Published in final edited form as:

Liver Int. 2012 February ; 32(2): 287–296. doi:10.1111/j.1478-3231.2011.02638.x.

Changes in vitamin D binding protein and vitamin D concentrations associated with liver transplantation

Peter P. Reese^{1,2,3}, Roy D. Bloom¹, Harold I. Feldman^{1,2,3}, Ari Huverserian⁴, Arwin Thomasson², Justine Shults², Takayuki Hamano², Simin Goral¹, Abraham Shaked⁵, Kimberly Olthoff⁵, Michael R. Rickels⁶, Melissa Bleicher¹, and Mary B. Leonard^{2,7}

¹ Renal Division, Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA

² Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA, USA

³ Leonard Davis Institute of Health Economics, University of Pennsylvania, Philadelphia, PA, USA

⁴ School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, USA

⁵ Transplant Institute, Department of Surgery, University of Pennsylvania, Philadelphia, PA, USA

⁶ Endocrine Division, Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA

⁷ Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

Abstract

Background—Vitamin D deficiency is associated with fractures, infections and death. Liver disease impairs vitamin D and vitamin D binding protein (DBP) metabolism.

Aims—We aimed to determine the impact of liver transplantation on vitamin D, particularly on DBP and free vitamin D concentrations.

Methods—Serum 25(OH)D, 1,25(OH)₂D and DBP concentrations were measured in 202 adults before liver transplantation and 3 months later in 155. Free vitamin D concentrations were estimated from these values. Risk factors for 25(OH)D deficiency (<20 ng/ml) and low 1,25(OH)₂D (<20 pg/ml) were examined with logistic regression, and changes in concentrations following transplantation with linear regression.

Results—Pretransplant, 84% were 25(OH)D deficient, 13% had 25(OH)D concentrations <2.5 ng/ml, and 77% had low 1,25(OH)₂D. Model for end-stage liver disease score ≥ 20 ($P < 0.005$) and hypoalbuminemia ($P < 0.005$) were associated with low 25(OH)D and 1,25(OH)₂D concentrations. Following transplantation, 25(OH)D concentrations increased a median of 17.8 ng/ml ($P < 0.001$). Albumin increased from a median of 2.7 to 3.8 g/dl ($P < 0.001$) and DBP from 8.6 to 23.8 mg/dl ($P < 0.001$). Changes in total 25(OH)D were positively and independently

Correspondence Peter P. Reese, MD, MSCE, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, School of Medicine, 917 Blockley Hall, 423 Guardian Drive, Philadelphia, PA 19104-6021, USA, Tel: +617 699 8848, Fax: +215 615 0349, peter.reese@uphs.upenn.edu.

An oral presentation based on this work was given at the American Transplant Congress in San Diego in May 2010.

Statement of conflicts of interest: The authors report no conflicts of interest relevant to this study.

associated with changes in DBP ($P < 0.05$) and albumin ($P < 0.001$). Free 25(OH)D concentrations rose from 6.0 to 9.7 pg/ml ($P < 0.001$). In contrast, total 1,25(OH)₂D concentrations rose only by 4.3 pg/ml ($P < 0.001$) and free 1,25(OH)₂D concentrations declined ($P < 0.001$).

Conclusions—Serum total and free 25(OH)D and DBP concentrations rose substantially following transplantation, while 1,25(OH)₂D concentrations showed modest changes and free 1,25(OH)₂D decreased. Studies of the effects of vitamin D status on diverse transplant complications are needed.

Keywords

liver transplantation; metabolism; vitamin D; vitamin D binding protein

Vitamin D deficiency is associated with mortality, cardiovascular disease, fractures, poor physical function, malignancy, infection and diabetes (1–5). Circulating vitamin D binds to albumin and vitamin D binding protein (DBP). Less than 1% of vitamin D is unbound and the biological activity of vitamin D may depend primarily on this free fraction (6). Individuals with advanced liver disease often have low concentrations of total vitamin D, which is attributed to malabsorption, failure of liver cells to 25-hydroxylate calciferol to 25(OH) vitamin D [25(OH)D], and decreased hepatic synthesis of albumin and DBP (7–11). On the other hand, the few studies that directly measured free vitamin D among patients with liver disease suggested that the low concentrations of carrier proteins in these patients may lead to normal concentrations of free vitamin D (12). Liver transplantation corrects many of the pathophysiologic mechanisms that impair vitamin D status. However, the impact of liver transplantation on vitamin D, particularly on DBP and free vitamin D, has not been established.

Liver transplant recipients experience a high burden of comorbidities associated with vitamin D deficiency, such as fractures, infections and diabetes (13, 14). Furthermore, a recent study suggested that vitamin D deficiency may contribute to cellular rejection of the hepatic allograft (7). Therefore, an assessment of changes in vitamin D status among liver transplant recipients may provide insight into the possible roles of vitamin D in promoting comorbidities before and after transplantation.

Prior studies of vitamin D metabolism in liver transplantation were limited by small size (most had <50 patients), lack of measurement of DBP, and did not estimate free vitamin D concentrations (11, 15–20). Many studies were also limited to measures of 25(OH)D, despite the high prevalence of renal disease among liver transplant recipients,(21) and evidence that 1,25 (OH)₂D exerts effects on multiple tissues (5, 22). Lastly, black race is associated with significantly greater risk of vitamin D deficiency,(5) but most prior studies of vitamin D in liver disease had no black participants and only one addressed the impact of race (11).

