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General Introduction
Skeletal complications before and after liver transplantation
INTRODUCTION

Skeletal pathology is common in patients with end-stage liver disease before and after orthotopic liver transplantation (OLT), and this is associated with significant morbidity and decreased quality of life. Significant bone loss occurs and the incidence of new fractures is high within the first year after OLT, despite recovery of liver function. (1-4) However, the pathophysiology of increased bone fragility in end-stage liver disease and after liver transplantation remains incompletely understood. A number of risk factors, such as vitamin D deficiency, hypogonadism, malnutrition, alcohol abuse and glucocorticoid use where applicable contribute to bone loss and increased fracture risk, but the persistence of increased fracture risk after correction of metabolic abnormalities after liver transplantation suggests that other factors, as yet to be identified, are likely to be involved in the pathophysiology of skeletal complications after OLT.

The aim of this Chapter is to provide an overview on the role of the liver in bone metabolism and on the pathophysiology of bone loss and increased fracture risk observed in liver diseases before and after liver transplantation.

NORMAL BONE REMODELING

The skeleton is constantly renewed by the process of bone remodeling which plays a major role in the preservation of calcium and phosphate homeostasis, and in the maintenance of the structural integrity of bone by preserving bone mass. Bone remodeling takes place in the so called bone multicellular units (BMU’s) through the coordinated activity of the 3 types of bone cells: osteoblasts, osteoclasts and osteocytes. The remodeling cycle starts with the resorption of bone by osteoclasts, followed by the formation of new bone by osteoblasts, which fills the resorption cavities created by the osteoclasts. Mineralization of the newly formed osteoid completes the bone remodeling cycle. (Figure 1)

Bone remodeling is regulated by several factors including hormones, signaling pathways and mechanical factors. Two signaling pathways are crucial for the differentiation, maturation, activation and survival of bone cells, the RANK/RANKL/OPG pathway for osteoclasts and the Wnt signaling pathway for osteoblasts. Differentiation of osteoclasts from hematopoietic stem cells is mediated through the RANK/RANKL/OPG pathway (5;6) in the presence of the macrophage-colony stimulating factor (M-CSF), which induces RANK expression in osteoclast progenitor cells. Binding of the ligand of RANK (RANKL), produced by the osteoblast, to its receptor RANK on the surface of pre-osteoblasts induces a cascade of signaling pathways, which affects all steps of osteoclastogenesis. All these steps are in turn inhibited by osteoprogerin (OPG), a natural decoy receptor also produced by the osteoblast, which binds to RANKL, and
preventing its binding to RANK, and thus inhibiting all steps of osteoclastogenesis when this is no longer required. (Figure 2)

The Wnt signaling pathway is a critical regulator of bone formation (6; 7), which when activated, results in binding between Wnt proteins (Figure 3), Frizzled and low density lipoprotein receptor-related protein 5 (LPR-5) or LPR-6 receptors. This results in inactivation of GSK-3beta, inhibition of beta-catenin degradation and accumulation of beta-catenin in the nucleus. (8) Beta-catenin binds to the TCF/LEF family of transcription factors and induces target gene expression, which is essential for the differentiation of mesenchymal stem cells to osteoblasts through effects on proliferation and maturation of osteoblast progenitor cells. The transcriptional factor RUNX2 is a target of beta-catenin for the stimulation of bone formation through the initiation of differentiation of multipotent mesenchymal stem cells into osteoblasts. (9) Osterix, another transcription factor, acts downstream of RUNX2 and is responsible for the transition from osteoprogenitor cells to pre-osteoblasts. Later in the process of osteoblast differentiation, beta-catenin influences bone resorption by decreasing osteoclastogenesis through stimulation of the expression of OPG. (10) The Wnt signaling pathway is antagonized by sclerostin, a protein produced by osteocytes, which is a product of the Sost-gene and binds to LPR-5 and LPR-6, thereby preventing the formation of the complex between Wnt proteins and Frizzled. (11) In addition to sclerostin, members of the Dickkopf (DKK) family such as DKK-1 also inhibit the Wnt signalling pathway by binding to the LPR-5 and LPR-6 receptors. (12) Secretion of sclerostin by the osteocyte is suppressed by mechanical stress, leading to increased bone formation. (6)
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Figure 2: The RANK/RANKL/OPG pathway. Binding of the ligand of RANK (RANKL), produced by the osteoblast, to its receptor RANK on the surface of pre-osteoblasts induces a cascade of signaling pathways, which affects all steps of osteoclastogenesis (upper panel, a). All these steps are in turn inhibited by OPG, a natural decoy receptor also produced by the osteoblast, which binds to RANKL, and preventing its binding to RANK, and thus inhibiting all steps of osteoclastogenesis when this is no longer required (lower panel, b). OPG = osteoprotegerin; RANK = receptor activator of nuclear factor kappa b; RANKL = receptor activator of nuclear factor kappa B- ligand; PTH = parathyroid hormone; PTHrP = parathyroid hormone related peptide; PGE-2 = prostaglandin E2; IL = interleukin; TNF = tumor necrosis factor; BMP = bone morphogenic protein; TGF-beta = transforming growth factor beta; TPO = thrombopoietin; PDGF = platelet derived growth factor (Adapted from Boyle WJ, Simonet WS, Lacey DL. Nature 2003; 423(6937):337-342, reproduced with permission)
The tightly regulated process of bone remodeling is thus controlled by a number of regulatory signals, which have been largely although as yet somewhat incompletely unraveled. Metabolic bone activity can be evaluated in the clinic by measuring circulating products of bone remodeling activity. Biochemical markers of bone

