ORIGINAL PAPER

Available from: http://www.jgld.ro/wp/archive/y2016/n2/a9
DOI: http://dx.doi.org/10.15403/jgld.2014.1121.252.cap

Effect of Short-Term Vitamin D Correction on Hepatic Steatosis as Quantified by Controlled Attenuation Parameter (CAP)

Ifigeneia Papapostoli, Frank Lammert, Caroline S. Stokes

Department of Medicine II,
Saarland University Medical Center,
Homburg, Germany.

Address for correspondence:
Dr. **Caroline S. Stokes**
Department of Medicine II
Saarland University Medical Center
Kirrberger Str. 100
66421 Homburg
Germany
caroline.stokes@uks.eu

Received: 29.01.2016
Accepted: 01.04.2016

ABSTRACT

Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Western countries. A meta-analysis has confirmed decreased serum 25-hydroxyvitamin D levels in NAFLD patients. This intervention study investigates whether vitamin D correction ameliorates hepatic steatosis.
Methods: We prospectively recruited 40 patients from an outpatient liver clinic with vitamin D deficiency (serum 25-hydroxyvitamin D < 20 ng/ml). Controlled attenuation parameter (CAP) during transient elastography quantified hepatic steatosis. Patients with significant liver fat accumulation were included, which was defined by a CAP value ≥ 280 dB/m. Patients received 20,000 IU vitamin D/week for six months, while vitamin D status, liver function tests (LFTs), CAP and body composition were monitored.
Results: The cohort comprised 47.5% women (age 54.9 ± 12.1 years; BMI 29.5 ± 3.0 kg/m^2). Mean serum vitamin D level was 11.8 ± 4.8 ng/ml. CAP decreased significantly from baseline (330 ± 32 vs. 307 ± 41 dB/m) during supplementation (P = 0.007). A mean CAP reduction relative to baseline was demonstrated at four weeks and three and six months: -5.3 ± 13.8%; -6.0 ± 14.6% and -6.4 ± 13.0%, respectively. During these time points, restoration of serum vitamin D levels was observed (34.6 ± 12.9, 36.3 ± 10.2, 34.8 ± 9.8 ng/ml; P < 0.0001). Liver function tests and body composition remained unchanged.
Conclusions: Hepatic steatosis, as assessed by CAP, significantly improves after only 4 weeks of vitamin D correction. Hepatic steatosis is a dynamic process, that can be monitored in the short-term using such non-invasive methods.

Key words: cholecalciferol – fatty liver – 25-hydroxyvitamin D – transient elastography.

Abbreviations: ALT: alanine aminotransferase; ANOVA: analysis of variance; AP: alkaline phosphatase; AST: aspartate aminotransferase; CAP: controlled attenuation parameter; CRP: C-reactive protein; FFA: free fatty acids; γ-GT: gamma-glutamyl transpeptidase; ITT: intention-to-treat; LFT: liver function test; LSM: liver stiffness measurement; NAFLD: non-alcoholic fatty liver disease; PPAR-γ: peroxisome proliferator-activated receptor gamma; PP: per protocol; PTH: parathyroid hormone; VDR: vitamin D receptor

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has a prevalence of 25 to 45% in Western countries and is one of the main causes of liver-related mortality, thus shifting the burden in hepatology in its direction [1]. NAFLD encompasses a spectrum of hepatic-associated conditions which are represented as a continuum. Hepatic steatosis transpires on account of repeated liver injury and the initiation of liver inflammation [2]. This can progress to non-alcoholic steatohepatitis (NASH), liver fibrosis and cirrhosis, as well as hepatocellular carcinoma [1, 3-5]. The pathophysiology of NALFD is multifactorial and is believed to result from 'multiple hits hypothesis' incorporating diverse genetic and environmental processes [6, 7]. Currently, no standard therapy for NALFD exists. Emerging pharmacotherapies such as obeticholic acid show promise, though long-term efficacy remains unknown [8]. Thus, most recent guidelines still advocate lifestyle interventions, focusing on weight loss through diet and physical activity as treatment targets [9-11].