The objectives of this study were to (i) identify risk factors for low 25(OH)D and 1,25(OH)₂D concentrations in a racially diverse population of liver disease patients prior to transplantation; (ii) characterise DBP concentrations and estimates of free vitamin D before

and after transplantation; and (iii) identify characteristics associated with increases in 25(OH)D and 1,25 (OH)₂D concentrations after transplantation.

Materials and methods

Subjects

This prospective cohort study was conducted in adults undergoing liver transplantation between June 2006 and October 2008 at the Hospital of the University of Pennsylvania (latitude 40°N). These patients were enrolled in the Gene Expression and Regenerative Pathways in Donor Livers study and had serum collected in the hours prior to transplantation and 3 months later. Prior publications in this cohort are limited to microarray profiles in 21 grafts (23).

All liver transplant recipients received intravenous methylprednisolone at surgery followed by a rapid taper of oral prednisone. By 3 months, the majority was maintained on a two or three drug immunosuppressive regimen that included tacrolimus and prednisone. All recipients were prescribed a daily multivitamin (brand 'Rugby'; Rugby Laboratories, Duluth, GA, USA) containing 400 IU of cholecalciferol. Medications were reviewed and adherence emphasised during meetings with a transplant pharmacist during the first month after transplantation.

This study was approved by the University of Pennsylvania Institutional Review Board and was conducted in accordance with the Helsinki Declaration of 1975. Informed consent was obtained from all participants.

Vitamin D measurement and categorisation

Serum 25(OH)D (ng/ml) and 1,25(OH)₂D (pg/ml) concentrations were quantified by radioimmunoassay with I¹²⁵-labelled tracer (Heartland Diagnostics, Aimes, IA, USA) (24). The intra-assay coefficient of variation for 25(OH)D was 2.2%, and for 1,25(OH)₂D was 7–11% (25, 26). The 25(OH)D assay had a lower limit of detection of 2.5 ng/ml therefore 1.5 ng/ml was substituted for 25(OH)D values below this limit. The 1,25(OH)₂D assay had a lower limit of detection of 2.5 pg/ml; 1.5 pg/ml was substituted for values below this limit. The vitamin D assays were completed more than 2 years after the blood specimens were obtained and the results were not provided to the treating physicians.

Because of evidence that 25(OH)D concentrations are lower in black patients, we presented 25(OH)D results both overall and stratified by black/non-black race in our tables.

Vitamin D binding protein was measured using a commercial enzyme linked immunosorbent assay kit (Catalog Number DVDBP0; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. All DBP samples were measured in duplicate and the intra-assay and inter-assay CV was 5.1–7.4%. The DBP assay has a lower limit of detection of 3.12 mg/dl therefore 1.5 mg/dl was substituted for DBP values below this limit.

Free 25(OH)D was estimated using the measured concentrations of total 25(OH)D, DBP, and albumin based on the affinity binding constants between 25(OH)D and these proteins. A similar approach was used to estimate 1,25(OH)₂D. These estimating equations were derived in prior studies by Bikle *et al.*, in which the free fractions of 25(OH)D and 1,25(OH)₂D were measured using centrifugal ultrafiltration (27, 28). Bikle's group reported a correlation of the measured free fraction to the calculated value of $r = 0.925$ ($P < 0.0001$) for 25(OH)D, and $r = 0.621$ ($P < 0.001$) for 1,25(OH)₂D (27, 28).

Ascertainment of participant characteristics

Liver transplant patient characteristics and laboratory results were obtained from the electronic medical record. Model for end-stage liver disease (MELD) score was calculated as $[10 (0.957 \ln(\text{serum creatinine in mg/dl}) + 0.378 \ln(\text{total bilirubin in mg/dl}) + 1.12 \ln(\text{international normalised ratio}) + 0.643]$ (29). Serum calcium concentrations were corrected for serum albumin as measured calcium + $[0.8 \times (4 - \text{albumin})]$ (30). Glomerular filtration rate (eGFR) was estimated using the four-variable Modification of Diet in Renal Disease (MDRD) equation, and chronic kidney disease was defined as either $\text{eGFR} < 60 \text{ ml/min/m}^2$ or dialysis therapy (31).

Statistical analyses

Analyses were completed using STATA 11.1 (Stata Corporation, College Station, TX, USA). Continuous variables were reported as median and interquartile range (IQR). The vitamin D, DBP and albumin concentrations were not normally distributed. Therefore, concentrations of these variables between baseline and 3 months were compared using the paired sign-rank test. Differences in binary outcomes such as deficiency in 25(OH)D between baseline and 3 months were assessed using McNemar's test.

We defined 25(OH)D deficiency as a concentration less than 20 ng/ml, based on the recent Institute of Medicine Report (22). 25(OH)D status was analysed as a binary rather than continuous outcome as it is unlikely that the clinical significance of vitamin D is linear across the range of concentrations. That is, we did not anticipate that a 10-point difference in 25(OH)D would have the same clinical effect for 50 vs. 60 ng/ml compared with 5 vs. 15 ng/ml. For analogous reasons, we analysed 1,25(OH)₂D as a binary outcome. Although there is no universal definition of 1,25(OH)₂D deficiency, we reviewed thresholds used by other investigators and categorised individuals with concentrations $< 20 \text{ pg/ml}$ as having low 1,25(OH)₂D (4, 32–34).