Figure 3: The Wnt signaling pathway. Activation of Wnt signaling results in binding between Wnt proteins, FZD and LRP5 or LRP6 receptors (right panel). This results in inactivation of GSK-3beta, inhibition of beta-catenin degradation and accumulation of beta-catenin in the nucleus. Beta-catenin binds to the TCF/LEF family of transcription factors and induces target gene expression, which is essential for the differentiation of mesenchymal stem cells to osteoblasts through effects on proliferation and maturation of osteoblast progenitor cells. The Wnt signaling pathway is antagonized by sclerostin, a protein produced by osteocytes, which is a product of the Sost-gene and binds to LPR-5 and LPR-6, thereby preventing the formation of the complex between Wnt proteins and FZD (left panel). In addition to sclerostin, members of the DKK family also inhibit the Wnt signaling pathway by binding to the LPR-5 and LPR-6 receptors. SFRP= secreted frizzled-related proteins; LRP= low density lipoprotein receptor-related protein; FZD =Frizzled; APC= adenomatous polyposis; P= phosphorylation; Ub = polyubiquitination; DVL = dishevelled; TCF/LEF = T cell factor/lymphoid enhancer factor; PTH= parathyroid hormone; DKK1 =Dickkopf-1; WIF1= Wnt inhibitory factor 1; DAAM1= dishevelled associated activator of morphogenesis 1; IP3 = inositol 1,4,5-trisphosphate, type 3; JNK = Jun kinase; ROCK = RHO-associated kinase; PPAR-gamma = peroxisome proliferator activated receptor-gamma; NFATc1 = nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 1; ROR = receptor-tyrosine-kinase-like orphan receptor; PLC = phospholipase C; DAG = dystroglycan; RUNX2 = Runx-related transcription factor 2. (Adapted from Baron R, Kneissel M. Nat Med 2013; 19(2):179-192, reproduced with permission)
remodeling activity, the bone turnover markers, include circulating crosslink fragments of degraded collagen: serum beta-crosslaps (CTX), as well as the urinary excreted crosslink fragments: hydroxyproline, pyridinoline, deoxypyridinoline and type 1 collagen cross-links (NTX and CTX). The products of various stages of bone formation, procollagen type 1 N-terminal propeptide (P1NP), osteocalcin and bone alkaline phosphatase (BALP), each representing a different stage of maturation of the osteoblast, can also be measured in serum. The gold standard of evaluation of metabolic bone disease remains the double tetracycline labeled bone biopsy, which provides information on bone cell activity as well as mineralization.

ROLE OF THE LIVER IN NORMAL BONE REMODELING

The two main physiological functions of the liver are metabolic and excretory, and various aspects of both functions play an important role in normal bone remodeling and thus in the maintenance of skeletal health.

