

# Hormonal and Cytokine Implications in the Pathophysiology of Osteoporosis Occurring in Chronic Liver Diseases

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## ABSTRACT

*In the last years there has been an increased awareness regarding the alterations of bone metabolism as a common complication of chronic liver that occurs regardless of the etiology: alcoholic, viral, autoimmune, whether or not associated with cholestasis.*

*The aim of this paper was to summarize the current understanding of bone metabolism and to point out the new discoveries that have been made in this field.*

*Bone density is maintained constant due to the equilibrium between bone formation bone resorbtion, under the control of hormonal and proinflammatory cytokines. The influence of sex hormones, parathyroid hormone, growth hormone, and insulin-like growth factor-1 and 2 on bone metabolism is discussed. The role of proinflammatory cytokines, CSF1-RANKL system, leptin and oncofetal fibronectin are also discussed.*

*Although the physiological mechanism of bone metabolism has been established, when it comes to pathological conditions, the hormones and cytokines have different new roles, or are associated with other factors having different influences.*

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## INTRODUCTION

In the last years there has been an increased awareness regarding the alterations of bone metabolism in gastrointestinal diseases and chronic liver diseases, such as inflammatory bowel diseases, celiac disease, malabsorption, gastrointestinal surgery, pancreatic insufficiency and liver transplantation. In chronic liver diseases, metabolic bone disease is a common complication that occurs regardless of the etiology: alcoholic, viral, autoimmune, whether or not associated with cholestasis.

In 2005, Schiefke et al. have demonstrated that patients with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection without cirrhosis already had significantly reduced bone mineral density (BMD) that correlated with the degree of fibrosis (1).

The etiology of reduced BMD is poorly understood and it is thought to vary according to the progression of liver disease, having also other risk factors: low sunlight exposure, reduced physical activity, low lean body mass, vitamin D deficiency, hypogonadism, alcohol abuse, or nutritional deficiencies. □

## BONE METABOLISM

The bone is a highly specialized tissue, characterized by its rigidity with implication in structural support, protection of organs, calcium homeostasis and environment for the marrow. It is composed of support cells (osteoblasts and osteocytes), remodeling cells (osteoclasts), matrix of collagen and noncollagenous proteins (osteoid), and anorganic mineral salts. Once the skeleton reaches maturity, regeneration continues in the form of periodic replacement of old bones tissue with new ones, and the temporal structural unit responsible for this is called basic multicellular unit (BMU). It has a lifespan of 6-9 months, length of about 1-2

mm, width 0.2 to 0.4 mm and longitudinal orientation (2). The structure is composed of a central blood capillary, and a team of osteoblasts and osteoclasts.

Osteoblasts derive from an undifferentiated mesenchymal cell common with chondrocytes, adipocytes and myocytes, under the influence of specific stimulator factors: Runt-related transcription factor 2 (RunX2; a key transcription factor associated with osteoblast differentiation), distal-less homeobox 2 (Dlx2; protects from TGFβ-induced cell-cycle arrest and apoptosis), msh homeobox 2 (Msx2; with a role in promoting cell growth and an important target for the RAS signaling pathways) and osterix.

Osteoclasts are large multinuclear cells, derived from the hematopoietic series, with the function of bone resorption (3). The molecular mechanism that controls osteoclastogenesis has been clearly established with the discovery of Receptor Activator of Nuclear Factor κ B (RANK) and its ligand (RANKL), which is a key factor for osteoclast differentiation and activation. Osteoprotegerin (OPG), produced by osteoblasts' precursors, is a potent inhibitor to osteoclastogenesis due to its ability to act as a RANK linking to RANKL (4).

Bone mass density is maintained constant due to the equilibrium between bone formation and bone resorption, under the control of multiple systemic and local factors such as: sex hormones, parathyroid hormone, growth hormone, and proinflammatory cytokines.

### Hormonal influence on the bone metabolism in liver diseases

The parathyroid hormone (PTH) is responsible for maintaining extracellular calcium level, and releasing calcium from the bone deposits in response to hypocalcaemia. It also increases renal tubular calcium reabsorption and renal calcitriol production (essential in intestinal calcium and phosphorus absorption)