Multivariable logistic regression was used to determine the odds ratio of 25(OH)D deficiency and low 1,25(OH)₂D prior to transplantation. For these models, we categorised the independent variables of pretransplant albumin and DBP into tertiles of approximately equal size ('xtile' command in STATA). The following potential risk factors for 25(OH)D deficiency and low 1,25(OH)₂D were evaluated: age, race (black vs. all others), gender, obesity, MELD score, hepatitis C virus (HCV), chronic kidney disease, 'summer' season at the time of measurement (May–September vs. October–April), albumin and DBP. MELD was skewed and was converted to a binary variable (elevated/not) for these analyses. Elevated MELD was defined as ≥ 20 , a cut-point that has been associated with worse

clinical outcomes among patients with end-stage liver disease (ESLD) (35). Obesity was defined by World Health Organization criteria ($\text{BMI} \geq 30 \text{ kg/m}^2$).

Multivariable analyses of changes in 25(OH)D and 1,25(OH)₂D concentrations after transplantation were performed using linear regression. These analyses were restricted to the 155 participants with vitamin D measurement at baseline and 3 months. For these models, we examined the same covariates listed in the previous paragraph. In addition, we examined whether there were associations between the outcome of change in vitamin D and season transition between transplantation and 3-month follow-up visit. This season variable was binary (transplant during 'non-summer' and follow-up visit during summer, vs. other season transitions). Since vitamin D is fat-soluble, we also examined whether there were associations between change in vitamin D and normalisation of serum bilirubin by the 3-month visit. Given the impact of renal disease and its therapies on 1,25(OH)₂D, the analyses of changes in 1,25(OH)₂D concentrations were restricted to the 76 participants with eGFR $\geq 60 \text{ ml/min/1.73m}^2$ at transplantation.

Multivariable models were fit in which independent variables that were nominally associated with the outcome in unadjusted analyses ($P < 0.15$) were initially entered. Variables that were significant ($P < 0.05$) in multivariate analyses or improved model fit as assessed through the likelihood ratio test were retained. For logistic models, goodness of fit was confirmed using the Hosmer–Lemeshow test.

Secondary analysis

The rank-sum test was used to examine univariate associations between death by 3 months and baseline 25 (OH)D and 1,25(OH)₂D concentrations.

Results

Subject characteristics

Two hundred and twenty-two liver transplant recipients were eligible for study participation. Thirteen did not provide consent, two did not have blood drawn, and five were excluded because they had a second transplant during the study period, leaving 202 with measures of vitamin D at transplantation. Table 1 reports subject characteristics. The median age was 53 years and 48 (24%) were black. The median MELD score was 24. The patients had significant comorbidities: 35% were diabetic and 10% were treated with haemodialysis. Among those not requiring haemodialysis, the median eGFR was $63.7 \text{ ml/min/1.73m}^2$ and 47% had an eGFR $< 60 \text{ ml/min/1.73m}^2$.

Ten patients died by 3 months and 37 did not have serum collected at the 3-month visit, leaving 155 (81% of the surviving cohort) with vitamin D measured at both time points. The 37 survivors without serum at 3 months were younger than the 155 recipients with complete data (median age 46 vs. 53 years, $P < 0.001$). However, the two groups did not differ according to gender, race, HCV prevalence, MELD score, or baseline 25(OH)D or 1,25(OH)₂D concentrations.

Among the 155 patients with vitamin D concentrations measured 3 months after transplantation, 150 (97%) were prescribed tacrolimus (median dose 8 mg/day), 146 (94%) were prescribed prednisone (median 5 mg/day) and 79 (51%) were prescribed mycophenolate mofetil (median 2000 mg/day). Only 11 patients (7%) were prescribed sirolimus (median 2 mg/day).

Concentrations of vitamin D binding protein, albumin and vitamin D at the time of liver transplantation

Table 2 displays concentrations of DBP, albumin, 25 (OH)D and 1,25(OH)₂D. DBP concentrations were skewed. The median pretransplant DBP concentration was 8.6 mg/dl. Median pretransplant serum albumin was 2.7 g/dl.

Baseline concentrations of 25(OH)D were extremely low and 27 (13%) patients had 25(OH)D concentrations <2.5 ng/ml. Median 25(OH)D concentrations were lower in black compared with non-black participants (4.9 vs. 7.6 ng/ml; $P = 0.006$); however, the prevalence of deficiency was identical in the black and non-black participants at 84%. Free 25(OH)D concentrations did not differ between the black and non-black participants ($P = 0.34$). Notably, no significant differences existed between the black and non-black patients in terms of age ($P = 0.84$), gender (0.94), BMI (0.46) or MELD score ($P = 0.48$).

The results of the multivariable logistic regression model of risk factors for 25(OH)D deficiency prior to liver transplantation are presented in Table 3. A MELD score ≥ 20 and a lower serum albumin concentration were significantly and independently associated with 25 (OH)D deficiency.

Overall, 77% of the patients prior to transplantation had low 1,25(OH)₂D concentrations. Among the 96 participants with eGFR ≥ 60 ml/min/1.73m² pretransplantation, 58 (60%) had low 1,25(OH)₂D concentrations.

The results of the logistic regression model for pretransplant 1,25(OH)₂D deficiency are presented in Table 3. MELD score ≥ 20 , male gender, lower serum albumin concentration and eGFR < 60 ml/min/m² were significantly and independently associated with low 1,25(OH)₂D.

Baseline vitamin D concentrations and mortality by 3 months

There were no significant differences in total 25(OH)D concentrations ($P = 0.43$), free 25(OH)D concentrations ($P = 0.83$), total 1,25(OH)₂D concentrations ($P = 0.59$), or free 1,25(OH)₂D concentrations ($P = 0.93$) between patients who did and did not die by 3 months.

