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Original Article

Bone Metabolism and Gonad Function in Male Patients Undergoing Liver Transplantation: A Two-Year Longitudinal Study

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Abstract. Osteodystrophy is a major complication of end-stage liver disease, especially in postmenopausal women. Our aim in this study was to evaluate bone metabolism and gonad function in men undergoing orthotopic liver transplantation (OLTx). Twenty-three consecutive men (mean age 48 ± 13 years) evaluated for OLTx were studied, assessing the following parameters at baseline and 3, 6, 12 and 24 months after OLTx: lumbar spine (L2–L4) bone mineral density (BMD), parathyroid hormone (PTH), osteocalcin (BGP), 25-hydroxyvitamin D (25OHD), free testosterone (FT) and gonadotropins (FSH, LH). At baseline, 12 patients (52%) had a *T*-score < -2.5 SD and the mean BMD was 0.806 ± 0.11 g/cm^2 (range 0.470–1.045 g/cm^2). The BMD was lower 3 months after OLTx and significantly higher 12 and 24 months after OLTx. A significant increase in serum BGP was observed at 6, 12 ($p < 0.05$) and 24 months ($p < 0.005$) after OLTx. The mean serum PTH level was 26.6 ± 3.1 pg/ml at baseline and increased significantly at 12 and 24 months (to 49.4 ± 9.9 and 61.2 ± 10.1 pg/ml, respectively; $p < 0.05$). 25OHD serum levels were low at baseline and returned to the normal range after 12 and 24 months (baseline, 8.73 ± 1.54 ng/ml; 12 months, 16.4 ± 2.6 ng/ml; 24 months, 17.67 ± 3.1 ng/ml; $p < 0.05$). FT was significantly lower at baseline than in a group of 10 healthy controls (5.09 ± 10.99, vs 10.3 ± 1.1 pg/ml; $p < 0.0001$). After OLTx a significant increase in FT was recorded at 6, 12 ($p < 0.05$) and 24 months ($p < 0.005$). FT was not correlated with BMD, however. After OLTx an increase in FSH and LH was observed (but failed to reach statistical significance) at 3 and 6 months, followed by a slight reduction at 12 and 24 months. Thus a high proportion of men with end-stage liver disease do have osteoporosis. After OLTx, an early recovery of gonad function is observed, followed by an increase in bone mass, which occurs from the sixth month onward.

Keywords: Bone metabolism; Gonad function; Liver transplantation; Osteoporosis

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Introduction

Osteodystrophy is a major complication of end-stage liver disease, especially in postmenopausal women [1,2]. Previous studies have indicated that there is no correlation between bone mineral density (BMD) and the etiology of the liver disease in cirrhotic patients undergoing orthotopic liver transplantation (OLTx) [3,4].

In women, estrogen deficiency is the most important factor for the onset of postmenopausal osteoporosis. In cirrhotic males, androgen deficiency is rather common [5–7]. Moreover, a relationship between hypogonadism and osteoporosis has been demonstrated in cirrhotic men [8,9], whose hypogonadism is probably caused by the combined effect of a central (hypothalamus–pituitary) and gonad failure [6,10]. A highly significant and quantitatively large increase in bone turnover in the first 3 months after liver transplantation was demonstrated by Vedi et al. [11] in a careful histomorphometric analysis. Previous studies concerning the effect of liver

