activity," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 118, no. 4-5, pp. 264–272, 2010.

[63] A. S. Chagin and L. Sävendahl, "Oestrogen receptors and linear bone growth," *Acta Paediatrica*, vol. 96, no. 9, pp. 1275–1279, 2007.

[64] J. D. Veldhuis, J. N. Roemmich, E. J. Richmond et al., "Endocrine control of body composition in infancy, childhood, and puberty," *Endocrine Reviews*, vol. 26, no. 1, pp. 114–146, 2005.

[65] V. Rochira, L. Zirilli, L. Maffei et al., "Tall stature without growth hormone: four male patients with aromatase deficiency," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 4, pp. 1626–1633, 2010.

[66] D. L. Metzger and J. R. Kerrigan, "Estrogen receptor blockade with tamoxifen diminishes growth hormone secretion in boys: evidence for a stimulatory role of endogenous estrogens during male adolescence," *The Journal of Clinical Endocrinology & Metabolism*, vol. 79, no. 2, pp. 513–518, 1994.

[67] M. Hero, E. Norjavaara, and L. Dunkel, "Inhibition of estrogen biosynthesis with a potent aromatase inhibitor increases predicted adult height in boys with idiopathic short stature: a randomized controlled trial," *The Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 12, pp. 6396–6402, 2005.

[68] O. Nilsson, D. Chrysis, O. Pajulo et al., "Localization of estrogen receptors-α and -β and androgen receptor in the human growth plate at different pubertal stages," *Journal of Endocrinology*, vol. 177, no. 2, pp. 319–326, 2003.

[69] S. G. Ren, S. Malozowski, P. Sanchez, D. E. Seet, D. L. Loriaux, and F. Cassorla, "Direct administration of testosterone increases rat tibial epiphyseal growth plate width," *Acta Endocrinologica*, vol. 121, no. 3, pp. 401–405, 1989.

[70] E. Nieschlag and H. Behre, "Clinical use of testosterone in hypogonadism and other conditions," in *Testosterone: Action, Deficiency, Substitution*, E. Nieschlag and H. Behre, Eds., chapter 14, pp. 292–308, Cambridge University Press, Cambridge, UK, 4th edition, 2012.

[71] M. Zachmann, A. Prader, E. H. Sobel et al., "Pubertal growth in patients with androgen insensitivity: indirect evidence for the importance of estrogens in pubertal growth of girls," *The Journal of Pediatrics*, vol. 108, no. 5, pp. 694–697, 1986.

[72] Y. Taes, B. Lapauw, S. Vandewalle et al., "Estrogen-specific action on bone geometry and volumetric bone density: longitudinal observations in an adult with complete androgen insensitivity," *Bone*, vol. 45, no. 2, pp. 392–397, 2009.

[73] S. Trabado, L. Maione, S. Salenave et al., "Estradiol levels in men with congenital hypogonadotropic hypogonadism and the effects of different modalities of hormonal treatment," *Fertility and Sterility*, vol. 95, no. 7, pp. 2324–2329, 2011.

[74] V. Rochira, A. Balestrieri, B. Madeo, L. Zirilli, A. R. M. Granata, and C. Carani, "Osteoporosis and male age-related hypogonadism: role of sex steroids on bone (patho) physiology," *European Journal of Endocrinology*, vol. 154, no. 2, pp. 175–185, 2006.

[75] M. Laurent, L. Antonio, M. Sinnesael et al., "Androgens and estrogens in skeletal sexual dimorphism," *Asian Journal of Andrology*, vol. 16, no. 2, pp. 213–222, 2014.

[76] J.-P. Bonjour, G. Theintz, B. Buchs, D. Slosman, and R. Rizzoli, "Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence," *The Journal of Clinical Endocrinology and Metabolism*, vol. 73, no. 3, pp. 555–563, 1991.

[77] D. Vanderschueren, M. R. Laurent, F. Claessens et al., "Sex steroid actions in male bone," *Endocrine Reviews*, 2014.

[78] L. Vico and J.-M. Vanacker, "Sex hormones and their receptors in bone homeostasis: insights from genetically modified mouse

models," *Osteoporosis International*, vol. 21, no. 3, pp. 365–372, 2010.

[79] F. Callewaert, S. Boonen, and D. Vanderschueren, "Sex steroids and the male skeleton: a tale of two hormones," *Trends in Endocrinology and Metabolism*, vol. 21, no. 2, pp. 89–95, 2010.

[80] M. Fukami, M. Shozu, and T. Ogata, "Molecular bases and phenotypic determinants of aromatase excess syndrome," *International Journal of Endocrinology*, vol. 2012, Article ID 584807, 8 pages, 2012.

[81] D. Yilmaz, B. Ersoy, E. Bilgin, G. Gümüşer, E. Onur, and E. D. Pinar, "Bone mineral density in girls and boys at different pubertal stages: relation with gonadal steroids, bone formation markers, and growth parameters," *Journal of Bone and Mineral Metabolism*, vol. 23, no. 6, pp. 476–482, 2005.

[82] B. M. Lapauw, Y. Taes, V. Bogaert et al., "Serum estradiol is associated with volumetric BMD and modulates the impact of physical activity on bone size at the age of peak bone mass: a study in healthy male siblings," *Journal of Bone and Mineral Research*, vol. 24, no. 6, pp. 1075–1085, 2009.

[83] B. L. Riggs, S. Khosla, and L. J. Melton III, "Sex steroids and the construction and conservation of the adult skeleton," *Endocrine Reviews*, vol. 23, no. 3, pp. 279–302, 2002.

[84] S. Khosla, M. J. Oursler, and D. G. Monroe, "Estrogen and the skeleton," *Trends in Endocrinology and Metabolism*, vol. 23, no. 11, pp. 576–581, 2012.

[85] S. C. Manolagas, C. A. O'Brien, and M. Almeida, "The role of estrogen and androgen receptors in bone health and disease," *Nature Reviews Endocrinology*, vol. 9, no. 12, pp. 699–712, 2013.

[86] A. Tomkinson, E. F. Gevers, J. M. Wit, J. Reeve, and B. S. Noble, "The role of estrogen in the control of rat osteocyte apoptosis," *Journal of Bone and Mineral Research*, vol. 13, no. 8, pp. 1243–1250, 1998.

[87] S. Kousteni, T. Bellido, L. I. Plotkin et al., "Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity," *Cell*, vol. 104, no. 5, pp. 719–730, 2001.

[88] S. Khosla, L. J. Melton III, and B. L. Riggs, "The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: is a revision needed?" *Journal of Bone and Mineral Research*, vol. 26, no. 3, pp. 441–451, 2011.

[89] S. Khosla, "Update on estrogens and the skeleton," *The Journal of Clinical Endocrinology & Metabolism*, vol. 95, no. 8, pp. 3569–3577, 2010.

[90] A. Idan, K. A. Griffiths, D. T. Harwood et al., "Long-term effects of dihydrotestosterone treatment on prostate growth in healthy, middle-aged men without prostate disease: a randomized, placebo-controlled trial," *Annals of Internal Medicine*, vol. 153, no. 10, pp. 621–632, 2010.

[91] A. Falahati-Nini, B. L. Riggs, E. J. Atkinson, W. M. O'Fallon, R. Eastell, and S. Khosla, "Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men," *The Journal of Clinical Investigation*, vol. 106, no. 12, pp. 1553–1560, 2000.

[92] G. A. Greendale, S. Edelstein, and E. Barrett-Connor, "Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo study," *Journal of Bone and Mineral Research*, vol. 12, no. 11, pp. 1833–1843, 1997.

[93] C. W. Slemenda, C. Longcope, L. Zhou, S. L. Hui, M. Peacock, and C. C. Johnston, "Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens," *The Journal of Clinical Investigation*, vol. 100, no. 7, pp. 1755–1759, 1997.

[94] S. Amin, Y. Zhang, C. T. Sawin et al., "Association of hypogonadism and estradiol levels with bone mineral density in elderly men from the Framingham study," *Annals of Internal Medicine*, vol. 133, no. 12, pp. 951–963, 2000.

[95] S. Khosla, L. J. Melton III, E. J. Atkinson, and W. M. O'Fallon, "Relationship of serum sex steroid levels to longitudinal changes in bone density in young versus elderly men," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 8, pp. 3555–3561, 2001.

[96] P. Szulc, F. Munoz, B. Claustrat et al., "Bioavailable estradiol may be an important determinant of osteoporosis in men: the MINOS study," *The Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 1, pp. 192–199, 2001.

[97] K. E. Ensrud, C. E. Lewis, L. C. Lambert et al., "Endogenous sex steroids, weight change and rates of hip bone loss in older men: the MrOS study," *Osteoporosis International*, vol. 17, no. 9, pp. 1329–1336, 2006.

[98] K. A. Ward, S. R. Pye, J. E. Adams et al., "Influence of age and sex steroids on bone density and geometry in middle-aged and elderly European men," *Osteoporosis International*, vol. 22, no. 5, pp. 1513–1523, 2011.

[99] E. Barrett-Connor, J. E. Mueller, D. G. von Mühlen, G. A. Laughlin, D. L. Schneider, and D. J. Sartoris, "Low levels of estradiol are associated with vertebral fractures in older men, but not women: the Rancho Bernardo Study," *Journal of Clinical Endocrinology and Metabolism*, vol. 85, no. 1, pp. 219–223, 2000.

[100] H. W. Goderie-Plomp, M. van der Klift, W. de Ronde, A. Hofman, F. H. de Jong, and H. A. P. Pols, "Endogenous sex hormones, sex hormone-binding globulin, and the risk of incident vertebral fractures in elderly men and women: the Rotterdam study," *The Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 7, pp. 3261–3269, 2004.

[101] S. Amin, Y. Zhang, D. T. Felson et al., "Estradiol, testosterone, and the risk for hip fractures in elderly men from the Framingham Study," *The American Journal of Medicine*, vol. 119, no. 5, pp. 426–433, 2006.

[102] D. Mellström, L. Vandenput, H. Mallmin et al., "Older men with low serum estradiol and high serum SHBG have an increased risk of fractures," *Journal of Bone and Mineral Research*, vol. 23, no. 10, pp. 1552–1560, 2008.

[103] S. Reutrakul, B. Ongphiphadhanakul, N. Piaseu et al., "The effects of oestrogen exposure on bone mass in male to female transsexuals," *Clinical Endocrinology*, vol. 49, no. 6, pp. 811–814, 1998.

[104] P. van Kesteren, P. Lips, L. J. G. Gooren, H. Asscheman, and J. Megens, "Long-term follow-up of bone mineral density and bone metabolism in transsexuals treated with cross-sex hormones," *Clinical Endocrinology*, vol. 48, no. 3, pp. 347–354, 1998.

[105] A. Mueller, R. Dittrich, H. Binder et al., "High dose estrogen treatment increases bone mineral density in male-to-female transsexuals receiving gonadotropin-releasing hormone agonist in the absence of testosterone," *European Journal of Endocrinology*, vol. 153, no. 1, pp. 107–113, 2005.

[106] K. Carlström, R. Stege, P. Henriksson, M. Grande, P. O. Gunnarsson, and A. Pousette, "Possible bone-preserving capacity of high-dose intramuscular depot estrogen as compared to orchidectomy in the treatment of patients with prostatic carcinoma," *Prostate*, vol. 31, no. 3, pp. 193–197, 1997.

[107] P. Taxel, D. G. Kennedy, P. M. Fall, A. K. Willard, J. M. Clive, and L. G. Raisz, "The effect of aromatase inhibition on sex steroids, gonadotropins, and markers of bone turnover in older men,"

The Journal of Clinical Endocrinology & Metabolism, vol. 86, no. 6, pp. 2869–2874, 2001.

[108] S.-A. M. Burnett-Bowie, E. A. McKay, H. Lee, and B. Z. Leder, "Effects of aromatase inhibition on bone mineral density and bone turnover in older men with low testosterone levels," *The Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 12, pp. 4785–4792, 2009.

[109] B. Ongphiphadhanakul, R. Rajatanavin, S. Chanprasertyothin, N. Piaseu, and L. Chailurkit, "Serum oestradiol and oestrogen-receptor gene polymorphism are associated with bone mineral density independently of serum testosterone in normal males," *Clinical Endocrinology*, vol. 49, no. 6, pp. 803–809, 1998.

[110] S. Khosla, B. L. Riggs, E. J. Atkinson et al., "Relationship of estrogen receptor genotypes to bone mineral density and to rates of bone loss in men," *The Journal of Clinical Endocrinology & Metabolism*, vol. 89, no. 4, pp. 1808–1816, 2004.

[111] I. van Pottelbergh, S. Goemaere, and J. M. Kaufman, "Bioavailable estradiol and an aromatase gene polymorphism are determinants of bone mineral density changes in men over 70 years of age," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 7, pp. 3075–3081, 2003.

[112] L. Gennari, L. Masi, D. Merlotti et al., "A polymorphic *CYP19* TTTA repeat influences aromatase activity and estrogen levels in elderly men: effects on bone metabolism," *The Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 6, pp. 2803–2810, 2004.

[113] V. Rochira, M. Faustini-Fustini, A. Balestrieri, and C. Carani, "Estrogen replacement therapy in a man with congenital aromatase deficiency: effects of different doses of transdermal estradiol on bone mineral density and hormonal parameters," *The Journal of Clinical Endocrinology and Metabolism*, vol. 85, no. 5, pp. 1841–1845, 2000.

[114] V. Rochira, L. Zirilli, B. Madeo et al., "Skeletal effects of long-term estrogen and testosterone replacement treatment in a man with congenital aromatase deficiency: evidences of a priming effect of estrogen for sex steroids action on bone," *Bone*, vol. 40, no. 6, pp. 1662–1668, 2007.

[115] L. Vandenput and C. Ohlsson, "Sex steroid metabolism in the regulation of bone health in men," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 121, no. 3–5, pp. 582–588, 2010.

[116] S. Khosla, "Estrogen and bone: Insights from estrogen-resistant, aromatase-deficient, and normal men," *Bone*, vol. 43, no. 3, pp. 414–417, 2008.

[117] E. Seeman, "From density to structure: growing up and growing old on the surfaces of bone," *Journal of Bone and Mineral Research*, vol. 12, no. 4, pp. 509–521, 1997.

[118] E. Seeman and P. D. Delmas, "Bone quality—the material and structural basis of bone strength and fragility," *The New England Journal of Medicine*, vol. 354, no. 21, pp. 2250–2261, 2006.

[119] C. Ohlsson, A. Darelid, M. Nilsson, J. Melin, D. Mellström, and M. Lorentzon, "Cortical consolidation due to increased mineralization and endosteal contraction in young adult men: a five-year longitudinal study," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 7, pp. 2262–2269, 2011.

[120] A. Darelid, C. Ohlsson, M. Nilsson, J. M. Kindblom, D. Mellström, and M. Lorentzon, "Catch up in bone acquisition in young adult men with late normal puberty," *Journal of Bone and Mineral Research*, vol. 27, no. 10, pp. 2198–2207, 2012.

[121] J. S. Walsh, M. A. Paggiosi, and R. Eastell, "Cortical consolidation of the radius and tibia in young men and women," *The Journal of Clinical Endocrinology & Metabolism*, vol. 97, no. 9, pp. 3342–3348, 2012.

[122] S. Bhasin, R. Jasuja, C. Serra et al., "Androgen effects on the skeletal muscle," in *Testosterone, Action, Deficiency, Substitution*, E. Nieschlag and H. Behre, Eds., vol. 14, Chapter 14, pp. 191–206, Cambridge University Press, Cambridge, UK, 4th edition, 2012.

[123] F. Rauch, D. A. Bailey, A. Baxter-Jones, R. Mirwald, and R. Faulkner, "The "muscle-bone unit" during the pubertal growth spurt," *Bone*, vol. 34, no. 5, pp. 771–775, 2004.

[124] E. van Caenegem, K. Wierckx, Y. Taes et al., "Bone mass, bone geometry, and body composition in female-to-male transsexual persons after long-term cross-sex hormonal therapy," *The Journal of Clinical Endocrinology and Metabolism*, vol. 97, no. 7, pp. 2503–2511, 2012.

[125] D. Vanderschueren, K. Venken, J. Ophoff, R. Bouillon, and S. Boonen, "Clinical review: Sex steroids and the periosteum—reconsidering the roles of androgens and estrogens in periosteal expansion," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 2, pp. 378–382, 2006.

[126] D. L. S. Danilovic, P. H. S. Correa, E. M. F. Costa, K. F. S. Melo, B. B. Mendonca, and I. J. P. Arnhold, "Height and bone mineral density in androgen insensitivity syndrome with mutations in the androgen receptor gene," *Osteoporosis International*, vol. 18, no. 3, pp. 369–374, 2007.

[127] T. S. Han, D. Goswami, S. Trikudanathan, S. M. Creighton, and G. S. Conway, "Comparison of bone mineral density and body proportions between women with complete androgen insensitivity syndrome and women with gonadal dysgenesis," *European Journal of Endocrinology*, vol. 159, no. 2, pp. 179–185, 2008.

[128] L. Vandenput, J. V. Swinnen, S. Boonen et al., "Role of the androgen receptor in skeletal homeostasis: the androgen-resistant testicular feminized male mouse model," *Journal of Bone and Mineral Research*, vol. 19, no. 9, pp. 1462–1470, 2004.

[129] F. C. W. Wu, A. Tajar, J. M. Beynon et al., "Identification of late-onset hypogonadism in middle-aged and elderly men," *The New England Journal of Medicine*, vol. 363, no. 2, pp. 123–135, 2010.

[130] G. Sartorius, S. Spasevska, A. Idan et al., "Serum testosterone, dihydrotestosterone and estradiol concentrations in older men self-reporting very good health: the healthy man study," *Clinical Endocrinology*, vol. 77, no. 5, pp. 755–763, 2012.

[131] L. Vandenput, M. Lorentzon, D. Sundh et al., "Serum estradiol levels are inversely associated with cortical porosity in older men," *The Journal of Clinical Endocrinology and Metabolism*, vol. 99, no. 7, pp. E1322–E1326, 2014.

[132] V. Rochira, A. Balestrieri, B. Madeo, L. Zirilli, A. R. Granata, and C. Carani, "Bone loss, sex steroids and male age related hypogonadism," *Journal of Endocrinological Investigation*, vol. 28, no. 11, pp. 46–48, 2005.

[133] E. Orwoll, L. C. Lambert, L. M. Marshall et al., "Endogenous testosterone levels, physical performance, and fall risk in older men," *Archives of Internal Medicine*, vol. 166, no. 19, pp. 2124–2131, 2006.

[134] L. Zirilli, L. Maffei, P. J. Meunier, P. Chavassieux, C. Carani, and V. Rochira, "The effects of long-term raloxifene and estradiol treatments on bone in a patient with congenital aromatase deficiency," *Bone*, vol. 45, no. 5, pp. 827–832, 2009.

[135] A. S. Chagin, E. Karimian, F. Zaman, M. Takigawa, D. Chrysis, and L. Sävendahl, "Tamoxifen induces permanent growth arrest through selective induction of apoptosis in growth plate chondrocytes in cultured rat metatarsal bones," *Bone*, vol. 40, no. 5, pp. 1415–1424, 2007.

[136] P. M. Doran, B. L. Riggs, E. J. Atkinson, and S. Khosla, "Effects of raloxifene, a selective estrogen receptor modulator, on bone turnover markers and serum sex steroid and lipid levels in elderly men," *Journal of Bone and Mineral Research*, vol. 16, no. 11, pp. 2118–2125, 2001.

[137] M. R. Smith, M. A. Fallon, H. Lee, and J. S. Finkelstein, "Raloxifene to prevent gonadotropin-releasing hormone agonist-induced bone loss in men with prostate cancer: a randomized controlled trial," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 8, pp. 3841–3846, 2004.

[138] N. Mauras, L. G. de Pijem, H. Y. Hsiang et al., "Anastrozole increases predicted adult height of short adolescent males treated with growth hormone: a randomized, placebo-controlled, multicenter trial for one to three years," *The Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 3, pp. 823–831, 2008.

[139] P. E. Løning and H. P. Eikesdal, "Aromatase inhibition 2013: clinical state of the art and questions that remain to be solved," *Endocrine-Related Cancer*, vol. 20, no. 4, pp. R183–R201, 2013.

[140] M. Hero, O. Mäkitie, H. Kröger, E. Nousiainen, S. Toiviainen-Salo, and L. Dunkel, "Impact of aromatase inhibitor therapy on bone turnover, cortical bone growth and vertebral morphology in pre- and peripubertal boys with idiopathic short stature," *Hormone Research*, vol. 71, no. 5, pp. 290–297, 2009.

[141] V. Rochira, "Aromatase inhibitors in pubertal boys: clinical implications," *The Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 4, pp. 1836–1838, 2001.

[142] M. E. Geffner, "Aromatase inhibitors to augment height: continued caution and study required," *Journal of Clinical Research in Pediatric Endocrinology*, vol. 1, no. 6, pp. 256–261, 2009.

[143] W. Rosner, S. E. Hankinson, P. M. Sluss, H. W. Vesper, and M. E. Wierman, "Challenges to the measurement of estradiol: an endocrine society position statement," *The Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 4, pp. 1376–1387, 2013.

[144] B. Madeo, L. Zirilli, G. Caffagni et al., "The osteoporotic male: overlooked and undermanaged?" *Clinical Interventions in Aging*, vol. 2, no. 3, pp. 305–312, 2007.

[145] S. Basaria, A. D. Coviello, T. G. Travison et al., "Adverse events associated with testosterone administration," *The New England Journal of Medicine*, vol. 363, no. 2, pp. 109–122, 2010.

[146] R. Vigen, C. I. O'Donnell, A. E. Barón et al., "Association of testosterone therapy with mortality, myocardial infarction, and stroke in men with low testosterone levels," *The Journal of the American Medical Association*, vol. 310, no. 17, pp. 1829–1836, 2013.

[147] J. M. Kaufman, "Mortality associated to late-onset hypogonadism: reasons not to treat with testosterone?" *Journal of Clinical Endocrinology and Metabolism*, vol. 99, no. 4, pp. 1161–1163, 2014.

[148] E. Hernlund, A. Svedbom, M. Ivergård et al., "Osteoporosis in the European Union: medical management, epidemiology and economic burden: a report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA)," *Archives of Osteoporosis*, vol. 8, no. 1-2, article 136, 2013.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2015, Article ID 758148, 7 pages
http://dx.doi.org/10.1155/2015/758148

Review Article

Skeleton and Glucose Metabolism: A Bone-Pancreas Loop

Maria Felicia Faienza,[1] Vincenza Luce,[1] Annamaria Ventura,[1] Graziana Colaianni,[2] Silvia Colucci,[2] Luciano Cavallo,[1] Maria Grano,[2] and Giacomina Brunetti[2]

[1]*Section of Pediatrics, Department of Biomedical Sciences and Human Oncology, University of Bari "A. Moro", 70124 Bari, Italy*
[2]*Section of Human Anatomy and Histology, Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari, 70124 Bari, Italy*

Correspondence should be addressed to Giacomina Brunetti; giacomina.brunetti@uniba.it

Received 6 August 2014; Revised 11 November 2014; Accepted 2 December 2014

Academic Editor: Andrea Del Fattore

Bone has been considered a structure essential for mobility, calcium homeostasis, and hematopoietic function. Recent advances in bone biology have highlighted the importance of skeleton as an endocrine organ which regulates some metabolic pathways, in particular, insulin signaling and glucose tolerance. This review will point out the role of bone as an endocrine "gland" and, specifically, of bone-specific proteins, as the osteocalcin (Ocn), and proteins involved in bone remodeling, as osteoprotegerin, in the regulation of insulin function and glucose metabolism.

1. Introduction

Bone is a dynamic structure that is constantly subject to remodeling by specialized cells, the osteoclasts (OCs), osteoblasts (OBs), and osteocytes. Bone remodeling consists of removal of mineralized bone tissue by OCs, to leave a resorptive cavity filled by the migration of OB precursors which differentiate into mature OBs. Osteocytes regulate both remodeling and mineralization processes and represent the terminal stage of the OB lineage embedded in the bone matrix. Osteocytes are also the source of molecules which control the production and activity of OCs, such as osteoprotegerin (OPG) and Receptor activator of nuclear factor kappa-B ligand (RANKL) [1].

Recently, bone has emerged as an endocrine "gland," and some key mediators of this alternative function have been identified.

This review focuses on the role of the skeleton as endocrine organ, its modulation of glucose tolerance by secretion of bone-specific proteins, in particular the osteocalcin (Ocn), and how proteins involved in bone remodeling, like OPG, are associated with impairment of insulin function.

2. The Role of Insulin in Regulating the Functions of Bone Cells

The regulation of glucose metabolism occurs through the interplay of multiple hormones which operate in many target organs. Insulin plays an important role in glucose regulation by promoting glucose uptake in adipose tissue and muscle and by suppressing gluconeogenesis in liver. To perform these functions, insulin binds to its receptor (InsR), a tyrosine kinase expressed in hepatocytes, adipocytes, myoblasts, and OBs.