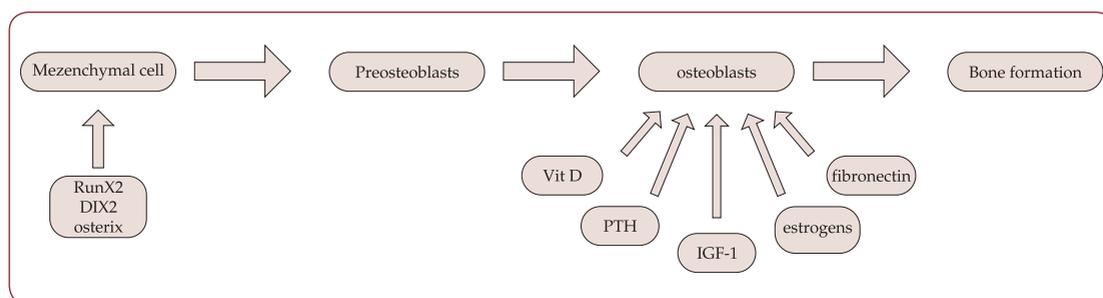
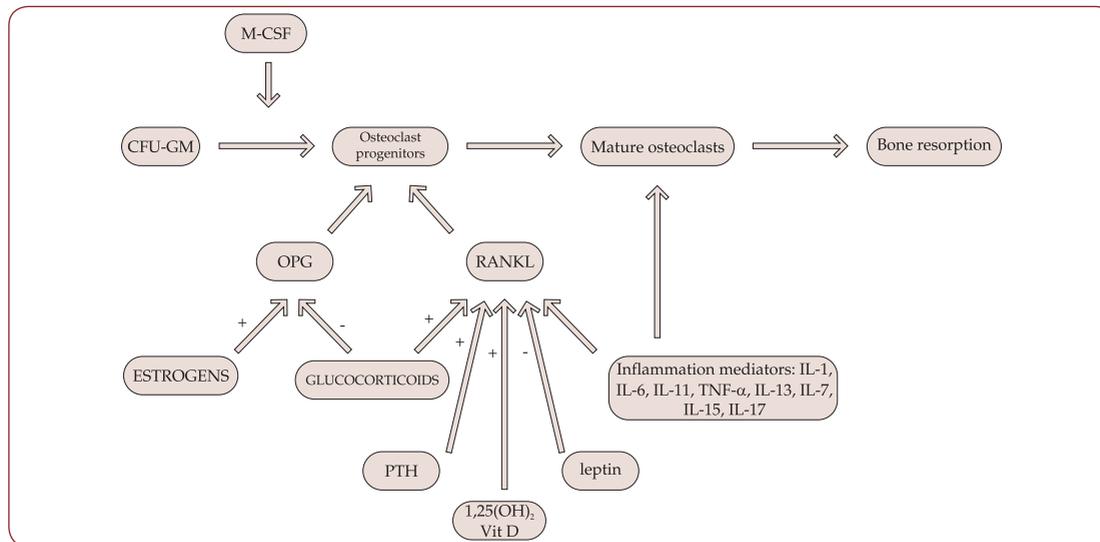


FIGURE 1. Bone formation.



**FIGURE 2.** Bone resorption.

(4). PTH acts on the bone cells through its specific receptor (PTH-R) that also possesses an endogenous ligand (PTH-rP). PTH-rP is expressed on the surface of the mature osteoblast, and could regulate entry of receptor-expressing osteoblast precursors into the compartment of bone forming cells, or their exit from this compartment through apoptosis (5). Studies in patients with primary biliary cirrhosis identified normal vitamin D levels even in advanced stages of disease (6). On the other hand studies in patients with viral and toxic hepatic disease proved that vitamin D level could be considered as a marker of liver function insufficiency. Fisher et al. evaluated vitamin D levels in 100 patients with liver disease (hepatitis or cirrhosis), including 36 patients with chronic hepatitis. The prevalence of vitamin D deficiency was significantly higher in cirrhotic than noncirrhotic subjects, and vitamin D levels were significantly decreased in patients with Child C class that in those with Child A class. Furthermore they considered that a vitamin D level below 25nmol/l could predict a higher INR (7). However Duarte et al. found no difference in mean vitamin D levels, but showed a lower serum level of calcium in cirrhotic versus noncirrhotic patients (8). These results were supported by several other studies evaluating vitamin D levels in chronic viral liver disease: Gallego-Rojo et al. published in 1998 (9) and one more recent study by Corey et al. published in 2012, that found no difference in vitamin levels between patients with and without progression of viral C chronic liver disease over the course of nearly 4 years (10).

All previous studies agreed that PTH levels are always normal, and vitamin D-receptor gene polymorphisms which can induce suppression of PTH secretion could be the explanation for this, but further studies are needed (11). Different therapies used in chronic hepatic diseases could interfere with bone metabolism such as: acid-binding agent cholestyramine that decreases vitamin D level and loop diuretics that can further promote renal calcium loss (12).