Table 1. Recent longitudinal studies of BMD after OLTx

First author	Year	Ref. no.	No. of patients	M/F	Follow-up (years)	Site of measurement	Outcome
Meys	1994	12	16	10/6	1	Lumbar spine and total body	29% prevalence of vertebral fracture in grafted patients
Valero	1995	13	120	78/42	1	Lumbar spine	BMD significantly lower in grafted patients compared with controls ($p<0.05$)
Abdelhadi	1995	14	9	6/3	1	Lumbar spine, proximal femur, distal radius	BMD decreased during the first 6 months and still below pre-OLTx values at 1 year
Riemens	1996	15	53	20/33	1	Lumbar spine and hip	BMD at lumbar spine decreased during the first 3 months, and then no changes; BMD at hip continued to fall during the first year
Floreani	1998	3	54	33/21	1	Lumbar spine	Significant reduction in BMD at 3 months, increase in BMD thereafter
Hussaini	1999	16	55	23/33	2	Lumbar spine and femoral neck	BMD reduction at 1 month up to 12 months; BMD reduction in femoral neck only after 6–9 months
Feller	1999	17	28	14/14	7	Lumbar spine	Significant increase in BMD at 7 years compared with 3 months after OLTx
Crosbie	1999	18	12	5/7	2	Lumbar spine and femoral neck	BMD significantly lower at the lumbar spine at 3 months, and at the femoral neck at 6 months; increase in BMD thereafter
Guardiola	1999	19	55	55/0	2	Lumbar spine	Bone loss at 3 months after OLTx
Hamburg	2000	20	66	19/47	5-15	Lumbar spine and hip	At lumbar spine, increasing of BMD during the second year; at hip, decreasing BMD during the second year
Ninkovic	2000	21	37	20/17	3 months	Lumbar spine and femoral neck	27% of new fractures at 3months
Giannini	2000	22	46	35/11	2	Lumbar spine and hip	BMD at hip decreased during the first year; partial recovery for both spine and femur after 24 months

transplantation on bone density have given conflicting results, but the majority of these studies did not analyze men and women separately (Table 1). The purpose of the present study was therefore to evaluate bone metabolism in males with end-stage liver disease before and after OLTx.

Materials and Methods

Patients

Twenty-three consecutive cirrhotic men evaluated for OLTx between 1996 and 1999 were included in the study. The mean age was 48.2 ± 12.8 years (range 20–61 years). All patients were asymptomatic for bone loss, and none were taking calcium and/or vitamin D supplements. There were 6 cases of alcoholic cirrhosis, 14 of viral cirrhosis, 1 of autoimmune hepatitis, 1 of primary sclerosing cholangitis, and 1 of Wilson's disease (Table 2).

Ethical approval was obtained from the hospital's ethics committee and informed consent was obtained from each patient prior to their inclusion in the study.

None of the patients suffered from renal failure. None of the patients was treated with steroids before OLTx, except for one patient (patient 15, Table 2) with autoimmune hepatitis, who had been taking prednisolone (7.5 mg daily) for 10 years before joining the study. The patients were not given hormone replacement. All patients were asymptomatic for bone disease before and after OLTx. All patients experienced ascites before transplantation and were on long-term treatment with diuretics. At the time of their inclusion in this study all of them had decompensated liver disease with ascites. They all had a normal liver function after liver tranplantation; in particular, we did not observe any episode of early graft dysfunction, defined as the presence of at least one of the following factors between 2 and 7 days after liver transplantation: serum bilirubin >10 mg/dl, prothrombin time $\geqslant 17$ s, and hepatic encephalopathy. After OLTx, patients were treated with the standard immunosuppressive treatment, including prednisone up to the third month after OLTx. The dose of prednisone was initially 200 mg/day and was tapered to 20 mg/day by the third month. Rejections were treated with 3 g of methylprednisolone administered intravenously over 3 days. Cyclosporin A (CyA) was the long-term therapeutic regimen for 19 patients, tacrolimus for 4. CyA was administered intravenously at a dosage of 2 mg/kg per day up to 7 days after surgery; thereafter, CyA was given by mouth at doses that would maintain serum levels of 150–350 μg/l (RIA; Incstar, Stillwater, MN).

Bone Assessment

The following parameters were assessed at baseline and 3, 6, 12 and 24 months after OLTx: bone mineral density (BMD) in the lumbar spine (L2–L4); parathyroid hormone (PTH), osteocalcin (BGP), 25-hydroxyvitamin D (25OHD), serum calcium corrected for serum albumin

Table 2. Characteristics of the study group

Patient no.	Age (years)	Etiology of cirrhosis	BMI (kg/m^2)	Body weight (kg)	Child–Pugh stage
1	57	PSC	26.8	67	C10
2	56	HBV-HCV	20.8	93	C10
3	22	HBV-HDV	20.4	59	C11
4	47	HCV	39.2	80	C10
5	41	Alcohol	25.2	66	C12
6	53	HBV	26	65	C10
7	58	HBV-HCV	33.4	75	C11
8	53	HCV	31	72	C10
9	56	HBV	22.5	61	B8
10	20	Wilson	19.2	57	C10
11	55	HCV	20.8	60	B9
12	58	HCV	25	66	C10
13	58	Alcohol	32.4	74	B9
14	38	Alcohol	29.7	73	C10
15	27	Autoimmune hepatitis	27.7	72	C11
16	54	Alcohol	20.8	60	C11
17	30	Alcohol	37.1	79	C10
18	61	HCV	25.7	63	C10
19	60	Alcohol	24.4	65	C10
20	38	HBV	41.5	84	C10
21	54	HCV	40.4	86	C11
22	54	HCV	37.6	80	C10
23	59	HCV	35.08	77	C11

PSC, primary sclerosing cholangitis; HBV, HCV and HDV, hepatitis B, C and D virus; Wilson, Wilson's disease.

