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Review Article: Non-alcoholic Fatty Liver Disease and Osteoporosis

Clinical and Molecular Crosstalk

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Abstract and Introduction

Abstract

Background Low bone mineral density (BMD) has been reported in both paediatric and adult patients with non-alcoholic fatty liver disease (NAFLD). The mechanisms behind the reduced BMD in NAFLD are still not completely understood.

Aim To provide a critical overview of the pathophysiological pathways linking NAFLD, reduced BMD and osteoporosis, with a special focus on the alterations of soluble mediators which could link fat accumulation in the liver with bone health. The MEDLINE database was searched by a combination of keywords: non-alcoholic fatty liver disease OR hepatic steatosis OR metabolic syndrome OR insulin resistance AND bone mineral density OR osteoporosis OR bone AND biomarkers OR serum marker.

Results Several factors that may influence bone mineralisation and the increased risk of osteoporosis in NAFLD can be discussed. These include the release of cytokines from the inflamed liver which may influence the bone microenvironment, vitamin D deficiency, and limited physical activity. Circulating markers of bone metabolism, including osteopontin, osteoprotegerin, osteocalcin and fetuin-A, have been found to be altered in patients with NAFLD.

Conclusion A better understanding of the mechanisms that link bone metabolism and the liver may open a new frontier to fight two highly prevalent conditions like NAFLD and osteoporosis.

Introduction

The term non-alcoholic fatty liver disease (NAFLD) refers to any fatty infiltration of the liver that is not caused by significant alcohol abuse.[1–8] From an epidemiological standpoint, the prevalence of NAFLD is twice as high in men than in women (42% vs. 24% respectively), but – similar to that of osteoporosis – increases significantly among postmenopausal women.[6] In addition, in accordance with the observed epidemiological figures for osteoporosis, the prevalence of NAFLD increases with age, from less than 20% in people under the age of 20 to more than 40% in people aged >60 years or higher.[6]

The metabolic syndrome (MS) is universally considered as the key factor in the pathogenesis of NAFLD.[9, 10] Interestingly, low bone mineral density (BMD) has been recognised as a potential health problem in both men and women suffering from the MS,[11–14] of which NAFLD is the hepatic manifestation.[9] Moreover, preliminary evidence seems to suggest that NAFLD may be associated with an increased risk of osteoporotic fractures.[15] Although the mechanisms behind the reduced BMD in NAFLD are still not completely understood, several factors that may influence bone health and mineralisation in NAFLD can be discussed. These include the chronic low-grade inflammation itself, which causes the release of cytokines from the inflamed liver, vitamin D deficiency, and limited physical activity. Circulating markers of bone metabolism, including osteopontin, osteoprotegerin, osteocalcin and fetuin-A, have been found to be altered in patients with NAFLD.

In the present review, we provide a critical overview of the potential pathways linking NAFLD with a reduced BMD, with a special focus on biochemical alterations of circulating bone-regulating markers. Toward this aim, the MEDLINE database was searched by a combination of keywords: non-alcoholic fatty liver disease OR hepatic steatosis OR metabolic syndrome OR insulin resistance AND bone mineral density OR osteoporosis OR bone AND biomarkers OR serum marker. A better understanding of the mechanisms that link bone metabolism and the liver may open a

new frontier to fight two highly prevalent conditions like NAFLD and osteoporosis.