Vitamin D holds potential as therapeutic target, given the presence of vitamin D receptor (VDR) in the liver and its multiple pleiotropic actions. For example, low vitamin D favours intrahepatic lipid accumulation due to increased circulation of free fatty acids (FFA), which is regulated via the

peroxisome proliferator-activated receptor gamma (PPAR-γ) [12, 13]. Although not all [14, 15], many observational studies report associations between vitamin D deficiency and hepatic steatosis [12, 16], the aggregate effect of which has been illustrated in a meta-analysis [17] confirming the presence of decreased serum 25-hydroxyvitamin D concentrations in such patients. Specifically, they were 26% more likely than healthy controls to be vitamin D deficient. Indeed, vitamin D deficiency has been associated with greater histological severity of hepatic steatosis in both experimental and human studies [18, 19].

Some studies have also inversely correlated serum 25-hydroxyvitamin D concentrations with elevated body fat, which is often seen in patients with comorbid hepatic steatosis [16, 20]. Indeed adipose tissue has been reported to sequester vitamin D and reduce its bioavailbility, thus exacerbating suboptimum vitamin D status [21]. As such, replacing inadequate vitamin D levels may induce biochemical and histological benefits to the host. Preclinical studies, notably that of Nakano et al. [22] reported a beneficial effect of phototherapy on NAFLD progression in rats, accompanied by increased serum concentrations of vitamin D metabolites.

In humans, the efficacy of vitamin D replacement in the presence of hepatic steatosis currently remains unknown. This pilot study investigates whether correction of vitamin D levels reduces the degree of hepatic steatosis. We hypothesised that hepatic steatosis may be attenuated by vitamin D correction in patients with vitamin D deficiency and significant hepatic fat accumulation, even in the absence of a weight loss co-intervention.

PATIENTS AND METHODS

Study patients

Adult outpatients attending the Department of Medicine II at Saarland University Medical Center were prospectively recruited for this intervention study. We included patients with vitamin D deficiency (serum 25-hydroxyvitamin D < 20 ng/ml) [23] and significant liver fat accumulation, defined as a value ≥ 280 dB/m (as previously reported [24]) using controlled attenuation parameter (CAP) during transient elastography [25]. Exclusion criteria were based on a previously published study in which patients with chronic liver diseases received the same vitamin D supplementation regimen (for full details see [26]). In brief, these included: pregnancy, hypercalciuria or hypercalcemia alone or with hyperparathyroidism, tendency for kidney stones; allergy or hypersensitivity to the supplement ingredients; sarcoidosis and stage IV or V chronic kidney disease. Additionally, patients with excessive alcohol intake (men > 30 g/day, women > 20 g/day) as determined with the Alcohol Use Disorders Identification Test (AUDIT) questionnaire [27] were excluded. The study was conducted in accordance with the Declaration of Helsinki. The protocol was based on the aforementioned published study [26] and the amendment was approved by the local Research Ethics Committee (Ärztekammer des Saarlandes, ref. 57/11). Written informed consent was a prerequisite for participation.

Study design and intervention

At baseline, patients were instructed to take a weekly single oral dose of 20,000 IU cholecalciferol/vitamin D3 (Dekristol®, Jenapharm, Jena, Germany) for six months. For the first seven days however, this dose was taken daily. All patients were followed up after four weeks, and at three and six months. Both the discrete interval pill-count method and increases in serum 25-hydroxyvitamin concentrations during follow-up were used to assess compliance. Patients were requested to refrain from taking other nutritional supplements during the study. The assessments below were carried out during all respective time points.

Quantification of hepatic steatosis

Hepatic steatosis was assessed non-invasively using vibration controlled transient elastography (Fibroscan, Echosens SA, Paris) with controlled attenuation parameter (CAP). This novel quantitative parameter measures liver fat contents based on the attenuation of the ultrasound signal by fat, the details of which have been previously described [28]. The CAP value is expressed in decibel per meter (dB/m) and ranges from 100 – 400 dB/m. Higher values denote greater liver fat contents. Transient elastrography also conducts liver stiffness measurements (LSM), based on the shear wave propagation speed, the results of which are reported in kilo Pascal (kPa) with a range of 2.5 – 75 kPa. The measurements were conducted with the M-probe placed on the skin, between the ribs at the midaxillary line vertical to the xyphoid process, with patients in the dorsal decubitus position and with the right arm stretched behind their head. CAP values were considered valid when at least 10 measurements per assessment were obtained from which the final value was based on. LSM values were included in the analyses, if the interquartile range /median LSM was ≤ 30% for values < 7.1 kPa [29].