However, deletion of the InsR in muscle, the most important site of glucose uptake, does not affect blood glucose levels, insulin concentration, and glucose tolerance, suggesting that other tissues, like bone, could be involved in glucose regulation [2, 3].

Insulin has been demonstrated to be an osteogenic hormone both *in vitro* and *in vivo*. OBs express abundant insulin receptors and respond to insulin treatment [4–6] by increasing cell proliferation [7, 8], collagen synthesis [5, 9–11], and glucose uptake [12, 13]. Mice knocked out for InsR in their OBs have decreased trabecular bone volume due

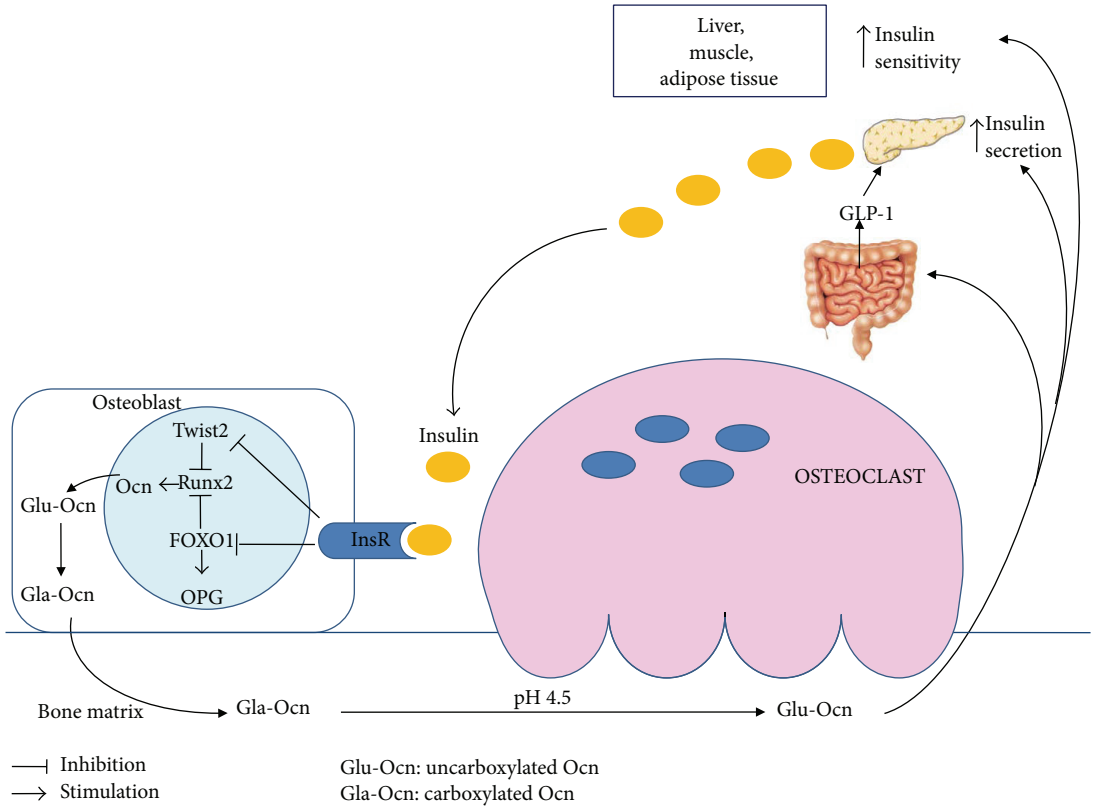


Figure 1: Interplay between Ocn and insulin secretion/sensitivity.

to reduced bone formation and poor numbers of OBs [3, 14]. In addition, these mutant mice show the reduction of OC erosion depth and low serum levels of cross-linked C-telopeptide (CTX) which indicate a decline of OC activity. Moreover, the treatment with insulin has been shown to be effective in determining the reversibility of skeletal alterations of rodent model with type 1 diabetes and also favoring the healing of fractures [15–19]. Based on these data, there are emerging studies which regard the skeleton as an important regulator of energy metabolism.

3. Osteocalcin and Glucose Metabolism: The Bone-Pancreas Loop

Recent investigations, particularly from the Karsenty group, have identified a crucial role for the Ocn in regulating insulin metabolism in a hormonal way [14]. Ocn is the major noncollagen protein secreted by the OBs and it is stored in the extracellular matrix of bone. Before its secretion, Ocn is carboxylated at the level of three Gla residues. This process of carboxylation confers high-affinity binding to hydroxyapatite, the mineral present in bone, and the attachment of carboxylated Ocn to the bone matrix [20]. Instead, when Ocn is uncarboxylated, its binding to hydroxyapatite is reduced, promoting the passage of Ocn into circulation. The involvement of undercarboxylated form of Ocn in a bone-pancreas loop has been demonstrated by previous studies. Ocn-deficient mice show few β cells, great fat mass, and decreased insulin sensitivity [21]. Conversely, the subcutaneous infusion of recombinant Ocn into wild-type mice enhances glucose tolerance and improves insulin sensitivity [22].

The decarboxylation of Ocn is dependent on bone resorption: insulin signaling in OBs favors the differentiation of OCs and the formation of resorption lacunae by inhibiting the expression of OPG [14]. The low pH present within these lacunae promotes the decarboxylation of Ocn and consequently its activation [14] (Figure 1). Conversely, a tyrosine phosphatase produced by *Esp* (*Ptprv*) gene blocks Ocn decarboxylation and decreases serum levels of active form of Ocn [21]. The human ortholog of *Esp* (OST-PTP, also called osteotesticular protein tyrosine phosphatase) is not active in humans but recent studies have shown that there are additional tyrosine phosphatases, such as TC-PTP1, expressed in OBs [21–24]. These phosphatases can regulate Ocn activity and glucose homeostasis by acting on the insulin signaling pathway in the OBs [21, 23, 24].

3.1. Uncarboxylated Osteocalcin Functions. The regulation of systemic glucose metabolism and insulin resistance by Ocn occurs in a hormonal manner [25].

Firstly, Ocn stimulates insulin secretion by β-cells both directly [26, 27] and indirectly promoting the secretion of gut glucagon-like peptide-1 (GLP-1) [28] (Figure 1). The effects of Ocn on activating ERK and insulin secretion are mediated by Ocn receptor, an orphan receptor belonging to the C

family of GPCRs, highly expressed in the mouse pancreatic β-cell line [29]. The Ocn-GPRC6A network has strong physiological effects in the mouse, but the clinical relevance of this endocrine pathway in humans is less certain. Up till now, no mutations or polymorphisms of *Osteocalcin* or *GPRC6A* genes have been reported in humans [27]. Secondly, Ocn promotes β-cell proliferation by increasing Ccnd2 and Cdk4 expression in β-cells [22]. Thirdly, Ocn increases insulin sensitivity in liver, muscle, and adipose tissue (Figure 1) by upregulation of adiponectin gene expression in adipocytes [21].

InsR signaling in OBs has a double and positive action on Ocn. On one side, InsR induces *Osteocalcin* gene expression in OBs by blocking the negative activity of the nuclear factor Twist2 on Runx2, the master gene of OB differentiation and Ocn expression [30]. Furthermore, InsR signal decreases the ability of FOXO1 to activate the OPG promoter (Figure 1), thus reducing the secretion of this inhibitor of OC function by OBs [31].

3.2. Clinical Relevance of Osteocalcin Glucose Regulation. A number of studies have established that numerous aspects of Ocn biology are similar in rodents and humans. There are several data indicating that serum levels of uncarboxylated Ocn negatively correlate with insulin resistance, obesity, diabetes, or markers of the metabolic syndrome (MetS) [32–35]. Interestingly, important weight loss causes a decrease of insulin resistance as well as an increase in Ocn levels in obese children [36], and acute aerobic exercise could increase serum uncarboxylated Ocn in obese subjects [37]. Furthermore, serum Ocn has also been positively correlated with improved glucose control in subjects with type 2 diabetes [38]. Women with gestational diabetes show high Ocn levels which correlate with insulin secretion parameters and return to normal values postpartum [39]. This raising of serum Ocn levels could represent an adaptive process to counteract glucose intolerance during gestational diabetes.

4. Osteoprotegerin

OPG is a soluble glycoprotein belonging to the tumor necrosis factor receptor superfamily which decreases bone resorption by inhibiting the differentiation and activation of OCs [40]. It acts as a decoy soluble receptor for RANKL, thus preventing RANKL binding with its receptor RANK on OCs, thus inhibiting osteoclastogenesis [41]. RANKL/RANK/OPG system mediates important and complex relations between the vascular, skeletal, and immune systems [42, 43]. OPG is mainly secreted by bone but it is produced also by different tissues, including endothelial and smooth muscle cells [43]. OPG improves endothelial cells survival but it may induce endothelial inflammation and proliferation of endothelial and vascular smooth muscle cells, thus promoting atherogenesis. OPG knockout mice show osteoporosis and vascular calcification, reintroducing the hypothesis that metabolic bone diseases and vascular diseases, for example, arterial calcification, share common pathways [44, 45]. OPG administration prevents calcification induced by Warfarin or high doses of vitamin D in rats, but the effects of OPG in humans are different from those in rodents [46]. In humans, high OPG levels have been found in patients with type 2 diabetes, coronary artery diseases, hypothyroidism, hypercholesterolemia, and obesity, as well as in aging men [47–51]. A population-based study has demonstrated that high serum OPG represents an independent risk factor for the progression of atherosclerosis, as well as of vascular mortality [52]. On the other hand, results of experimental studies suggest that OPG has also vasoprotective properties through reduction of vascular calcification [53]. Recent data have indicated a role of OPG as metabolic biomarker [54]. In obese subjects, OPG has been found to be positively associated with insulin resistance [55, 56]. Furthermore, high OPG levels have been associated with risk of metabolic syndrome and microvascular complications in type 2 diabetes patients [57].

5. Other Regulators of Bone-Pancreas Loop

5.1. Vitamin D. Vitamin D is recognized as a key regulator of bone and mineral metabolism. Vitamin D signaling is mediated by binding of the physiologically active form 1α,25-dihydroxyvitamin D3 (1,25D3) to its intracellular receptor (VDR) which, after translocation to the nucleus, binds to vitamin D response elements (VDREs) of target genes involved in different pathways (cell proliferation, differentiation, and immunomodulation) [58].

1,25D3 has an indirect effect on bone formation through intestinal and renal regulation of calcium levels. However, the presence of VDRs in OBs suggests a direct role of vitamin D in bone metabolism, supported by gene expression profiling studies examining mRNA in OBs treated with 1,25D3 [59–62]. Moreover, data from *in vitro* and *in vivo* models have shown that 1,25D3 can exert catabolic or anabolic actions on bone, depending on species and/or environmental context, in order to control the plasma calcium homeostasis [63]. In particular, 1,25D3 showed stimulatory effects on human and rat OBs and inhibitory effects on murine OBs. Generally, in condition of negative calcium balance, VDR signaling in OBs enhances bone resorption stimulating the expression of RANKL [64] and suppresses bone mineralization by inducing expression of Ocn and osteopontin [65, 66].

The identification of VDRs in different organs and tissues including the prostate, brain, colon, breast, immune cells, and pancreas underlines the extra skeletal effects of vitamin D [67]. In particular, vitamin D regulates glucose homeostasis and insulin secretion by binding to its VDR in pancreatic β-cells [68]. Vitamin D deficiency has been associated with insulin resistance in nondiabetic subjects and with a reduced insulin production in type 2 diabetics [69].

The role of vitamin D in regulation of insulin production by pancreatic β-cells is supported by the presence of VDREs in the human *InsR* gene promoter [70]. Moreover, several studies have shown that polymorphisms of *VDR* gene may affect insulin release and insulin sensitivity [71, 72]. In addition, pancreatic β-cells express a plasma membrane VDR, which seems to mediate an insulinotropic rapid effect of

vitamin D, independent of mRNA transcription and protein translation [73].

5.2. Gastric Inhibitory Polypeptide (GIP). Gastric inhibitory polypeptide (GIP) is a 42-amino-acid hormone, secreted from K cells of duodenum and proximal jejunum. The main function of GIP is the stimulation of the postprandial insulin secretion from the pancreatic islets [74]. GIP exerts its effects by binding to the GIP receptor (GIPR) and stimulates insulin secretion by β-cells in a glucose-dependent manner [75]. GIPRs are present on OBs, OCs, osteocytes, and chondrocytes [76, 77] and GIP signaling has an anabolic action on bone. In fact, several studies using *in vitro* and animal models demonstrated an antiapoptotic and stimulating effect on OBs [76, 78, 79] and a direct antiresorptive activity probably mediated by cAMP. [77]. GIP is designed as a member of the "entero-osseous axis," responsible for the postprandial reduction of bone resorption [78, 80]. This is supported by a recent study of Nissen et al. showing a reduction of CTX plasma levels after infusion with GIP, both during euglycemia and hyperglycemia [81].

5.3. Adiponectin. Adiponectin is a 28 kDa protein produced by differentiated adipocytes and is abundantly present in plasma [82–84]. The biological actions of adiponectin are mediated through the two adiponectin receptors (AdipoR) 1 and 2 and comprise regulation of glucose and lipid metabolism, inflammation, and energy balance [85].

Adiponectin controls glucose homeostasis by enhancing insulin sensitivity and maintaining a functional β-cell mass [86]. In particular, adiponectin stimulates muscle glucose utilization [87, 88] and exerts a cytoprotective and antiapoptotic effect on β-cells [89]. Moreover, adiponectin influences bone metabolism, even if the mechanisms mediating this effect are controversial. *In vitro* experiments showed that adiponectin promotes proliferation of OBs in human [90] and inhibits osteoclastogenesis, increasing bone mass [91].

Conversely, Shinoda et al. [92] demonstrated that high level of circulating adiponectin represents a risk factor for fractures independent of body composition and BMD [92].

This effect could be the consequence of the stimulation of RANKL and inhibition of OPG expression by adiponectin in OBs [93].

Moreover, a recent study has shown that adiponectin inhibits OB proliferation and induces OB apoptosis in young animals, whereas in older animals it increases the bone mass [94]. Thus, according to this study, adiponectin has opposite influences on bone mass, a local negative action on OBs (inhibition of OB proliferation and induction of OB apoptosis), and an indirect effect through a central signaling that decreases sympathetic tone, leading to increase of bone formation and bone mass [94].

6. Conclusions

Recent advances highlighted the role of the bone in modulating metabolic functions. The identification of Ocn as a hormone that stimulates insulin sensitivity in peripheral tissues and insulin secretion by the pancreas has opened the way for new fields of research. Nevertheless, the interactions between bone, pancreas, and probably other organs need to be further explored. There are conflicting results on the effects of antiresorptive drugs for osteoporosis, like bisphosphonates and denosumab, on glucose metabolism. Bisphosphonates and denosumab reduce circulating levels of total Ocn and in particular of the undercarboxylated, active form. However, although in mouse models the suppression of bone turnover with antiresorptive drugs determines important effects on fasting glucose, weight, and diabetes incidence, randomized placebo-controlled trials have demonstrated that the reduction of bone turnover and low levels of undercarboxylated Ocn are not involved in the regulation of insulin sensitivity in humans. Thus, patients receiving such osteoporosis treatments would not be at risk of impaired glucose metabolism or diabetes.

These observations suggest that the bone pancreas loop is more complex than currently known and additional studies will be necessary to evaluate the impact of the connection between the skeleton and metabolism in humans.

Development of new drugs that simultaneously target the skeleton, the glucose metabolism, and the adipose tissue are certain to be considered a future perspective.

Insulin signaling in OBs decreases the expression of OPG, inhibiting FOXO1, and induces Ocn expression, blocking the negative activity of Twist2 on Runx2. Reduction of OPG favors the differentiation of OCs and the low pH of resorption lacunae promotes the decarboxylation of Ocn and consequently its activation. The undercarboxylated Ocn was released into the circulation and stimulates β-cells insulin secretion both directly and indirectly by promoting the secretion of gut GLP-1. Moreover, active Ocn increases insulin sensitivity in liver, muscle, and adipose tissue.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] G. Brunetti, A. Di Benedetto, and G. Mori, "Bone remodeling," in *Imaging of Prosthetic Joints—A Combined Radiological and Clinical Perspective*, C. Albanese and C. Faletti, Eds., pp. 27–37, Springer, New York, NY, USA, 2014.

[2] J. C. Brüning, M. D. Michael, J. N. Winnay et al., "A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance," *Molecular Cell*, vol. 2, no. 5, pp. 559–569, 1998.

[3] K. Fulzele, R. C. Riddle, D. J. DiGirolamo et al., "Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition," *Cell*, vol. 142, no. 2, pp. 309–319, 2010.

[4] J. R. Levy, E. Murray, S. Manolagas, and J. M. Olefsky, "Demonstration of insulin receptors and modulation of alkaline phosphatase activity by insulin in rat osteoblastic cells," *Endocrinology*, vol. 119, no. 4, pp. 1786–1792, 1986.

[5] K. K. Pun, P. Lau, and P. W. M. Ho, "The characterization, regulation, and function of insulin receptors on osteoblast-like clonal osteosarcoma cell line," *Journal of Bone and Mineral Research*, vol. 4, no. 6, pp. 853–862, 1989.

[6] D. M. Thomas, D. K. Hards, S. D. Rogers, K. W. Ng, and J. D. Best, "Insulin receptor expression in bone," *Journal of Bone and Mineral Research*, vol. 11, no. 9, pp. 1312–1320, 1996.

[7] M. Hashizume and M. Yamaguchi, "Stimulatory effect of β-alanyl-L-histidinato zinc on cell proliferation is dependent on protein synthesis in osteoblastic MC3T3-E1 cells," *Molecular and Cellular Biochemistry*, vol. 122, no. 1, pp. 59–64, 1993.

[8] J. E. Wergedal and D. J. Baylink, "Characterization of cells isolated and cultured from human bone," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 176, no. 1, pp. 60–69, 1984.

[9] E. M. Canalis, J. W. Dietrich, D. M. Maina, and L. G. Raisz, "Hormonal control of bone collagen synthesis in vitro. Effects of insulin and glucagon," *Endocrinology*, vol. 100, no. 3, pp. 668–674, 1977.

[10] D. M. Rosen and R. A. Luben, "Multiple hormonal mechanisms for the control of collagen synthesis in an osteoblast-like cell line, MMB-1," *Endocrinology*, vol. 112, no. 3, pp. 992–999, 1983.

[11] B. E. Kream, M. D. Smith, E. Canalis, and L. G. Raisz, "Characterization of the effect of insulin on collagen synthesis in fetal rat bone," *Endocrinology*, vol. 116, no. 1, pp. 296–302, 1985.

[12] T. J. Hahn, S. L. Westbrook, T. L. Sullivan, W. G. Goodman, and L. R. Halstead, "Glucose transport in osteoblast-enriched bone explants: characterization and insulin regulation," *Journal of Bone and Mineral Research*, vol. 3, no. 3, pp. 359–365, 1988.

[13] E. A. Ituarte, L. R. Halstead, A. Iida-Klein, H. G. Ituarte, and T. J. Hahn, "Glucose transport system in UMR-106-01 osteoblastic osteosarcoma cells: regulation by insulin," *Calcified Tissue International*, vol. 45, no. 1, pp. 27–33, 1989.

[14] M. Ferron, J. Wei, T. Yoshizawa et al., "Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism," *Cell*, vol. 142, no. 2, pp. 296–308, 2010.

[15] N. Follak, I. Klöting, and H. Merk, "Influence of diabetic metabolic state on fracture healing in spontaneously diabetic rats," *Diabetes/Metabolism Research and Reviews*, vol. 21, no. 3, pp. 288–296, 2005.

[16] N. Follak, I. Klöting, E. Wolf, and H. Merk, "Delayed remodelling in the early period of fracture healing in spontaneously diabetic BB/OK rats depending on the diabetic metabolic state," *Histology and Histopathology*, vol. 19, no. 2, pp. 473–486, 2004.

[17] J. C.-H. Hou, R. F. Zernicke, and R. J. Barnard, "Effects of severe diabetes and insulin on the femoral neck of the immature rat," *Journal of Orthopaedic Research*, vol. 11, no. 2, pp. 263–271, 1993.

[18] K. M. Thrailkill, L. Liu, E. C. Wahl et al., "Bone formation is impaired in a model of type 1 diabetes," *Diabetes*, vol. 54, no. 10, pp. 2875–2881, 2005.

[19] A. Gandhi, H. A. Beam, J. P. O'Connor, J. R. Parsons, and S. S. Lin, "The effects of local insulin delivery on diabetic fracture healing," *Bone*, vol. 37, no. 4, pp. 482–490, 2005.

[20] P. V. Hauschka, J. B. Lian, D. E. Cole, and C. M. Gundberg, "Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone," *Physiological Reviews*, vol. 69, no. 3, pp. 990–1047, 1989.

[21] N. K. Lee, H. Sowa, E. Hinoi et al., "Endocrine regulation of energy metabolism by the skeleton," *Cell*, vol. 130, no. 3, pp. 456–469, 2007.

[22] M. Ferron, E. Hinoi, G. Karsenty, and P. Ducy, "Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 13, pp. 5266–5270, 2008.

[23] W. Cousin, A. Courseaux, A. Ladoux, C. Dani, and P. Peraldi, "Cloning of hOST-PTP: the only example of a protein-tyrosine-phosphatase the function of which has been lost between rodent and human," *Biochemical and Biophysical Research Communications*, vol. 321, no. 1, pp. 259–265, 2004.

[24] T. Zee, C. Settembre, R. L. Levine, and G. Karsenty, "T-cell protein tyrosine phosphatase regulates bone resorption and whole-body insulin sensitivity through its expression in osteoblasts," *Molecular and Cellular Biology*, vol. 32, no. 6, pp. 1080–1088, 2012.

[25] M. Pi and L. D. Quarles, "Novel bone endocrine networks integrating mineral and energy metabolism," *Current Osteoporosis Reports*, vol. 11, no. 4, pp. 391–399, 2013.

[26] E. Hinoi, N. Gao, D. Y. Jung et al., "The sympathetic tone mediates leptin's inhibition of insulin secretion by modulating osteocalcin bioactivity," *The Journal of Cell Biology*, vol. 183, no. 7, pp. 1235–1242, 2008.

[27] M. Pi, Y. Wu, and L. D. Quarles, "GPRC6A mediates responses to osteocalcin in beta-cells *in vitro* and pancreas *in vivo*," *Journal of Bone and Mineral Research*, vol. 26, no. 7, pp. 1680–1683, 2011.

[28] A. Mizokami, Y. Yasutake, J. Gao et al., "Osteocalcin induces release of glucagon-like peptide-1 and thereby stimulates insulin secretion in mice," *PLoS ONE*, vol. 8, no. 2, Article ID e57375, 2013.

[29] M. Pi, L. Chen, M.-Z. Huang et al., "GPRC6A null mice exhibit osteopenia, feminization and metabolic syndrome," *PLoS ONE*, vol. 3, no. 12, Article ID e3858, 2008.

[30] P. Ducy, R. Zhang, V. Geoffroy, A. L. Ridall, and G. Karsenty, "*Osf2/Cbfa1*: a transcriptional activator of osteoblast differentiation," *Cell*, vol. 89, no. 5, pp. 747–754, 1997.

[31] P. Puigserver, J. Rhee, J. Donovan et al., "Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1α interaction," *Nature*, vol. 423, no. 6939, pp. 550–555, 2003.

[32] J. M. Kindblom, C. Ohlsson, O. Ljunggren et al., "Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men," *Journal of Bone and Mineral Research*, vol. 24, no. 5, pp. 785–791, 2009.

[33] M. Zhou, X. Ma, H. Li et al., "Serum osteocalcin concentrations in relation to glucose and lipid metabolism in Chinese individuals," *European Journal of Endocrinology*, vol. 161, no. 5, pp. 723–729, 2009.

[34] A. G. Pittas, S. S. Harris, M. Eliades, P. Stark, and B. Dawson-Hughes, "Association between serum osteocalcin and markers of metabolic phenotype," *The Journal of Clinical Endocrinology & Metabolism*, vol. 94, no. 3, pp. 827–832, 2009.

[35] I. Kanazawa, T. Yamaguchi, M. Yamamoto et al., "Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus," *The Journal of Clinical Endocrinology & Metabolism*, vol. 94, no. 1, pp. 45–49, 2009.

[36] T. Reinehr and C. L. Roth, "A new link between skeleton, obesity and insulin resistance: relationships between osteocalcin, leptin and insulin resistance in obese children before and after weight loss," *International Journal of Obesity*, vol. 34, no. 5, pp. 852–858, 2010.

[37] I. Levinger, R. Zebaze, G. Jerums, D. L. Hare, S. Selig, and E. Seeman, "The effect of acute exercise on undercarboxylated

osteocalcin in obese men," *Osteoporosis International*, vol. 22, no. 5, pp. 1621–1626, 2011.