Changes in vitamin D binding protein and vitamin D at 3 months after liver transplantation

Transplantation resulted in substantial increases in serum DBP and albumin. The median DBP increase was 12.5 mg/dl ($P < 0.001$). The median albumin increase was 1.0 g/dl (IQR 0.3–1.6) with a median concentration of 3.8 g/dl (IQR 3.4–4.1) at 3 months ($P < 0.001$).

Total 25(OH)D also increased markedly ($P < 0.001$). The median change in 25(OH)D was 17.8 ng/ml (IQR 8.60–25.9). Notably, the median serum 25(OH)D concentration at follow-up was not different between blacks and non-blacks ($P = 0.10$). Free 25(OH)D concentrations increased significantly following liver transplantation, and, the percent free 25(OH)D decreased from 8.0 to 3.1% ($P < 0.001$). Figure 1 displays the distributions of total and free 25(OH)D before and after transplantation.

The multivariable linear regression model shown in Table 4 demonstrates that lower baseline 25(OH)D concentrations, greater increases in serum albumin and DBP concentrations by 3 months, and normalisation of bilirubin concentration by 3 months were associated with greater increases in serum 25(OH)D concentrations. Additionally, individuals who underwent transplantation during the non-summer months (October through April) but had follow-up during summer had greater increases in 25(OH)D compared to individuals whose transplantation and follow-up took place during other season transitions. BMI ≥ 30 kg/m² at transplantation was associated with a lesser increase in 25(OH)D.

Liver transplantation resulted in improvements in 1,25(OH)₂D concentrations ($P < 0.001$) in the entire cohort. The median increase was 4.3 pg/ml (IQR: -3.0, 17.6) and the median post-transplant concentration was 20.2 pg/ml (IQR: 8.6, 31.1). Despite the increase in concentrations, 76 (49%) of liver recipients overall had low 1,25(OH)₂D at 3 months, and among those without CKD prior to transplant, 34% had low 1,25(OH)₂D. Free concentrations of 1,25(OH)₂D fell significantly from a median of 137 fg/ml pretransplant to a median of 98 fg/ml post-transplantation (Fig. 2); the percent free 1,25(OH)₂D fell from 1.2 to 0.5% by 3 months after transplantation.

We fit a multivariable linear regression model for change in 1,25(OH)₂D restricted to the 76 participants with eGFR ≥ 60 ml/min/m² prior to transplantation. Only change in DBP ($\beta = 0.27$ per mg/dl increase; 95% CI 0.04, 0.50; $P = 0.02$) and age less than 45 years ($\beta = 11.26$; CI 3.42, 19.1; $P = 0.005$) were associated with greater increases in 1,25(OH)₂D concentrations. Low baseline 1,25(OH)₂D defined as <20 pg/ml was not significantly associated with the outcome ($\beta = 6.37$ for concentrations <20 pg/ml; 95% CI -0.50, 13.23; $P = 0.07$).

Renal function and calcium concentration after transplantation

Many liver disease patients had abnormal renal function even after transplantation. A total of 73 (47%) had eGFR ≥ 30 and <60 ml/min/m², while 9 (6%) had GFR < 30 ml/min/m².

Median uncorrected calcium rose substantially after transplantation (8.4 mg/dl pretransplant to 9.1 mg/dl post-transplantation, $P < 0.001$). Corrected calcium was slightly lower after transplant ($P = 0.01$), although the difference was not clinically important (9.5 mg/dl versus 9.3 mg/dl after transplantation).

Discussion

This study reveals that liver transplantation resulted in substantial rises in total 25(OH)D concentrations, and that the increases in serum albumin and DBP concentrations were

powerful predictors of increases in 25 (OH)D concentrations. By contrast, total 1,25(OH)₂D rose modestly after transplantation, free concentrations of 1,25(OH)₂D fell, and low concentrations of total 1,25 (OH)₂D after transplantation were not explained by reduced eGFR. Given these substantial changes in concentrations of vitamin D and its binding proteins, studies that examine the potential relationship of vitamin D to post-transplant outcomes represent an important research opportunity.

Vitamin D affects multiple organ systems through durable influences on gene transcription mediated by the vitamin D receptor (36). Studies of the general population have generated mounting evidence that vitamin D deficiency is associated with diabetes, fractures, poor physical function, bacterial infections and malignancies (1, 2, 4, 37, 38). These comorbidities also commonly complicate liver disease and liver transplantation (13, 14). Additionally, low 25(OH)D concentrations may be a risk factor for liver fibrosis among hepatitis C infected patients,(35, 39) and an observational study of Italian liver transplant recipients suggested that low pretransplant 25(OH)D was associated with a higher risk of rejection (7). These results indicate that vitamin D status could predict or contribute to complications of liver transplantation.

Our study extends the findings of smaller studies showing that low concentrations of 25(OH)D and 1,25 (OH)₂D are common among liver transplant candidates (11, 13, 15–18). Using an established assay, we found that 25(OH)D concentrations were undetectable in 13% of our cohort prior to transplantation. Deficiency in 25 (OH)D was present in 84% of patients and did not differ across black and non-black patients. However, unlike most prior work, our study prospectively followed a large and diverse cohort of patients with ESLD. Most prior investigators reported on cohorts of ~50 patients; most either did not report race or included no black patients. Additionally, we prospectively measured DBP, which allowed us to show the large increases in DBP after transplantation, and to estimate changes in free vitamin D concentrations.