Biochemical measurements and clinical assessments

Serum 25-hydroxyvitamin D was measured using chemiluminescence immunoassay LIAISON® 25-OH Vitamin D TOTAL Assay (DiaSorin, Minnesota, USA). Liver function tests (LFTs) including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (γ-GT), alkaline phosphatase (AP), as well as bilirubin and albumin were measured using standard clinical-chemical assays, as were serum creatinine, urea, parathyroid hormone (PTH), calcium, phosphate, and urine calcium and phosphate levels. A medical history captured current and previous medications and medical conditions. Body composition was assessed with bioelectrical impedance analysis (Tanita, Sindelfingen). A self-report questionnaire based on the European Prospective Investigation into Cancer and Nutrition (EPIC) study captured physical activity and classified patients into the following physical activity index groups: inactive, moderately inactive, moderately active, and active [30, 31].

Outcomes and statistical analyses

The primary outcome was to evaluate absolute and relative (percent) changes in liver fat contents from baseline, using CAP values during vitamin D supplementation. Prespecified secondary outcomes included changes in liver fat contents and in liver biochemistry at the individual time points (four weeks, three and six months of treatment). Parametric statistics

were predominantly used since the primary outcome variable was normally distributed. Non-parametric descriptives were employed for variables that were not normally distributed. Student's *t* and Mann-Whitney U tests, as well as the χ^2 test were used to assess differences between continuous unpaired data and between categorical variables, respectively.

Per-protocol (PP) analyses were conducted when comparing related samples (i.e. pre and post-intervention). These were corroborated with the intention-to-treat (ITT) principle with last observation carried forward. Repeated measures analysis of variance (ANOVA) with Bonferroni correction, or the non-parameteric Friedman test were employed to asses changes over time. If significant, then post-hoc paired *t* tests or Wilcoxon signed rank tests were used to compare absolute and relative change between two time points. We also examined the influence of seasonal variation on vitamin D levels by comparing samples collected in summer and autumn (associated with higer serum concentrations) with those taken during the winter and spring, and conducted comparisons based on baseline vitamin D status (deficient versus severely deficient). A two-sided P value < 0.05 defined statistical significance. All statistical analyses were performed with SPSS 20.0 (IBM Munich) and GraphPad Prism 5.0 (GraphPad Software Inc., California). Results are presented as means ± standard deviations or medians with ranges, depending on data distribution.

The trial was registered in the German Trials Registry (DRKS), registration No. DRKS00007816.

RESULTS

Patient characteristics

A total of 40 patients participated in this study, the main characteristics for whom are summarised in Table I. Follow-up data were obtained for all 40 patients at four weeks. Seven patients were lost to follow-up at three months and one at six months. In brief, 19 (47.5%) were women, the mean age was 54.9 ± 12.1 years, and BMI was 29.5 ± 3.0 kg/m^2. Visceral fat index levels were above normal values for 39.5% where a cut-off of 12 differentiates normal from elevated levels. Most patients were moderately inactive (42.5%) or moderately active (37.5%), as classified per the physical activity index groups [30, 31]. The remaining 20% were active. Eight patients had an infection with viral hepatitis C (n = 1) or B (n = 7).

The mean baseline CAP value was 330 ± 32 dB/m, and the mean serum 25-hydroxyvitamin D was 11.8 ± 4.8 ng/ml (range 4 – 20). Forty percent of patients were sampled in summer or autumn with a mean vitamin D level of 13.9 ± 5.3 ng/ml. This was significantly higher than the 60% of patients sampled in winter or spring (10.4 ± 3.9 ng/ml; P = 0.022). A higher non-significant CAP value was present in the 17 (42.5%) patients with severe vitamin D deficiency (< 10 ng/ml) as compared to the 23 (57.5%) with moderate deficiency (339 ± 32 vs. 323 ± 30 dB/m). Moreover, patients with severe vitamin D deficiency displayed higher baseline serum PTH values when compared to those with vitamin D deficiency (51 vs. 33 pg/ml; P = 0.001), and higher concentrations of LFTs, though this was

Table I. Baseline and follow-up characteristics of 19 women and 21 men (mean age 54.9 ± 12.1 years) based on a per protocol analysis