[38] Y. Q. Bao, M. Zhou, J. Zhou et al., "Relationship between serum osteocalcin and glycaemic variability in Type 2 diabetes," *Clinical and Experimental Pharmacology and Physiology*, vol. 38, no. 1, pp. 50–54, 2011.

[39] Y. Winhofer, A. Handisurya, A. Tura et al., "Osteocalcin is related to enhanced insulin secretion in gestational diabetes mellitus," *Diabetes Care*, vol. 33, no. 1, pp. 139–143, 2010.

[40] G. Mori, P. D'Amelio, R. Faccio, and G. Brunetti, "The interplay between the bone and the immune system," *Clinical and Developmental Immunology*, vol. 2013, Article ID 720504, 16 pages, 2013.

[41] L. C. Hofbauerand and A. E. Heufelder, "The role of receptor activator of nuclear factor-κB ligand and osteoprotegerin in the pathogenesis and treatment of metabolic bone diseases," *The Journal of Clinical Endocrinology & Metabolism*, vol. 85, no. 7, pp. 2355–2363, 2000.

[42] U. M. Malyankar, M. Scatena, K. L. Suchland, T. J. Yun, E. A. Clark, and C. M. Giachelli, "Osteoprotegerin is an alpha vbeta 3-induced, NF-kappa B-dependent survival factor for endothelial cells," *The Journal of Biological Chemistry*, vol. 275, no. 28, pp. 20959–20962, 2000.

[43] P. Collin-Osdoby, L. Rothe, F. Anderson, M. Nelson, and W. Maloney, "Receptor activator of NF-B and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis," *The Journal of Biological Chemistry*, vol. 276, no. 23, pp. 20659–20672, 2001.

[44] N. Bucay, I. Sarosi, C. R. Dunstan et al., "Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification," *Genes & Development*, vol. 12, no. 9, pp. 1260–1268, 1998.

[45] J. K. Min, Y. M. Kim, E. C. Kim et al., "Vascular endothelial growth factor up-regulates expression of receptor activator of NF-κB (RANK) in endothelial cells. Concomitant increase of angiogenic responses to rank ligand," *Journal of Biological Chemistry*, vol. 278, no. 41, pp. 39548–39557, 2003.

[46] P. A. Price, H. H. June, J. R. Buckley, and M. K. Williamson, "Osteoprotegerin inhibits artery calcification induced by warfarin and by vitamin D," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 21, no. 10, pp. 1610–1616, 2001.

[47] W. S. Browner, L.-Y. Lui, and S. R. Cummings, "Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women," *The Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 2, pp. 631–637, 2001.

[48] M. Schoppet, A. M. Sattler, J. R. Schaefer, M. Herzum, B. Maisch, and L. Hofbauer, "Increased osteoprotegerin serum levels in men with coronary artery disease," *The Journal of Clinical Endocrinology & Metabolism*, vol. 88, no. 3, pp. 1024–1028, 2003.

[49] S. Jono, Y. Ikari, A. Shioi et al., "Serum osteoprotegerin levels are associated with the presence and severity of coronary artery disease," *Circulation*, vol. 106, no. 10, pp. 1192–1194, 2002.

[50] M. Schoppet, J. R. Schaefer, and L. C. Hofbauer, "Low serum levels of soluble RANK ligand are associated with the presence of coronary artery disease in men," *Circulation*, vol. 107, no. 11, article e76, 2003.

[51] L. M. Rasmussen and T. Ledet, "Osteoprotegerin and diabetic macroangiopathy," *Hormone and Metabolic Research*, vol. 37, no. 1, pp. 90–94, 2005.

[52] S. Kiechl, G. Schett, G. Wenning et al., "Osteoprotegerin is a risk factor for progressive atherosclerosis and cardiovascular disease," *Circulation*, vol. 109, no. 18, pp. 2175–2180, 2004.

[53] S. Morony, Y. Tintut, Z. Zhang et al., "Osteoprotegerin inhibits vascular calcification without affecting atherosclerosis in ldlr$^{(-/-)}$ mice," *Circulation*, vol. 117, no. 3, pp. 411–420, 2008.

[54] C. E. Pepene, I. R. Ilie, I. Marian, and I. Duncea, "Circulating osteoprotegerin and soluble receptor activator of nuclear factor κB ligand in polycystic ovary syndrome: relationships to insulin resistance and endothelial dysfunction," *European Journal of Endocrinology*, vol. 164, no. 1, pp. 61–68, 2011.

[55] M.-H. Gannagé-Yared, C. Yaghi, B. Habre et al., "Osteoprotegerin in relation to body weight, lipid parameters insulin sensitivity, adipocytokines, and C-reactive protein in obese and non-obese young individuals: results from both cross-sectional and interventional study," *European Journal of Endocrinology*, vol. 158, no. 3, pp. 353–359, 2008.

[56] J. Sulibursk a, P. Bogdanski, E. Gajewska, G. Kalmus, M. Sobieska, and W. Samborski, "The association of insulin resistance with serum osteoprotegerin in obese adolescents," *Journal of Physiology and Biochemistry*, vol. 69, no. 4, pp. 847–853, 2013.

[57] S. Tavintharan, L. T. Pek, J. J. Liu et al., "Osteoprotegerin is independently associated with metabolic syndrome and microvascular complications in type 2 diabetes mellitus," *Diabetes & Vascular Disease Research*, vol. 11, no. 5, pp. 359–362, 2014.

[58] M. R. Haussler, G. K. Whitfield, C. A. Haussler et al., "The nuclear vitamin D receptor: biological and molecular regulatory properties revealed," *Journal of Bone and Mineral Research*, vol. 13, no. 3, pp. 325–349, 1998.

[59] T. S. Lisse, R. F. Chun, S. Rieger, J. S. Adams, and M. Hewison, "Vitamin D activation of functionally distinct regulatory miRNAs in primary human osteoblasts," *Journal of Bone and Mineral Research*, vol. 28, no. 6, pp. 1478–1488, 2013.

[60] P. Tarroni, I. Villa, E. Mrak et al., "Microarray analysis of 1,25$(OH)_2D_3$ regulated gene expression in human primary osteoblasts," *Journal of Cellular Biochemistry*, vol. 113, no. 2, pp. 640–649, 2012.

[61] M. B. Meyer, P. D. Goetsch, and J. W. Pike, "Genome-wide analysis of the VDR/RXR cistrome in osteoblast cells provides new mechanistic insight into the actions of the vitamin D hormone," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 121, no. 1-2, pp. 136–141, 2010.

[62] V. J. Woeckel, R. D. A. M. Alves, S. M. A. Swagemakers et al., "1α,25-$(OH)_2D_3$ acts in the early phase of osteoblast differentiation to enhance mineralization via accelerated production of mature matrix vesicles," *The Journal of Cellular Physiology*, vol. 225, no. 2, pp. 593–600, 2010.

[63] H. A. Morris, "Vitamin D activities for health outcomes," *Annals of Laboratory Medicine*, vol. 34, no. 3, pp. 181–186, 2014.

[64] L. Lieben and G. Carmeliet, "Vitamin D signaling in osteocytes: effects on bone and mineral homeostasis," *Bone*, vol. 54, no. 2, pp. 237–243, 2013.

[65] L. Lieben, R. Masuyama, S. Torrekens et al., "Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D-induced inhibition of bone mineralization," *The Journal of Clinical Investigation*, vol. 122, no. 5, pp. 1803–1815, 2012.

[66] M. Noda, R. L. Vogel, A. M. Craig, J. Prahl, H. F. Deluca, and D. T. Denhardt, "Identification of a DNA sequence responsible

for binding of the 1,25-dihydroxyvitamin D3 receptor and 1,25-dihydroxyvitamin enhancement of mouse secreted phosphoprotein 1 (SPP-1 or osteopontin) gene expression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 24, pp. 9995–9999, 1990.

[67] B. Bouvard, C. Annweiler, A. Sallé et al., "Extraskeletal effects of vitamin D: facts, uncertainties, and controversies," *Joint, Bone, Spine: Revue du Rhumatisme*, vol. 78, no. 1, pp. 10–16, 2011.

[68] A. W. Norman, B. J. Frankel, A. M. Heldt, and G. M. Grodsky, "Vitamin D deficiency inhibits pancreatic secretion of insulin," *Science*, vol. 209, no. 4458, pp. 823–825, 1980.

[69] A. Esteghamati, Z. Aryan, A. Esteghamati, and M. Nakhjavani, "Vitamin D deficiency is associated with insulin resistance in nondiabetics and reduced insulin production in type 2 diabetics," *Hormone and Metabolic Research*, 2014.

[70] B. Maestro, N. Dávila, M. C. Carranza, and C. Calle, "Identification of a Vitamin D response element in the human insulin receptor gene promoter," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 84, no. 2-3, pp. 223–230, 2003.

[71] J.-Y. Oh and E. Barrett-Connor, "Association between vitamin D receptor polymorphism and type 2 diabetes or metabolic syndrome in community-dwelling older adults: the Rancho Bernardo study," *Metabolism*, vol. 51, no. 3, pp. 356–359, 2002.

[72] M. T. Malecki, J. Frey, D. Moczulski, T. Klupa, E. Kozek, and J. Sieradzki, "Vitamin D receptor gene polymorphisms and association with type 2 diabetes mellitus in a Polish population," *Experimental and Clinical Endocrinology & Diabetes*, vol. 111, no. 8, pp. 505–509, 2003.

[73] M. Kajikawa, H. Ishida, S. Fujimoto et al., "An insulinotropic effect of vitamin D analog with increasing intracellular Ca^{2+} concentration in pancreatic β-cells through nongenomic signal transduction," *Endocrinology*, vol. 140, no. 10, pp. 4706–4712, 1999.

[74] L. L. Baggio and D. J. Drucker, "Biology of incretins: GLP-1 and GIP," *Gastroenterology*, vol. 132, no. 6, pp. 2131–2157, 2007.

[75] D. J. Drucker, "The biology of incretin hormones," *Cell Metabolism*, vol. 3, no. 3, pp. 153–165, 2006.

[76] R. J. Bollag, Q. Zhong, P. Phillips et al., "Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors," *Endocrinology*, vol. 141, no. 3, pp. 1228–1235, 2000.

[77] Q. Zhong, T. Itokawa, S. Sridhar et al., "Effects of glucose-dependent insulinotropic peptide on osteoclast function," *American Journal of Physiology: Endocrinology and Metabolism*, vol. 292, no. 2, pp. E543–E548, 2007.

[78] K. Tsukiyama, Y. Yamada, C. Yamada et al., "Gastric inhibitory polypeptide as an endogenous factor promoting new bone formation after food ingestion," *Molecular Endocrinology*, vol. 20, no. 7, pp. 1644–1651, 2006.

[79] Q. Zhong, K.-H. Ding, A. L. Mulloy, R. J. Bollag, and C. M. Isales, "Glucose-dependent insulinotropic peptide stimulates proliferation and TGF-β release from MG-63 cells," *Peptides*, vol. 24, no. 4, pp. 611–616, 2003.

[80] N. H. Bjarnason, E. E. G. Henriksen, P. Alexandersen, S. Christgau, D. B. Henriksen, and C. Christiansen, "Mechanism of circadian variation in bone resorption," *Bone*, vol. 30, no. 1, pp. 307–313, 2002.

[81] A. Nissen, M. Christensen, F. K. Knop, T. Vilsbøll, J. J. Holst, and B. Hartmann, "Glucose-dependent insulinotropic polypeptide inhibits bone resorption in humans," *Journal Clinical Endocrinology Metabolism*, vol. 99, no. 11, pp. E2325–E2329, 2014.

[82] P. E. Scherer, S. Williams, M. Fogliano, G. Baldini, and H. F. Lodish, "A novel serum protein similar to C1q, produced exclusively in adipocytes," *The Journal of Biological Chemistry*, vol. 270, no. 45, pp. 26746–26749, 1995.

[83] K. Maeda, K. Okubo, I. Shimomura, T. Funahashi, Y. Matsuzawa, and K. Matsubara, "cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (adipose most abundant gene transcript 1)," *Biochemical and Biophysical Research Communications*, vol. 221, no. 2, pp. 286–289, 1996.

[84] Y. Nakano, T. Tobe, N.-H. Choi-Miura, T. Mazda, and M. Tomita, "Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma," *The Journal of Biochemistry*, vol. 120, no. 4, pp. 803–812, 1996.

[85] R. Ye and P. E. Scherer, "Adiponectin, driver or passenger on the road to insulin sensitivity?" *Molecular Metabolism*, vol. 2, no. 3, pp. 133–141, 2013.

[86] C. Tao, A. Sifuentes, and W. L. Holland, "Regulation of glucose and lipid homeostasis by adiponectin: effects on hepatocytes, pancreatic β cells and adipocytes," *Best Practice & Research: Clinical Endocrinology & Metabolism*, vol. 28, no. 1, pp. 43–58, 2014.

[87] A. R. Nawrocki, M. W. Rajala, E. Tomas et al., "Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor γ agonists," *The Journal of Biological Chemistry*, vol. 281, no. 5, pp. 2654–2660, 2006.

[88] T. Yamauchi, J. Kamon, Y. Minokoshi et al., "Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase," *Nature Medicine*, vol. 8, no. 11, pp. 1288–1295, 2002.

[89] W. L. Holland, R. A. Miller, Z. V. Wang et al., "Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin," *Nature Medicine*, vol. 17, no. 1, pp. 55–63, 2011.

[90] X.-H. Luo, L.-J. Guo, L.-Q. Yuan et al., "Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway," *Experimental Cell Research*, vol. 309, no. 1, pp. 99–109, 2005.

[91] K. Oshima, A. Nampei, M. Matsuda et al., "Adiponectin increases bone mass by suppressing osteoclast and activating osteoblast," *Biochemical and Biophysical Research Communications*, vol. 331, no. 2, pp. 520–526, 2005.

[92] Y. Shinoda, M. Yamaguchi, N. Ogata et al., "Regulation of bone formation by adiponectin through autocrine/paracrine and endocrine pathways," *Journal of Cellular Biochemistry*, vol. 99, no. 1, pp. 196–208, 2006.

[93] X. H. Luo, L. J. Guo, H. Xie et al., "Adiponectin stimulates RANKL and inhibits OPG expression in human osteoblasts through the MAPK signaling pathway," *Journal of Bone and Mineral Research*, vol. 21, no. 10, pp. 1648–1656, 2006.

[94] D. Kajimura, H. W. Lee, K. J. Riley et al., "Adiponectin regulates bone mass via opposite central and peripheral mechanisms through FoxO1," *Cell Metabolism*, vol. 17, no. 6, pp. 901–915, 2013.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2015, Article ID 206853, 7 pages
http://dx.doi.org/10.1155/2015/206853

Review Article

Pituitary Disorders and Osteoporosis

Marek Bolanowski, Jowita Halupczok, and Aleksandra Jawiarczyk-Przybyłowska

Department of Endocrinology, Diabetes and Isotope Therapy, Wroclaw Medical University, Pasteura 4, 50-367 Wroclaw, Poland

Correspondence should be addressed to Marek Bolanowski; marek.bolanowski@umed.wroc.pl

Received 29 June 2014; Revised 17 September 2014; Accepted 30 September 2014

Academic Editor: Cristina Sobacchi

Various hormonal disorders can influence bone metabolism and cause secondary osteoporosis. The consequence of this is a significant increase of fracture risk. Among pituitary disorders such effects are observed in patients with Cushing's disease, hyperprolactinemia, acromegaly, and hypopituitarism. Severe osteoporosis is the result of the coexistence of some of these disorders and hypogonadism at the same time, which is quite often.

1. Introduction

Osteoporosis is defined as a loss of bone mass and strength that leads to fragility fractures [1]. Secondary osteoporosis results from a large and diverse group of medical problems, including endocrine disorders, adverse effects of medications, immobilization, disorders of the gastrointestinal or biliary tract, renal disease, and cancer. Some pituitary diseases may influence bone mineral density (BMD) and bone turnover causing osteopenia, osteoporosis, and fractures [2]. Moreover, in some cases fractures occur despite normal bone density. In our review we have focused on the association between osteoporosis and pituitary disorders such as Cushing's disease, hyperprolactinemia, acromegaly, and hypopituitarism. The mechanisms of bone effects of certain hormones involved in pituitary disorders are shown in Table 1, and their influence on bone turnover, bone density, and fracture risk is shown in Table 2.

2. Cushing's Disease

In current review we discuss both pituitary-dependent Cushing's syndrome (Cushing's disease) and adrenal-dependent Cushing's syndrome, since the final bone effects of both are caused by hypercorticism. Glucocorticoid-induced osteoporosis (GIO) is the most common cause of secondary osteoporosis. Hypercorticism affects bone metabolism in a variety of mechanisms [3]. The most significant influences of glucocorticoids (GCs) are their inhibitory effects on bone formation, acting directly and indirectly by regulating the synthesis of factors present in the bone microenvironment [4]. GCs reduce replication and prevent the terminal differentiation of bone forming cells and additionally promote apoptosis of osteoblasts and osteocytes by activating caspase 3. Besides decreasing the overall number of osteoblasts, GCs also exert effects on their function by inhibiting the synthesis of type I collagen, the major component of bone extracellular matrix, with a consequent decrease in bone matrix available for mineralization. Furthermore, alkaline phosphatase and osteocalcin serum levels are decreased in patients with Cushing's syndrome. Indirect actions of GCs involve decreased production of insulin-like growth factors (IGFs) and shifting transforming growth factor-β (TGF-β) binding [4, 5]. GCs enhance osteoclastogenesis by increasing the production of RANKL (receptor activator of NF-kappaB (RANK) ligand) and reducing the synthesis of osteoprotegerin in stromal and osteoblastic cells [3, 5, 6]. GCs prolong the life span of mature osteoclasts by acting directly or through the increased expression of macrophage colony-stimulating factor (M-CSF) [3]. Impaired intestinal calcium absorption and renal calcium reabsorption affect bone metabolism in patients with Cushing's syndrome as well. In addition, GCs decrease the release of growth hormone and gonadotropins which may also play a role in bone turnover [7]. The catabolic effects of GCs on muscle could contribute to the fracture risk because muscle weakness increases the incidence of falls. Furthermore myopathy and muscle weakness induce bone loss by removing the stimulating activity on the bone [5, 8].

Table 1: Mechanism of bone effects of certain hormones acting in pituitary disorders.

Hormone	Physiological effect	Effect of hormone excess	Effect of hormone deficit
GH, IGF-1	Anabolic effect, influence bone size, OB stimulation, OC promotion, necessary for attaining normal PBM	Thickening of bone, ↑cortical BMD	↓PBM, ↓BMD
Prolactin	None	OC stimulation, ↓trabecular BMD	None
Cortisol	None	OB inhibition, OC stimulation, ↓trabecular BMD, ↓PBM	None
Sex steroids	Necessary for attaining normal PBM	None	OC stimulation, ↓trabecular BMD
Thyroxine	Necessary for bone growth and attaining normal PBM	↓PBM, OC stimulation	↓PBM

OB—osteoblast; OC—osteoclast; PBM—peak bone mass; BMD—bone mineral density.

Table 2: The influence of pituitary disorders on bone turnover, bone density and fracture risk.

Disorder	Bone turnover	Bone mineral density	Fracture risk
Acromegaly	Increase	Increase/decrease	Increase
Hyperprolactinemia	Increase	Decrease	Increase
Cushing's Disease	Decrease	Decrease	Increase
Hypogonadism	Increase	Decrease	Increase
Hypopituitarism	Decrease	Decrease/normal	Increase
GHD	Decrease	Decrease	Increase

GHD-growth hormone deficiency.

GIO occurs in two phases, a rapid early phase in which BMD is reduced as a result of bone resorption, followed by a slower, progressive phase in which BMD declines because of impaired bone formation [3]. In patients with endogenous or exogenous hypercortisolism bone loss is more severe in trabecular bone than in cortical bone. This is explained by greater surface-to-volume ratio of trabecular bone compared with cortical bone; thus trabecular bone responds more rapidly to stimulation than cortical bone [7]. It is well known that patients with Cushing's syndrome may have lower BMD and increased osteoporotic fracture risk [6, 7, 9]. A study on 58 patients with pituitary-dependent and 21 patients with adrenal-dependent Cushing's syndrome showed low mean BMD before treatment. BMD values were lower in the lumbar spine than in the femoral neck, although the difference was not statistically significant. According to World Health Organization (WHO) criteria 82% patients were diagnosed with osteopenia. Interestingly, the prevalence of osteopenia and osteoporosis was independent of age. There was no significant difference in BMD between patients with Cushing's syndrome of different origins, neither in the whole group nor in male or female patients separately. Factors such as type of Cushing's syndrome, mean 24-hour serum level of cortisol, and duration of symptomatic glucocorticoid overproduction had no influence on *Z-scores*. Body mass index (BMI) and menstrual status did not show a correlation with *Z-score*. In males as well as in females, there was positive correlation between age and *Z-score* at both the lumbar spine and the femoral neck [7].

Endogenous hypercortisolism changes bone structure and causes vertebral fractures in up to 70% of patients. Factors associated with fracture risk include age at the onset, span and severity of the disease, and individual susceptibility to GCs [3]. In patients with endogenous Cushing's syndrome fractures occur most commonly at the thoracic and lumbar vertebrae, hip, ribs, and pelvis, not infrequently developing spontaneously or after low-energy trauma [10]. The prevalence of osteoporosis and atraumatic fractures is higher in adrenal Cushing's syndrome than in pituitary Cushing's syndrome [11]. In both endogenous and exogenous hypercortisolism fractures might be asymptomatic and occur in patients with normal or only slightly decreased BMD values [3, 10, 12]. It is thought that even subclinical hypercortisolism (SH) might be the cause of osteoporosis and increased risk of vertebral fractures [3, 13]. Morelli et al. studied the risk of new vertebral fractures in patients with adrenal incidentaloma (AI). BMD in subjects with and without SH was comparable; however the prevalence of vertebral fracture and spinal deformity index (SDI) were significantly higher in SH subjects than in the group of patients without SH. The presence of SH was related with worsening of SDI and occurrence of a new vertebral fracture or the progression of the grade of a preexisting vertebral deformity regardless of age, gender, BMI, years since menopause, lumbar spine BMD, and basal SDI. Patients with SH showed higher fracture incidence in spite of stable BMD throughout the observation [13]. This suggests that factors other than BMD, such as bone quality, are associated with increased risk of fracture [7, 13]. Although dual-energy X-ray absorptiometry (DXA) is considered a gold-standard measurement of BMD, it cannot be employed to assess bone microarchitecture, the most important factor in determining bone quality [10, 14]. The trabecular bone score (TBS) has been recently introduced as a method of bone quality estimation in GIO [10, 15]. TBS can be used in addition to BMD to divide patients according to their fracture risk. The patients with low BMD and low TBS will present fractures more likely than patients with low BMD and high TBS [16]. Eller-Vainicher et al. showed that, in AI patients with subclinical hypercortisolism, TBS is reduced

and correlates with the number and severity of vertebral fractures and with the degree of cortisol excess [16].

The aim of medical therapy is to prevent fractures [3]. Initially, factors influencing the fracture risk should be recognized. Patients with GIO and all patients exposed to glucocorticoid excess should be treated with appropriate supplementation of vitamin D and calcium [12]. Administration of calcium (1500 mg daily) and vitamin D (800 IU daily) is recommended [3]. The management in patients with GH and sex steroids deficiencies may include replacement therapy with adequate hormones. It seems that treatment of GHD protects the skeleton from the adverse effects of glucocorticoid overtreatment in hypopituitary patients [3, 17]. A proactive preventive approach including lifestyle changes, such as tobacco cessation, reduction in alcohol consumption, exercise program, and restriction of sodium intake in the presence of hypercalciuria, should be implemented [5]. Antiosteoporotic drugs may be needed in patients in whom fractures risk could not be normalized by correction of hypercortisolism. Proposed treatment protocol in patients with endogenous hypercortisolism comprises bone active treatment for postmenopausal women or men older than 50 years with a 10-year risk for fractures of at least 20% (calculated by FRAX) or in patients older than 70 and/or with BMD $<$ -1.5 SD and/or prevalent fractures and/or more severe hypercortisolism. Young patients (premenopausal women or men aged less than 50 years) with long-term pituitary hypercortisolism, even subclinical, not adequately corrected by surgery should be treated with antiosteoporotic drugs [12]. Bisphosphonates ought to be considered in patients with Cushing's syndrome who have a decreased BMD, regardless of age [7]. Administration of alendronate or clodronate provides faster BMD recovery in comparison with untreated patients [3]. Fracture risk (FRAX score) and BMD should be assessed few months after resolution of hypercortisolism. Based on that, clinician is able to make a decision about cessation of antiosteoporotic therapy [12].