NAFLD and Its Association With Bone Mineral Density: Epidemiological Evidence

Growing evidence suggests the presence of a complex interplay between the skeleton and numerous homeostatic processes, including energy balance, insulin resistance, obesity and the MS. Recent years have also witnessed an increased awareness of the clinical and epidemiological association between NAFLD and bone health (both in terms of reduced BMD and an increased risk of osteoporosis); at present, such an association has been independently reported by at least four studies in both children and adults.[16–19] In a case–control study of 82 obese children and 30 normal-weight controls, Pirgon *et al*.[16] demonstrated a negative association between BMD and insulin resistance in obese adolescents both with and without NAFLD. Moreover, obese children with NAFLD had a lower BMD than their non-NAFLD counterparts. The authors concluded that NAFLD could exert a negative impact on BMD in obese adolescents, probably via an increased insulin resistance.[16] Campos *et al*.[17] enrolled 40 postpuberty obese adolescents who were divided into two subgroups according to the presence or absence of NAFLD diagnosis using ultrasonography. Following a weight loss therapy, the authors measured the changes in BMD using dual-energy X-ray absorptiometry (DEXA). The authors found a positive correlation between the changes in BMD and the changes in fat mass, whereas a negative association with the changes in insulin resistance and leptin was found.[17] An important study by Pardee *et al*.[18] demonstrated that obese children with NAFLD had significantly lower bone mineral density *Z*-scores than obese children without fatty liver. In addition, 45% of children with NAFLD had low BMD adjusted for age, compared to none of the children without NAFLD.[18] Recently, Moon and coworkers[19] have examined the association between BMD and NAFLD in 381 pre and postmenopausal women. Lumbar BMD was measured using DEXA, whereas NAFLD was screened by means of liver ultrasonography. The results indicated that the mean lumbar BMD was lower in subjects with NAFLD than those without NAFLD in postmenopausal women even after adjusting for the presence of the MS. The authors considered BMD as related to NAFLD *per se* (i.e. independently of the MS) in postmenopausal females, and suggested that postmenopausal women with NAFLD may have a greater risk of osteoporosis than those without.[19] Taken together, these results indicated that NAFLD is associated with poor bone health both in obese children and postmenopausal women. In addition, a more severe liver disease seems to be associated with lower bone mineralisation.[19] This evidence is intriguing and set the stage for longitudinal investigations of BMD changes over the course of fatty liver. Future studies should explore whether the presence of more severe liver histology (hepatocellular ballooning, necroinflammation and fibrosis) could be associated with a poorer bone health, and whether this in turn relates to an increased risk of osteoporotic fractures.[15]

Decreased BMD in NAFLD: Pathogenetic Insights

Although there is evidence that NAFLD could be related to a reduced BMD from a clinical standpoint,[16–19] the pathogenesis of decreased BMD in NAFLD is currently unclear. However, this link is likely multifactorial and may include one or more of the following mechanisms, that is the release of cytokines and other bone-influencing molecules from the inflamed liver, vitamin D deficiency and reduced physical activity. We will therefore provide an overview of the mechanistic correlations between fatty liver and the osseous system – first focusing on the molecules that communicate between the liver and the skeleton.

Release of Cytokines and Other Bone-influencing Molecules From the Inflamed Liver

Tumour Necrosis Factor (TNF)-α

The observation that low BMD is already present in patients with newly diagnosed NAFLD prior to any treatment[16–19] suggests that the inflammatory process itself may play a role in the pathogenesis of disturbed bone mineralisation in this patient group. One of the potential key contributors in both hepatic inflammation and the pathophysiology of bone loss is tumour necrosis factor (TNF)-α. An increase in circulating levels of TNF-α has been reported in NAFLD patients by several independent investigators.[20–22] Of note, evidence suggests that increased TNF-α levels are both involved in the stimulation of osteoclastogenesis and in the inhibition of the activation of osteoblasts from their

progenitor cells.[23] Furthermore, TNF-α stimulates the expression of genes that amplify osteoclastogenesis (interleukin-6, macrophage colony-stimulating factor) but also inhibits those that are involved in bone formation (alkaline phosphatase, vitamin D receptor, parathyroid hormone receptor).[24] Importantly, an inverse association between TNF-α and vitamin D levels has been reported,[25] which could in turn affect skeletal metabolism.