(Ca), phosphorus (P), serum albumin, serum creatinine, free testosterone (FT), serum gonadotropins (FSH, LH).

BMD was assessed by dual-energy X-ray absorptiometry (DXA; Hologic QDR 4500 C, Waltham, MA). The manufacturer's reference database was obtained by measuring more than 500 healthy male volunteers at the University of California (San Diego, CA) and at other QDR-1000 installations across the United States. Reference data collected on Hologic systems in Europe, including Italy, have demonstrated a close correlation with US values. In our hands, the long-term (1 year) coefficient of variation in vitro with a Hologic phantom is 0.44%; the short-term (1 week) coefficient of variation in vivo, with five consecutive measurements performed in 10 healthy volunteers, is 1.2%.

PTH was tested by IRMA (Allegro Intact, Nichols Institute, S. Juan Capistrano, CA). This method detects the biologically intact 1–84 amino acid chain of PTH. The normal range is 10–55 ng/l. The intra-assay coefficient of variation (CV) was 3.6% and the inter-assay CV 5.8%. BGP was assayed by IRMA (Cis BioInternational, Italy). The normal range is 4–26 μg/l. The intra-assay CV was 3.1% and inter-assay Cv 5.7%. 25OHD was determined by RIA (Incstar, Stillwater, MN). The normal range is 25–150 nmol/l. The intra-assay CV was 5.1% and the inter-assay CV 9.7%. Calcium, phosphorus, albumin, and creatinine were measured by automated standard laboratory methods (Olympus AU 5000 autoanalyzer). Calcium corrected for albumin (Ca) was obtained according to the following formula:

$$\text{Corrected Ca} = \text{total calcium (mg/dl)} - \text{albumin (g/dl)} + 4$$

FT was determined by RIA (Biochem Immunosystem, Milan, Italy). The normal range is 14–41 pg/ml. The intra-assay CV was 4.3% and the intra-assay CV 5.5%. FSH and LH were assayed by IRMA (MAJA Clone, Serono Diagnostics, Turin, Italy). The normal range for FSH is 1–14 mU/ml. The intra-assay CV was 4.7% and the inter-assay CV 5.4%. The normal range for LH is 1.5–9.2 mU/ml. The intra-assay CV was 10% and the inter-assay CV 12.5%.

Statistical Analysis

Results were expressed as the mean ± standard error. Student's *t*-test for paired data and the linear regression test were used, as appropriate.

Results

Twelve patients (52%) had a BMD *T*-score < -2.5 SD, compatible with the WHO definition of osteoporosis. The mean BMD at baseline was 0.806 ± 0.11 g/cm^2 (range 0.470–1.045 g/cm^2). Three months after OLTx a 2.6% drop in BMD was observed (from 0.806 ± 11 to 0.785 ± 0.14 g/cm^2), though this was not statistically significant. However, a significant rise in BMD was observed after 12 months ($p < 0.05$) and 24 months ($p < 0.01$), with a BMD gain of 3.9% and 9.8% respectively versus baseline (Fig. 1). Seven patients did not show any significant change in BMD after OLTx, whereas 6 of 23 patients showed an increase in BMD ranging from 7% to 61% with respect to the lowest value detected at the 3 months after OLTx. No correlation was

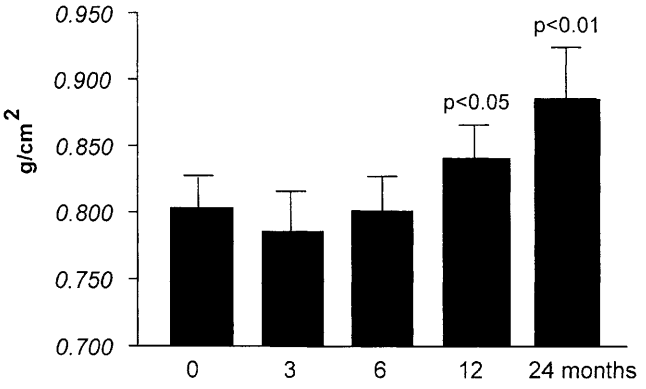

Fig. 1. Bone mineral density before and after liver transplantation.

found between cumulative dose of methylprednisolone and changes in BMD after OLTx.