3. Hyperprolactinemia

Hyperprolactinemia causes secondary hypogonadism and may have sex hormone-independent effects on bone metabolism [17, 18]. Estrogen deficiency in approximately 50% of premenopausal women with secondary amenorrhea is attributable to acquired gonadotropin-releasing hormone (GnRH) dysregulation, comprising hyperprolactinemia. The effects of estrogen deficiency on bone include an acceleration of bone turnover with higher resorption than formation [19]. In cases of functional GnRH deficiency the pattern of GnRH secretion is altered, involving reduced frequency of pulsations and decreased pulse amplitude [4, 5]. Trabecular bone is more affected than cortical. A study on hyperprolactinemic males showed low BMD at the lumbar spine in 80% of patients, whereas only 30% subjects had a reduced BMD at the femoral neck. Similar finding was reported in women [20].

Increase in bone resorption and low BMD occur in males and females with hyperprolactinemia [18, 20–23]. Authors reported that the patients with prolactinomas also presented with osteopenia or osteoporosis and the risk factors of bone loss were disease duration and hypogonadism [21]. A significant negative correlation was found between lumbar spine and femoral neck BMD values and both prolactin (PRL) levels and disease duration, which suggests that the duration of the disease is strongly correlated with the severity of bone loss [20, 22]. In addition, patients with a longer duration of hypogonadism are likely to have lower bone density [19, 22]. In men with prolactinoma the absence of detectable levels of estradiol was correlated with lower BMD in all analyzed sites. It is not clear if testosterone deficiency or estradiol deficiency is mostly responsible for bone loss in men with hypogonadism [22]. The importance of achievement of adequate peak bone mass is considered the best protection against age-related bone loss. The disturbances impeding the acquirement of peak bone mass severely influence future bone health. Patients who developed hyperprolactinemia in childhood or adolescence have more severe bone impairment than subjects who developed hyperprolactinemia in adulthood [20, 22]. The evaluation of bone markers can be useful in diagnosing an early bone turnover alteration before a change in BMD becomes apparent. In one study, in all patients, osteocalcin (OC) levels were significantly lower and urinary cross-linked N-telopeptide of type I collagen (Ntx) levels were significantly higher; however BMD values were in normal ranges in 20% of the patients [20].

Untreated prolactinomas were associated with a significant increase in fracture risk attributed to gonadotropins and sex steroids deficiency or hyperprolactinemia per se [17]. Mazziotti et al. have shown that higher prevalence of radiological vertebral fractures (VFs) occurs in women and men with PRL-secreting adenomas compared to controls [18, 24]. Males with prolactinomas who suffered fractures had lower BMD *T-score* and longer duration of the disease, independently of the effects of age, serum IGF-1 and PRL values, frequency of macroadenomas, adrenal insufficiency, hypothyroidism, diabetes insipidus, parental history of fractures, cigarette smoking, and excessive alcohol consumption [18]. In female patients prevalence of VFs was associated with the duration of the disease independently of the effects of hypopituitarism, age, BMD, serum PRL levels, and treatment with dopaminergic drugs [24]. Fractures were more frequent in patients with untreated hyperprolactinemia in comparison with patients treated with cabergoline [18, 24]. Dopamine agonist therapy restores gonadal function and increases vertebral BMD in most hyperprolactinemic women [19, 20]. History of amenorrhea may be a cause of an increased fracture risk despite resumption of menses [19]. A progressive significant increase in serum OC levels and a significant decrease in Ntx levels were noted after 6, 12, and 18 months of treatment in the 3 groups of patients. 18-month treatment with one of the dopamine agonists (bromocriptine, quinagolide, or cabergoline) normalized serum PRL and OC levels and gonadal function, although it was unable to completely restore lumbar spine and femoral neck BMD and normalize Ntx levels [20].

4. Acromegaly

Acromegaly is characterized by growth hormone (GH) excessive secretion and in most cases is caused by the presence of a

somatotroph tumor of the pituitary gland [25, 26]. GH and its main peripheral mediator insulin-like growth factor-1 (IGF-1) stimulate proliferation, differentiation, and extracellular matrix production in osteoblastic cells. GH and IGF-1 also promote osteoclast recruitment and bone resorption activity [27–30]. In prepubertal period anabolic action of GH is responsible for longitudinal bone growth, whereas during the adolescence and early adulthood it induces skeletal maturation until the achievement of peak bone mass. In adulthood GH regulates bone turnover, thus maintaining bone mass [27]. In patients with acromegaly changes in bone size and density and arthropathy are common problems [31–33]. Acromegaly is known to be a secondary cause of osteoporosis [27].

Information about the influence of GH excess on BMD varies in the literature. In some studies BMD was reported to be increased or within the reference ranges, while in other papers it was decreased [17, 26, 34–37]. Conflicting data on bone mineral density might be related to gonadal status, different skeletal sites being measured, various techniques employed, subject's gender, age, and activity of the disease [28, 36, 38]. Hypogonadal patients with acromegaly are at higher risk of osteoporosis, particularly at sites composed predominantly of trabecular bone, which has more intimate contact with the circulation and is influenced by sex steroids to a greater extent [34, 39, 40]. Normal or increased bone mass is attributable to anabolic effect of GH, although BMD measurements may be overestimated due to structural modification of the spine [27]. Hypogonadism occurs frequently in patients with acromegaly as a consequence of PRL and GH hypersecretion or hypopituitarism due to local mass effects [25]. Impaired gonadal status including hypogonadism and hyperprolactinemia is thought to be a cause of BMD decline [28, 34]. In one study, forearm and vertebral BMD values changed in opposite directions. It seems that forearm BMD was increased because of GH and IGF-1 action and vertebral BMD was low due to hypogonadism [41]. Axial skeleton is composed of 70% trabecular bone, while appendicular skeleton of 90% cortical bone [40]. Cancellous bone is more susceptible to rapid resorption than cortical bone. Patients with acromegaly generally demonstrate increased cortical bone mass, whereas trabecular BMD is more variable [23, 35, 41, 42]. Influence of gender and disease activity on BMD has been also widely studied [36, 40, 43]. Scillitani et al. reported that the anabolic effect of GH excess on bone in acromegalic patients is gender independent, evident only at the spine in eugonadal patients regardless of disease activity and present only at femoral neck during active disease regardless of gonadal status [36].

It is well known that in patients with acromegaly bone turnover is increased [28, 29, 35]. A specific marker of bone formation, osteocalcin, is elevated in subjects with active disease [28, 29, 35, 40, 43]. Indicators of bone resorption such as urinary hydroxyproline/creatinine, urinary type I collagen cross-linked N-telopeptide, and serum C-terminal collagen type I cross-links are in higher concentrations than in controls [28, 29, 35, 40, 43]. Additionally, serum calcium and phosphate values and 24-hour urinary calcium are elevated in patients with active disease [27, 29, 34, 35, 41]. Rise in serum calcium levels may be caused by increased intestinal calcium absorption, while high serum phosphate levels may be due to GH-mediated increased intestinal and renal absorption [27].

Bone quality depends not only on bone density and bone turnover but also on collagen integrity and the macrostructure and microstructure of bone [26]. Quantified ultrasound (QUS) measurements of skeletal properties provide important information on bone strength and resistance to fractures. This method is performed at hand phalanges and at the heel in patients with acromegaly [28, 33]. High-resolution peripheral quantitative computed tomography (HR-pQCT) permits in vivo assessment of the bone microarchitecture and the volumetric BMD in the distal radius and tibia [26]. DXA is currently the most prominent clinical tool used to assess bone health. However, DXA and QUS methods are not sufficient for identifying patients at risk for fracture, due to the many possible interferences including bone deformities, osteoarthritis, joint rigidity, and soft tissue thickening [26, 27]. Patients with acromegaly have an increased risk of fractures, which might be correlated with insufficient quality of bone [26, 27, 39, 42]. The reported prevalence of VFs varies between 39% and 59% [27, 42]. In a prospective study the incidence of VFs was significantly higher in patients with active disease as compared with those who had controlled/cured acromegaly at the beginning of the study [38]. Claessen et al. showed that VFs tend to progress in the long term in 20% of patients with biochemically controlled acromegaly in the absence of osteoporosis or osteopenia. This is suggestive of an abnormal bone quality persisting after remission, possibly due to pretreatment long-term exposure to high circulating levels of GH [30]. VFs are often asymptomatic and are largely underdiagnosed. Fractures occur more frequently in men than women. They are also more severe and often multiple in men [27, 42]. Vertebral fractures may be present in patients with normal or slightly decreased BMD, which makes BMD a poor fracture predictor [27, 30, 39, 42]. In patients with acromegaly a spine X-ray should be performed, both at diagnosis and during follow-up in order to reveal possible VFs [39, 42]. The early diagnosis and the effective control of acromegaly seem to be the most important tools in reducing the risk of VFs [38].

5. Hypopituitarism and Growth Hormone Deficiency

Patients with isolated GH deficiency (GHD) and with multiple pituitary deficiencies usually have low BMD. However, in some cases normal BMD can be also observed. In patients with childhood onset (CO) GHD there seems to be a clear reduction in BMD, which may present the potential role of GH in the achievement of peak bone mass [44–49]. Other authors claim that there is no evidence supporting association between isolated CO GHD and increased fracture risk or low bone density [50]. Studies have shown that GHD itself is crucial for the development of osteopenia in hypopituitary patients [44, 49, 51]. A direct relation between GHD and decreased bone mass in hypopituitarism is supported

by the fact that GH replacement therapy improves BMD values [51, 52]. The causes of reduced BMD in patients with hypopituitarism and untreated GHD are not fully explained. According to the literature BMD might be affected by many factors such as age at onset, gender, body mass index, gonadal status, and the severity of growth hormone deficiency [47, 49, 51]. Pituitary hormone deficiencies causing hypogonadism, hypothyroidism, or hypoadrenalism may also contribute to bone loss. The mechanisms underlying the association between either ACTH or TSH deficiency and lower BMD are not clarified and might be a consequence of either deficiencies of pituitary hormones or excessive hormone replacement [47, 53, 54]. It is important to take into consideration possible interactions between replaced hormones [53].

It has been reported that GHD and hypopituitarism are associated with increased fracture risk [17, 44, 51, 55]. Studies suggest that fracture rate is rather attributable to GHD alone than other pituitary hormone deficiencies or their replacement therapy [51]. In hypopituitary adult males with untreated GH deficiency vertebral fractures occurred more frequently in patients who received higher cumulative and current doses of cortisone than in patients who received lower doses [54]. In one nationwide study of patients suffering from GHD the risk of fracture was significantly increased in adult onset (AO) GHD females, but not in males. This was explained by a reduced percentage of properly treated females with hypogonadism compared to men [56]. Holmer et al. studied fracture incidence in GHD patients on complete hormone replacement therapy. They demonstrated that women with CO GHD had more than doubled risk for nonosteoporotic fractures. In contrast, a significantly decreased incidence of fractures was observed in AO GHD men. These findings were justified by the interaction between oral estrogen and the GH-IGF-1 axis in women and by the adequate substitution of testosterone and GH in men [44].

Recent meta-analysis revealed that administration of recombinant human growth hormone (rhGH) resulted in a significant increase in BMD in randomized/controlled studies of more than 12 months. Similar outcomes were observed in prospective studies. This beneficial effect of rhGH replacement therapy is affected by gender, age, and treatment duration. The doses used in studies varied from 0.2 to 0.96 mg/daily with higher doses prescribed to women and patients with CO GHD [57]. BMD within the first months of treatment decreased which was followed by a subsequent increase after at least one year of therapy. GH replacement promotes bone turnover by stimulating both bone formation and bone resorption, which is prominent during the first phase of GH treatment. After this period bone turnover slows down and bone mass increases [48, 57, 58]. Some studies suggest that GH replacement therapy may reduce the risk of fracture in patients with hypopituitarism and GHD [51, 59].

6. Conclusions

Secondary osteoporosis and fragility fractures can be a result of Cushing's syndrome, hyperprolactinemia, acromegaly, and reduced production of sex steroids (hypogonadism). Hypopituitarism might also be a cause of bone loss due to deficiencies of several pituitary hormones, especially growth hormone or excessive replacement therapy. The key is early diagnosis and effective management of the underlying disease. In addition, primary prevention of fractures and proper treatment of osteoporosis are very important, especially in patients unsuccessfully treated with basic disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] L. G. Raisz, "Pathogenesis of osteoporosis: concepts, conflicts, and prospects," *Journal of Clinical Investigation*, vol. 115, no. 12, pp. 3318–3325, 2005.

[2] L. A. Fitzpatrick, "Secondary causes of osteoporosis," *Mayo Clinic Proceedings*, vol. 77, no. 5, pp. 453–468, 2002.

[3] G. Kaltsas and P. Makras, "Skeletal diseases in Cushing's syndrome: osteoporosis versus arthropathy," *Neuroendocrinology*, vol. 92, no. 1, pp. 60–64, 2010.

[4] E. Canalis and A. M. Delany, "Mechanisms of glucocorticoid action in bone," *Annals of the New York Academy of Sciences*, vol. 966, pp. 73–81, 2002.

[5] G. Mazziotti, A. Angeli, J. P. Bilezikian, E. Canalis, and A. Giustina, "Glucocorticoid-induced osteoporosis: an update," *Trends in Endocrinology and Metabolism*, vol. 17, no. 4, pp. 144–149, 2006.

[6] T. Lekva, T. Ueland, H. Byøum, J. A. Evang, K. Godang, and J. Bollerslev, "TXNIP is highly regulated in bone biopsies from patients with endogenous Cushing's syndrome and related to bone turnover," *European Journal of Endocrinology*, vol. 166, no. 6, pp. 1039–1048, 2012.

[7] A. W. Van der Earden, M. Den Heijer, W. J. Oyen, and A. R. Hermus, "Cushing's syndrome and bone mineral density: lowest Z scores in young patients," *Netherlands Journal of Medicine*, vol. 65, no. 4, pp. 137–141, 2007.

[8] T. Mancini, M. Doga, G. Mazziotti, and A. Giustina, "Cushing's syndrome and bone," *Pituitary*, vol. 7, no. 4, pp. 249–252, 2004.

[9] O. M. Dekkers, E. Horváth-Puhó, J. O. L. Jørgensen et al., "Multisystem morbidity and mortality in cushing's syndrome: a cohort study," *The Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 6, pp. 2277–2284, 2013.

[10] M. Tóth and A. Grossman, "Glucocorticoid-induced osteoporosis: lessons from Cushing's syndrome," *Clinical Endocrinology*, vol. 79, pp. 1–11, 2013.

[11] N. Ohmori, K. Nomura, K. Ohmori, Y. Kato, T. Itoh, and K. Takano, "Osteoporosis is more prevalent in adrenal than in pituitary Cushing's syndrome," *Endocrine Journal*, vol. 50, no. 1, pp. 1–7, 2003.

[12] A. Scillitani, G. Mazziotti, C. di Somma et al., "Treatment of skeletal impairment in patients with endogenous hypercortisolism: when and how?" *Osteoporosis International*, vol. 25, no. 2, pp. 441–446, 2014.

[13] V. Morelli, C. Eller-Vainicher, A. S. Salcuni et al., "Risk of new vertebral fractures in patients with adrenal incidentaloma with and without subclinical hypercortisolism: a multicenter longitudinal study," *Journal of Bone and Mineral Research*, vol. 26, no. 8, pp. 1816–1821, 2011.

[14] V. Bousson, C. Bergot, B. Sutter, P. Levitz, and B. Cortet, "Trabecular bone score (TBS): available knowledge, clinical relevance, and future prospects," *Osteoporosis International*, vol. 23, no. 5, pp. 1489–1501, 2012.

[15] F. M. Ulivieri, B. C. Silva, F. Sardanelli, D. Hans, J. P. Bilezikian, and R. Caudarella, "Utility of the trabecular bone score (TBS) in secondary osteoporosis," *Endocrine*, 2014.

[16] C. Eller-Vainicher, V. Morelli, F. M. Ulivieri et al., "Bone quality, as measured by trabecular bone score in patients with adrenal incidentalomas with and without subclinical hypercortisolism," *Journal of Bone and Mineral Research*, vol. 27, no. 10, pp. 2223–2230, 2012.

[17] P. Vestergaard, J. O. L. Jørgensen, C. Hagen et al., "Fracture risk is increased in patients with GH deficiency or untreated prolactinomas—a case-control study," *Clinical Endocrinology*, vol. 56, no. 2, pp. 159–167, 2002.

[18] G. Mazziotti, T. Porcelli, M. Mormando et al., "Vertebral fractures in males with prolactinoma," *Endocrine*, vol. 39, no. 3, pp. 288–293, 2011.

[19] K. K. Miller and A. Klibanski, "Clinical review 106: amenorrheic bone loss," *The Journal of Clinical Endocrinology and Metabolism*, vol. 84, no. 6, pp. 1775–1783, 1999.

[20] C. di Somma, A. Colao, A. di Sarno et al., "Bone marker and bone density responses to dopamine agonist therapy in hyperprolactinemic males," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 3, pp. 807–813, 1998.

[21] Y. Zhao, X. Gan, P. Luo et al., "The risk of osteopenia in premenopausal women with various sellar tumors," *Gynecological Endocrinology*, vol. 28, no. 12, pp. 945–948, 2012.

[22] E. C. O. Naliato, M. L. F. Farias, G. R. Braucks, F. S. R. Costa, D. Zylberberg, and A. H. D. Violante, "Prevalence of osteopenia in men with prolactinoma," *Journal of Endocrinological Investigation*, vol. 28, no. 1, pp. 12–17, 2005.

[23] J. Matsuyama, N. Eshima, T. Fukunaga, S. Hori, H. Kobayashi, and M. Isono, "Various risks of osteoporosis in patients with pituitary adenomas," *Journal of Bone and Mineral Metabolism*, vol. 21, no. 2, pp. 91–97, 2003.

[24] G. Mazziotti, T. Mancini, M. Mormando et al., "High prevalence of radiological vertebral fractures in women with prolactin-secreting pituitary adenomas," *Pituitary*, vol. 14, no. 4, pp. 299–306, 2011.

[25] L. Katznelson, D. Kleinberg, M. L. Vance et al., "Hypogonadism in patients with acromegaly: data from the multi-centre acromegaly registry pilot study," *Clinical Endocrinology*, vol. 54, no. 2, pp. 183–188, 2001.

[26] M. Madeira, L. V. Neto, F. de Paula Paranhos Neto et al., "Acromegaly has a negative influence on trabecular bone, but not on cortical bone, as assessed by high-resolution peripheral quantitative computed tomography," *Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 4, pp. 1734–1741, 2013.

[27] G. Padova, G. Borzì, L. Incorvaia et al., "Prevalence of osteoporosis and vertebral fractures in acromegalic patients," *Clinical Cases in Mineral and Bone Metabolism*, vol. 8, no. 3, pp. 37–43, 2011.

[28] M. Bolanowski, D. Jędrzejuk, A. Milewicz, and A. Arkowska, "Quantitative ultrasound of the heel and some parameters of bone turnover in patients with acromegaly," *Osteoporosis International*, vol. 13, no. 4, pp. 303–308, 2002.

[29] S. Ezzat, S. Melmed, D. Endres, D. R. Eyre, and F. R. Singer, "Biochemical assessment of bone formation and resorption in acromegaly," *Journal of Clinical Endocrinology and Metabolism*, vol. 76, no. 6, pp. 1452–1457, 1993.

[30] K. M. J. A. Claessen, H. M. Kroon, A. M. Pereira et al., "Progression of vertebral fractures despite long-Term biochemical control of acromegaly: a prospective follow-up study," *The Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 12, pp. 4808–4815, 2013.

[31] A. L. Barkan, "Acromegalic arthropathy," *Pituitary*, vol. 4, no. 4, pp. 263–264, 2001.

[32] R. Scarpa, D. de Brasi, R. Pivonello et al., "Acromegalic axial arthropathy: a clinical case-control study," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 2, pp. 598–603, 2004.

[33] M. Bolanowski, W. Pluskiewicz, P. Adamczyk, and J. Daroszewski, "Quantitative ultrasound at the hand phalanges in patients with acromegaly," *Ultrasound in Medicine & Biology*, vol. 32, no. 2, pp. 191–195, 2006.

[34] M. J. Kayath and J. G. H. Vieira, "Osteopenia occurs in a minority of patients with acromegaly and is predominant in the spine," *Osteoporosis International*, vol. 7, no. 3, pp. 226–230, 1997.

[35] H. Kotzmann, P. Bernecker, P. Hubsch et al., "Bone mineral density and parameters of bone metabolism in patients with acromegaly," *Journal of Bone and Mineral Research*, vol. 8, no. 4, pp. 459–465, 1993.

[36] A. Scillitani, C. Battista, I. Chiodini et al., "Bone mineral density in acromegaly: the effect of gender, disease activity and gonadal status," *Clinical Endocrinology*, vol. 58, no. 6, pp. 725–731, 2003.

[37] M. Bolanowski, W. Wielgus, A. Milewicz, and R. Marciniak, "Axial bone mineral density in patients with acromegaly," *Academic Radiology*, vol. 7, no. 8, pp. 592–594, 2000.

[38] G. Mazziotti, A. Bianchi, T. Porcelli et al., "Vertebral fractures in patients with acromegaly: a 3-year prospective study," *The Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 8, pp. 3402–3410, 2013.

[39] G. Mazziotti, A. Bianchi, S. Bonadonna et al., "Prevalence of vertebral fractures in men with acromegaly," *The Journal of Clinical Endocrinology & Metabolism*, vol. 93, no. 12, pp. 4649–4655, 2008.

[40] T. Ueland, S. L. Fougner, K. Godang, T. Schreiner, and J. Bollerslev, "Serum GH and IGF-I are significant determinants of bone turnover but not bone mineral density in active acromegaly: a prospective study of more than 70 consecutive patients," *European Journal of Endocrinology*, vol. 155, no. 5, pp. 709–715, 2006.

[41] T. Diamond, L. Nery, and S. Posen, "Spinal and peripheral bone mineral densities in acromegaly: the effects of excess growth hormone and hypogonadism," *Annals of Internal Medicine*, vol. 111, no. 7, pp. 567–573, 1989.

[42] M. J. E. Wassenaar, N. R. Biermasz, N. A. T. Hamdy et al., "High prevalence of vertebral fractures despite normal bone mineral density in patients with long-term controlled acromegaly," *European Journal of Endocrinology*, vol. 164, no. 4, pp. 475–483, 2011.

[43] M. Bolanowski, J. Daroszewski, M. Mędraś, and B. Zadrozna-Śliwka, "Bone mineral density and turnover in patients with acromegaly in relation to sex, disease activity, and gonadal function," *Journal of Bone and Mineral Metabolism*, vol. 24, no. 1, pp. 72–78, 2006.

[44] H. Holmer, J. Svensson, L. Rylander et al., "Fracture incidence in GH-deficient patients on complete hormone replacement including GH," *Journal of Bone and Mineral Research*, vol. 22, no. 12, pp. 1842–1850, 2007.

[45] M. Degerblad, B.-A. Bengtsson, M. Bramnert et al., "Reduced bone mineral density in adults with growth hormone (GH) deficiency: increased bone turnover during 12 months of GH substitution therapy," *European Journal of Endocrinology*, vol. 133, no. 2, pp. 180–188, 1995.

[46] A. A. Toogood, J. E. Adams, P. A. O'Neill, and S. M. Shalet, "Elderly patients with adult-onset growth hormone deficiency are not osteopenic," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 5, pp. 1462–1466, 1997.

[47] N. A. Tritos, S. L. Greenspan, D. King et al., "Unreplaced sex steroid deficiency, corticotropin deficiency, and lower IGF-I are associated with lower bone mineral density in adults with growth hormone deficiency: a KIMS database analysis," *The Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 5, pp. 1516–1523, 2011.

[48] J. M. Gómez, N. Gómez, J. Fiter, and J. Soler, "Effects of long-term treatment with GH in the bone mineral density of adults with hypopituitarism and GH deficiency and after discontinuation of GH replacement," *Hormone and Metabolic Research*, vol. 32, no. 2, pp. 66–70, 2000.

[49] A. Colao, C. D. I. Somma, R. Pivonello et al., "Bone loss is correlated to the severity of growth hormone deficiency in adult patients with hypopituitarism," *The Journal of Clinical Endocrinology and Metabolism*, vol. 84, no. 6, pp. 1919–1924, 1999.

[50] W. Högler and N. Shaw, "Childhood growth hormone deficiency, bone density, structures and fractures: scrutinizing the evidence," *Clinical Endocrinology*, vol. 72, no. 3, pp. 281–289, 2010.

[51] C. Wüster, R. Abs, B.-A. Bengtsson et al., "The influence of growth hormone deficiency, growth hormone replacement therapy, and other aspects of hypopituitarism on fracture rate and bone mineral density," *Journal of Bone and Mineral Research*, vol. 16, no. 2, pp. 398–405, 2001.