Interestingly, in multivariable analysis, albumin was associated with 25(OH)D deficiency prior to liver transplantation, while DBP was not. Both albumin and DBP are synthesised by the liver. The low concentrations of these proteins prior to transplantation are likely driven both by impaired liver function as well as poor nutritional status. For patients who underwent paracentesis, albumin and DBP (as well as vitamin D) could have been lost in the ascites. Important work by Bikle *et al.* (among others) showed that approximately 85–90% and 10–15% of circulating 25(OH)D in the serum is bound to DBP and albumin respectively (27). Thus, low serum concentrations of DBP and albumin could lead to migration of lipophilic 25(OH)D into adipose tissue and to lower 25(OH)D in the serum. However, the studies by Bikle *et al.* suggest that DBP binds a higher proportion of 25(OH)D than albumin, raising the question of why albumin was more associated with 25(OH)D deficiency in our cohort. It is possible that albumin was more closely associated with 25(OH)D deficiency than DBP because protein binding of vitamin D is only one factor contributing to serum 25(OH)D concentrations in ESLD. Other factors, such as overall nutritional and functional status may be important determinants of 25(OH)D concentrations, and albumin may be a more sensitive indicator of overall health than DBP.

We propose three possible explanations for the dramatic improvements in 25(OH)D concentrations following transplantation. First, the allograft was able to 25-hydroxylate calciferol. Second, restoration of hepatic synthetic function with improvements in serum albumin and DBP concentrations facilitated 25(OH)D movement from adipose tissue to the serum. Third, all transplant recipients were prescribed a multivitamin – although the vitamin D dose was quite small.

The persistently low 1,25(OH)₂D concentrations after transplantation may be explained by the fact that a majority of the liver recipients had chronic kidney disease stage 3 or greater (a finding that is consistent with other liver transplant cohorts) after transplant (21). Kidney disease reduces 1,25(OH)₂D concentrations via impaired 1-alpha hydroxylation of vitamin D (5). However, even among liver transplant recipients with a pretransplant eGFR 60 ml/min/m², the 1,25(OH)₂D concentrations were low at 3 months. This may indicate that the MDRD equation overestimated GFR among these liver recipients (due, for instance, to low muscle mass) or that factors besides renal function diminished 1,25(OH)₂D production.

We also found that although estimated free 25(OH)D concentrations rose after liver transplantation, free concentrations of 1,25(OH)₂D fell substantially as the concentrations of albumin and DBP rose. These findings draw attention to the many unanswered questions about which component(s) of vitamin D drives the end-organ biological effects of the hormone in humans. It is believed that free vitamin D is the metabolically active component (the 'free hormone hypothesis'), and that therefore low concentrations of DBP may liberate greater fractions of free vitamin D and thus preserve the hormone's action (27). For instance, a murine model of DBP deficiency showed that the mice had normal calcium concentrations and relatively intact expression of genes involved in calcium regulation (40). Likewise, a recent study provided evidence of the impact of free 25 (OH)D on bone mineral density in healthy young adults. In this cross-sectional study by Powe *et al.*, free 25(OH)D but not total 25(OH)D concentrations were strongly correlated with bone density in the lumbar spine (6).

ESLD may offer a useful model in which to examine the biological effects of vitamin D, since total concentrations prior to transplantation are extremely low, but the percent free concentrations are likely to be normal or high (27). Furthermore, liver transplant recipients suffer a high burden of comorbidities, such as diabetes, immune dysfunction and infection, that are believed to be affected by vitamin D metabolism. Interestingly, corrected calcium concentrations in our cohort were relatively preserved pretransplantation (median 9.5 mg/dl). This finding suggests the possibility that serum total vitamin D concentrations (as opposed to the free concentrations) may not represent the same functional deficiency as observed in other populations (8). Our findings of sometimes divergent changes in total versus free vitamin D concentrations also suggest the importance of assessing changes in total versus free forms of vitamin D when investigating the relationship of vitamin D to health outcomes in transplantation.

This study's strengths include use of a high quality vitamin D assay and measurements pre- and post-transplantation in a large cohort. This study also has limitations. First, missing data at 3 months in 20% of the surviving cohort may introduce bias. However, the participants

with and without follow up were similar with regards to baseline vitamin D concentrations and most participant characteristics. Second, the conduct of this study in a single centre may limit generalisability. Third, we do not have data on albumin infusions prior to transplantation. We found significant relationships between albumin concentrations and the outcomes of both pretransplant vitamin D and change in 25(OH)D after transplantation. As albumin binds vitamin D in the serum, these relationships are biologically plausible. As a result of the high transvascular escape rate of albumin in patients with cirrhosis, albumin infusions would be likely to increase measured serum concentrations to a modest extent and for a brief period (41, 42). For these reasons, we believe that our results concerning albumin and vitamin D are valid. Fourth, this study is limited by lack of outcomes of vitamin D deficiency such as PTH. Finally, these inferences about free vitamin D rely on estimating equations. However, the direct measurement of free vitamin D is highly challenging and not commercially available currently. Prior studies have demonstrated that these estimates of free vitamin D are correlated with free concentrations, and a better understanding of changes in free levels may be important to relating changes in vitamin D concentrations to important outcomes after transplantation in future work (27, 28).

Conclusions

Vitamin D deficiency is extremely common among liver transplant candidates and strongly associated with MELD score and serum albumin. After transplantation, serum 25(OH)D and DBP concentrations improve substantially, while low 1,25(OH)₂D concentrations persist in the majority of recipients. Free concentrations of 1,25 (OH)₂D in this cohort fell after liver transplant. Further studies are needed to determine how vitamin D status contributes to complications of transplantation, and how liver-synthesised proteins including DBP may influence the biological effects of vitamin D.