Serum PTH was 26.6 ± 3.1 pg/ml at baseline, increasing progressively after OLTx by months 6 ($p < 0.05$), 12 ($p < 0.05$), and 24 ($p < 0.01$). Similarly, BGP was 5.01 ± 0.78 ng/ml at baseline and showed a significant increase by months 6, 12 and 24 after OLTx ($p < 0.05$, $p < 0.05$, and $p < 0.01$, respectively). The same situation was observed for 25OHD, which was 8.7 ± 1.54 ng/ml at baseline and increased significantly at the same intervals after OLTx. The serum PTH, BGP and 25OHD levels at baseline and after OLTx are summarized in

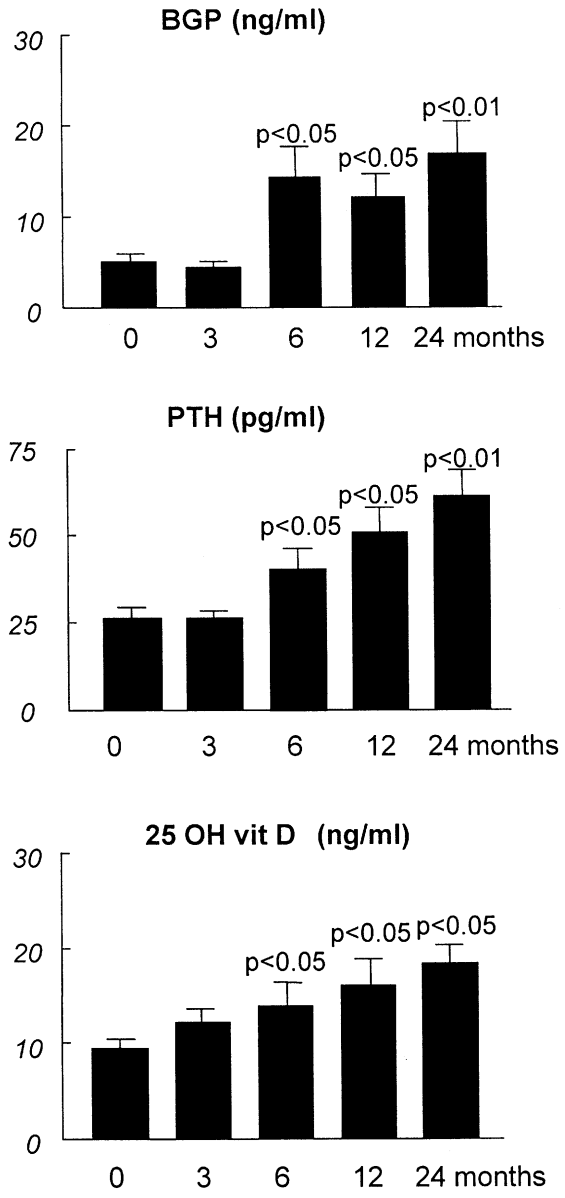

Fig. 2. Serum BGP, PTH and 25OHD levels before and after liver transplantation.