[52] C. Clanget, T. Seck, V. Hinke, C. Wüster, R. Ziegler, and J. Pfeilschifter, "Effects of 6 years of growth hormone (GH) treatment on bone mineral density in GH-deficient adults," *Clinical Endocrinology*, vol. 55, no. 1, pp. 93–99, 2001.

[53] H. Filipsson and G. Johannsson, "GH replacement in adults: interactions with other pituitary hormone deficiencies and replacement therapies," *European Journal of Endocrinology*, vol. 161, supplement 1, pp. S85–S95, 2009.

[54] G. Mazziotti, T. Porcelli, A. Bianchi et al., "Glucocorticoid replacement therapy and vertebral fractures in hypopituitary adult males with GH deficiency," *European Journal of Endocrinology*, vol. 163, no. 1, pp. 15–20, 2010.

[55] T. Rosén, L. Wilhelmsen, K. Landin-Wilhelmsen, G. Lappas, and B.-Å. Bengtsson, "Increased fracture frequency in adult patients with hypopituitarism and GH deficiency," *European Journal of Endocrinology*, vol. 137, no. 3, pp. 240–245, 1997.

[56] K. Stochholm, T. Laursen, A. Green et al., "Morbidity and GH deficiency: a nationwide study," *European Journal of Endocrinology*, vol. 158, no. 4, pp. 447–457, 2008.

[57] M. Barake, A. Klibanski, and N. A. Tritos, "Effects of recombinant human growth hormone therapy on bone mineral density in adults with growth hormone deficiency: a meta-analysis," *The Journal of Clinical Endocrinology and Metabolism*, vol. 99, no. 3, pp. 852–860, 2014.

[58] F. Rota, M. C. Savanelli, L. Tauchmanova et al., "Bone density and turnover in young adult patients with growth hormone deficiency after 2-year growth hormone replacement according with gender," *Journal of Endocrinological Investigation*, vol. 31, no. 2, pp. 94–103, 2008.

[59] G. Mazziotti, A. Bianchi, S. Bonadonna et al., "Increased prevalence of radiological spinal deformities in adult patients with GH deficiency: influence of GH replacement therapy," *Journal of Bone and Mineral Research*, vol. 21, no. 4, pp. 520–528, 2006.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2015, Article ID 372156, 8 pages
http://dx.doi.org/10.1155/2015/372156

Review Article

Osteopetrosis and Its Relevance for the Discovery of New Functions Associated with the Skeleton

Amélie E. Coudert,[1] Marie-Christine de Vernejoul,[2] Maurizio Muraca,[3] and Andrea Del Fattore[3]

[1]*Institut National de la Santé et de la Recherche Médicale U1138, Centre de Recherche des Cordeliers, Paris, France*
[2]*Institut National de la Santé et de la Recherche Médicale U1132, Hôpital Lariboisière, Paris, France*
[3]*Regenerative Medicine Unit, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165 Rome, Italy*

Correspondence should be addressed to Andrea Del Fattore; andrea.delfattore@opbg.net

Received 6 August 2014; Revised 16 October 2014; Accepted 30 October 2014

Academic Editor: Cristina Sobacchi

Osteopetrosis is a rare genetic disorder characterized by an increase of bone mass due to defective osteoclast function. Patients typically displayed spontaneous fractures, anemia, and in the most severe forms hepatosplenomegaly and compression of cranial facial nerves leading to deafness and blindness. Osteopetrosis comprises a heterogeneous group of diseases as several forms are known with different models of inheritance and severity from asymptomatic to lethal. This review summarizes the genetic and clinical features of osteopetrosis, emphasizing how recent studies of this disease have contributed to understanding the central role of the skeleton in the whole body physiology. In particular, the interplay of bone with the stomach, insulin metabolism, male fertility, the immune system, bone marrow, and fat is described.

1. Introduction

Bone is a dynamic tissue which undergoes continuous self-renewal, and bone homeostasis relies on functional equilibrium among three types of cells: osteoclasts essential for bone resorption, osteoblasts responsible for bone matrix formation, and osteocytes involved in the reception and transduction of mechanical stimuli and in the regulation of osteoclast/osteoblast differentiation and function [1]. The balance between bone synthesis and resorption is finely tuned and any perturbations of this balance in adults trigger bone disease. Human osteopetrosis was first described by Albers-Schönberg in 1904 [2]. Osteopetrosis (*osteo*: bone and *petros*: stone) regroups a set of rare, heterogeneous, and inherited bone diseases characterized by increased bone mass. Osteopetrosis is therefore an osteocondensing disease. In principle, two causes could give rise to this osteocondensing phenotype: increased bone formation or failure of resorption by osteoclasts. However, osteopetrosis is known to result from defective osteoclast differentiation or function [3, 4].

Important progress has been made during the past decades in understanding the molecular mechanisms underlying the development of hereditary diseases characterized by increased bone mass [3, 5].

Our objective in this review is not to give a detailed description of all the sclerosing bone diseases; such information can be found in other reviews [3, 4, 6, 7]. Instead, we discuss recent findings regarding osteopetrosis and how the study of this disease has contributed to new understanding of functions associated with the skeleton [8–10].

2. Osteoclasts

Osteoclasts are highly specialized cells responsible for the dissolution of bone mineral and for the degradation of organic matrix. This activity is essential to bone remodeling and mineral homeostasis [8].

Osteoclasts are multinucleated cells (containing up to 50 nuclei), derived from the fusion of mononuclear cells belonging to the monocyte-macrophage lineage. Under the

Table 1: Genes mutated in osteopetrotic patients.

Osteopetrosis form	Genetic transmission	Gene	Mutation type	Protein
ARO	Autosomal recessive	*TCIRG1*	Loss of function	α3 subunit V-ATPase
		CLCN7	Loss of function	Chloride channel 7
		OSTM1	Loss of function	Osteopetrosis associated transmembrane protein
		PLEKHM1	Loss of function	Pleckstrin homology domain containing family M, member I
		SNX10	Loss of function	Sorting nexin 10
		TNFSF11	Loss of function	Receptor activator for nuclear factor κB ligand
		TNFRSF11A	Loss of function	Receptor activator for nuclear factor κB
IRO	Autosomal recessive	*CAII*	Loss of function	Carbonic anhydrase II
ADO II	Autosomal dominant	*CLCN7*	Dominant negative	Chloride channel 7

influence of factors secreted by osteoblasts and/or stromal cells present in the bone microenvironment, these precursors differentiate into osteoclasts [1].

The osteoclast differentiation pathway and the molecules involved are now well established. M-CSF (macrophage colony stimulating factor) is expressed by osteoblasts and binds the c-fms receptor on osteoclast precursors, stimulating their proliferation and the expression of RANK (receptor activator of NF-κB) receptor. The interaction of RANK-L, expressed and secreted by osteoblasts and stromal cells, with its receptor propels the fusion of osteoclast progenitors to form a giant multinucleated cell. Osteoprotegerin inhibits osteoclast differentiation by acting as a receptor decoy for RANK-L [1].

A terminally differentiated osteoclast is able to degrade extracellular bone matrix by the action of specific proteins. To resorb bone matrix, osteoclasts must be perfectly polarized with a ruffle border and a sealing zone. These two features allow the creation of a resorption lacuna into which H^+ ions are actively secreted in order to acidify it, leading to dissolution of bone matrix hydroxyapatite [1]. Creation of the acidic compartment requires a continuous source of protons. Type II carbonic anhydrase (CAII) hydrates CO_2 to form carbonic acid, which spontaneously dissociates into protons and HCO_3^- ions. The protons are actively transported into the resorption lacuna by a vacuolar ATPase proton pump located in the ruffled border domain [1, 11]. The HCO_3^- ion is exchanged with Cl^- by a bicarbonate/chloride antiport on the basolateral membrane of the cell. The chloride ion is translocated into the resorption lacuna through chloride channel 7 (ClCn7), recently reclassified as chloride/proton antiport. The acidic environment promotes the dissolution of inorganic content and also exposes the organic matrix, which is then ready to be digested by secreted proteolytic enzymes [1, 11].

The collagenous bone matrix is dissolved by two groups of enzymes, the matrix metalloproteases and the lysosomal cathepsins. Cathepsin K especially has been identified as a key enzyme in osteoclast function. This enzyme is secreted into the resorption lacuna and degrades type I collagen in the acidic environment [12].

The acquisition and maintenance of osteoclast membrane polarity require a complex system of vesicle trafficking and ongoing cytoskeletal renewal [1]. One of the proteins involved in these processes is Plekhm1 (pleckstrin homolog domain containing family M with run domain member 1). This protein plays a crucial role in acidification and trafficking of intracellular vesicles [13, 14]. A recently discovered protein important for osteoclast trafficking activity is Snx10 (sorting nexin 10). Snx10 belongs to a family of about 30 proteins sharing the PX (phox homology) phospholipid binding domain and is involved in protein trafficking and osteoclast differentiation/function [15, 16].

3. Osteopetrosis

Osteopetrosis is a generic name for a group of rare genetic bone diseases characterized by osteoclast failure [6]. Several forms are known with different models of inheritance and severity. The adult autosomal dominant type II form or Albert-Schönberg disease classified as mild is sometimes associated with bone symptoms. This is the most frequent form of osteopetrosis observed by rheumatologists. In contrast, the infantile recessive osteopetroses are severe forms and usually lethal in childhood without treatment [3, 5–7, 17]. Mutations in at least 8 genes (Table 1) have been identified as being responsible for osteopetrosis pathogenesis in humans.

3.1. Autosomal Recessive Osteopetrosis. Autosomal recessive osteopetrosis is a severe disease diagnosed in the first months of life owing to a variety of problems [3]. Patients are treated in pediatrics or hematology departments. Sick children have recurrent infections. They also show bruising and frequent bleeding secondary to medullar hyperplasia caused by bony invasion of the medullar space. Cranial nerve compressions can occur leading to blindness and deafness. Neurological defects may also be observed in some patients independently of nerve compressions. X-ray analysis reveals dense bones which are characterized by extreme brittleness. Untreated children usually die during their decade from hemorrhage, pneumonia, anemia, or infection. Hematopoietic stem cell

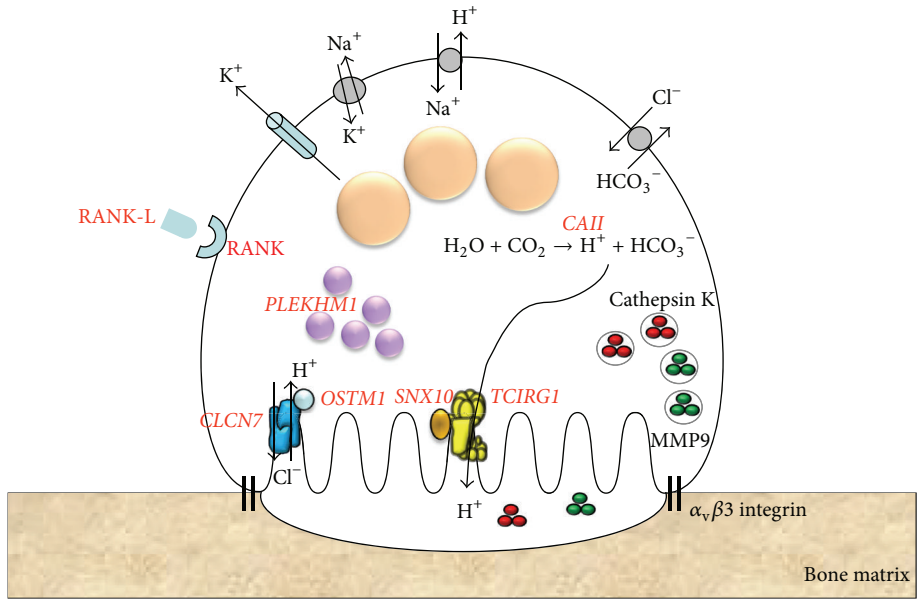


Figure 1: Schematic diagram showing an osteoclast and the involved genes in osteopetrosis. Cellular localization and protein involved in osteoclast differentiation and function. The genes mutated in human osteopetrosis are red in bold.

transplantation (HSCT) is the only treatment option currently far available [3, 5, 18].

Several biological abnormalities can cause this pathology. Generally in ARO, the number of osteoclasts is normal or high, but their acidifying activity, compulsory for bone resorption, is impaired [17]. Several genes are known to be involved in this form of osteopetrosis (Table 1, Figure 1). About 50% of ARO patients harbors loss-of-function mutations of *TCIRG1* which codes for the proton pump V-ATPase α3 subunit [17, 19]. Loss-of-function mutations of the *CLCN7* gene, coding for chloride channel 7, have also been described in ~10% of ARO patients [20]. Mutations in *OSTM1* (osteopetrosis associated transmembrane protein 1), coding for a protein involved in transport of ClCn7 to the ruffled border (and considered as a β subunit of ClCn7), have been described as causing severe osteopetrosis in ~5% of patients [21–23]. Primary neurological defects can also be present in patients bearing *OSTM1* or *CLCN7* mutations [20, 23].

Two cases of intermediate forms of ARO caused by *PLEKHM1* mutations have been described. An "Erlenmeyer flask" deformity of the distal femora, bone pain, and chondrolysis of the left hip were described in one patient. Interestingly, a brother with the same mutation showed no clinical signs [14]. Recently, a mutation in the *SNX10* gene was found in 15 families in which the patients displayed a heterogeneous phenotype. Mild growth retardation, hypocalcemia, hydrocephalus, severe hematological abnormalities, and visual impairment have been described in patients with loss of function mutations of *SNX10* [3, 15, 16, 24].

Less than 4% of ARO patients harbors loss-of-function mutations of *TNFSF11*, encoding RANK-L, or of *TNFRSF11A*, encoding RANK receptor, and constitute a distinct subgroup of recessive osteopetrosis. Indeed, bone biopsies from these patients revealed a complete lack of osteoclasts [25–27]. In addition, patients with *TNFSF11* mutations exhibit some immune abnormalities and not palpable lymph nodes, but B and T lymphocyte numbers are normal. By contrast, most of the patients with *TNFRSF11A* mutations have a more severe immunological phenotype with a defect in memory B lymphocyte differentiation and a reduction in immunoglobulins levels [25–27].

Treatment of most recessive forms of osteopetrosis includes HSCT, which restores osteoclast function. However, osteopetrosis caused by *TNFSF11* mutations cannot be treated by HSCT, because an osteoblast defect is the basis of this pathology [28]. In practice, a molecular genetic diagnosis should be made before transplantation to ensure that the pathology is not due to a RANK-L mutation.

3.2. A Specific Intermediate Recessive Osteopetrosis (IRO): Type II Carbonic Anhydrase Deficiency. In 1983, an autosomal recessive osteopetrosis syndrome associated with renal tubular acidosis was described [29]. The clinical signs of the affected patients are highly variable. Mental deficiency is frequent, but not always present. Optical nerve compression and dental malocclusions can occur. Renal tubular acidosis can explain the hypotonia, apathy, and muscular weakness occurrence in some patients. By radiography CAII deficiency resembles other forms of osteopetrosis, but brain calcifications can develop during childhood and osteosclerosis and bone modeling spontaneously decrease instead of increasing in the course of pathology evolution. Metabolic acidosis occurs during the neonatal period, and renal tubular acidosis, both proximal and distal, has been described [29, 30].

CAII is expressed in many different tissues including brain, kidney, red blood cells, cartilage, lung, and digestive mucosa. All patients with this pathology have a selective defect involving CAII expressed in erythrocytes [31].

3.3. Type II Autosomal Dominant Osteopetrosis (ADOII Also Known as Albert-Schönberg Disease). ADO II is commonly called benign osteopetrosis but presents with an extremely heterogeneous course from asymptomatic to rarely fatal. Prevalence of the pathology has been estimated at 5 per 100 000 [32].

ADOII clinical and radiological signs occur quite late in childhood or in the teens, although earlier occurring has sometimes been reported. ADOII patients usually displayed osteosclerosis at the vertebral level (so-called sandwich vertebrae) and also a bone in bone aspect observed mainly in the iliac bones, but sometimes in other epiphyses. An increase in cranial bone density can also occur. In addition, on radiography, alternating dense and light bands are often seen in iliac bones and at the extremities of long bones [7, 33].

The main ADOII complications involve the skeleton [34]. Bone fractures occur in 80% of patients, with a mean of 3 fractures per patient. A few patients have had more than 10 fractures. The femur is the most fractured bone in this pathology, but fractures can occur on any long bones and even at the posterior arch of the vertebrae, which often leads to a spondylolisthesis. Scoliosis is not rare. Hip arthritis is frequent (in 50% of the cases) and could be due to excessive stiffness of the subchondral bone. Arthritis can occur in other locations as well. Mandibular osteomyelitis is often associated with dental abscess or carious cavity. Cranial nerve compressions caused by osteosclerosis are rare. Auditory or visual impairment occurs in less than 5% of affected individuals [7, 33].

Orthopedic treatment is often necessary to treat fractures and arthritis. Arthropathies are technically difficult and postsurgical complications, such as strengthening delay, infections, and pseudoarthritis are frequent (50% of cases) due to bone stiffness. The penetrance of ADOII is 60–90%. Disease severity is highly variable, even within the same family [33]. For example, in 3 families in which most of the affected individuals expressed only a mild form of ADOII, some members exhibited anemia and blindness caused by optical nerve compression. This phenotype has been called intermediate osteopetrosis because of its overlap with that of mild ARO [33].

About 70% of patients affected by ADOII harbors heterozygous dominant negative mutations of the *CLCN7* gene (Figure 1, Table 1) [33]. In the remaining ~30% of cases, no mutations in *CLCN7* gene sequences were found, suggesting involvement of further genes in the pathogenesis of this form of osteopetrosis [33].

4. The Relevance of Osteopetrosis Studies to New Understanding of Functions Associated with the Skeleton

4.1. Osteopetrosis and the Bone-Stomach Interaction. Osteopetrorickets is a bone disorder characterized by increase of bone mass with a defect of skeletal mineralization. Schinke and coauthors performed histological analysis of undecalcified bone biopsies of 21 patients who received a diagnosis of osteopetrosis. In patients with loss-of-function mutations in the *TCIRG1* gene, an increase of unmineralized bone matrix osteoid was observed. The same pathological enrichment of osteoid was confirmed in *oc/oc* mice carrying a loss-of-function mutation of the *tcirg1* gene, while no increase was revealed in osteopetrotic $scr^{-/-}$ mice [35].

The increase of osteoid volume was associated with hypocalcemia, due to a defect of intestinal calcium uptake. Indeed it was shown that *TCIRG1* is also expressed in the fundus, a region of the stomach involved in gastric acidification, and loss-of-function mutations induce hypochlorhydria and reduced intestinal calcium uptake in both humans and mice [35].

This study was fundamental in demonstrating a physiological link between the stomach and bone. Gastric acidification is a prerequisite for efficient intestinal calcium uptake; in hypochlorhydria, intestinal calcium uptake is lowered leading to parathyroin hormone (PTH)-dependent activation of osteoclasts and an osteoporosis phenotype. In the case of loss-of-function mutation of *TCIRG1*, intestinal calcium uptake is reduced and PTH-dependent stimulation of bone resorption is blocked, resulting in an osteopetrorickets phenotype [35].

Barvencik et al. performed histomorphometric analysis of bone biopsies of 9 osteopetrotic patients with loss-of-mutation in the *TCIRG1*, *CLCN7,* and *TNFSF11A* genes [36]. Pathological enrichment of nonmineralized bone matrix was observed in all cases with *TCIRG1* mutations. In contrast, there was no sign of osteopetrorickets in patients with *CLCN7* and *TNFSF11A* gene mutations [35–37].

4.2. Osteopetrosis and Insulin Metabolism. Osteopetrosis studies were fundamental to understand the link between bone and osteocalcin signaling. Osteocalcin is a small protein embedded in bone matrix. Osteocalcin can exist in two different forms, undercarboxylated and carboxylated on 3 glutamic acid residues [10, 38]. The carboxylated form has high affinity for the hydroxyapatite, facilitating its engraftment in the bone matrix. It was shown that acidic pH can decarboxylate proteins [39]. Ferron et al. investigated whether acidic bone resorption lacuna promotes the decarboxylation of osteocalcin. Indeed they observed that in *oc/oc* mice the levels of undercarboxylated osteocalcin were reduced by 30% compared to wild-type animals. Similar features were observed in wild-type mice that received fetal liver hematopoietic stem cells (HSCs) from *oc/oc* mice confirming the relevance of osteoclast function in osteocalcin-insulin signaling. Moreover they observed that *oc/oc* mice were glucose intolerant, with reduced serum insulin levels, pancreas insulin content, and insulinexpression in the pancreas [40, 41].

Interestingly, it was shown that osteopetrotic patients affected by autosomal dominant osteopetrosis with osteoclast acidification defects have lower levels of insulin and a lower undercarboxylated/carboxylated osteocalcin ratio but diabetes was not reported [40, 41].

4.3. Osteopetrosis and Male Fertility. Osteocalcin is very important for the cross talk between bone and the systems responsible for male fertility [42, 43]. Karsenty's group showed that osteocalcin is able to stimulate, in a cAMP

response element binding (CREB) protein-dependent manner, the production of testosterone by testes. This function is mediated by the interaction of osteocalcin with GPRC6A, a G-coupled receptor expressed in Leydig cells [42, 43].

In 1997 Cohen et al. [44] showed that op/op mice (which lack colony stimulating factor 1, CSF-1) have reduced mating ability, low sperm numbers, and low serum testosterone levels due to decreased Leydig cell steroidogenesis. The study also showed how CSF-1 is essential for the development and function of the hypothalamic-pituitary-gonadal axis. Further studies in osteopetrotic animal models will be important to confirm the interaction between bone and male fertility.

4.4. Osteopetrosis and the Immune System. It now well established that there is a tight correlation between bone and the immune system, which has led to a new discipline called osteoimmunology. This research area is just now expanding and we are beginning to better understand the relevance of this interplay in bone diseases [45].

Many osteopetrotic animals are characterized by immunological defects. Associated defects in B cell function were attributed to mutations in genes involved in osteoclast differentiation or function or to an abnormal medullary microenvironment. *oc/oc* mice display a block at the pro-B to pre-B cell transition, which is due to a defect of the bone microenvironment rather than to a cell autonomous defect of B cells, because *in vitro* experiments showed that B cell progenitors isolated from osteopetrotic mice were able to differentiate into immature B cells [46].

Moreover it was shown that *rankl*$^{-/-}$ mice display an immunological defects. Apart the alterations of B cell differentiation, Kong and coauthors described a reduction in thymus size and a block of thymocyte development at the $CD4^{-}CD8^{-}CD44^{-}CD25^{+}$ stage [47]. These effects are correlated with many functions exerted by RANKL in the immune system [48].

Many studies have been published regarding the effects on osteoclast differentiation and function following alterations of immune cells [45]. The involvement of T regulatory cells (Treg) is still under investigation. In particular, it was shown that animal overexpressing the transcription factor FoxP3 (forkhead box P3) displayed osteopetrotic phenotype with increased bone mass and reduced osteoclast number and activity [49]. Moreover *in vitro* experiments suggested that Treg cells could inhibit osteoclast differentiation and function by suppression of cytoskeletal reorganization [50].

4.5. Osteopetrosis and Bone Marrow. Bone and bone marrow can be considered as two distinct compartments of the same functional unit, the bone-bone marrow organ. Perturbations to one of the compartments typically affect the other as well [51].

Indeed it was shown that dysfunction of osteoclast activity results in aberrant formation of the HSC niche, leading to retention of HSC in the spleen. The frequency and absolute number of $LinnegSca1^{+}cKit^{+}$ (LSK cells) were decreased by 90% and 99.8%, respectively, in the bone marrow of *oc/oc* mice compared to controls. This alteration was associated with a defect of mesenchymal stem cells to differentiate into osteoblasts. The effect was revealed by a dramatic reduction in the expression of the osteoblast markers *Runx2*, *Alp*, *Osteocalcin* and *Bsp* and a reduced proportion of cells expressing CD51 and the integrin $\alpha 5$ (CD49e). The study showed that osteoclasts promote the formation of the HSC niche, regulating the osteoblast differentiation important for the niche [52]. Indeed the authors showed that the absence of osteoclast activity affects formation of the bone marrow HSC niche and impairs ability of mesenchymal stem/stromal cells to recruit hematopoietic progenitor cells. Moreover the restoration of osteoclast function by treatment with $CD45^{+}Sca1^{+}$ cells reestablishes normal levels of hematopoietic progenitors in the bone marrow [46, 53].

4.6. Osteopetrosis and Fat. The relationship between bone and adipose tissue is an area of intensive investigations because molecules involved in bone-fat interactions could be used as pharmacological targets to prevent osteoporosis and bone fractures [54]. In particular, the involvement of peroxisome proliferator-activated receptor-γ (PPAR-γ) was studied. PPAR-γ is a nuclear receptor and acts as a heterodimer with retinoid X receptor. Ligands for PPAR-γ include long-chain fatty acid and synthetic compounds such as thiazolidinedione [55]. PPAR-γ functions are associated with activation of the adipogenesis and inhibition of the osteoblastogenesis [56, 57].