Acknowledgements

Financial support: This work was supported by NIH grants K23 - DK078688-01 (Dr Reese), K24 - DK002651 (Dr Feldman), R01 DK073192 (Drs Olthoff and Shaked), K24 DK076808 (Dr Leonard) and R01 DK064966 (Dr Leonard).

Abbreviations

1,25(OH)₂D	1,25 dihydroxy-vitamin D
25 (OH)D	25(OH)-vitamin D
ALT	alanine aminotransferase
AST	aspartate transaminase
BMI	body mass index
DBP	vitamin D binding protein
eGFR	estimated glomerular filtration rate
ESLD	end-stage liver disease

HCV	hepatitis C virus
INR	international normalised ratio of prothrombin time
IQR	interquartile range
MDRD	modification of diet in renal disease
MELD	model for end-stage liver disease

References

1. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care*. 2004; 27:2813–8. [PubMed: 15562190]
2. Giovannucci E, Liu Y, Rimm EB, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J Natl Cancer Inst*. 2006; 98:451–9. [PubMed: 16595781]
3. Gombart AF. The vitamin D-antimicrobial peptide pathway and its role in protection against infection. *Future Microbiol*. 2009; 4:1151–65. [PubMed: 19895218]
4. Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med*. 2008; 168:1629–37. [PubMed: 18695076]
5. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007; 357:266–81. [PubMed: 17634462]
6. Powe CE, Ricciardi C, Berg AH, et al. Vitamin D binding protein modifies the vitamin D-bone mineral density relationship. *J Bone Miner Res*. 2011; 26:1609–16. [PubMed: 21416506]
7. Bitetto D, Fabris C, Falletti E, et al. Vitamin D and the risk of acute allograft rejection following human liver transplantation. *Liver Int*. 2009; 30:417–44. [PubMed: 19849776]
8. Bikle DD, Gee E, Halloran B, Haddad JG. Free 1,25-dihydroxyvitamin D levels in serum from normal subjects, pregnant subjects, and subjects with liver disease. *J Clin Invest*. 1984; 74:1966–71. [PubMed: 6549014]
9. Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. *Dig Dis Sci*. 2009; 55:2624–8. [PubMed: 19960254]
10. Bikle DD. Vitamin D insufficiency/deficiency in gastrointestinal disorders. *J Bone Miner Res*. 2007; 22(Suppl 2):V50–4. [PubMed: 18290722]
11. Stein EM, Cohen A, Freeby M, et al. Severe vitamin D deficiency among heart and liver transplant recipients. *Clin Transplant*. 2009; 23:861–5. [PubMed: 19453643]
12. Bikle DD, Halloran BP, Gee E, Ryzen E, Haddad JG. Free 25-hydroxyvitamin D levels are normal in subjects with liver disease and reduced total 25-hydroxyvitamin D levels. *J Clin Invest*. 1986; 78:748–52. [PubMed: 3745436]
13. Guichelaar MM, Schmoll J, Malinchoc M, Hay JE. Fractures and avascular necrosis before and after orthotopic liver transplantation: long-term follow-up and predictive factors. *Hepatology*. 2007; 46:1198–207. [PubMed: 17654700]
14. Oufroukhi L, Kamar N, Muscari F, et al. Predictive factors for posttransplant diabetes mellitus within one-year of liver transplantation. *Transplantation*. 2008; 85:1436–42. [PubMed: 18497684]
15. Monegal A, Navasa M, Guanabens N, et al. Bone disease after liver transplantation: a long-term prospective study of bone mass changes, hormonal status and histomorphometric characteristics. *Osteoporos Int*. 2001; 12:484–92. [PubMed: 11446565]
16. Compston JE, Greer S, Skingle SJ, et al. Early increase in plasma parathyroid hormone levels following liver transplantation. *J Hepatol*. 1996; 25:715–8. [PubMed: 8938550]
17. Rabinovitz M, Shapiro J, Lian J, et al. Vitamin D and osteocalcin levels in liver transplant recipients. Is osteocalcin a reliable marker of bone turnover in such cases? *J Hepatol*. 1992; 16:50–5. [PubMed: 1484167]
18. Crosbie OM, Freaney R, McKenna MJ, Curry MP, Hegarty JE. Predicting bone loss following orthotopic liver transplantation. *Gut*. 1999; 44:430–4. [PubMed: 10026333]