Metabolic functions of the liver of relevance for skeletal health

Vitamin D metabolism

The liver plays an important role in skeletal and calcium homeostasis through its role in vitamin D metabolism. Vitamin D, a fat soluble vitamin, is synthesized in the skin by the effect of UV radiation from sunlight on 7-dehydrocholesterol. Intestinal absorption of dietary vitamin D is a much less important source of vitamin D as this is mainly found in fatty fish and essentially scarce in a normal diet. Dietary vitamin D is absorbed by enterocytes, incorporated into micelles, packaged into chylomicrons and transported to the liver via the portal circulation. Vitamin D is metabolically converted in the liver to the pro-vitamin D hormone, 25-hydroxy vitamin (25(OH)D), by cytochrome p450 enzymes, which exhibit a high capacity for the substrate vitamin D3. 25(OH)D is converted to the biologically active ligand for the widely distributed vitamin D receptor (VDR) through activity of the renal one-alpha hydroxylase (CYP27B1) enzyme. The proximal renal tubular epithelial cell is the largest source of the 1-alpha hydroxylase enzyme but the enzyme has also been found to be present in a large number of cells resulting in the extra-renal intracellular production of biologically active 1,25(OH)2D (calcitriol), which is primarily governed by the availability of extracellular 25(OH)D as substrate. The VDR is expressed in virtually all nucleated cells and influences a wide variety of processes such as proliferation, differentiation, apoptosis, mineral homeostasis, immune response and metabolism.(6;13) The vitamin D-binding protein (DBP), a member of the albumin family of proteins, is mainly produced by the liver and
is essential for binding of vitamin D and its metabolites in serum and for delivery of its biologically active form 1,25(OH)₂D to target tissues expressing the VDR. The most important biological action of vitamin D in the control of calcium homeostasis is the promotion of enterocyte differentiation, the intestinal absorption of calcium and the suppression of parathyroid hormone (PTH) synthesis and release by the parathyroid gland. In bone, the VDR regulates osteoblast and osteoclast function and is also important for bone mineralization.(6;14;15) VDR controls bone remodeling by regulating the production of RANKL and OPG by osteoblasts and osteocytes, thus playing a major role in the coupling of bone formation and resorption and in the maintenance of bone mass and integrity. Bone resorption is decreased by VDR activity by suppression of the RUNX2 gene transcription in the Wnt signaling pathway. Activation of the VDR also leads to production of osteopontin (OPN) by the osteoblasts, a peptide believed to be involved in bone mineralization and remodeling by anchoring osteoblasts to the mineral matrix.(6;15) VDR also affects the osteocalcin gene, the function of which in bone is incompletely understood, (6;15) although it has been suggested this osteoblast-specific protein may contribute to the density and structural integrity of bone.

A feedback loop is formed by the formation of fibroblast growth factor (FGF-23) by VDR signaling in the osteocyte.(6;15) FGF-23 inhibits the renal 1-alpha-hydroxylase enzyme and thereby the conversion of calcidiol to the biologically active calcitriol (1,25OH)₂D), simultaneously inducing the degradation of 1,25(OH)₂D to the biologically inactive metabolite 24,25(OH)₂D by increasing the expression of the 24-alpha-hydroxylase enzyme.

Metabolism of gonadal steroids

Gonadal steroids play an important regulatory role on bone and mineral metabolism. Sex steroid deficiency results in high bone turnover in favor of increased bone resorption, leading to loss of bone mass and bone strength and to an increase in fracture risk. The liver is the main site for peripheral inactivation and clearance of estrogens. Furthermore, sex-hormone binding globulin (SHBG) and albumin, the most important serum binding proteins for testosterone and estradiol, are synthesized in the liver.

Insulin-like growth factor 1

Bone remodeling is influenced by insulin-like growth factor-1 (IGF-1), a growth factor synthesized in the liver under the influence of growth hormone (GH).(16-18) To a lesser extent, IGF-1 is also produced in bone under the influence of GH as well as of local factors, with bone-derived IGF-1 also playing an important role in bone remodeling.(17) IGF-1 circulates mostly as a complex of IGF-1 bound to IGF-binding-protein-3 (IGFBP-3), one of the IGF-binding-proteins (IGFBP’s).(19) IGFBP-concentrations and IGF-1 expression are regulated by various hormones, including GH, testosterone, estrogen and thyroid
hormones. IGF-1 stimulates osteoblast function and bone formation by mitogenic activity and by stabilization of beta-catenin, thereby enhancing Wnt-dependent activity. On the other hand, IGF-1 also induces RANK-L synthesis thereby promoting osteoclastogenesis and osteoclast function. Furthermore, IGF-1 maintains the bone matrix by influencing collagen transcription and collagenase production. IGF-1 is also believed to be necessary for the action of PTH on bone; PTH increases IGF-1 expression in osteoblasts and IGF-1 in turn mediates the anabolic action of PTH on the skeleton. Systemic IGF-1 appears to contribute more to cortical bone integrity, whereas locally bone-derived IGF-1 is thought to play a more important role in the maintenance of trabecular bone integrity.

**Excretory function of the liver**

One of the main excretory functions of the liver is the synthesis and excretion of bile salts, which contribute significantly to normal calcium and bone homeostasis as they are essential for the absorption of the lipid soluble vitamin D. Bile acid synthesis starts with the synthesis of the two primary bile acids, cholic acid and chenodeoxycholic acid, by the process of oxidation of cholesterol in liver cells. These bile acids are secreted into the lumen of the intestine, where intestinal bacteria partly dehydroxylate them to the secondary bile acids, deoxycholic acid and lithocholic acid. The secondary bile acids are in turn reabsorbed in the ileum and transported to the liver via the portal circulation, and are re-secreted, creating an enterohepatic circulation. Bile acids are secreted after conjugation with glycine or taurine, forming a total of eight possible conjugated bile acids. These are referred to as bile salts. Bile salts are more water-soluble than bile acids. They are able to emulsify lipids and fat-soluble vitamins (such as vitamin D) in the intestine, to form micelles that can be transported to the liver.