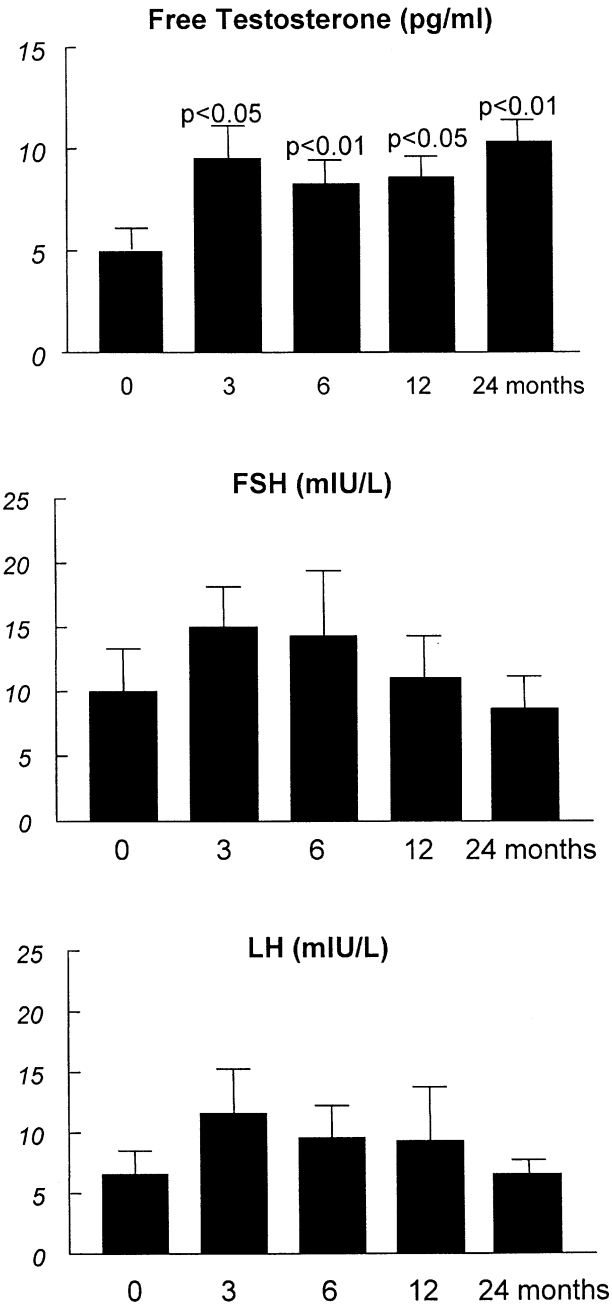

Fig. 3. FT, FSH, and LH levels before and after liver transplantation.

Table 3. Serum calcium, phosphorus, albumin and creatinine levels recorded during the study

Parameter	Normal range	Baseline	3 months	6 months	12 months	24 months
Calcium	2.10–2.6 mmol/l	2.07 ± 0.19	2.19 ± 0.11	2.21 ± 0.10	2.22 ± 0.21	2.24 ± 0.21
Phosphorus	0.87–1.45 mmol/l	1.14 ± 0.34	1.27 ± 0.33	1.24 ± 0.21	1.18 ± 0.24	1.08 ± 0.18
Albumin	39.9–53 g/l	33.55 ± 16	40.14 ± 5.89	42.65 ± 2.85	40.88 ± 3.60	42.62 ± 2.62
Creatinine	62–115 μmol/l	82.42 ± 19.59	91.77 ± 0.18	91.26 ± 14.07	101.7 ± 21.8	117.7 ± 39.09

Fig. 2. FT serum levels were significantly lower at baseline than in healthy controls (5.09 ± 0.99 vs 10.3 ± 4.4 pg/ml, $p < 0.05$). No correlation was found between FT and age. A significant increase in FT serum levels was observed after OLTx, i.e., +79% at 3months ($p < 0.05$), 65% at 6 months ($p < 0.01$), 68% at 12 months ($p < 0.05$), and 102% at 24 months ($p < 0.01$). A negative correlation was found between FT and LH at 6 months ($r^2 = 0.49$, $p < 0.02$) (Fig. 3). The mean ± SE was within the normal range for FSH and LH at baseline (9.8 ± 3.4 and 6.44 ± 2.04 mIU/ml, respectively). After OLTx an increase was observed, which was not statistically significant, at 3 and 6 months, followed by a slight decline at 12 and 24 months (Fig. 3). Calcium and phosphorus levels did not change during the study. Creatinine increased significantly at 3 months post-OLTx and even more so at 24 months ($p < 0.01$). Serum albumin, which was 33.5 ± 16 g/l at baseline, increased significantly after 6 months post-OLTx, confirming stable values at the subsequent intervals (Table 3).