Moreover Wan et al. investigated PPAR-γ function in osteoclasts [58]. The authors used TieCre/flox mice to delete PPAR-γ in osteoclasts. These mice developed increased bone mass with a parallel reduction of bone marrow cavities and extramedullary hematopoiesis. Indeed deletion of PPAR-γ resulted in impaired osteoclast differentiation and activity, since it regulates c-fos expression involved in RANKL signaling [58].

Moreover Cock et al. demonstrated that the absence of PPAR-γ in white adipose tissue led to lipodystrophy, increased bone mineral density, and extramedullary hematopoiesis in spleen [59]. This interplay between bone and adipose tissue has clinical important implications, since a long-term treatment with the PPAR-γ agonist rosiglitazone in patients affected by type 2 diabetes could result in osteoporosis and bone fractures [54, 58].

5. Conclusion

Rare hereditary diseases inducing a bone condensation have shed new light on several aspects of bone cellular biology that were not well known. Indeed the study of these diseases allowed the identification of new mechanisms of osteoclast differentiation and function and the discovery of new functions associated with the skeleton. Much evidence suggests that the skeleton has a central role in bone physiology since bone disorders usually impact other organs [8, 9, 42, 43, 45, 53, 54, 60]. Osteopetrosis studies were essential to demonstrate these interactions. However, there are some features of these diseases that require further investigation. For example, as in other monogenic diseases, the genotype-phenotype correlation is not always clear and consistent. Indeed, the

same mutations can give rise to different phenotypes, as exemplified by the *CLCN7* gene heterozygous mutations. Moreover, the mutations identified to date explain only 70% of osteopetrosis cases. Efforts to identify the mutations responsible for the remaining 30% are on-going [33].

From a pathophysiological point of view, it is worth noting that the pathologies caused by reduced osteoclastic activity such as osteopetrosis lead to frequent fractures. This might be linked to a skeleton elasticity defect, but also to an inability to repair micro damage in bones because of a lower rate of bone turnover. This situation illustrates the well-known discrepancy between the bone quantity and its resistance to mechanical stress. In contrast, pathologies caused by an increase in bone formation due to increased activity of the Wnt signaling pathway (striated osteopathy) or to TGFβ activating mutations (Camurati Engelmann disease) are not associated with an increased incidence of fractures [7].

In conclusion, further study of osteopetrosis will allow us to better understand the physiology of bone and its impact on the whole body. Moreover our challenge for the future will be to identify new therapeutic approaches for this disabling disease, particularly for those forms for which only palliative intervention is currently available.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] A. del Fattore, A. Teti, and N. Rucci, "Bone cells and the mechanisms of bone remodelling," *Frontiers in Bioscience*, vol. 4, no. 6, pp. 2302–2321, 2012.

[2] H. E. Albers-Schönberg, "Röntgenbilder einer seltenen Knockenerkrankung," *Münchener Medizinische Wochenschrift*, vol. 5, pp. 365–368, 1904.

[3] C. Sobacchi, A. Schulz, F. P. Coxon, A. Villa, and M. H. Helfrich, "Osteopetrosis: genetics, treatment and new insights into osteoclast function," *Nature Reviews Endocrinology*, vol. 9, no. 9, pp. 522–536, 2013.

[4] Z. Stark and R. Savarirayan, "Osteopetrosis," *Orphanet Journal of Rare Diseases*, vol. 4, no. 1, article 5, 2009.

[5] M. H. Helfrich, "Osteoclast diseases," *Microscopy Research and Technique*, vol. 61, no. 6, pp. 514–532, 2003.

[6] M. C. de Vernejoul and O. Bénichou, "Human osteopetrosis and other sclerosing disorders: recent genetic developments," *Calcified Tissue International*, vol. 69, no. 1, pp. 1–6, 2001.

[7] M.-C. de Vernejoul and U. Kornak, "Heritable sclerosing bone disorders: presentation and new molecular mechanisms," *Annals of the New York Academy of Sciences*, vol. 1192, pp. 269–277, 2010.

[8] J. F. Charles and A. O. Aliprantis, "Osteoclasts: more than 'bone eaters'," *Trends in Molecular Medicine*, vol. 20, no. 8, pp. 449–459, 2014.

[9] A. R. Guntur and C. J. Rosen, "Bone as an endocrine organ," *Endocrine Practice*, vol. 18, no. 5, pp. 758–762, 2012.

[10] D. J. DiGirolamo, T. L. Clemens, and S. Kousteni, "The skeleton as an endocrine organ," *Nature Reviews Rheumatology*, vol. 8, no. 11, pp. 674–683, 2012.

[11] K. Henriksen, J. Bollerslev, V. Everts, and M. A. Karsdal, "Osteoclast activity and subtypes as a function of physiology and pathology—implications for future treatments of osteoporosis," *Endocrine Reviews*, vol. 32, no. 1, pp. 31–63, 2011.

[12] V. Everts, W. Korper, K. A. Hoeben et al., "Osteoclastic bone degradation and the role of different cysteine proteinases and matrix metalloproteinases: differences between calvaria and long bone," *Journal of Bone and Mineral Research*, vol. 21, no. 9, pp. 1399–1408, 2006.

[13] A. del Fattore, L. van Wesenbeeck, F. de Freitas et al., "A new heterozygous mutation (R714C) of the osteopetrosis gene, pleckstrin homolog domain containing family M (with run domain) member 1 (PLEKHM1), impairs vesicular acidification and increases TRACP secretion in osteoclasts," *Journal of Bone and Mineral Research*, vol. 23, no. 3, pp. 380–391, 2008.

[14] L. van Wesenbeeck, P. R. Odgren, F. P. Coxon et al., "Involvement of PLEKHM1 in osteoclastic vesicular transport and osteopetrosis in incisors absent rats and humans," *The Journal of Clinical Investigation*, vol. 117, no. 4, pp. 919–930, 2007.

[15] M. Aker, A. Rouvinski, S. Hashavia et al., "An SNX10 mutation causes malignant osteopetrosis of infancy," *Journal of Medical Genetics*, vol. 49, no. 4, pp. 221–226, 2012.

[16] A. Mégarbané, A. Pangrazio, A. Villa et al., "Homozygous stop mutation in the SNX10 gene in a consanguineous Iraqi boy with osteopetrosis and corpus callosum hypoplasia," *European Journal of Medical Genetics*, vol. 56, no. 1, pp. 32–35, 2013.

[17] C. Sobacchi, A. Frattini, P. Orchard et al., "The mutational spectrum of human malignant autosomal recessive osteopetrosis," *Human Molecular Genetics*, vol. 10, no. 17, pp. 1767–1773, 2001.

[18] M.-C. de Vernejoul, "Sclerosing bone disorders," *Best Practice and Research: Clinical Rheumatology*, vol. 22, no. 1, pp. 71–83, 2008.

[19] A. Frattini, P. J. Orchard, C. Sobacchi et al., "Defects in TCIRG1 subunit of the vacuolar proton pump are responsible for a subset of human autosomal recessive osteopetrosis," *Nature Genetics*, vol. 25, no. 3, pp. 343–346, 2000.

[20] U. Kornak, D. Kasper, M. R. Bösl et al., "Loss of the CIC-7 chloride channel leads to osteopetrosis in mice and man," *Cell*, vol. 104, no. 2, pp. 205–215, 2001.

[21] N. Chalhoub, N. Benachenhou, V. Rajapurohitam et al., "Grey-lethal mutation induces severe malignant autosomal recessive osteopetrosis in mouse and human," *Nature Medicine*, vol. 9, no. 4, pp. 399–406, 2003.

[22] P. F. Lange, L. Wartosch, T. J. Jentsch, and J. C. Fuhrmann, "ClC-7 requires Ostm1 as a β-subunit to support bone resorption and lysosomal function," *Nature*, vol. 440, no. 7081, pp. 220–223, 2006.

[23] A. Pangrazio, P. L. Poliani, A. Megarbane et al., "Mutations in *OSTM1* (grey lethal) define a particularly severe form of autosomal recessive osteopetrosis with neural involvement," *Journal of Bone and Mineral Research*, vol. 21, no. 7, pp. 1098–1105, 2006.

[24] A. Pangrazio, A. Fasth, A. Sbardellati et al., "SNX10 mutations define a subgroup of human autosomal recessive osteopetrosis with variable clinical severity," *Journal of Bone and Mineral Research*, vol. 28, no. 5, pp. 1041–1049, 2013.

[25] A. Pangrazio, B. Cassani, M. M. Guerrini et al., "RANK-dependent autosomal recessive osteopetrosis: characterization of five new cases with novel mutations," *Journal of Bone and Mineral Research*, vol. 27, no. 2, pp. 342–351, 2012.

[26] C. Sobacchi, A. Frattini, M. M. Guerrini et al., "Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL," *Nature Genetics*, vol. 39, no. 8, pp. 960–962, 2007.

[27] A. Villa, M. M. Guerrini, B. Cassani, A. Pangrazio, and C. Sobacchi, "Infantile malignant, autosomal recessive osteopetrosis: the rich and the poor," *Calcified Tissue International*, vol. 84, no. 1, pp. 1–12, 2009.

[28] N. Lo Iacono, H. C. Blair, P. L. Poliani et al., "Osteopetrosis rescue upon RANKL administration to Rankl$^{-/-}$ mice: a new therapy for human RANKL-dependent ARO," *Journal of Bone and Mineral Research*, vol. 27, no. 12, pp. 2501–2510, 2012.

[29] W. S. Sly, D. Hewett-Emmett, M. P. Whyte, Y. S. Yu, and R. E. Tashian, "Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 80, no. 9, pp. 2752–2756, 1983.

[30] W. S. Sly, M. P. Whyte, V. Sundaram et al., "Carbonic anhydrase II deficiency in 12 families with the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification," *The New England Journal of Medicine*, vol. 313, no. 3, pp. 139–145, 1985.

[31] R. L. Jilka, J. I. Rogers, R. G. Khalifah, and H. K. Vaananen, "Carbonic anhydrase isozymes of osteoclasts and erythrocytes of osteopetrotic microphthalmic mice," *Bone*, vol. 6, no. 6, pp. 445–449, 1985.

[32] J. Bollerslev, K. Henriksen, M. F. Nielsen, K. Brixen, and W. Van Hul, "Autosomal dominant osteopetrosis revisited: lessons from recent studies," *European Journal of Endocrinology*, vol. 169, no. 2, pp. R39–R57, 2013.

[33] M. C. de Vernejoul, A. Schulz, and U. Kornak, "CLCN7-related osteopetrosis," in *GeneReviews*, 2007.

[34] O. D. Bénichou, J. D. Laredo, and M. C. de Vernejoul, "Type II autosomal dominant osteopetrosis (Albers-Schonberg disease): clinical and radiological manifestations in 42 patients," *Bone*, vol. 26, no. 1, pp. 87–93, 2000.

[35] T. Schinke, A. F. Schilling, A. Baranowsky et al., "Impaired gastric acidification negatively affects calcium homeostasis and bone mass," *Nature Medicine*, vol. 15, no. 6, pp. 674–681, 2009.

[36] F. Barvencik, I. Kurth, T. Koehne et al., "CLCN7 and TCIRG1 mutations differentially affect bone matrix mineralization in osteopetrotic individuals," *Journal of Bone and Mineral Research*, vol. 29, no. 4, pp. 982–991, 2014.

[37] C. Supanchart, L. Wartosch, C. Schlack et al., "ClC-7 expression levels critically regulate bone turnover, but not gastric acid secretion," *Bone*, vol. 58, pp. 92–102, 2014.

[38] P. V. Hauschka, J. B. Lian, D. E. Cole, and C. M. Gundberg, "Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone," *Physiological Reviews*, vol. 69, no. 3, pp. 990–1047, 1989.

[39] J. A. Engelke, J. E. Hale, J. W. Suttie, and P. A. Price, "Vitamin K-dependent carboxylase: utilization of decarboxylated bone Gla protein and matrix Gla protein as substrates," *Biochimica et Biophysica Acta*, vol. 1078, no. 1, pp. 31–34, 1991.

[40] M. Ferron and J. Lacombe, "Regulation of energy metabolism by the skeleton: osteocalcin and beyond," *Archives of Biochemistry and Biophysics*, vol. 561, pp. 137–146, 2014.

[41] M. Ferron, J. Wei, T. Yoshizawa et al., "Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism," *Cell*, vol. 142, no. 2, pp. 296–308, 2010.

[42] F. Oury, "A crosstalk between bone and gonads," *Annals of the New York Academy of Sciences*, vol. 1260, no. 1, pp. 1–7, 2012.

[43] F. Oury, G. Sumara, O. Sumara et al., "Endocrine regulation of male fertility by the skeleton," *Cell*, vol. 144, no. 5, pp. 796–809, 2011.

[44] P. E. Cohen, M. P. Hardy, and J. W. Pollard, "Colony-stimulating factor-1 plays a major role in the development of reproductive function in male mice," *Molecular Endocrinology*, vol. 11, no. 11, pp. 1636–1650, 1997.

[45] U. Lange, J. Teichmann, G. Schett, E. Neumann, and U. Müller-Ladner, "Osteoimmunology: how inflammation influences bone metabolism," *Deutsche Medizinische Wochenschrift*, vol. 138, no. 37, pp. 1845–1849, 2013.

[46] C. Blin-Wakkach, A. Wakkach, P. M. Sexton, N. Rochet, and G. F. Carle, "Hematological defects in the *oc/oc* mouse, a model of infantile malignant osteopetrosis," *Leukemia*, vol. 18, no. 9, pp. 1505–1511, 2004.

[47] Y.-Y. Kong, H. Yoshida, I. Sarosi et al., "OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis," *Nature*, vol. 397, no. 6717, pp. 315–323, 1999.

[48] N. lo Iacono, A. Pangrazio, M. Abinun et al., "RANKL cytokine: from pioneer of the osteoimmunology era to cure for a rare disease," *Clinical and Developmental Immunology*, vol. 2013, Article ID 412768, 9 pages, 2013.

[49] M. M. Zaiss, K. Sarter, A. Hess et al., "Increased bone density and resistance to ovariectomy-induced bone loss in FoxP3-transgenic mice based on impaired osteoclast differentiation," *Arthritis and Rheumatism*, vol. 62, no. 8, pp. 2328–2338, 2010.

[50] Z. S. Buchwald, J. R. Kiesel, R. DiPaolo, M. S. Pagadala, and R. Aurora, "Osteoclast activated FoxP3$^+$ CD8$^+$ T-cells suppress bone resorption *in vitro*," *PLoS ONE*, vol. 7, no. 6, Article ID e38199, 2012.

[51] A. Del Fattore, M. Capannolo, and N. Rucci, "Bone and bone marrow: the same organ," *Archives of Biochemistry and Biophysics*, vol. 503, no. 1, pp. 28–34, 2010.

[52] A. Mansour, G. Abou-Ezzi, E. Sitnicka, S. E. W. Jacobsen, A. Wakkach, and C. Blin-Wakkach, "Osteoclasts promote the formation of hematopoietic stem cell niches in the bone marrow," *The Journal of Experimental Medicine*, vol. 209, no. 3, pp. 537–549, 2012.

[53] C. Blin-Wakkach, M. Rouleau, and A. Wakkach, "Roles of osteoclasts in the control of medullary hematopoietic niches," *Archives of Biochemistry and Biophysics*, vol. 561, pp. 29–37, 2014.

[54] B. Lecka-Czernik and L. A. Stechschulte, "Bone and fat: a relationship of different shades," *Archives of Biochemistry and Biophysics*, vol. 561, pp. 124–129, 2014.

[55] S. A. Kliewer, B. M. Forman, B. Blumberg et al., "Differential expression and activation of a family of murine peroxisome proliferator-activated receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 15, pp. 7355–7359, 1994.

[56] T. Akune, S. Ohba, S. Kamekura et al., "PPARγ insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors," *Journal of Clinical Investigation*, vol. 113, no. 6, pp. 846–855, 2004.

[57] E. D. Rosen, P. Sarraf, A. E. Troy et al., "PPARγ is required for the differentiation of adipose tissue in vivo and in vitro," *Molecular Cell*, vol. 4, no. 4, pp. 611–617, 1999.

[58] Y. Wan, L.-W. Chong, and R. M. Evans, "PPAR-γ regulates osteoclastogenesis in mice," *Nature Medicine*, vol. 13, no. 12, pp. 1496–1503, 2007.

[59] T.-A. Cock, J. Back, F. Elefteriou et al., "Enhanced bone formation in lipodystrophic PPAR$\gamma^{hyp/hyp}$ mice relocates haematopoiesis to the spleen," *EMBO Reports*, vol. 5, no. 10, pp. 1007–1012, 2004.

[60] A. Neve, A. Corrado, and F. P. Cantatore, "Osteocalcin: skeletal and extra-skeletal effects," *Journal of Cellular Physiology*, vol. 228, no. 6, pp. 1149–1153, 2013.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2014, Article ID 820615, 10 pages
http://dx.doi.org/10.1155/2014/820615

Review Article

Osteoporosis, Fractures, and Diabetes

Peter Jackuliak and Juraj Payer

5th Department of Internal Medicine, Comenius University Faculty of Medicine and University Hospital in Bratislava, Ruzinovska 6, 826 06 Bratislava, Slovakia

Correspondence should be addressed to Peter Jackuliak; peter.jackuliak@gmail.com

Received 8 March 2014; Revised 19 April 2014; Accepted 9 June 2014; Published 23 June 2014

Academic Editor: Andrea Del Fattore

It is well established that osteoporosis and diabetes are prevalent diseases with significant associated morbidity and mortality. Patients with diabetes mellitus have an increased risk of bone fractures. In type 1 diabetes, the risk is increased by ~6 times and is due to low bone mass. Despite increased bone mineral density (BMD), in patients with type 2 diabetes the risk is increased (which is about twice the risk in the general population) due to the inferior quality of bone. Bone fragility in type 2 diabetes, which is not reflected by bone mineral density, depends on bone quality deterioration rather than bone mass reduction. Thus, surrogate markers and examination methods are needed to replace the insensitivity of BMD in assessing fracture risks of T2DM patients. One of these methods can be trabecular bone score. The aim of the paper is to present the present state of scientific knowledge about the osteoporosis risk in diabetic patient. The review also discusses the possibility of problematic using the study conclusions in real clinical practice.

1. Introduction

Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fractures. Many of these fractures are associated with significant morbidity and mortality. Diabetes is also an increasingly prevalent disease, with significant associated morbidity and mortality. Additionally, it has become apparent in recent years that both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are associated with an increased risk of osteoporosis-associated fractures [1–3]. Authors present the overview of factors involved in the risk of osteoporosis and fractures in both types of diabetes.

2. Diabetes Mellitus

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs. Diabetes mellitus is a common disease in most parts of the world. Worldwide 382 million people have diabetes and by 2035 this will rise to 592 million. A further 316 million with impaired glucose tolerance are at high risk from the disease—an alarming number, that is, set to reach 471 million by 2035. The number of people with type 2 diabetes is increasing in every country. Diabetes caused 5.1 million deaths in 2013; every six seconds a person dies from diabetes [4]. Well known late complications of diabetes are microvascular disease including nephropathy, retinopathy, neuropathy, and macrovascular disease such as acute coronary syndrome, claudicatio intermittens, and stroke [5]. However, the bone turnover and thus the skeletal integrity may also be affected by diabetes, and diabetic bone disease can represent an overlooked complication of diabetes [6].

3. Osteoporosis

Osteoporosis is defined as a combination of reduced bone mass and altered bone quality, with microarchitectural abnormalities, resulting in decreased bone strength with an increased risk of fractures [7]. Based on the present definition, both bone density and quality, which encompass the structural and material properties of bone, are important factors in the determination of bone strength. Twenty-two

million women and 5,5 million men in the 27 countries of the European Union (EU27) were estimated to have osteoporosis, and 3,5 million new fragility fractures were sustained, comprising 610,000 hip fractures, 520,000 vertebral fractures, 560,000 forearm fractures, and 1,800,000 other fractures (i.e., fractures of the pelvis, rib, humerus, tibia, fibula, clavicle, scapula, sternum, and other femoral fractures) [8].

At present, the diagnosis of osteoporosis rests on areal bone mineral density (BMD) measurement using dual-energy X-ray absorptiometry (DXA). The results are reported as the difference, in standard deviations (SDs), with the peak bone mass (T-score). The World Health Organization (WHO) defines osteoporosis as a BMD T-score of −2.5 or less [9]. Low BMD has been recognized as a good predictor of osteoporotic fracture risk [10]. Nevertheless, although widely used, a major limitation of BMD measurement is that a substantial degree of BMD overlap exists between subjects with and without subsequent fractures [11, 12]. An additional explanation for this is that BMD does not capture all of the factors that contribute to bone strength. Among these factors is trabecular bone microarchitecture, which also appears to be a significant determinant of bone strength and is complementary to bone density [13, 14]. Another limitation of BMD measurements is that they disproportionately evaluate cortical bone depending on the skeletal site measured, which has a relatively slow rate of turnover [15]. In addition to BMD, several other parameters that can be measured during the same DXA scan may help to identify patients at high risk of fractures, such as the femoral neck length, the neck-diaphysis angle, the cross-sectional moment of inertia, and the cortical thicknesses. In addition, they reflect bone geometry or macroarchitecture, which is influenced by BMD [16]. To partially answer this problem the current osteoporosis classification criteria drafted by the World Health Organization (WHO) are currently revised to include clinical risk factors (http://www.shef.ac.uk/FRAX/).

4. Diabetes and Osteoporosis

Diabetes itself is associated with increased risk of fracture, although T2DM is often characterized by normal or high bone mineral density (BMD). Thus, diabetes may be associated with a reduction of bone strength, that is, not reflected in the measurement of BMD [17]. Diabetic osteopathy is a significant comorbidity of both forms of diabetes and is characterized by microarchitectural changes that decrease bone quality leading to an increased risk of bone fracture in both types of diabetes [18, 19].

T2DM is associated with an increased risk of hip fracture in both men, summary relative risk (RR) 2.8, and women, summary RR 2.1. Results are consistent between studies of men and women and between studies conducted in the United States and Europe. The association between type of diabetes and hip fracture incidence is stronger for T1DM, summary RR 6.3, than for T2DM, summary RR 1.7 [3]. In other meta-analyses, Vestergaard estimated a risk ratio for diabetes and hip fracture of 1.38 (95% CI, 1.25–1.53) for T2DM and 6.94 (95% CI, 3.25–14.78) for T1DM [17]. Increased risk of nonspine fractures in general has also been reported for T2DM [20].

The Nurses' Health Study with 109,983 women aged 34–59 years and followup of 22 years for the occurrence of hip fracture indicated that both type 1 and type 2 diabetes are associated with an increased risk of hip fracture. The results of this study highlight the need for fracture prevention strategies in all patients with diabetes [21].

4.1. The Pathogenesis of Bone Changes in Diabetes. Although the relationship between diabetes and osteoporosis has been widely investigated, it remains controversial. Diabetes could impact bone through several mechanisms, some of which may have contradictory effects.

The fracture risk of T1DM increases because of a decrease of BMD. In patients with T1DM the impaired bone formation is a result of absolute deficiency of insulin and insulin-like growth factor-1 (IGF-1), which leads to lower values of peak bone mass. In type 2 diabetes, obesity, increased load on bone, and insulin resistance resulting in hyperinsulinaemia lead to increased bone formation [22]. The coexistence of other autoimmune diseases with T1DM can lead to an additional risk factor for osteoporosis and increased fracture risk (secondary osteoporosis) in T1DM [23].

In both types of diabetes, bone displays inferior quality and strength [23]. T2DM reduces bone quality rather than BMD. Several risk factors for osteoporotic fractures are known and they are listed in Table 1.

4.1.1. Hyperglycemia. Hyperglycemia resulting from impaired secretion and/or action of insulin acts on bone tissue cells through an increased production of interleukin-6 (IL-6) in osteoblast line cells. IL-6 stimulates osteoclasts to resorb bone. The accumulation of advanced glycation end products (AGEs) in collagen leads to inferior bone quality and strength. Furthermore, glycated collagen inhibits expression in osteoblasts [24]. The relationship between hyperglycemia and fracture risk does not appear to be linear. Studies have reported no increase in risk [25], or even decreased risk [26], comparing those with impaired glucose tolerance to those with normoglycemia. Another indirect effect of hyperglycemia is glycosuria, which causes hypercalciuria, leading to decreased levels of calcium in the body and poor bone quality, and fastens bone loss [27]. Among those with diabetes, there is not an established relationship between glycated hemoglobin (A1C) and fracture risk. Most observational studies have found no effect [28].

4.1.2. Hypoglycemia. The risk of fractures in diabetes is also affected by the incidence of hypoglycemic episodes, if they especially are not preceded by prodromal symptoms. Although hypoglycemia can occur with sulfonylurea use, an increased risk of falls with low A1C levels is associated mainly with insulin use [29].