**Vitamin K metabolism and bone**

Bile salts are necessary for the absorption of vitamin K, a fat-soluble vitamin necessary for the production of osteocalcin in bone. Vitamin KI is mainly found in leafy vegetables and absorbed in the small intestine, and vitamin KII is synthesized by bacterial flora and found in animal liver and fermented foods. Absorption of vitamin K is dependent on pancreatic function and on the presence of bile salts and it is excreted as its catabolic product in bile and urine. A proportion of vitamin K is stored in the liver. Osteocalcin is the most abundant non-collagenous protein in bone, which is produced by the osteoblasts and incorporated into the bone matrix, where it is involved in bone calcification. Vitamin K is essential for the post-translational carboxylation of glutamic acid, which is necessary for binding of calcium to the proteins found in bone such as osteocalcin. Vitamin K has also been shown to inhibit osteoclast formation by affecting
RANK-L production, and to stimulate bone formation by enhancing osteoblastogenesis and promoting osteoblast to osteocyte formation.(27;28)

**FACTORS AFFECTING BONE REMODELING IN LIVER DISEASE**

In liver disease, the metabolic and excretory functions of the liver are compromised to a lesser or greater extent by the underlying pathology, leading to disturbances in calcium and bone metabolism. Viral pathogens, toxins such as alcohol or accumulated bile salts, and medication potentially used to treat the liver disease such as corticosteroids in auto-immune hepatitis may also exert direct deleterious effects on bone independently of the disturbed hepatobiliary function.

**Vitamin D deficiency**

Vitamin D deficiency is common in chronic liver disease, with a prevalence ranging from 64-92%, which increases with the severity the disease.(29;30) In liver disease, the pathogenesis of vitamin D deficiency is multifactorial, including poor nutritional status, decreased sun light exposure due to chronic illness and decreased vitamin D absorption in cholestatic liver diseases. In the late stages of liver disease, the production of the 25 hydroxylase enzyme is also impaired and increased breakdown of 25(OH)D to biologically inactive metabolites decreases substrate availability for the renal production of the biologically active 1,25(OH)2D. This leads to decreased intestinal absorption of calcium, hypocalcemia, and secondary hyperparathyroidism.(31) Decreased VDR signaling results in further stimulation of PTH synthesis and increased bone turnover in favour of bone resorption. Bone mineralization is also significantly compromised by vitamin D deficiency, resulting first in a decrease in bone mass and eventually in case of longstanding severe vitamin D deficiency to osteomalacia.

In patients with chronic liver disease, vitamin D deficiency is mainly due to decreased sunlight exposure and malnutrition.(32) 25-hydroxylation of vitamin D remaining generally intact until the late stages of liver failure when more than 90% of liver function is lost.(30;33) Cholestasis such as occurs in primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and overlap syndromes with auto-immune hepatitis (AIH), negatively influences vitamin D metabolism.(32;34;35) In these diseases, in which transportation of bile salts to the intestine is compromised, the resulting decrease in bile salts leads to defective absorption of fat-soluble vitamins including dietary vitamin D.(32;34;35) In patients with PSC the often concomitant inflammatory bowel disease, Crohn’s disease, may in its own right contribute to decreased intestinal re-absorption of bile salts and of vitamin D through mucosal inflammation or surgical resection of significant length of the small intestine to control disease activity.(35) In addition to
the effect of cholestasis on the intestinal absorption of vitamin D, cholestatic toxins may also directly influence bone remodeling through a direct toxic effect on various steps of the RANK/RANKL signaling pathway and on mediators of osteoblast function, thereby inhibiting bone formation and increasing bone resorption. A large fraction of vitamin D is bound to DBP and albumin so that reduced production of these proteins in the liver also leads to a decrease in circulating vitamin D and in its availability for metabolic functions, particularly in the late stages of liver failure.

**Hypogonadism**

Gonadal hormone deficiency is common in liver disease. Estrogen and testosterone attenuate the maturation of osteoclasts and inhibit bone resorption by the stimulation of osteoclast apoptosis while preventing osteoblast and osteocyte apoptosis thus maintaining a healthy balance between bone resorption and formation leading to preservation of bone mass and integrity. A decrease or loss of sex steroids therefore leads to increased osteoclast activity and accelerated bone resorption.

However, the aetiology of hypogonadism in liver failure is not completely understood. It may develop as a result of primary gonadal failure as well as a result of failure of the pituitary-hypothalamic gonadal axis. Alcohol abuse is associated with central as well as primary gonadal hypogonadism, independently of cirrhosis or nutritional status. Alcohol also accelerates the metabolic clearance of testosterone, leading to decreased circulating testosterone levels. Some studies also suggest that alcohol increases hepatic aromatization of androgens to estrogen in men, although this would not be deleterious to the skeleton. In hereditary and secondary hemochromatosis, pituitary iron deposition leads to destruction of gonadotroph cells with consequent secondary hypogonadism. In patients with end-stage liver disease, testosterone levels decline while SHBG increases, leading to decreased free testosterone levels.