	Baseline (n=40)	At 4 weeks (n=40)	At 3 months (n=33)	At 6 months (n=32)
Transient elastography				
CAP (dB/m)	330 ± 32*	311 ± 40	307 ± 48	307 ± 41
LSM (kPa), median (range)	6.8 (4.3 – 12.8)	6.4 (4.0 – 18.6)	6.4 (4.4 – 14.6)	6.7 (3.6 – 12.6)
Body composition				
BMI (kg/m^2)	29.5 ± 3.0	29.1 ± 2.8	29.0 ± 2.9	28.9 ± 3.1
Fat-free mass (%)	67.9 ± 8.0	68.0 ± 7.9	66.1 ± 13.7	69.7 ± 9.3
Fat-mass (%)	32.3 ± 8.2	31.7 ± 7.8	32.6 ± 11.8	30.1 ± 9.0
Visceral fat index < 12, n (%)	23 (60.5)	23 (60.5)	24 (75.0)	23 (74.2)
Biochemical serum markers				
25-hydroxyvitamin D (ng/ml)	11.8 ± 4.8**	34.6 ± 12.9	36.3 ± 10.2	34.8 ± 9.8
ALT (U/l)	45 (13 – 131)	41 (15 – 89)	36 (12 – 96)	39 (11 – 133)
AST (U/l)	34 (18 – 60)	31 (18 – 70)	30 (16 – 70)	32 (15 – 94)
γ-GT (U/l)	50 (21 – 292)	44 (18 – 183)	41 (18 – 225)	41 (17 – 151)
AP (U/l)	75 ± 22	75 ± 20	73 ± 20	70 ± 18
Total bilirubin (mg/dl)	0.5 (0.1 – 1.2)	0.4 (0.2 – 2.4)	0.5 (0.2 – 1.7)	0.5 (0.2 – 1.6)
Albumin (g/l)	45.9 ± 2.4	45.5 ± 2.8	46.6 ± 2.3	46.3 ± 2.4
Creatinine (mg/dl)	0.88 (0.06 – 1.12)	0.88 (0.60 – 1.16)	0.89 (0.58 – 1.19)	0.89 (0.60 – 1.20)
Urea (mg/dl)	28.4 ± 7.1	30.3 ± 8.6	30.5 ± 8.0	31.2 ± 5.5
Calcium (mmol/l)	2.4 (2.2 – 2.6)	2.4 (2.2 – 2.6)	2.4 (2.2 – 2.6)	2.4 (2.2 – 2.5)
Phosphate (mg/dl)	3.1 ± 0.5	3.3 ± 0.6	3.2 ± 0.6	3.3 ± 0.6
PTH (pg/ml)	41.5 (21.0 – 107.0)**	34.5 (21.0 – 95.0)	34.0 (9.0 – 67.0)	30.5 (16.0 – 52.0)

Significant differences from baseline using repeated measures ANOVA are denoted with *(P < 0.05),** (P < 0.001).
ALT: alanine aminotransferase; AST: aspartate aminotransferase; AP: alkaline phosphatase; BMI: body mass index; CAP: controlled attenuation parameter; γ-GT: gamma-glutamyl transpeptidase; PTH: parathyroid hormone.

only significant for γ-GT (65 vs. 37 U/l; P = 0.015). Overall, 51%, 23%, 43% and 8% of all patients had elevated baseline ALT, AST, γ-GT and AP activities, respectively. A significant inverse correlation between serum 25-hydroxyvitamin D and PTH concentrations at baseline was also observed for the cohort (R^2 = 0.196, P = 0.004).

Serum 25-hydroxyvitamin D levels and pill counts at follow-up revealed a high rate of adherence to vitamin D substitution. Mean compliance with expected capsules was 99.6% (75 – 100%). No adverse events occurred during this study. As such, all patients were included in the follow up data analyses. Six LSM values (two at baseline, three at the four-week follow-up and one at the final follow-up) from different patients were outside of the prespecified thresholds for inclusion, and were therefore excluded from LSM-related analyses.

Vitamin D supplementation reduces liver fat quantitatively

Table I summarises absolute values for CAP and serum 25-hydroxyvitamin D concentrations for each study visit (including other biochemical and anthropological data). A significant decrease in CAP values over time (four weeks, three and six months) occurred, with an overall absolute mean decrease of 23 dB/m, from 330 ± 32 to 307 ± 41 dB/m (P = 0.007; Fig. 1). When assessing the specific follow ups, the mean CAP decrease relative to baseline at four weeks and at three and six months was: -5.3 ± 13.8%, (P = 0.012), -6.0 ± 14.6% (P = 0.024) and -6.4 ± 13.0%, (P = 0.008), respectively. Thus, improvements were observed as early as four weeks of supplementation. Figure 2A displays the individual values for each patient.