Osteopontin

Osteopontin (OPN) is a T-helper 1 cytokine that enhances the viability and growth of liver progenitors cells by binding to its specific receptor CD44.[26] It acts as a profibrogenic cytokine and activates the hedgehog signalling pathway in the liver, a molecular cascade which is increased in parallel with fibrosis stage in non-alcoholic steatohepatitis (NASH).[27] In addition, OPN exacerbates inflammation in several chronic inflammatory diseases,[28] including NAFLD. A role for OPN in the pathogenesis of hepatic inflammation has been hypothesised from its proinflammatory actions and its effects on macrophages.[29] In a seminal study, Lima-Cabello *et al*.[30] demonstrated that the OPN gene is abundantly expressed in the liver of patients with NAFLD and chronic hepatitis C. Using a transgenic mouse model, Syn *et al*.[27] reported that OPN directly promotes fibrosis progression in NASH. Consistent with its putative role in NAFLD, genetic OPN deficiency has been recently shown to protect from obesity-induced hepatic steatosis by downregulating hepatic triacylglycerol synthesis in the mice.[31] Interestingly, Chang *et al*.[32] have reported that same OPN-deficient mice are resistant to ovariectomy-induced osteoporosis. Taken together, this evidence suggests that OPN overexpression found in NAFLD may be associated with less resistance to postmenopausal osteoporosis. This would in keeping with the results by Chang *et al*.[33] who showed that high serum OPN levels >14.7 ng/mL can be considered a significant risk factor for menopausal osteoporosis. OPN, hyperexpressed in NAFLD,[27] is known to exert a direct effect on the osseous system by acting as a noncollagenous bone matrix protein.[34] In addition, OPN is known to modulate both osteoblastic and osteoclastic functions by inhibiting mineral crystal growth both *in vivo* and *in vitro*.[35] Taken together, these results suggest that OPN may have a role as a shared cytokine in the pathogenesis of NAFLD and osteoporosis.

Osteoprotegerin

Osteoprotegerin (OPG), a member of the TNF-receptor superfamily is a decoy receptor for the receptor activator for nuclear factor-κB (RANK) ligand and TNF-related apoptosis-inducing ligand.[36, 37] OPG is not only one of the major player in the balance between bone formation and bone resorption, but it also has important metabolic effects. In this regard, evidence suggests that obesity and insulin resistance are associated with reduced OPG concentrations.[38] In addition, serum OPG levels have been associated with several components of the MS in apparently healthy women.[39] In a pilot study, we have shown that concentrations of OPG are significantly lower in patients with definite NASH and borderline NASH compared with healthy individuals.[40] The area under the ROC curve for distinguishing between steatohepatitis (definite NASH plus borderline NASH) from healthy controls using OPG was 0.82 in our sample.[40] Although the potential usefulness of reduced serum OPG concentrations for identifying patients with the most severe forms of NAFLD needs to be corroborated by independent studies, these preliminary data could prompt further research on the role played by OPG in the crosstalk between the NAFLD spectrum and the bone. In the skeletal system, OPG exerts osteoprotective effects by inhibiting osteoclast differentiation and activation and promoting osteoclast apoptosis.[37] Nevertheless, in relation to bone disease in the clinical setting, the association between OPG, bone density and fragility fractures remains controversial. In men, increased OPG levels have been associated with higher BMD of the lumbar spine,[41, 42] whereas other studies found a negative correlation[43, 44] or no correlation at all.[45] In women, both low[46] and high[47] OPG levels have been associated with vertebral fractures. Given these discrepant findings, future studies should clarify the relationship between serum OPG and BMD in NAFLD patients and to evaluate the role of OPG in bone status in this group of patients.

Osteocalcin

Osteocalcin is a 49-amino acid bone matrix noncollagen protein expressed mainly by osteoblasts. It acts as a specific marker of bone formation and it is involved in calcium homeostasis.[48] Recently, osteocalcin has been recognised as a bone-derived hormone to regulate energy metabolism.[48, 49] Osteocalcin−/− knockout mice exhibits