Hypoglycemic treatments could modulate the risk of fractures in many ways. Insulin-sensitizing treatment with metformin is not associated with a higher incidence of bone fractures [30]. Fracture rates are higher among all patients taking glitazones (TZDs). TZDs act as stimulators

Table 1: Risk factors for osteoporotic fractures in diabetes (modified according to [23]).

Risk for osteoporosis
Directly due to diabetes
(i) Diabetes mellitus types 1 and 2
(ii) Poor glycemic control and hyperglycemia
(iii) Hypoglycemia (due to DM treatment)
Due to complications of diabetes
(i) Nephropathy and other kidney diseases
(ii) Neuropathy
(iii) Diabetic diarrhea
Due to diseases associated with diabetes
(i) Thyroid gland dysfunction (Grave's disease)
(ii) Intestinal bowel diseases and celiac sprue
(iii) Amenorrhea
(iv) Delayed puberty
(v) Eating disorders
Risk of falls
(i) Episodes of hypoglycemia due to medication (mostly insulin)
(ii) Episodes of nocturia (during uncompensated DM)
(iii) Poor vision due to retinopathy or cataracts
(iv) Poor balance due to neuropathy, foot ulcers, or amputations of diabetic foot
(v) Orthostatic hypotension (due to cardiac autonomic neuropathy)
(vi) Impaired joint motility due to cheiropathy and arthropathy

of the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and could reduce bone density through the inhibition of osteoblast differentiation and activity. In fact, PPAR-γ activation induces the differentiation of multipotent mesenchymal stem cells into adipocytes, rather than osteoblasts, and increases osteoblast apoptosis [31, 32]. On the other hand, the insulin-sensitizing effect of TZDs reduces circulating insulin levels and therefore the insulin anabolic effect on the bone [18]. Higher incidence of fractures has been reported in insulin-treated patients in comparison with noninsulin-treated individuals [33]. In addition, the recently introduced class of incretin-based drugs (i.e., GLP-1 receptor agonists and DPP-4 inhibitors) is expected to exert potentially beneficial effects on bone health, possibly due to a bone anabolic activity of GLP-1 that can be either direct or indirect through the involvement of thyroid C cells [34]. C cells are mainly known for producing calcitonin, a hypocalcemic and hypophosphatemic hormone. Calcitonin suppresses resorption of bone by inhibiting the activity of osteoclasts. Some studies found that GLP-1 and other incretin hormones, such as GIP or GLP-2, could have positive effects on bone through antiresorptive and anabolic properties, suggesting beneficial effects of antidiabetic drugs like GLP-1R agonists or DPP-4 inhibitors on bone metabolism. The molecular mechanisms involved Wnt/beta-catenin pathway, OPG/RANKL ratio, and sclerostin levels [35].

4.1.3. Insulin. Insulin is an anabolic hormone, which acts on bone through insulin receptors expressed by osteoblasts—IRS-1 and IRS-2 (insulin-like substrate). Stimulation of IRS-1 affects bone turnover, while stimulation of IRS-2 shifts the balance between bone formation and resorption towards the former. Insulin stimulates osteoblast proliferation, inactivates p27 (responsible for osteoblastogenesis), promotes collagen synthesis, and increases glucose uptake [18]. In T1DM, the deficiency of insulin and IGF-1, which is present since the diagnosis, leads to impaired bone formation, abnormal mineralisation, abnormal bone microarchitecture, increased fragility of the bone, and reduced peak bone mass [36]. In T2DM hyperinsulinism (the stimulatory effects of insulin on bone formation) coupled with insulin resistance increases bone mass through effects on bone formation via IRS-1 and IRS-2 surface receptors on osteoblasts and by reducing the concentration of sex-hormone binding globulin (SHBG), which leads to increased concentrations of estradiol and testosterone [37].

4.1.4. Genetic Factors. Bone mineral density is affected by genetic factors. The A1-type-1-collagen (COL1A1) gene polymorphism in patients with T1DM is associated with reduced BMD at femoral neck and reduced serum vitamin D levels versus controls [38]. Vitamin D receptor gene polymorphism has also effect on BMD in diabetics [39].

4.1.5. Alterations in Collagen Cross-Link Formation. Bone matrix consists of a two-phase composite material—the mineral phase (provides stiffness) and collagen fibers (provide tensile strength, ductility, and toughness) [40]. Collagen cross-linking plays an important role in bone strength [41]. Collagen cross-links can be divided into lysyl-hydroxylase- and lysyl-oxidase-mediated enzymatic immature divalent cross-links, mature trivalent cross-links, and glycation- or oxidation-induced nonenzymatic cross-links (AGEs) such as pentosidine [42]. These types of cross-links differ in the mechanism of formation and in function [43]. Not only hyperglycemia but also oxidative stress induces the reduction in enzymatic beneficial cross-links and the accumulation of disadvantageous AGEs in bone.

4.1.6. Changes in Bone Turnover Markers (BTM). Bone turnover is a dual relationship between the process of bone formation by osteoblasts (creation of new bone) and the process of bone resorption by osteoclasts (removal of old bone) [44]. Bone markers are subdivided into bone formation and bone resorption markers. Bone formation markers consist of osteocalcin (OC), bone-specific alkaline phosphatase (BAP), alkaline phosphatase (AP), procollagen type 1 amino terminal propeptide (P1NP), and procollagen type 1 carboxyl terminal propeptide (P1CP), while resorptive markers consist of N-terminal cross-linked telopeptide of type-I collagen (NTX) and C-terminal cross-linked telopeptide of type-I collagen (CTX) [45]. Several markers, especially OC, CTX, and P1NP, may also vary with blood glucose or glucose intake, making them perhaps less markers of bone turnover in diabetics and more markers of alterations in glucose metabolism. In most studies of bone turnover markers, osteocalcin, a marker of

formation, is decreased with T2DM [46]. However, other formation markers are not consistently different in diabetic patients [47]. Resorption markers have been reported as increased, decreased, or not different in those with diabetes [48]. Another issue is kidney function, which may influence the measurement of several biochemical markers of bone turnover and also influence histomorphometry of the bone. The lack of a difference in bone turnover markers indicates that T1DM and T2DM are not different regarding the effect on bone markers, although Scl levels are higher in T2DM, proposing that bones are affected through an antagonizing effect on the WNT pathway in T2DM, but not in T1DM [49].

Osteocalcin (OC), one of the osteoblast-specific secreted proteins, has several hormonal features and is secreted in the general circulation from osteoblastic cells [50]. Recent animal studies have shown that uncarboxylated OC (ucOC) action is related to bone metabolism and glucose metabolism and fat mass [51, 52]. Pittas et al. have shown that serum OC concentration is inversely associated with fasting plasma glucose, fasting insulin, homeostasis model assessment for insulin resistance, high-sensitivity C-reactive protein, interleukin-6, body mass index, and body fat in cross-sectional analyses [53].

4.1.7. Vitamin D. Most studies across a variety of geographic locations suggest that vitamin D insufficiency is more common in individuals with diabetes compared to the general population [54, 55]. Proposed mechanisms for vitamin D deficiency in diabetes include genetic predisposition (T1DM), increased BMI (T2DM), concurrent albuminuria (T1DM or T2DM), or exaggerated renal excretion of vitamin D metabolites or vitamin D-binding protein (T1DM, T2DM) [56, 57].

4.1.8. Osteoprotegerin and RANK. Serum osteoprotegerin (OPG) is significantly increased in diabetic patients, prompting expanded investigation of the correlation between OPG production/release and glycemic levels [58]. Osteoprotegerin is a protein belonging to the family of tumour necrosis factor receptors (TNFR) capable of binding with receptor activator of nuclear factor kappa B ligand (RANKL), which prevents RANKL from binding to receptor activator of nuclear factor kappa B (RANK) and results in the suppression of osteoclastogenesis. Elevated osteoprotegerin in patients with T1DM may be the body's response to increased bone resorption [59]. Serum levels of OPG, but not of its cognate ligand receptor activator of nuclear factor kappa B ligand (RANKL), are significantly increased also in T2DM patients compared with healthy blood donors [58].

4.1.9. Wnt Signaling Pathway. Wnt signaling is also thought to be a pathogenetic feature of osteoporosis in DM. In particular, Wnt signaling has been shown as an important regulatory pathway in the osteogenic differentiation of mesenchymal stem cells not only in the embryonic development but also in the maintenance and differentiation of the stem cells in adulthood. Induction of the Wnt signaling pathway promotes bone formation while inactivation of the pathway leads to osteopenic states. Activating and inactivating aberrations of the canonical Wnt signaling pathway in osteogenesis result in sclerosteosis and osteoporosis, respectively. Mani et al. have shown that a single missense mutation in low-density lipoprotein receptor-related protein 6, the coreceptor for the Wnt signaling pathway, is genetically linked to osteoporosis as well as DM, dyslipoproteinemia, and coronary artery disease [60]. In addition, several studies have documented that T-cell-specific transcription factor- (TCF-) 4, the partner of β-catenin in the canonical Wnt signaling pathway, is the strongest T2DM susceptibility gene [61–63].

4.1.10. Obesity and BMI. A low BMI is associated with the decreased BMD, the increased possibility of osteoporosis, and the risk of fracture [64]. A meta-analysis demonstrated that BMI is also an important predictor of BMD in T2DM [19]. Overweight and obesity are believed to be protective factors of BMD [65, 66]. Obesity, widespread in T2DM, is strongly associated with higher BMD probably through mechanical loading and hormonal factors including insulin, estrogen, and leptin [67]. Recently published results from the Global Longitudinal Study of Osteoporosis in Women (GLOW) demonstrated that association between fracture risk, height, weight, and BMI differs according to fracture site—there is an inverse linear association between BMI and wrist fractures, positive linear association between BMI and spine fractures, and no significant association between BMI and upper leg fractures [68].

4.1.11. Complications of DM. A few studies have reported on diabetes-related complications as risk factors of fracture in those with T2DM, but results have not been consistent. The development of osteoporosis in both types of diabetes is also promoted by the coexistence of chronic microvascular complications, which also affect the bone marrow blood vessels [69].

4.2. Problems in Clinical Practice. Mainly older adults with T2DM are more likely to fall, but little is known about risk factors of falls in this population [70]. A higher risk of falls and the resulting fractures in patients with diabetes may result from the presence of diabetic retinopathy or cataracts, which impair visual acuity. In patients with coexisting sensory motor neuropathy and diabetic foot, balance disorders and falls are also observed [56]. Patel et al. suggest that reduced vibration perception (a measure of peripheral neuropathy) is an important risk factor for falling. The authors conclude also that quantitative ultrasound (QUS), as opposed to DXA, may be a more useful method for fracture risk prediction in older women with type 2 DM [71]. Insulin therapy is also associated with increased falls, possibly because of more severe disease and/or hypoglycemic episodes [72].

In T1DM, the increased risk of fractures may result from reduced BMD, so the basic diagnostic procedure and gold standard for diagnosis of osteoporosis and risk groups of diabetic patients are DXA. In T2DM the higher risk for osteoporotic fractures may be a consequence of poorer bone quality, impaired micro- and macroarchitecture, and increased tendency to fall. There is a need to clarify the use of standard methods for assessing fracture risk in T2DM.

The 10-year absolute risks of hip and osteoporotic fracture can be calculated using the FRAX algorithm. The FRAX scores are designed to predict the absolute 10-year risks of hip and osteoporotic fracture using hip BMD and other clinical risk factors of fracture [73]. The FRAX algorithm includes femoral neck BMD T-score, age, sex, body mass index, previous history of fracture, parental history of hip fracture, current smoking, recent use of corticosteroids, presence of rheumatoid arthritis, and at least 3 alcoholic beverages per day [73–75]. Schwartz et al. showed in a study that femoral neck BMD T-score and FRAX score are both associated with fracture risk in older adults with T2DM and both methods appear to be useful for clinical evaluation of fracture risk. They also warn that, at any given T-score or FRAX score, fracture risk was higher in those with diabetes [76].

To improve the management of osteoporosis, bone turnover biomarkers can be used. They can assess, directly or indirectly, bone development or bone resorption activity. According to the level of bone turnover we can estimate the fracture risk and evaluate the effect of treatment. These markers are measured in serum, plasma, and urine [77]. The ability to measure these markers has led to major advances in clinical research. Unfortunately, for reasons of availability, cost, and reproducibility, biological markers of bone turnover are not commonly measured among nonspecialists of bone diseases.

4.2.1. Bone Quality. Bone must be stiff and able to resist deformation, so that loading is possible. Bone must also be flexible and able to deform to allow energy absorption during impact loading. Bone must also be light to allow movement [78]. The balance between bone's material stiffness and its flexibility is achieved by varying its mineral content. The greater the mineral content, the greater the material stiffness and the lower the flexibility [26]. Bone strength, one of its major determinants, is dependent both on bone mass, reflected by bone mineral density (BMD), and on bone microarchitecture [79]. Thus, bone strength arises from both bone quantity and bone quality. Bone quality encompasses the geometric and material factors that contribute to fracture resistance [80]. Bone quality is not precisely defined. It is described as an amalgamation of all the factors that determine how well the skeleton can resist to fractures, such as the microarchitecture of the bone, the accumulated microscopic damages, the quality of collagen, the size of mineral crystals, and the rate of bone turnover [16, 26].

In fact, BMD explains only 70–75% of the variance in bone strength, while the rest could be related to other factors such as the accumulation of microfractures, the altered bone microarchitecture, the disordered bone remodeling, or the influence of extraskeletal risk factors [81].

Diabetes showing hyperglycemia and oxidative stress deteriorates bone material properties in terms of collagen posttranslational modification such as enzymatic immature and mature cross-links and nonenzymatic AGEs formation. The adverse effects of AGEs on bone cells accelerate bone fragility and impaired bone quality in diabetes [27].

Despite the use of BMD, biomarkers, and fracture clinical risk factors, many patients at risk for fractures are not detected and many fractures are not explained. BMD is only an assessment of bone mass. It does not provide information on bone quality, another key parameter describing bone. Fracture clinical risk factors (FRAX tool) are an indirect assessment of bone quality [82].

The presence of prevalent vertebral fractures (VFs) could also be used for the assessment of bone quality in individual patients, because a large study on the incidence of VFs in postmenopausal osteoporosis has shown that patients with previous VFs were more likely to suffer from new VFs [83, 84].

One important way to describe bone quality is to assess its microarchitecture. Bone microarchitecture contributes to the mechanical strength of bone and, thus, to its ability to withstand fractures. Bone loss is often accompanied by deterioration in bone architecture, resulting from a decrease in the number of trabeculae of cancellous bone, increased intertrabecular distances, and a loss of trabecular connectivity. In addition, a reduction in the thickness of cortical bone and an increase in its porosity of trabecular bone can result in fragility of the femoral neck [85]. Osteoporotic bone is, hence, called "porous." Although no single method can completely characterize bone quality, current noninvasive imaging techniques can be combined with ex vivo mechanical and compositional techniques to provide a comprehensive understanding of bone quality [86].

A variety of imaging techniques allow characterization of bone geometry and microarchitecture from the macroscale to the microscale and also to nanoscale. Methods for characterizing bone geometry and microarchitecture include quantitative CT, high-resolution peripheral quantitative CT, high-resolution MRI, and micro-CT. Macroscopic assessment of three-dimensional (3D) bone geometry can be performed in vivo using quantitative CT (QCT), but an important drawback of QCT is its delivery of ionizing radiation to patients [87]. The advent of high-resolution peripheral QCT (HR-pQCT) scanners with isotropic resolution of approximately 80 μm has enabled in vivo imaging of 3D trabecular morphology at peripheral sites such as the distal radius. These measurements are largely restricted to peripheral sites but have the concomitant benefit of reduced radiation doses relative to those from whole-body QCT scans [88, 89]. High-resolution MRI (HR-MRI) allows nonionizing 3D imaging of the trabecular network at peripheral sites. A critical advantage of this technique is its ability to generate 3D images of bone geometry and microarchitecture without ionizing radiation, but the disadvantages include the long scan times required for high-resolution images of trabecular bone [90].

To meet the need for a clinical tool capable of assessing bone microarchitecture, the trabecular bone score (TBS) was developed [91, 92].

4.2.2. Trabecular Bone Score. Trabecular bone score (TBS) is a novel noninvasive modality designed to assess the trabecular microarchitecture parameters derived from DXA images. The proponents described the TBS as a texture parameter that reflects pixel gray level variations in DXA images [93]. These variations may reflect microarchitecture, but the pixel size of currently available DXA machines is about four times larger

Table 2: Levels of degradation of trabecular structure according to TBS.

Degradation	Description	TBS range
Normal		Above 1.350
Moderate	Grade 1	1.300–1.350
	Grade 2	1.250–1.300
	Grade 3	1.200–1.250
Degraded	Severe	1.100–1.200
	Highly degraded	Below 1.100

than the mean trabecular size. TBS development is based on the following facts [94–96].

(i) A healthy patient has well- and dense-structured trabecular bone at the vertebral level (high connectivity, high trabecular number, and small spaces between trabeculae). If we project this structure onto a plane, we obtain an image containing a large number of pixel value variations, but the amplitudes of these variations are small.

(ii) Conversely, an osteoporotic patient has an altered and porous trabecular bone structure (low connectivity, low trabecular number, and wide spaces between trabeculae). If we project this structure onto a plane, we obtain an image containing a low number of pixel value variations, but the amplitudes of these variations are high.

The amount of trabecular bone lost during aging in women and men is similar or only slightly less in men than women [97]. Strength of the vertebrae is compromised more by loss of connectivity than by trabecular thinning [98].

TBS measures the level of degradation of trabecular structure. We get a result, which can be evaluated according to Table 2 (temporary consensus available on the website: http://www.medimapsgroup.com/).

Large retrospective study with more than 29,000 postmenopausal women showed that TBS independently predicts fractures in a subpopulation of patients with diabetes [99].

We performed a retrospective cohort study using BMD results from clinical registry of our department. We evaluated the ability of lumbar spine TBS to account the increased risk of fractures in T2DM in 56 postmenopausal women patients with T2DM and 61 women patients without DM or IGT. T2DM was associated with higher BMD (1.155 versus 1.048 g/cm^2 in average, $P < 0.05$) at all sites but lower lumbar spine TBS (1.211 versus 1.295 in average, $P < 0.05$). The adjusted odds ratio (aOR) for a measurement in the lowest versus the highest tertile was less than 1 for BMD ($P < 0.05$) but was increased for lumbar spine TBS (aOR 2.39, 95% confidence interval (CI) 2.22–2.81). Also according to these results lumbar spine TBS predicts osteoporotic fractures in those with diabetes and captures a larger portion of the diabetes-associated fracture risk than BMD [100].

From a clinical point of view TBS is able to predict future fracture risk [101], in combination with BMD. Using TBS we can increase the number of patients with a well identified risk, to improve the management of patients in which bone quality has a greater impact than bone quantity [102–104]. This method allows us to follow the evolution of a patients' trabecular bone texture over time and to monitor the effects of antiresorptive or anabolic treatment [105, 106].

TBS can be used in diabetology as a diagnostic toll and it can be used also to evaluate the effect of treatment. It is necessary to keep in mind that TBS is not intended to replace existing tools but rather to supplement them and assist clinicians in our medical decisions. BMD and TBS are two independent parameters reflecting different bone properties: quantity and quality, respectively. Both BMD and TBS are very important in assessing bone strength. Bone strength status is important to evaluate the risk of fracture as well as to make decision about the type of treatment [107].

5. Conclusions

Given the current data, which suggest that diabetic patients are at a higher risk of fracture, it would be reasonable to screen diabetic patients for osteoporosis. The current osteoporosis guidelines for screening can be used for patients with T1DM and also T2DM, but it is important to bear in mind that DM is a risk factor for osteoporosis and fracture and that fracturing can occur at higher BMD levels in patients with DM [108].

As a result of the ineffectiveness of BMD in assessing fracture risks in T2DM, the major clinical problems are how to assess the risks and when to start therapy for preventing fractures in daily practice. Although there are potential candidates (osteocalcin, AGEs, and insulin) for such purposes, it is unclear whether or not they could predict the occurrence of new fractures in T2DM patients in a prospective fashion and can be used in daily practice.

A simple and recommended procedure for all physicians who are engaged in T2DM treatment is to question the patients about their fracture histories. It is likely that about half of them will be identified as those who have bone fragility and need osteoporosis treatment for fracture prevention. Also if T2DM patients undergo spinal X-ray examination we should directly look for vertebral fractures.

Recently, the fracture risk assessment (FRAX) algorithm has been developed by the WHO, which could assess the fracture risk of an individual even if BMD is not measured [109]. This algorithm integrates the influence of several well-validated risk factors for fractures that are independent of BMD and therefore it might be useful for the case-finding strategy that identifies diabetic patients at high risk for fracture.

The TBS is a new parameter, that is, determined from grey level analysis of DXA images. The TBS meets the need for a noninvasive method for assessing bone microarchitecture—key determinant of bone strength. In addition, the TBS can be estimated very simply, using widely available DXA machines and during the same procedure as BMD measurement. The TBS is a quantitative value, that is, reproducible and easy to handle.

Therapeutic considerations in diabetic patients with osteoporosis are recommendations from good clinical practice, rather than evidence-based studies (Table 3).

Table 3: General management of osteoporosis in diabetic patients.

(i) To avoid glitazones
(ii) Good glycaemic control
(iii) Minimizing of hypoglycemic episodes
(iv) Prevention of diabetic complications, especially kidney disease
(v) To assess and prevent falls
(vi) Supplementation with calcium and vitamin D
(vii) Specific antiporotic medication (antiresorptive or osteoanabolic treatment)

All patients with DM should be counseled regarding their risk of osteoporosis and fractures. They should also be advised on adequate calcium (at least 100–1200 mg/day) and vitamin D (800–1000 IU/day) intake [110, 111]. When a patient meets guidelines for treatment, there are several options including antiresorptive medications such as bisphosphonates, denosumab, SERM, and anabolic agent teriparatide. There are no long-term data on the effectiveness of either of these types of medications in patients with diabetes [112]. Achieving adequate glycaemic control in patients with diabetes is especially important as there are data to suggest that the microvascular complications of DM, such as retinopathy and neuropathy, which arise from less than ideal glycaemic control, can lead to falls and subsequent fractures.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] S. Kurra and E. Siris, "Diabetes and bone health: the relationship between diabetes and osteoporosis-associated fractures," *Diabetes/Metabolism Research and Reviews*, vol. 27, no. 5, pp. 430–435, 2011.

[2] A. V. Schwartz and D. E. Sellmeyer, "Diabetes, fracture, and bone fragility," *Current Osteoporosis Reports*, vol. 5, no. 3, pp. 105–111, 2007.

[3] M. Janghorbani, R. M. van Dam, W. C. Willett, and F. B. Hu, "Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture," *American Journal of Epidemiology*, vol. 166, no. 5, pp. 495–505, 2007.

[4] International Diabetes Federation, *IDF Diabetes Atlas*, International Diabetes Federation, Brussels, Belgium, 6th edition, 2013.

[5] American Diabetes Association, "Diagnosis and classification of diabetes mellitus," *Diabetes Care*, vol. 35, supplement 1, pp. S64–S71, 2012.

[6] C. Hamann, S. Kirschner, K.-P. Günther, and L. C. Hofbauer, "Bone, sweet bone—osteoporotic fractures in diabetes mellitus," *Nature Reviews Endocrinology*, vol. 8, no. 5, pp. 297–305, 2012.

[7] NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy, "Osteoporosis prevention, diagnosis and therapy," *The Journal of the American Medical Association*, vol. 285, no. 6, pp. 785–795, 2001.

[8] E. Hernlund, A. Svedbom, M. Ivergård et al., "A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Osteoporosis in the European Union: medical management, epidemiology and economic burden," in *Archives of Osteoporosis*, vol. 8, pp. 136–256, 2013.

[9] J. A. Kanis, J.-P. Devogelaer, and C. Gennari, "Practical guide for the use of bone mineral measurements in the assessment of treatment of osteoporosis: a position paper of the European Foundation for Osteoporosis and Bone Disease," *Osteoporosis International*, vol. 6, no. 3, pp. 256–261, 1996.

[10] O. Johnell, J. A. Kanis, A. Oden et al., "Predictive value of BMD for hip and other fractures," *Journal of Bone and Mineral Research*, vol. 20, no. 7, pp. 1185–1194, 2005.

[11] M. R. McClung, "Do current management strategies and guidelines adequately address fracture risk?" *Bone*, vol. 38, no. 2, pp. S13–S17, 2006.

[12] H. Johansson, J. A. Kanis, A. Oden, O. Johnell, and E. McCloskey, "BMD, clinical risk factors and their combination for hip fracture prevention," *Osteoporosis International*, vol. 20, no. 10, pp. 1675–1682, 2009.