**Renal impairment**

Renal disease is common in patients with liver disease with a reported prevalence of 20-25% of patients. The later stages of liver failure may be associated with renal failure: the hepatorenal syndrome. Renal IgA deposition is often seen in liver disease and may result in IgA nephropathy, especially in the case of alcoholic liver disease. Viral hepatitis is associated with membranous and membranoproliferative glomerulonephritis and focal segmental glomerulosclerosis. In patients with diabetes and steatohepatitis, co-existing diabetic nephropathy may also lead to renal impairment.

Renal impairment has complex deleterious effects on bone, on one hand through phosphate retention and consequent progressive stimulation of FGF-23 production, and on the other hand through chronic stimulation of the parathyroids resulting in hyperplasia and proliferation of these cells. Secondary hyperparathyroidism...
results from phosphate retention and from decreased calcium- and calcitriol concentrations. (43) Calcitriol concentrations are reduced due to suppressed renal 1-alpha hydroxylase activity, which result from phosphate retention and increased FGF-23 concentrations. (43; 44) In severely impaired renal function, loss of functional renal mass leads to decreased 1-alpha hydroxylase production, also contributing to the decline in calcitriol concentration. (44) Nutritional vitamin D deficiency and loss of 25(OH)D are also very common in renal failure, also contributing to secondary hyperparathyroidism. High or low bone turnover may both be associated with osteoporosis and increased fracture risk in patients with renal failure. (45-48)

IGF-1

Liver diseases are associated with low IGF-1 levels, and these have been shown to be associated with low bone mass and increased fracture risk in growth hormone deficiency states. (49) The contribution of low IGF-1 levels to decreased bone mass in patients with liver disease has not however been adequately addressed so far. (50) A decrease in IGF-1 levels is observed in the early stages of liver disease, such as fibrosis and steatosis, and in alcoholics (51-53) and IGF-1 levels progressively decline with further deterioration in liver function. (52-57) In liver diseases, the aetiology of decreased IGF-1 levels is multifactorial. A decrease in hepatocyte function leads to a decrease in hepatic IGF-1 and IGFBP-3 secretion. (50-56; 58-63) Interestingly, GH levels have been shown to be increased in these patients (50-56; 58-63), suggesting hepatic GH-resistance, which may be related to malnutrition. (59). Low IGF-1 levels are also common in inflammatory bowel disease, such as Crohn’s disease which is associated with PSC. Moreover, IGF-1 production may be inhibited by inflammatory cytokines such as IL-6, which are released during infections such as spontaneous bacterial peritonitis. (64) Although hormone deficiencies such as testosterone, insulin and thyroxine deficiencies which are prevalent in liver diseases may lead to an increase if IGFBP-3 and therefore to an increase in unbound IGF-1, a decrease of IGFBP-3 is actually observed in most patients with compromised liver function.

Vitamin K

In liver disease, vitamin K deficiency may occur as a result of malnutrition and of decreased intestinal absorption of vitamin K in cholestatic disease due to a decrease in bile salt excretion in the intestine. Small-intestine pathology such as Crohn’s disease may also lead to decreased vitamin K absorption. In case of vitamin K insufficiency, osteocalcin fails to become carboxylated in bone, and it is released from the osteoblasts into the circulation. (65) Low circulation levels of vitamin K have been associated with low bone mineral density (BMD) and osteoporotic fractures. (27; 65; 66)
**Other factors associated with skeletal complications in liver disease**

Pro-inflammatory cytokines, such as IL-1 and TNF-alpha, are increased in patients with liver cirrhosis and stimulate osteoclast activity and thereby bone resorption. Hyperhomocysteinemia may occur in patients with liver disease as a result of stimulation of the hepatic production of homocysteine by a number of factors such as nutritional vitamin deficiencies and cigarette smoking or because of accumulation of the protein due to reduced excretion in case of renal impairment. Hyperhomocysteinemia has been associated with increased risk for fractures independently of BMD, possibly by altering bone quality through abnormal collagen crosslinking.

**Effect of underlying liver disease on the skeleton**

*Alcoholic liver disease*

Alcohol abuse is associated with decreased bone mass and increased fracture risk. (67-70) Ethanol exerts direct toxic effects on osteoblasts, resulting in decreased bone formation.(71) In addition, ethanol also stimulates osteoclast activity through stimulating the RANK/RANK-L system.(72;73) Ethanol also alters the metabolism of several hormones, including vitamin D, IGF-1 and cortisol. In alcoholics, malnutrition may also contribute to bone loss when alcohol represents the main caloric intake.(74) Heavy alcohol intake is also associated with increased risk of falls and thereby increased risk of fracture.(75)