Patients with alcohol abuse have additional risk factors like malnutrition, reduced sun exposure, altered biliary secretion and the negative effect of alcohol on vitamin D absorption. Alcohol itself is responsible for a resistance to the skeletal actions of PTH. Transient episodes of hypocalcaemia have also been observed in patients with alcohol abuse followed by compensatory changes in parathyroid hormone (PTH) and vitamin D metabolism. Alcohol abuse reduces bone formation in a dose dependent manner (13), especially by a toxic effect on osteoblast function, and indirectly on PTH, IGF-1, leptin, IL-1 (14).

Calcium and vitamin D supplements have no effect on low BMD, but 1,25(OH)vitamin D increases BMD in men with cirrhosis and slows bone loss in women with cirrhosis. Human PTH the only anabolic agent approved by FDA for osteoporosis stimulates osteoblastogenesis. Its use, although with good results in animal studies, is put under question because of the increased number of bone malignant tumors (15).

The growth hormone together with insulin-like growth factor-1 and 2 (IGF-1 and IGF-2)

has a very important role in maintaining BMD (16). More than 90% of IGF-1 is synthesized in the liver and declines in the early stages of cirrhosis even before other markers of liver function (albumin, bilirubin, prothrombin) do (17). Its level is even lower as the liver disease is more severe and there is an inverse correlation between these two. The implication of IGF-1 in osteopenia associated with chronic liver disease has been investigated. It has been proved that in patients with viral hepatic disease, a low level of IGF-1 is correlated to a low BMD (18). Such correlations have not been demonstrated in patients with other hepatic diseases, although low levels have been highlighted in alcoholic liver disease.

Glucocorticoids stimulate both bone resorption and bone formation. They promote mature osteoblast synthesis, but they have also an inhibitory effect on their activity. They also increase osteolysis and augment osteoclast recruitment (19). Corticosteroid therapy is widely used in some hepatic diseases. Besides its beneficial effect on the liver, changes in bone metabolism should not be neglected. Corticosteroid therapy exacerbates existing osteopenia caused by underlying liver disease. More than 50% of the patients have bone loss after 1 year of corticosteroid therapy, and this is more pronounced in the spine than in other regions because of the high trabecular bone content and turnover at this site. The use of bisphosphonates in patients treated with corticoids may protect those from osteoporosis, even if they have normal BMD, but this hypothesis needs further study (20).

It is supposed that the effects of sex steroids, especially estrogens, are mediated by two types of receptors on the surface of osteoblasts and osteoclasts. The first one that is only on the surface of osteoblasts carries the hormone into the nucleus and activates different genes. The second one found on the surface of osteoblasts, osteoclasts and osteocytes is connected to cytoplasmatic triggers like src, erk and through ERK-1 influence indirectly ADN transcription. Estrogens have an opposite effect on the lifespan of osteoblasts, osteoclasts and osteocytes (an antiapoptotic effect on the former and a proapoptotic effect on the others) and thus they maintain the balance between bone resorption and formation. In addition estrogens suppress the production of IL-6, a pro-osteoclastogenic cytokine. This is actually very important in all diseases associated with hypogonadism, like

liver cirrhosis. Alcohol abuse also affects the levels of estrogen, testosterone and their bioavailability in a dose-dependent manner, even in the absence of cirrhosis. It was proved that hormone replacement therapy increased BMD, but with all the beneficial effect, its use is limited by the increased incidence of hepatocellular carcinoma (21).

Patients with cholestatic liver disease present malabsorption of vitamin K, a vitamin important in carboxylation of bone proteins. A low vitamin K level is associated in healthy population with osteoporosis and a high risk of fractures (22).

Considering that unconjugated bilirubin reduces osteoblast proliferation in a dose-dependent manner *in vitro*, it was thought for a while that retained substances in hepatic disease may reduce bone formation. Further studies on patients undergoing liver transplantation, could not find any statistical correlation between serum bilirubin and low BMD. Moreover treatment with ursodeoxycholic acid improved biochemical parameters of cholestasis, but had no effect on bone density. Iron and copper might also directly affect bone formation and a correlation between iron overload and reduced BMD has been identified not only in hepatic diseases but also in other conditions with iron overload like thalassemia (23).

The molecular mechanisms implied in bone resorption and bone formation are RANK-RANKL and OPG. RANKL, expressed on the surface of preosteoblasts binds to RANK on the osteoclastic precursor cells and is critical for the differentiation, activation, and survival of osteoclastic cells (24). OPG blocks the entire system due to its capacity to act like a RANKL and to bind RANK (25). In patients with hepatic disorders levels of RANKL have been shown to be decreased, increased, or not different from those of the normal population, and most studies found no correlation between RANKL levels and BMD.