glucose intolerance, increased fat mass, insulin resistance, decreased expression of insulin target genes in liver and muscle and decreased adiponectin gene expression in the adipose tissue.[50] In contrast, administration of recombinant osteocalcin increases insulin secretion, decreased blood glycemia and contrasts the development of obesity in experimental studies.[51] In the clinical setting of chronic liver diseases, a pilot study reported decreased serum OCN levels in patients with primary biliary cirrhosis and in those with chronic alcoholic liver disease.[52] In a study of 28 obese patients, Fernández-Real *et al*.[53] have shown that circulating OCN concentrations are negatively associated with blood markers of liver injury and liver disease, including alanine transaminase (ALT) and aspartate transaminase (AST). In addition, the changes in ALT levels following weight loss in obese individuals were linearly associated with changes in OCN concentrations.[53] Recently, we found that patients with biopsy-proven NAFLD have significant reductions in serum OCN concentrations (vs. matched controls) which were weakly, but significantly associated with the extent of hepatocyte ballooning, independent of insulin resistance and the MS.[54] In studies of bone metabolism, serum OCN levels have emerged as a sensitive marker of bone production;[55] of note, reduced OCN concentrations have been associated with postmenopausal osteoporosis.[56] Therefore, it is feasible that reduced OCN observed in patients with NAFLD could be one of the potential links between the presence of hepatic steatosis and a reduced BMD.

Fetuin-A

Fetuin-A, also known as alpha-2-HS (Heremans-Schmid) glycoprotein, is produced in the liver and can be found in relatively high concentrations in human serum.[57] Fetuin-A is involved in the molecular mechanisms of insulin resistance by inhibiting the insulin receptor tyrosine kinase in skeletal muscle and hepatocytes, ultimately resulting in insulin resistance in these target tissues.[57] The fetuin-A null mice are insulin-sensitive and resistant to weight gain following a high fat diet.[58] In humans, higher fetuin-A levels associate with obesity and insulin resistance in the general population and are independently associated with the MS and its components.[59, 60] Moreover, high fetuin-A levels also associate with NAFLD and short-term diet and exercise interventions result in declines in serum fetuin-A levels which parallel the improvement in NAFLD and the reduction in body weight.[61–63] Fetuin-A has also recently emerged as a mineral carrier protein and a systemic inhibitor of pathological mineralisation.[64] In this regard, several lines of evidence have also indicated that circulating fetuin-A levels could be associated with markers of bone turnover.[65, 66] Animal studies have shown that the fetuin-A null mouse displays growth plate defects, increased bone formation with age and enhanced cytokine-dependent osteogenesis.[67] In addition, Chailurkit *et al*.[66] showed that circulating fetuin-A is related to bone mass and bone resorption markers in elderly women. It can be therefore hypothesised that alterations in circulating fetuin-A levels can lead to deficits in bone mineral density in subjects with NAFLD; whether these changes are of clinical significance and possibly influencing fracture risk over the lifetime in these subjects deserve further investigation.

Vitamin D, the Osseous System and NAFLD

Besides its role in calcium and bone metabolism, vitamin D exerts multiple pleiotropic effects in many tissues (e.g. antiproliferative, prodifferentiative and immunomodulatory actions).[68] In addition, vitamin D receptors are present in several cell types, including pancreatic beta cells.[69] The main source of vitamin D is endogenous generation of cholecalciferol (vitamin D3) from 7-dehydrocholesterol in skin keratinocytes through exposure to medium-wavelength ultraviolet light (UVB) from the sun.[70] Some vitamin D also derives from dietary intake and vitamin D supplements. Vitamin D is either stored in adipose tissue or converted to 25-hydroxyvitamin D (25(OH)D or calcidiol) in the liver.[68] The serum concentration of 25(OH)D, the major circulating form of vitamin D, is considered to reflect the total production of vitamin D from both endogenous and exogenous sources and constitutes the best clinical measure of vitamin D stores.[68] The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D or calcitriol), is primarily generated in the kidney from 25(OH)D. 1,25(OH)2D functions as a steroid hormone regulating the transcription of numerous genes.[71] A number of clinical studies have shown that vitamin D deficiency can play a role in a number of different metabolic derangements, including type-1 and type-2 diabetes, obesity, dyslipidemia, hypertension and the MS.[72–74] Recent years have also witnessed a significant scientific interest into the potential role played by vitamin D in liver pathophysiology and NAFLD. An experimental study by Nakano *et al*.[75] showed that phototherapy may be