*Cholestatic liver disease*

Cholestasis, as seen in cholestatic liver diseases such as primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and overlap syndromes with auto-immune hepatitis (AIH), negatively influences vitamin D metabolism.(32;34;35) A decrease in bile salts availability is associated with defective absorption of dietary vitamin D when transportation of bile salts to the intestine is compromised.(32;34;35) In patients with PSC a concomitant inflammatory bowel disease, such as Crohn’s disease, may in its own right contribute to decreased intestinal re-absorption of bile salts and vitamin D through mucosal inflammation or surgical resection of significant length of the small intestine to control disease activity.(35) In addition to the effect of cholestasis on absorption of vitamin D, cholestatic toxins may also directly influence bone remodeling through an effect on the RANK/RANKL pathway and on mediators of osteoblast function, thus contributing to decreased bone formation and increased bone resorption and thereby to bone loss.(30;76;77)
Viral hepatitis
Chronic hepatitis B and C infections (HBV and HCV) have been reported to be associated with bone loss, regardless of their effect on liver function (78-81). High bone turnover and increased bone resorption have been observed in viral liver diseases possibly related to the inflammatory process associated with increased levels of cytokines, leading to stimulation of the RANK/RANKL pathway and of all aspects of osteoclastogenesis. Higher levels of soluble tumor necrosis factor (TNF) receptor-55 have also been shown to be associated with increased bone resorption and lower BMD in patients with HCV.(81) Fracture rate has also been shown to decline after virus clearance.(82) Antiviral therapy used to treat viral hepatitis may also affect bone turnover. Ribavirin has been shown to impair osteoblast proliferation, with one study showing lower BMD in patients treated with this agent (83), although this deleterious effect of this antiviral agent on bone is controversial, as other studies have showed stable or increasing BMD during ribavirin therapy.(84-86)

Iron storage disease
In haemochromatosis, an autosomal recessive disease with a HFE-gene mutation leading to increased iron absorption due to inappropriate hepcidin secretion (87), iron excess is associated with low bone mass and increased risk of fractures.(38;40;88;89) The main mechanism of action of iron overload is to impair osteoblast activity.(38-40) Iron overload may also contribute to the development of central hypogonadism due to pituitary iron deposition, with associated increased bone resorption and decreased bone formation.(38-40)

SKELETAL COMPLICATIONS AFTER LIVER TRANSPLANTATION
Bone loss and fractures are common after solid organ transplantation, including liver transplantation, and these skeletal complications are associated with significant morbidity and mortality. The pathogenesis of these skeletal complications remains incompletely understood. Corticosteroids and other drugs used for immunosuppression play an important role. Main additional contributing factors include the underlying primary liver pathology and skeletal status at the time of transplantation. Potential other contributing factors include persistent vitamin D deficiency and hypogonadism or high bone turnover.

Significant bone loss at the lumbar spine (LS) as well as the femoral neck (FN) occurs during the first six months after liver transplantation with a more variable course thereafter. (90-98) Fracture incidence is very high despite recovery of liver function, with new fractures occurring in 15-34% of patients within the first year after OLT, mainly
vertebral and rib fractures. (1-4) LS BMD has been shown to spontaneously recover thereafter, reaching pre-transplant values within two years after OLT. (4;99-103) FN BMD has been reported to remain low in most studies, with BMD stabilizing or even decreasing further beyond the first year after OLT. (4;102;104;105)

Underlying liver disease

Significant differences in degree of bone loss and in fracture incidence have been reported after OLT according to type of liver disease (2;102;103;105;106;106-108), with patients with cholestatic liver disease being at higher risk for fractures than patients with other liver diseases.

Underlying skeletal pathology

Low bone mass and fractures are already highly prevalent before liver transplantation. Osteoporosis is thus reported in 15-40% of patients with end-stage liver disease (94;95;107;109;110) and fractures are reported to be prevalent in 6-30% of patients (107;111-113). Whereas pre-existing low bone mass is a risk factor for fractures after transplantation (1;2;4;114-116), fractures have also been shown to occur in patients with osteopenia or normal bone mineral density (BMD).