No significant changes in LSM values were observed during the study; however, the CAP reductions coincided with vitamin

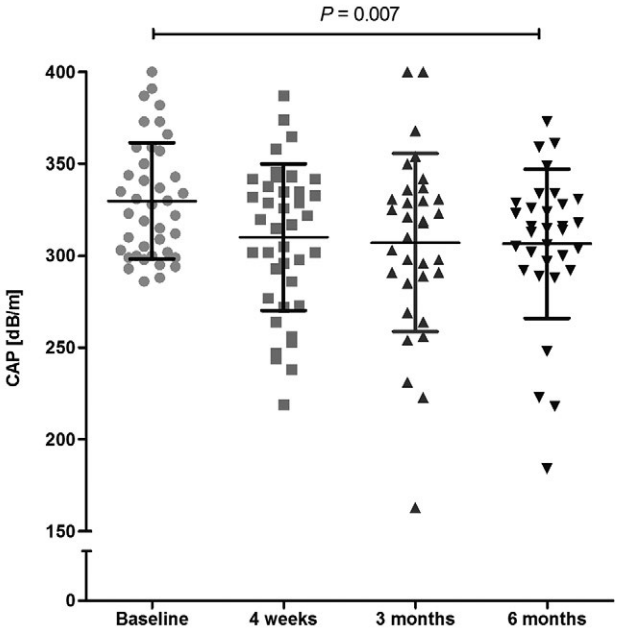

Fig. 1. Absolute CAP values during vitamin D supplementation. Repeated measures ANOVA demonstrated a significant reduction of CAP (plotted together with mean ± standard deviation) over time. A significant mean CAP decrease relative to baseline was demonstrated at four weeks as well as at three and six months: -5.3 ± 13.8% (P = 0.012; n = 40), -6.0 ± 14.6% (P = 0.024; n = 33) and -6.4 ± 13.0% (P = 0.007; n = 32), respectively.

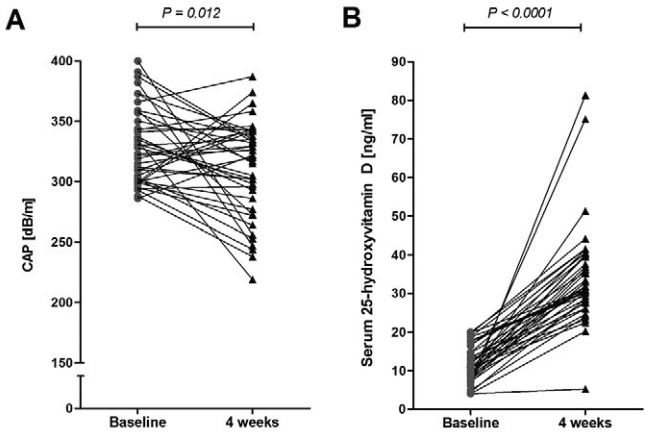

Fig. 2. Individual patient CAP values (A) and serum vitamin D concentrations (B) are plotted for baseline and for the first follow-up four weeks post vitamin D supplementation. Significant differences between these time points in both parameters were observed.

D levels improving significantly (P < 0.0001) at four weeks, with 27 (68%) patients displaying normal serum 25-hydroxyvitamin D concentrations (> 30 ng/ml). Figure 2B depicts the individual vitamin D concentrations for patients at baseline and at four weeks. Overall, mean serum vitamin D levels at four weeks and at three and six months were: 34.6 ± 12.9, 36.3 ± 10.2 and 34.8 ± 9.8 ng/ml, respectively. There were no significant changes in vitamin D levels or CAP values between the three follow-ups.

When assessing overall CAP response at the end of the study, a total of 21 patients (66%) displayed reductions in CAP values after six months of supplementation (final follow-up). We categorised these patients as CAP responders. No within or between group differences in sex, body composition or in LFTs were detected at baseline or at follow-up between these patients and those who did not display decreases of CAP. However, a significant inverse correlation was noted for baseline CAP values and relative CAP change at six months for the overall cohort (R^2 = 0.175, P = 0.02).