19. Feller RB, McDonald JA, Sherbon KJ, McCaughan GW. Evidence of continuing bone recovery at a mean of 7 years after liver transplantation. *Liver Transpl Surgery*. 1999; 5:407–13.
20. Guichelaar MM, Kendall R, Malinchoc M, Hay JE. Bone mineral density before and after OLT: long-term follow-up and predictive factors. *Liver Transpl*. 2006; 12:1390–402. [PubMed: 16933236]
21. Bloom RD, Reese PP. Chronic kidney disease after nonrenal solid-organ transplantation. *J Am Soc Nephrol*. 2007; 18:3031–41. [PubMed: 18039925]
22. Institute of Medicine. Dietary Reference Intakes Calcium Vitamin D. National Academies Press; Washington, DC: 2011.
23. De Jonge J, Kurian S, Shaked A, et al. Unique early gene expression patterns in human adult-to-adult living donor liver grafts compared to deceased donor grafts. *Am J Transplant*. 2009; 9:758–72. [PubMed: 19353763]
24. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD, Napoli JL. Determination of vitamin D status by radioimmunoassay with an 125I-labeled tracer. *Clin Chem*. 1993; 39:529–33. [PubMed: 8448871]
25. Clive DR, Sudhaker D, Giacherio D, et al. Analytical and clinical validation of a radioimmunoassay for the measurement of 1,25 dihydroxy vitamin D. *Clin Biochem*. 2002; 35:517–21. [PubMed: 12493579]
26. Hollis BW. Comparison of commercially available (125)I-based RIA methods for the determination of circulating 25-hydroxyvitamin D. *Clin Chem*. 2000; 46:1657–61. [PubMed: 11017946]
27. Bikle DD, Siiteri PK, Ryzen E, Haddad JG. Serum protein binding of 1,25-dihydroxyvitamin D: a reevaluation by direct measurement of free metabolite levels. *J Clin Endocrinol Metab*. 1985; 61:969–75. [PubMed: 3840175]
28. Bikle DD, Gee E, Halloran B, et al. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. *J Clin Endocrinol Metab*. 1986; 63:954–9. [PubMed: 3745408]
29. UNOS. MELD/PELD calculator documentation. Available at http://www.unos.org/SharedContentDocuments/MELD_PELD_Calculator_Documentation.pdf. Accessed 12 July 2009
30. Eknoyan G, Levin A, Levin N. Bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis*. 2003; 42:S1–201. [PubMed: 14520607]
31. Levey AS, Coresh J, Greene T, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med*. 2006; 145:247–54. [PubMed: 16908915]
32. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. *N Engl J Med*. 1998; 338:777–83. [PubMed: 9504937]
33. Zittermann A, Schleithoff SS, Frisch S, et al. Circulating calcitriol concentrations and total mortality. *Clin Chem*. 2009; 55:1163–70. [PubMed: 19359534]
34. Evenepoel P, Naesens M, Claes K, Kuypers D, Vanrenterghem Y. Tertiary ‘hyperphosphatonism’ accentuates hypophosphatemia and suppresses calcitriol levels in renal transplant recipients. *Am J Transplant*. 2007; 7:1193–200. [PubMed: 17359508]
35. Merion RM. Current status and future of liver transplantation. *Semin Liver Dis*. 2010; 30:411–21. [PubMed: 20960380]
36. Haussler M, Whitfield G, Haussler C, et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J Bone Miner Res*. 1998; 13:325–49. [PubMed: 9525333]
37. Wolff AE, Jones AN, Hansen KE. Vitamin D and musculoskeletal health. *Nat Clin Pract Rheumatol*. 2008; 4:580–8. [PubMed: 18852718]
38. Stubbs JR, Idiculla A, Slusser J, Menard R, Quarles LD. Cholecalciferol supplementation alters calcitriol-responsive monocyte proteins and decreases inflammatory cytokines in ESRD. *J Am Soc Nephrol*. 2010; 21:353–61. [PubMed: 20007751]
39. Petta S, Camma C, Scazzone C, et al. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. *Hepatology*. 2010; 51:1158–67. [PubMed: 20162613]

40. Zella LA, Shevde NK, Hollis BW, Cooke NE, Pike JW. Vitamin D-binding protein influences total circulating levels of 1,25-dihydroxyvitamin D3 but does not directly modulate the bioactive levels of the hormone in vivo. *Endocrinology*. 2008; 149:3656–67. [PubMed: 18372326]
41. Gines P, Arroyo V. Is there still a need for albumin infusions to treat patients with liver disease? *Gut*. 2000; 46:588–90. [PubMed: 10764695]
42. Ohnishi K, Kawaguchi A, Nakajima S, Mori H, Ueshima T. A comparative pharmacokinetic study of recombinant human serum albumin with plasma-derived human serum albumin in patients with liver cirrhosis. *J Clin Pharmacol*. 2008; 48:203–8. [PubMed: 18199895]

Fig. 1.

Changes in total and free 25(OH)D concentrations pre- and post-liver transplantation. The figure shows data for 202 patients with end-stage liver disease prior to transplantation, and 155 liver transplant recipients. The dashed line indicates deficiency in total 25(OH)D.

Fig. 2. Changes in total and free 1,25(OH)₂D pre- and post-liver transplantation. The figure shows data for 202 patients with end-stage liver disease prior to transplantation, and 155 liver transplant recipients.

Table 1

Subject characteristics of liver transplant recipients prior to transplantation *

<i>n</i>	202
Age, year	53 (48–59)
Male, <i>n</i> (%)	149 (73.8)
Race, <i>n</i> (%)	
White	141 (69.8)
Black	48 (23.8)
Other	13 (6.4)
BMI, kg/m ²	27.4 (24.2–30.7)
Summer season, <i>n</i> (%)	90 (44.6)
Cause of liver disease, <i>n</i> (%)	
Hepatitis C	91 (45.1)
Hepatitis C and hepatocellular carcinoma	27 (13.4)
Alcohol	20 (9.9)
Cryptogenic cirrhosis	10 (5.0)
Primary sclerosing cholangitis	10 (5.0)
Autoimmune	8 (4.0)
Non-alcoholic steatohepatitis	7 (3.5)
Hepatitis B	6 (3.0)
Other/aetiology unknown	5 (2.5)
Drug toxicity	4 (2.0)
Hepatitis B and hepatocellular carcinoma	4 (2.0)
Cystic fibrosis	2 (1.0)
Amyloid	1 (0.5)
Glycogen storage disease	1 (0.5)
Polycystic liver disease	1 (0.5)
Secondary biliary cirrhosis	1 (0.5)
Trauma	1 (0.5)
Diabetes, <i>n</i> (%)	71 (35.1)
MELD score	24 (17–29)
Receiving dialysis treatment, <i>n</i> (%)	21 (10)
eGFR, ml/min/1.73 m ² †	63.7 (34.1–95.8)
INR, INR units	1.6 (1.3–2.0)
Serum AST, U/L	90 (52–150)
Total bilirubin, mg/dl	4.0 (1.4–12.9)
Donor type, <i>n</i> (%)	
Deceased donor	198 (98.0)
Live donor	1 (0.5)
Split	3 (1.5)
Kidney-liver transplant, <i>n</i> (%)	9 (4.5)

AST, aspartate aminotransferase; BMI, body mass index; eGFR, estimated glomerular filtration rate; INR, international normalised ratio; MELD, model for end-stage liver disease score.