Corticosteroids

Corticosteroids are used postoperatively in almost all immunosuppressive protocols after solid organ transplantation. Corticosteroids exert their deleterious effects on bone through direct and indirect effects on bone cells, mainly leading to suppression of bone formation, but also to a certain extent by increasing bone resorption, with the resulting bone loss being more prominent at trabecular sites. The mechanism bone of loss due to corticosteroids is complex. Glucocorticoids lead to osteoblast apoptosis and to suppression of osteoblast differentiation by suppression of markers genes such as Run2x and Wnt/beta catenin signaling, leading to decreased bone formation. (117) On the other hand, glucocorticoids also induce bone resorption by reducing osteoclast apoptosis, resulting in net bone loss. Glucocorticoids also indirectly contribute to bone loss by reducing intestinal calcium absorption and increasing urinary calcium excretion. Vitamin D metabolism does not appear to be influenced by glucocorticoids. (118) Corticosteroids also decrease the stimulatory effect of IGF-1 on osteoblasts. The effect of glucocorticoids on bone have been shown to be dose dependent (4;105;119), which may explain the bone loss and increased fractures risk observed in liver transplant recipients particularly within the first few months after transplantation.
Other immunosuppressive agents
A number of immunosuppressive agents may also have adverse effects on bone with a tendency for calcineurin inhibitors such as cyclosporine or tacrolimus to cause rapid and profound bone loss. High bone turnover and increase in bone turnover markers of bone formation and resorption have been demonstrated in experimental animal studies of calcineurin use and also confirmed by histomorphometric studies of bone biopsies, with greater bone loss shown with tacrolimus than with cyclosporine A. However, data on the effects of calcineurin inhibitors in humans are scarce and results are conflicting. Several studies have shown a less deleterious effect of tacrolimus on bone compared to cyclosporine A. A histomorphometric study showed earlier recovery of bone turnover after OLT in patients treated with tacrolimus compared to those treated with cyclosporine A, which was confirmed by two other studies, showing less bone loss in patients treated with tacrolimus and an earlier recovery of bone mass to pre-transplant values. Small subgroup analysis in one study and an observational study indeed show that incident fractures occur less in OLT recipients treated with tacrolimus compared to cyclosporine A. In contrast, another study showed no difference in bone mass between cyclosporine A and tacrolimus treated patients. Taken together, these conflicting data suggest that the exact effect of tacrolimus on the skeleton remains to be established in patients with a liver transplantation. Mycophenolate mofetil (MMF) has not been shown thus far to have a deleterious effect on bone. Data on the effect of sirolimus on bone turnover are conflicting, with studies showing increased as well as decreased osteoclast activity.

Renal failure
A decline in renal function is common after OLT and this may contribute to further bone loss. During the early post-operative period, acute kidney injury occurs in up to 64% of patients. Pre-existing renal impairment and comorbidity lead to increased susceptibility to kidney injury. Several factors may lead to acute kidney injury after transplantation, including acute tubular necrosis, calcineurin inhibitor toxicity and sepsis. In the long-term, some degree of chronic renal impairment is highly prevalent in OLT recipients. One study showed a prevalence of moderate renal impairment in over 50% of OLT recipients at twenty years after OLT, and the cumulative incidence of renal failure (glomerular filtration rate of 29 ml/minute/1.73m² body surface or less) is reported to be 18% at five years after OLT. As previously mentioned, renal impairment contributes to bone loss and to mineralization disorders due to abnormalities such as phosphate retention and calcitriol deficiency leading to secondary hyperparathyroidism.
Other factors
Recovery of liver function leads to reversal of cholestasis and reversal of metabolic disturbances early after transplantation. IGF-1 and IGFBP-3 levels increase rapidly after OLT, normalizing within the first week after successful transplantation.(60;62;138) Gonadal hormones have been shown to increase (4;100;139) and 25(OH)D levels normalize in the majority of patients(100;104;134;139;140). The introduction of new immunosuppressive regimens has allowed early tapering of corticosteroids (141-145), substantially contributing to recovery of bone mass.

Vitamin D deficiency
Even though vitamin D status improves after OLT, as shown by post-transplantation increases in 25(OH)D (100;100;104;134;139;140), vitamin D levels do not normalize in all patients, and a degree of vitamin D deficiency persist in a number of patients. Furthermore, even though 25(OH)D levels increase after transplantation, this does not always translate in increases in the metabolically active calcitriol.(4;100)

Persistent hypogonadism
Gonadal function improves after transplantation, with marked increases in free testosterone levels observed within the first year after OLT.(4) However, recovery to normal levels is not achieved in all patients, with hypogonadism persisting in a substantial proportion of OLT recipients.(4;100;139)

THERAPEUTIC CONSIDERATIONS
In solid organ transplantation, prevention of post-transplantation bone disease involves optimization of pre-transplant skeletal health and prophylaxis of post-transplantation bone loss. Patients with end-stage liver disease should be screened for skeletal pathology and treated accordingly. Life-style measures, including cessation of smoking and of alcohol use, good nutrition and weight bearing exercise, are to be advised to all patients. Calcium and vitamin D should be adequately supplemented where required. Vitamin D deficiency should be corrected to levels above 50 nmol/L, using follow-up measurements of 25(OH)D. This is the level suggested by the Institute of Medicine (146) although the International Osteoporosis Foundation (IOF) suggests a higher threshold of 75 nmol/L for men and women above the age of 60 and those with osteoporosis (147). It is to be noted that in clinical practice, it is sometimes difficult to reach the threshold of 75 nmol/L with the recommended daily use of 800 units of colecalciferol. At the moment, there is still no consensus on the desirable cut-off point of 25(OH)D to reach in liver transplant recipients but in view of the important role of the liver in vitamin
D metabolism, it remains highly recommended to measure these 25(OH)D levels in patients with chronic liver disease before and after liver transplantation.