[13] T. M. Link and S. Majumdar, "Current diagnostic techniques in the evaluation of bone architecture," *Current Osteoporosis Report*, vol. 2, no. 2, pp. 47–52, 2004.

[14] C. D. Rubin, "Emerging concepts in osteoporosis and bone strength," *Current Medical Research and Opinion*, vol. 21, no. 7, pp. 1049–1056, 2005.

[15] K. Sawada, K.-I. Morishige, M. Ohmichi et al., "Peripheral quantitative computed tomography (pQCT) is useful for monitoring bone mineral density of the patients who receive hormone replacement therapy," *Maturitas*, vol. 56, no. 4, pp. 343–349, 2007.

[16] E. Seeman, "Is a change in bone mineral density a sensitive and specific surrogate of anti-fracture efficacy?" *Bone*, vol. 41, no. 3, pp. 308–317, 2007.

[17] P. Vestergaard, "Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis," *Osteoporosis International*, vol. 18, no. 4, pp. 427–444, 2007.

[18] J. S. Nyman, J. L. Even, C.-H. Jo et al., "Increasing duration of type 1 diabetes perturbs the strength-structure relationship and increases brittleness of bone," *Bone*, vol. 48, no. 4, pp. 733–740, 2011.

[19] K. M. Thrailkill, C. K. Lumpkin Jr., R. C. Bunn, S. F. Kemp, and J. L. Fowlkes, "Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues," *American Journal of Physiology: Endocrinology and Metabolism*, vol. 289, no. 5, pp. E735–E745, 2005.

[20] L. J. Melton III, C. L. Leibson, S. J. Achenbach, T. M. Therneau, and S. Khosla, "Fracture risk in type 2 diabetes: update of a population-based study," *Journal of Bone and Mineral Research*, vol. 23, no. 8, pp. 1334–1342, 2008.

[21] M. Janghorbani, D. Feskanich, W. C. Willett, and F. Hu, "Prospective study of diabetes and risk of hip fracture," *Diabetes Care*, vol. 29, no. 7, pp. 1573–1578, 2006.

[22] A. Milczarczyk and E. Franek, "Osteoporosis and bone fractures in patients with diabetes mellitus," *Diabetologia Doświadczalna i Kliniczna*, vol. 8, no. 2, pp. 63–67, 2008.

[23] S. A. Brown and J. L. Sharples, "Osteoporosis: an underappreciated complication of diabetes," *Clinical Diabetes*, vol. 22, no. 1, pp. 10–20, 2004.

[24] S. Yamagishi, K. Nakamura, and H. Inoue, "Possible participation of advanced glycation end products in the pathogenesis of osteoporosis in diabetic patients," *Medical Hypotheses*, vol. 65, no. 6, pp. 1013–1015, 2005.

[25] E. S. Strotmeyer, J. A. Cauley, A. V. Schwartz et al., "Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults: the health, aging, and body composition study," *Archives of Internal Medicine*, vol. 165, no. 14, pp. 1612–1617, 2005.

[26] C. Gagnon, D. J. Magliano, P. R. Ebeling et al., "Association between hyperglycaemia and fracture risk in non-diabetic middle-aged and older Australians: a national, population-based prospective study (AusDiab)," *Osteoporosis International*, vol. 21, no. 12, pp. 2067–2074, 2010.

[27] F. Gregorio, S. Cristallini, F. Santeusanio, P. Filipponi, and P. Fumelli, "Osteopenia associated with non-insulin-dependent diabetes mellitus: what are the causes?" *Diabetes Research and Clinical Practice*, vol. 23, no. 1, pp. 43–54, 1994.

[28] M. Viégas, C. Costa, A. Lopes, L. Griz, M. A. Medeiro, and F. Bandeira, "Prevalence of osteoporosis and vertebral fractures in postmenopausal women with type 2 diabetes mellitus and their relationship with duration of the disease and chronic complications," *Journal of Diabetes and Its Complications*, vol. 25, no. 4, pp. 216–221, 2011.

[29] S. A. V. de Vittinghoff E Sellmeyer, K. R. Feingold, N. de Rekeneire et al., "Diabetes-related complications, glycemic control, and falls in older adults," *Diabetes Care*, vol. 31, no. 5, pp. 391–396, 2008.

[30] M. Monami, B. Cresci, A. Colombini et al., "Bone fractures and hypoglycemic treatment in type 2 diabetic patients," *Diabetes Care*, vol. 31, no. 2, pp. 199–203, 2008.

[31] M. A. Sorocéanu, D. Miao, X.-Y. Bai, H. Su, D. Goltzman, and A. C. Karaplis, "Rosiglitazone impacts negatively on bone by promoting osteoblast/osteocyte apoptosis," *Journal of Endocrinology*, vol. 183, no. 1, pp. 203–216, 2004.

[32] J. Payer, P. Jackuliak, and Z. Killinger, "Risk of osteoporotic fractures in diabetic patients treated with glitazones treatment," *Osteologicky Bulletin*, vol. 15, no. 2, pp. 57–60, 2010.

[33] K. K. Nicodemus and A. R. Folsom, "Type 1 and type 2 diabetes and incident hip fractures in postmenopausal women," *Diabetes Care*, vol. 24, no. 7, pp. 1192–1197, 2001.

[34] P. Marchetti, R. Lupi, M. Bugliani et al., "A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets," *Diabetologia*, vol. 55, no. 12, pp. 3262–3272, 2012.

[35] B. Nuche-Berenguer, P. Moreno, P. Esbrit et al., "Effect of GLP-1 treatment on bone turnover in normal, type 2 diabetic, and insulin-resistant states," *Calcified Tissue International*, vol. 84, no. 6, pp. 453–461, 2009.

[36] G. Valerio, A. del Puente, A. Esposito-del Puente, P. Buono, E. Mozzillo, and A. Franzese, "The lumbar bone mineral density is affected by long-term poor metabolic control in adolescents with type 1 diabetes mellitus," *Hormone Research*, vol. 58, no. 6, pp. 266–272, 2002.

[37] A. Räkel, O. Sheehy, E. Rahme, and J. LeLorier, "Osteoporosis among patients with type 1 and type 2 diabetes," *Diabetes and Metabolism*, vol. 34, no. 3, pp. 193–205, 2008.

[38] G. Hampson, C. Evans, R. J. Petitt et al., "Bone mineral density, collagen type 1 α 1 genotypes and bone turnover in premenopausal women with diabetes mellitus," *Diabetologia*, vol. 41, no. 11, pp. 1314–1320, 1998.

[39] H. Bid, R. Konwar, C. Aggarwal et al., "Vitamin D receptor (FokI, BsmI and TaqI) gene polymorphisms and type 2 diabetes mellitus : a North Indian Study," *Indian Journal of Medical Sciences*, vol. 63, no. 5, pp. 187–194, 2009.

[40] E. Seeman and P. D. Delmas, "Bone quality—the material and structural basis of bone strength and fragility," *The New England Journal of Medicine*, vol. 354, no. 21, pp. 2212–2261, 2006.

[41] M. Saito and K. Marumo, "Bone quality in diabetes," *Frontiers in Endocrinology*, vol. 4, article 72, pp. 1–9, 2013.

[42] M. Brownlee, "Advanced protein glycosylation in diabetes and aging," *Annual Review of Medicine*, vol. 46, pp. 223–234, 1995.

[43] D. Vashishth, G. J. Gibson, J. I. Khoury, M. B. Schaffler, J. Kimura, and D. P. Fyhrie, "Influence of nonenzymatic glycation on biomechanical properties of cortical bone," *Bone*, vol. 28, no. 2, pp. 195–201, 2001.

[44] P. Garnero, "Bone markers in osteoporosis," *Current Osteoporosis Reports*, vol. 7, no. 3, pp. 84–90, 2009.

[45] S. Vasikaran, R. Eastell, O. Bruyère et al., "Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards," *Osteoporosis International*, vol. 22, no. 2, pp. 391–420, 2011.

[46] J. M. Kindblom, C. Ohlsson, O. Ljunggren et al., "Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men," *Journal of Bone and Mineral Research*, vol. 24, no. 5, pp. 785–791, 2009.

[47] P. Gerdhem, A. Isaksson, K. Åkesson, and K. J. Obrant, "Increased bone density and decreased bone turnover, but no evident alteration of fracture susceptibility in elderly women with diabetes mellitus," *Osteoporosis International*, vol. 16, no. 12, pp. 1506–1512, 2005.

[48] H. Dobnig, J. C. Piswanger-Sölkner, M. Roth et al., "Type 2 diabetes mellitus in nursing home patients: effects on bone turnover, bone mass, and fracture risk," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 9, pp. 3355–3363, 2006.

[49] L. Gennari, D. Merlotti, R. Valenti et al., "Circulating Sclerostin levels and bone turnover in type 1 and type 2 diabetes," *Journal of Clinical Endocrinology and Metabolism*, vol. 97, no. 5, pp. 1737–1744, 2012.

[50] P. V. Hauschka, J. B. Lian, D. E. Cole, and C. M. Gundberg, "Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone," *Physiological Reviews*, vol. 69, no. 3, pp. 990–1047, 1989.

[51] N. K. Lee, H. Sowa, E. Hinoi et al., "Endocrine regulation of energy metabolism by the skeleton," *Cell*, vol. 130, no. 3, pp. 456–469, 2007.

[52] M. Ferron, E. Hinoi, G. Karsenty, and P. Ducy, "Osteocalcin differentially regulates β cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 13, pp. 5266–5270, 2008.

[53] A. G. Pittas, S. S. Harris, M. Eliades, P. Stark, and B. Dawson-Hughes, "Association between serum osteocalcin and markers of metabolic phenotype," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 3, pp. 827–832, 2009.

[54] B. M. Svoren, L. K. Volkening, J. R. Wood, and L. M. B. Laffel, "Significant vitamin D deficiency in youth with type 1 diabetes mellitus," *The Journal of Pediatrics*, vol. 154, no. 1, pp. 132–134, 2009.

[55] A. A. Tahrani, A. Ball, L. Shepherd, A. Rahim, A. F. Jones, and A. Bates, "The prevalence of vitamin D abnormalities in South Asians with type 2 diabetes mellitus in the UK," *International Journal of Clinical Practice*, vol. 64, no. 3, pp. 351–355, 2010.

[56] K. Bućan, M. Ivanišević, T. Zemunik et al., "Retinopathy and nephropathy in type 1 diabetic patients—association with polymorphysms of vitamin D-receptor, TNF, Neuro-D and Il-1 receptor 1 genes," *Collegium Antropologicum*, vol. 33, no. 2, pp. 99–105, 2009.

[57] V. A. Diaz, A. G. Mainous III, P. J. Carek, A. M. Wessell, and C. J. Everett, "The association of vitamin D deficiency and insufficiency with diabetic nephropathy: implications for health disparities," *Journal of the American Board of Family Medicine*, vol. 22, no. 5, pp. 521–527, 2009.

[58] P. Secchiero, F. Corallini, A. Pandolfi et al., "An increased osteoprotegerin serum release characterizes the early onset of diabetes mellitus and may contribute to endothelial cell dysfunction," *American Journal of Pathology*, vol. 169, no. 6, pp. 2236–2244, 2006.

[59] L. M. Rasmussen, L. Tarnow, T. K. Hansen, H.-H. Parving, and A. Flyvbjerg, "Plasma osteoprotegerin levels are associated with glycaemic status, systolic blood pressure, kidney function and cardiovascular mordibity in type 1 diabetic patients," *European Journal of Endocrinology*, vol. 154, no. 1, pp. 75–81, 2006.

[60] A. Mani, J. Radhakrishnan, H. Wang et al., "LRP6 mutation in a family with early coronary disease and metabolic risk factors," *Science*, vol. 315, no. 5816, pp. 1278–1282, 2007.

[61] U. Smith, "TCF7L2 and type 2 diabetes—we WNT to know," *Diabetologia*, vol. 50, no. 5, pp. 5–7, 2007.

[62] K. R. Owen and M. I. McCarthy, "Genetics of type 2 diabetes," *Current Opinion in Genetics and Development*, vol. 17, no. 3, pp. 239–244, 2007.

[63] T. Jin, "The WNT signalling pathway and diabetes mellitus," *Diabetologia*, vol. 51, no. 10, pp. 1771–1780, 2008.

[64] M. Espallargues, L. Sampietro-Colom, M. D. Estrada et al., "Identifying bone-mass-related risk factors for fracture to guide bone densitometry measurements: a systematic review of the literature," *Osteoporosis International*, vol. 12, no. 10, pp. 811–822, 2001.

[65] M. C. Wang, L. K. Bachrach, M. van Loan, M. Hudes, K. M. Flegal, and P. B. Crawford, "The relative contributions of lean tissue mass and fat mass to bone density in young women," *Bone*, vol. 37, no. 4, pp. 474–481, 2005.

[66] G. Barrera, D. Bunout, V. Gattás, M. P. de la Maza, L. Leiva, and S. Hirsch, "A high body mass index protects against femoral neck osteoporosis in healthy elderly subjects," *Nutrition*, vol. 20, no. 9, pp. 769–771, 2004.

[67] D. T. Felson, Y. Zhang, M. T. Hannan, and J. J. Anderson, "Effects of weight and body mass index on bone mineral density in men and women: the Framingham Study," *Journal of Bone and Mineral Research*, vol. 8, no. 5, pp. 567–573, 1993.

[68] J. Compston, J. Flahive, D. W. Hosmer et al., "Relationship of weight, height and body mass index with fracture risk at different sites in postmenopausal women: the Global Longitudinal Study of Osteoporosis in Women (GLOW)," *Journal of Bone Mineral Research*, vol. 29, no. 2, pp. 487–493, 2014.

[69] K. Suzuki, C. Sugimoto, M. Takizawa et al., "Correlations between bone mineral density and circulating bone metabolic markers in diabetic patients," *Diabetes Research and Clinical Practice*, vol. 48, no. 3, pp. 185–191, 2000.

[70] M. S. Maurer, J. Burcham, and H. Cheng, "Diabetes mellitus is associated with an increased risk of falls in elderly residents of a long-term care facility," *The Journals of Gerontology A: Biological Sciences and Medical Sciences*, vol. 60, no. 9, pp. 1157–1162, 2005.

[71] S. Patel, S. Hyer, K. Tweed et al., "Risk factors for fractures and falls in older women with type 2 diabetes mellitus," *Calcified Tissue International*, vol. 82, no. 2, pp. 87–91, 2008.

[72] S. Volpato, S. G. Leveille, C. Blaum, L. P. Fried, and J. M. Guralnik, "Risk factors for falls in older disabled women with diabetes: the Women's Health and Aging Study," *The Journals of Gerontology A: Biological Sciences and Medical Sciences*, vol. 60, no. 12, pp. 1539–1545, 2005.

[73] J. A. Kanis, A. Oden, O. Johnell et al., "The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women," *Osteoporosis International*, vol. 18, no. 8, pp. 1033–1046, 2007.

[74] K. E. Ensrud, L.-Y. Lui, B. C. Taylor et al., "A comparison of prediction models for fractures in older women: is more better?" *Archives of Internal Medicine*, vol. 169, no. 22, pp. 2087–2094, 2009.

[75] J. A. Kanis, O. Johnell, A. Oden, H. Johansson, and E. McCloskey, "FRAXŮ and the assessment of fracture probability in men and women from the UK," *Osteoporosis International*, vol. 19, no. 4, pp. 385–397, 2008.

[76] A. V. Schwartz, E. Vittinghoff, D. C. Bauer et al., "Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes," *The Journal of the American Medical Association*, vol. 305, no. 21, pp. 2184–2192, 2011.

[77] K. Naylor and R. Eastell, "Bone turnover markers: use in osteoporosis," *Nature Reviews Rheumatology*, vol. 8, no. 7, pp. 379–389, 2012.

[78] P. Chavassieux, E. Seeman, and P. D. Delmas, "Insights into material and structural basis of bone fragility from diseases associated with fractures: how determinants of the biomechanical properties of bone are compromised by disease," *Endocrine Reviews*, vol. 28, no. 2, pp. 151–164, 2007.

[79] J. C. Rice, S. C. Cowin, and J. A. Bowman, "On the dependence of the elasticity and strength of cancellous bone on apparent density," *Journal of Biomechanics*, vol. 21, no. 2, pp. 155–168, 1988.

[80] M. L. Bouxsein, "Bone quality: where do we go from here?" *Osteoporosis International*, vol. 14, supplement 5, pp. S118–S127, 2003.

[81] A. Sanyal, A. Gupta, H. H. Bayraktar, R. Y. Kwon, and T. M. Keaveny, "Shear strength behavior of human trabecular bone," *Journal of Biomechanics*, vol. 45, no. 15, pp. 2513–2519, 2012.

[82] J. A. Kanis, D. Hans, C. Cooper et al., "Interpretation and use of FRAX in clinical practice," *Osteoporosis International*, vol. 22, no. 9, pp. 2395–2411, 2011.

[83] H. K. Genant, C. Y. Wu, C. van Kuijk, and M. C. Nevitt, "Vertebral fracture assessment using a semiquantitative technique," *Journal of Bone and Mineral Research*, vol. 8, no. 9, pp. 1137–1148, 1993.

[84] D. M. Black, N. K. Arden, L. Palermo, J. Pearson, and S. R. Cummings, "Prevalent vertebral deformities predict hip fractures and new vertebral deformities but not wrist fractures," *Journal of Bone and Mineral Research*, vol. 14, no. 5, pp. 821–828, 1999.

[85] J. P. Roux, J. Wegrzyn, and S. Boutroy, "Relationship between Trabecular Bone Score (TBS), Bone Mass and Microarchitecture in Human vertebrae: an ex vivo study," *Osteoporosis International*, vol. 23, supplement 2, pp. S385–S386, 2012.

[86] E. Donnelly, "Methods for assessing bone quality: a review," *Clinical Orthopaedics and Related Research*, vol. 469, no. 8, pp. 2128–2138, 2011.

[87] H. K. Genant, K. Engelke, and S. Prevrhal, "Advanced CT bone imaging in osteoporosis," *Rheumatology*, vol. 47, no. 4, pp. iv9–iv16, 2008.

[88] G. J. Kazakia and S. Majumdar, "New imaging technologies in the diagnosis of osteoporosis," *Reviews in Endocrine and Metabolic Disorders*, vol. 7, no. 1-2, pp. 67–74, 2006.

[89] J. A. MacNeil and S. K. Boyd, "Accuracy of high-resolution peripheral quantitative computed tomography for measurement of bone quality," *Medical Engineering and Physics*, vol. 29, no. 10, pp. 1096–1105, 2007.

[90] J. Carballido-Gamio and S. Majumdar, "Clinical utility of microarchitecture measurements of trabecular bone," *Current Osteoporosis Reports*, vol. 4, no. 2, pp. 64–70, 2006.

[91] L. Pothuaud, P. Carceller, and D. Hans, "Correlations between grey-level variations in 2D projection images (TBS) and 3D microarchitecture: applications in the study of human trabecular bone microarchitecture," *Bone*, vol. 42, no. 4, pp. 775–787, 2008.

[92] B. Rabier, A. Héraud, C. Grand-Lenoir, R. Winzenrieth, and D. Hans, "A multicentre, retrospective case-control study assessing the role of trabecular bone score (TBS) in menopausal Caucasian women with low areal bone mineral density (BMDa): analysing the odds of vertebral fracture," *Bone*, vol. 46, no. 1, pp. 176–181, 2010.

[93] L. Pothuaud, N. Barthe, M.-A. Krieg, N. Mehsen, P. Carceller, and D. Hans, "Evaluation of the potential use of trabecular bone score to complement bone mineral density in the diagnosis of osteoporosis: a preliminary spine BMD-matched, Case-Control Study," *Journal of Clinical Densitometry*, vol. 12, no. 2, pp. 170–176, 2009.

[94] D. Hans, N. Barthe, S. Boutroy, L. Pothuaud, R. Winzenrieth, and M.-A. Krieg, "Correlations between trabecular bone score, measured using anteroposterior dual-energy X-ray absorptiometry acquisition, and 3-dimensional parameters of bone microarchitecture: an experimental study on human cadaver vertebrae," *Journal of Clinical Densitometry*, vol. 14, no. 3, pp. 302–312, 2011.

[95] R. Dufour and A. Heraud, "Lumbar spine micro-architecture in french women derived from DXA: TBS normative data," *Journal of Clinical Densitometry*, vol. 12, no. 3, pp. 377–378, 2009.

[96] J. Wegrzyn, J.-P. Roux, M. E. Arlot et al., "Role of trabecular microarchitecture and its heterogeneity parameters in the mechanical behavior of ex vivo human L3 vertebrae," *Journal of Bone and Mineral Research*, vol. 25, no. 11, pp. 2324–2331, 2010.

[97] Y. Duan, X.-F. Wang, A. Evans, and E. Seeman, "Structural and biomechanical basis of racial and sex differences in vertebral fragility in Chinese and Caucasians," *Bone*, vol. 36, no. 6, pp. 987–998, 2005.

[98] J. C. van der Linden, J. Homminga, J. A. N. Verhaar, and H. Weinans, "Mechanical consequences of bone loss in cancellous bone," *Journal of Bone and Mineral Research*, vol. 16, no. 3, pp. 457–465, 2001.

[99] W. D. Leslie, B. Aubry-Rozier, O. Lamy, and D. Hans, "TBS (trabecular bone score) and diabetes-related fracture risk," *Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 2, pp. 602–609, 2013.

[100] P. Jackuliak and J. Payer, "Fracture Risk in Type 2 Diabetes and Trabecular Bone Score," Accepted for a Poster Presentation at the American Diabetes Association's 74th Scientific Sessions, San Francisco, Calif, USA, June 2014.

[101] S. Boutroy, D. Hans, E. Sornay-Rendu, N. Vilayphiou, R. Winzenrieth, and R. Chapurlat, "Trabecular bone score improves fracture risk prediction in non-osteoporotic women: the OFELY Study," *Osteoporosis International*, vol. 24, no. 1, pp. 77–85, 2013.

[102] O. Lamy, M. Metzger, M.-A. Krieg, B. Aubry-Rozier, D. Stoll, and D. Hans, "OsteoLaus: prediction of osteoporotic fractures by clinical risk factors and DXA, IVA and TBS," *Revue Medicale Suisse*, vol. 7, no. 315, pp. 2130–2136, 2011.

[103] J. Vasic, F. Gojkovic, T. Petranova et al., "Spine microarchitecture estimation (TBS) discriminates major osteoporotic fracture from controls equally well than site matched BMD and independently: the eastern europe TBS Study," *Osteoporosis International*, vol. 23, supplement 2, p. S337, 2012.

[104] A. W. Popp, S. Meer, M. A. Krieg, R. Perrelet, D. Hans, and K. Lippuner, "Bone mineral density (BMD) combined with micro-architecture parameters (TBS) significantly improves the indentification of women at high risk of fracture: the SEMOF cohort study," *Osteoporosis International*, vol. 23, supplement 2, p. S327, 2012.

[105] A. W. Popp, S. Guler, O. Lamy et al., "Effects of zoledronate versus placebo on spine bone mineral density and microarchitecture assessed by the trabecular bone score in postmenopausal women with osteoporosis: a three-year study," *Journal of Bone and Mineral Research*, vol. 28, no. 3, pp. 449–454, 2013.

[106] M. Kužma, Z. Kužmová, Z. Zelinková et al., "Impact of the growth hormone replacement on bone status in growth hormone deficient adults," *Growth Hormone and IGF Research*, vol. 24, no. 1, pp. 22–28, 2014.

[107] K. Briot, S. Paternotte, S. Kolta et al., "Added value of trabecular bone score to bone mineral density for prediction of osteoporotic fractures in postmenopausal women: the OPUS Study," *Bone*, vol. 57, no. 1, pp. 232–236, 2013.

[108] National Osteoporosis Foundation, *Clinician's Guide to Prevention and Treatment of Osteoporosis. National Osteoporosis Foundation*, National Osteoporosis Foundation, Washington, DC, USA, 2010.

[109] J. A. Kanis, A. Oden, H. Johansson, F. Borgström, O. Ström, and E. McCloskey, "FRAX and its applications to clinical practice," *Bone*, vol. 44, no. 5, pp. 734–743, 2009.

[110] B. Dawson-Hughes, A. Mithal, J.-P. Bonjour et al., "IOF position statement: vitamin D recommendations for older adults," *Osteoporosis International*, vol. 21, no. 7, pp. 1151–1154, 2010.

[111] R. Rizzoli, S. Boonen, M.-L. Brandi et al., "Vitamin D supplementation in elderly or postmenopausal women: a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO)," *Current Medical Research and Opinion*, vol. 29, no. 4, pp. 305–313, 2013.

[112] J. A. Kanis, E. V. McCloskey, H. Johansson, C. Cooper, R. Rizzoli, and J.-Y. Reginster, "European guidance for the diagnosis and management of osteoporosis in postmenopausal women," *Osteoporosis International*, vol. 24, no. 1, pp. 23–57, 2013.