* Continuous variables are presented as median (interquartile range).

[†] Excludes the patients treated with haemodialysis prior to transplantation.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2

Vitamin D binding protein, albumin, vitamin D and calcium concentrations before and after liver transplantation*

	Pretransplant (<i>n</i> = 202)	Post-transplant (<i>n</i> = 155)	P-value [†]
Vitamin D binding protein (mg/dl)	8.6 (5.5–14.5)	23.8 (11.1–33.5)	<0.001
Non-black race	9.7 (6.7–14.8)	23.8 (11.1–32.9)	<0.001
Black race	5.2 (3.8–10.6)	23.8 (10.6–35.8)	<0.001
Serum albumin, g/dl	2.7 (2.2–3.3)	3.8 (3.5–4.1)	<0.001
Total 25(OH)D, ng/ml	6.7 (3.7–13.7)	27.4 (20.4–36.4)	<0.001
Non-black race	7.6 (4.3–14.2)	28.3 (21.2–37.2)	<0.001
Black race	4.9 (1.5–10.7)	24.4 (17–34)	<0.001
Total 25(OH)D <20 ng/ml, <i>n</i> (%)	170 (84)	37 (23.9)	<0.001
Non-black race	132 (84)	24 (19.8)	<0.001
Black race	38 (84)	13 (38.2)	<0.001
Free 25(OH)D, pg/ml	5.8 (3.0–10.4)	8.7 (6.3–15.4)	<0.001
Non-black race	6.1 (3.2–10.4)	9.7 (6.4–15.6)	<0.001
Black race	4.5 (2.0–10.6)	7.5 (5.2–12.4)	0.51
Percent free 25(OH)D (%)	8.0 (5.2–10.8)	3.1 (2.3–6.1)	<0.001
Non-black race	7.1 (5.0–9.7)	3.1 (2.3–6.0)	<0.001
Black race	11.5 (6.4–15.3)	3.2 (2.1–6.3)	<0.001
Total 1,25(OH) ₂ D, pg/ml	11.4 (8.4–18.6)	20.2 (8.6–31.1)	<0.001
Total 1,25(OH) ₂ D <20 pg/ml, <i>n</i> (%)	155 (77)	76 (49)	<0.001
Free 1,25(OH) ₂ D, fg/ml	138 (87–214)	98 (53–173)	<0.001
Percent free 1,25(OH) ₂ D	1.2 (0.8–1.5)	0.5 (0.4–0.9)	<0.001
Serum calcium, mg/dl	8.4 (8.1–9.0)	9.1 (8.8–9.5)	<0.001
Serum calcium (albumin corrected), mg/dl	9.5 (9.0–9.9)	9.3 (9.0–9.6)	0.01

* Continuous variables are presented as median (interquartile range).

[†] Represents change pre- and post-transplantation.

Table 3

Multivariable logistic regression models for the outcomes of deficiency in 25(OH)D (<20 ng/ml) and low 1,25(OH)₂D (<20 pg/ml) in liver transplant recipients prior to transplantation

	Deficiency in 25 (OH)D			Low 1,25(OH) ₂ D		
	OR	CI	P	OR	CI	P
MELD score ≥ 20	5.0	2.10–11.9	<0.001	4.63	1.78–12.0	0.002
Serum albumin						
<2.5 g/dl		Reference			Reference	
2.5 & <3.0 g/dl	0.16	0.03–0.77	0.02	0.34	0.10–1.12	0.077
>3 g/dl	0.08	0.02–0.38	0.001	0.14	0.05–0.42	<0.001
Male *				3.20	1.26–8.14	0.015
Obese *				2.57	0.94–7.00	0.066
Chronic kidney disease (eGFR < 60 ml/min/m ²) *				4.48	1.60–12.56	0.004

* These covariates were not included in the regression on 25(OH)D deficiency because they either were not significant or did not improve model fit. OR, odds ratio.

Table 4

Multivariable linear regression model of changes in 25(OH) vitamin D concentrations following liver transplantation

	β (95% CI)	<i>P</i> -value
Pretransplant 25(OH)D concentration <20 ng/ml (vs. ≥ 20)	10.9 (6.62, 15.09)	<0.001
Change in vitamin D binding protein by 3 months (per mg/dl increase)	0.11 (0.00, 0.20)	0.049
Change in serum albumin concentration by 3 months (per g/dl increase)	3.72 (1.77, 5.67)	<0.001
BMI ≥ 30 kg/m ² at transplantation (vs. <30)	-4.50 (-7.92, -1.08)	0.01
Non-summer season at transplant and summer season at 3-month follow-up (vs. other season transitions)	4.84 (1.32, 8.36)	0.007
Normal bilirubin concentration by 3 months (vs. bilirubin >1.2 mg/dl)	6.76 (2.62, 10.91)	0.002
Constant	-1.66 (-6.52, 3.19)	0.50