**Bisphosphonates**

Most studies showed a short-term beneficial effect of bisphosphonate treatment on BMD when treatment is initiated at or shortly after OLT.(148-155) Bisphosphonates used in these studies were largely nitrogen-containing bisphosphonates including intravenously administered zoledronate, ibandronate and pamidronate (148-150,154-163) and orally administered alendronate and risedronate (149,151-153). Time of initiation of treatment was at or shortly after OLT in most studies (148-152,154-157,162), and 1-2 years after OLT in 3 studies (158,161,163) and patients were usually treated for one year (148-150,152,155-157,161) or 2-3 years (151,153,158,163). Most studies showed a decrease of biochemical markers of both bone formation, including procollagen type 1 N-terminal propeptide (PINP), bone alkaline phosphatase and osteocalcin, and of those of bone resorption, including beta crosslaps (CTX) and urinary deoxyhydroxyproline, after initiation of treatment with bisphosphonates in some (148,151,152,155,158,163) but not all studies.(149,150,157,160) A decrease in bone turnover was also demonstrated in two bone histomorphometry studies.(156,162) The authors of a meta-analysis published in 2011 concluded that bisphosphonate treatment administered during the first year after OLT appeared to reduce accelerated bone loss and to improve LS BMD.(164) Data on long-term effect of treatment with bisphosphonates administered shortly after OLT on BMD are scarce and conflicting, with two studies showing a persistent beneficial effect on BMD (150,153), whereas this was not confirmed in a second study.(157) Three studies in which treatment was initiated after the first year after OLT show a beneficial effect on BMD.(158,161,163) Treatment with bisphosphonates in the form of pamidronate administered as a single infusion before OLT did not prevent post-transplantation bone loss.(160) Data on both short-term and long-term effect of bisphosphonate treatment on fracture risk are conflicting, with studies showing a decreased fracture incidence in treated patients with oral as well as intravenous bisphosphonates, initiated before, at or shortly after and 1-2 years after OLT and for a duration of 9-24 months (149,150,152,153,158,159), whereas other studies did not show any effect on fracture risk. (151,154,157,160,163) In the only published meta-analysis, the effect of bisphosphonate treatment on fracture risk could not be analysed because of heterogeneity of the available data.(164)

Whereas bisphosphonates have thus been shown to reduce post-transplantation bone loss and to some degree fracture risk in most but not all studies, there is still no consensus on which patients should be treated with these agents. Antiresorptive therapy should be considered in patients with low bone mass particularly in the presence of fractures. However, since pre-transplant BMD poorly identifies patients at
risk for fractures, and these have been shown to occur in patients with normal BMD (2;114;165), the question raised is whether all liver transplant recipients should be treated with antiresorptive agents at time of transplantation.

CONCLUSION

The metabolic and excretory functions of the liver play an important role in normal bone remodeling. In liver diseases, disturbances in both physiological functions of the liver are associated with loss of bone mass as well as bone quality, leading to increased fracture risk. Significant bone loss occurs after liver transplantation despite recovery of the metabolic and excretory functions of the liver, mainly due to immunosuppressive regimens including glucocorticoids. The incidence of new fractures is also very high during the first year after liver transplantation, with fractures occurring in up to a third of transplant recipients, leading to significant morbidity and to a decrease of quality of life. The aetiology of bone loss and increased fracture risk observed in OLT recipients is multifactorial and still incompletely understood. It has thus been proven difficult to identify patients at risk. Moreover, data on efficacy of bisphosphonate treatment after liver transplantation are scarce and heterogeneous.

Awareness of the magnitude of skeletal pathology in patients with liver disease remains a clinically unmet need before and after liver transplantation. Candidates for liver transplantation and patients with liver diseases need to be thoroughly screened for low bone mass and prevalent fractures and disturbances in calcium and mineral homeostasis need to be adequately corrected in order to prevent further bone loss and decrease post-transplantation fracture risk.

QUESTIONS TO BE ADDRESSED

Mechanisms leading to bone loss in patients with end-stage liver disease need to be further elucidated. Since immunosuppressive regimens have changed during the last decade with the introduction of new immunosuppressive agents and early tapering of corticosteroids, the magnitude of bone loss and fracture risk in OLT recipients under current transplantation protocols needs to be re-examined. Risk factors for post-transplantation bone loss and fractures need to be elucidated in order to identify patients at risk. The value of bone turnover markers, BMD measurements and prevalent fractures in the identification of patients at risk need to be examined. Furthermore, the optimal time of initiation of treatment, the modality of treatment and its duration
remain to be established in OLT recipients. Finally, it needs to be clarified whether the persistent high fracture rate a year after OLT may be decreased or prevented.
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