Discussion

Our study group comprised 23 men undergoing OLTx, whose graft survived in the long term. Our findings indicate that more than 50% of cirrhotic men undergoing OLTx have osteoporosis; a similar rate is reported in the literature in series that do not distinguish between men and women. Three months after OLTx there was a reduction in BMD compared with baseline values, confirming other reports [14,15,18,19]. This decrease in BMD was probably related to steroid therapy and immobility. Another important factor which might influence bone loss after liver transplantation is vitamin D receptor gene polymorphism. In fact, patients carrying the bb genotype are, to some extent, protected against post-transplantation bone loss [19]. It must be pointed out that all our patients before OLTX had ascites, which could have affected the accuracy of the lumbar spine measurement. In our previous study [23], the effect on BMD of the progressive increase in depth of a water bath on a self-made phantom built with three femoral heads was examined. BMD progressively decreased with increasing water bath depth: when the distance between phantom and water surface reached 20 cm, approximately the same mean value observed between the lumbar spine and the superior limit of ascites in decompensated liver disease, BMD decrease to 12.1% below the value observed with a minimal water depth. The first control at 3 months after OLTx was carried out in patients without ascites. Thus the bone loss observed in this period could be underestimated. In contrast, BMD values obtained at 6, 12 and 24 months after OLTx could be overestimated compared with the basal value, but give suitable results when compared with the 3 month values, due to the absence of ascites during this period.

The increase in BGP serum levels after OLTx suggests a renewal of osteoblastic activity, which may be due to the increase in bone turnover, partly related to cyclosporin treatment. A positive correlation between cumulative intake of CyA and bone alkaline phosphatase as well as CyA serum levels and hydroxyproline excretion was observed by Giannini et al. [22] in a longitudinal study of 21 liver transplant patients. The increase in bone mass might also be due to an increase in physical activity, though the majority of patients showed a slow return to physical activity starting 5–6 weeks after OLTx. The improvement in vitamin D status together with the increase in FT might contribute to the increase in bone mass. 25OHD significantly increases after OLTx, probably due to an improvement of hepatic 25-hydroxylation of vitamin D.

We found significantly lower serum levels of FT in our patients than in healthy controls. Several pathogenetic mechanisms play a part in this reduction, including a direct toxic effect of alcohol on gonad function, dysregulation of the hypothalamus–pituitary–gonadal axis, and increased peripheral aromatization of androgens to estrogens [6,8]. In addition, the increase in SHBG (commonly seen in chronic liver failure) leads to a stronger binding with testosterone, with a consequent decline in the free, biologically active proportion [24,25]. The normalization of liver function following OLTx is characterized by an early rise in FT, though the mean values in our patients are lower than in other series [25,26]. In a recent study, Jin et al. [27] did not find any significant change in either total or free testosterone in a small number of patients 3–6 months after OLTx. Similarly, serum gonadotropins showed a slight increase soon after OLTx but this failed to reach statistical significance, probably due to the small size of our sample. An indirect sign of the recovery of LH secretion induced by testosterone is the negative correlation between FT and serum LH 6 months after OLTx.

Our data confirm previous findings on the behavior of PTH after OLTx, and particularly the significant increase at 6 months [3,14,17,28]. The increase in PTH may suggest secondary hyperparathyroidism, due to cyclosporin-induced renal function impairment. However,

though serum creatinine was significantly higher in our patients after OLTx, we failed to demonstrate any correlation between serum PTH and creatinine. The increase in creatinine levels might be independent of the renal function impairment, but could also be due to the improvement in muscle function secondary to the return to a normal vitamin D status. In fact, in a previous study, Bertoli et al. [29] demonstrated that the increase in serum creatinine in patients with chronic renal failure treated with calcitriol was due not to impaired kidney function but to improved muscle function. Secondary hyperparathyroidism might also be due to a reduced renal synthesis of 1,25-dihydroxyvitamin D, despite the increase in 25OHD, due to a cyclosporin-induced deterioration in renal function. We can neither confirm nor refute this hypothesis because we did not test 1,25-dihydroxyvitamin D in our patients.

In conclusion, a high proportion of men with end-stage liver disease have osteoporosis. Sex hormone changes in these patients are related to liver dysfunction. After OLTx, an early recovery of gonad function is observed, followed by an increase in bone mass, which occurs from the sixth month onward. This behaviour suggests that the presence of osteoporosis, at least in men, does not constitute a contraindication to liver transplantation. Hormone replacement therapy or other alternative therapies, such as bisphosphonates, should be considered for patients with low bone mass and insufficient recovery of BMD after OLTx.

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