a good complementary therapy for NASH because of its regulation on vitamin D3. Roth *et al*.[76] recently reported that vitamin D deficiency in obese rats exacerbates NAFLD and increases the hepatic expression of genes involved in inflammatory pathways. With regard to clinical studies, Targher *et al*.[77] reported that NAFLD patients have a marked reduction in serum 25(OH)D levels compared with controls; this decrease was closely associated with the histopathological features of NAFLD. These results were independently confirmed by Barchetta *et al*.,[78] who showed that low 25(OH)D levels were associated with the presence of ultrasound-diagnosed NAFLD independently from metabolic syndrome, diabetes and insulin resistance. However, Katz *et al*.[79] did not confirm an independent association between vitamin D status and suspected NAFLD after adjusting for obesity in adolescents. Future studies are needed to clarify whether the vitamin D status should be routinely checked in NAFLD patients and deficiency corrected if present. In addition, the consequences of addressing hypovitaminosis D on both liver histology and bone health in this group of patients need to be determined in longitudinal clinical trials.

Limited Physical Activity in the Link Between NAFLD and Reduced BMD

Physical activity is an important predictor of health in older adults, although the mechanisms remain poorly understood. Physical activity is currently considered as a cornerstone for the prevention of both NAFLD[80] and osteoporosis.[81] Various training studies have shown that exercise reduces levels of visceral adiposity in the absence of weight loss, which might be a key mechanism in protection from the MS and NAFLD.[82] Therefore, lifestyle modifications with weight loss and exercise are regarded as first line treatments in patients with hepatic fat accumulation. Besides its positive effects on the cardiometabolic function, exercise is thought to strengthen skeletal bones through gravitational forces or muscle pull producing strains within the skeleton.[83] If a strain is detected as greater than the optimum strain, then bone formation will occur. There is strong evidence that aerobic and strength/resistance activities are effective forms of weight-bearing exercise to minimise bone loss and osteoporosis.[83] Consistent data from randomised controlled trials show that exercise training programmes can prevent or reverse almost 1% of bone loss per year in both pre and postmenopausal women.[84] These observations point to the direction that a reduction in physical activity could lead both at a decrease in BMD at one hand, but may also metabolically affect tissues, such as the liver, thus resulting in insulin resistance and ectopic fat accumulation.

Conclusions

Besides the well-described links between inflammatory bowel diseases, coeliac disease and osteoporosis,[85–87] recent studies have also suggested that NAFLD may act as a risk factor for a reduced BMD in both children and adult populations. In this review, various pathophysiological mechanisms – including a variety of soluble mediators (Table 1) – that can play a role in the link between NAFLD and the osseous system have been discussed. The list of identified mechanisms is already long and still growing, although future research should aim to confirm the physiological relevance of NAFLD in relation to the risk of osteoporosis-related bone fractures. A better understanding of the mechanisms that link bone metabolism and the liver may open a new frontier to fight two highly prevalent conditions like NAFLD and osteoporosis.

Table 1. Soluble mediators involved in the link between NAFLD and osteoporosis

Molecule	Purported role in NAFLD	Purported role in osteoporosis	Change in NAFLD	References
Tumour necrosis factor (TNF)-α	Key mediator of hepatic inflammation	Amplifies osteoclastogenesis and inhibits bone formation	↑	[21, 22]
Osteopontin	Molecular mediator of hepatic inflammation and fibrosis	Modulates both osteoblastic and osteoclastic functions by inhibiting mineral crystal growth	↑	[30]

Osteoprotegerin	Reduced levels of this molecule are associated with obesity and insulin resistance	Major player in the balance between bone formation and bone resorption	↓	[40]
Osteocalcin	Increases insulin secretion	Marker of bone production	↓	[52–54]
Fetuin-A	Inhibits the insulin receptor tyrosine kinase and is involved in insulin resistance	Mineral carrier protein and systemic inhibitor of pathological mineralization	↑	[61–63]

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