Osteoprotegerin is synthesized in the liver and that is why it was considered to have a major role in the development of osteoporosis in patients with hepatic diseases, actually supported by the high level in these pathologies, compared with levels in healthy controls, although no significant correlation was proved between OPG and BMD. According to the same hypothesis a high level of OPG should determine an increased BMD, but this has not been proven (26). All these findings about

RANK-RANKL-OPG system demonstrate that it has a limited role in the bone loss secondary to liver diseases.

Macrophage colony-stimulating factors (M-CSF) together with its specific receptor on preosteoclastic cells influence the osteoclastic development. Circulating levels of CSF1 were increased in patients with cholestatic liver disease and osteoporosis compared with levels in patients without osteoporosis, raising the possibility that CSF1 may suggest that there is a RANKL-independent pathway responsible for bone resorption in patients with cholestasis (27). Inflammatory changes secondary hepatic C viral infection appears to be responsible for changes in OPG, M-CSF, but the exact mechanism should be clarified.

Hormones have, beside the direct effect on bone cells, an indirect effect on bone metabolism through RANK-RANKL-OPG system. PTH, 1,25(OH)<sub>2</sub> vitamin D<sub>3</sub>, glucocorticoids increase RANKL level and decrease OPG level, and estrogen have no effect on RANKL level but increases the OPG level (28).

### Cytokine influence on bone metabolism in liver diseases

Cytokines influence BMD in both physiological and pathological conditions. Interleukin-6 (IL-6) synthesized in osteoblasts, osteoclasts, and stromal cells is a potent activator of osteoclasts and bone resorption, but also promotes osteoblast generation in conditions of high bone turnover. Similarly other cytokines, like IL-1, IL-11, and TNF- $\alpha$  influence osteoclast function. IL-8, besides its pro-inflammatory role, causes an increase in PTH level (29). Prostaglandins, especially PGE<sub>2</sub>, stimulates both bone formation (in response to mechanical stress) and bone resorption. Cyclooxygenase 2 (COX2) is needed for PGE<sub>2</sub> synthesis, and so non-steroidal anti-inflammatory drugs that inhibit COX-2, decrease osteosynthesis.

Activation of inflammatory cells in patients with these conditions induces the production of proinflammatory cytokines such as TNF, IL-1, IL-13, IL-6, IL-7, IL-11, IL-15 and IL-17. These cytokines can increase bone loss either by the direct activation of osteoclast precursors, or by inducing the production of RANKL by osteoblasts (30). Ethanol seems to stimulate IL-6 production, causing, via induction of RANKL, activation of osteoclastogenesis. Also, serum levels of TNF are elevated in patients with alcoholic

or viral liver disease, and serum levels of the TNF receptor, TNF-R1, correlate with severity of liver disease.

Leptin, mainly produced by adipocytes, has a role not only in regulating hunger and satiety but also in maintaining BMD by central and peripheral mechanism. Hypothalamic neurons involved in leptin antiosteogenic central function are distinct from the neurons responsible for the regulation of energy metabolism (31). Peripheral leptin increases osteoblast proliferation, suppresses RANKL production and stimulates the synthesis of bone matrix. As a consequence, all these actions increase BMD. From the molecular point of view, leptin has immunomodulatory effect stimulating in turn the synthesis of other cytokines such as IL-1 and TNF $\alpha$  (32). A study of Ducy et al. raised the hypothesis that leptin-deficient mice have increased bone mass because of the protective effect of obesity against osteoporosis, but also considered leptin's central effect as a neural control circuit for regulation of the osteoblast (33).

Leptin production is increased in activated stellate cells, in patients with chronic hepatitis C, and its level correlate with fibrosis scores (34). On the contrary, in patients with cholestatic liver disease, leptin level is decreased compared to healthy controls. These data suggest that although the physiological mechanism of action on bone metabolism is unknown, in patients with liver disease of various etiologies leptin level is not the only determinant of BMD.

It has been demonstrated *in vitro* that fibronectin is necessary for the osteoblast functions. Plasma fibronectin is produced by the liver and patients with liver disease exhibit altered fibronectin production (35) represented by a large number of isoforms. Circulating fibronectin isoforms produced by activated stellate cells represent a viable marker for the presence or not of significant fibrosis in patients with chronic hepatitis C (36). □

### CONCLUSION

Although the physiological mechanism of bone metabolism has been established, when it comes to pathological conditions, the hormones and cytokines have different new roles, or are associated with other factors having different influences. Further studies are needed to clarify the complex mechanism of bone loss in hepatic disease.

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