Anthropometry and biochemistry remain stable during supplementation

No alterations to body composition occurred during the study, and as anticipated, a reduction in serum PTH concentrations during follow-up was observed (P < 0.0001; Table I). A subgroup comparison showed LFT activities remained stable during the six months in patients with normal levels, and a non-signficant improvement was observed during the follow-up in patients with elevated baseline activities (Table II). Specifically, fewer patients presented with elevated aminotransferase activities at the final follow-up (34.4% and 12.5% for ALT and AST, respectively).

No follow-up differences in absolute CAP values or vitamin D levels were noted for patients with moderate (10 – 20 ng/ml) versus severe (< 10 ng/ml) vitamin D deficiency at baseline. Additionally, no significant differences in relative CAP changes between these two groups were observed and no effect of seasonal variation during the study was illustrated. Moreover, no differences in CAP response were observed in patients with and without a viral hepatitis infection.

Table II. Liver function tests based on a per protocol analysis of patients with normal versus elevated activities

	ALT (U/l)		AST (U/l)		γ-GT (U/l)		AP (U/l)	
	Normal	High	Normal	High	Normal	High	Normal	High
Baseline	26 (13 – 46)	58 (36 – 131)	30 (18 – 47)	48 (36 – 60)	33 (21 – 59)	96 (50 – 292)	71 ± 18	121 ± 16
n (%)	19 (48.7)	20 (51.3)	31 (77.5)	9 (22.5)	23 (57.5)	17 (42.5)	37 (92.5)	3 (7.5)
4 weeks	29 (15 – 52)	51,5 (32 – 89)	28 (18 – 48)	40 (33 – 70)	32 (18 – 92)	85 (43 – 183)	72 ± 17	112 ± 16
n (%)	21 (52.5)	19 (47.5)	31 (77.5)	9 (22.5)	24 (60.0)	16 (40.0)	38 (95.0)	2 (5.0)
3 months	28 (12 – 51)	48 (23 – 96)	29 (16 – 50)	41.5 (28 – 55)	32 (18 – 66)	96 (28 – 225)	71 ± 19	97 ± 31
n (%)	21 (63.6)	12 (36.4)	29 (87.9)	4 (12.1)	19 (57.6)	14 (42.4)	31 (93.9)	2 (6.1)
6 months	32 (11 – 43)	44 (28 – 133)	30 (15 – 94)	33 (26 – 41)	31 (17 – 72)	81 (22 – 151)	69 ± 17	105 (n=1)
n (%)	21 (65.6)	11 (34.4)	28 (87.5)	4 (12.5)	18 (56.2)	14 (43.8)	31 (96.9)	1 (3.1)

Data are presented as medians with ranges for ALT, AST and γ-GT and as means and standard deviations for AP (based on data distribution).
ALT: alanine aminotransferase; AP: alkaline phosphatase; AST: aspartate aminotransferase; γ-GT: gamma-glutamyl transpeptidase.

DISCUSSION

This study shows that correcting a vitamin D deficiency attenuates the degree of hepatic steatosis, as assessed using CAP, supporting the study hypothesis. The mean decrease in CAP of 23 dB/m corresponded to approximately a reduction of half a steatosis grade, as per the cut-offs determined by de Ledinghen, which compared CAP to liver biopsy [32]. The largest CAP reductions occurred after only four weeks of vitamin D replacement, coinciding with the restoration of vitamin D concentrations. Indeed, Heaney [33] suggested that the efficacious effects of vitamin D supplementation occur during the process of restoring serum vitamin D to normal levels (i.e. when correcting a deficiency). Once serum adequacy has been attained, further vitamin D supplementation may provide little gains. This may explain why the greatest improvements occurred during the initial four-week vitamin D replenishment period, although further modest reductions in CAP ensued at the three and six-month follow-ups, with absolute values significantly lower than baseline.

We recently noted such an effect in liver in the widely used animal model of chronic sclerosing cholangitis [34], when comparing *Abcb4-/-* mice receiving a vitamin D deficient diet to those given a normal or high vitamin D diet. The vitamin D deficient mice displayed the most advanced fibrosis and the greatest collagen accumulation in comparison to mice with normal or elevated vitamin D levels. Moreover, the control mice showed reductions in chronic liver injury whereas no major benefits from high dose supplementation were observed. Thus, these findings also support the notion that supplementing above and beyond the acquisition of normal vitamin D levels may offer no major benefits, in contrast to the clear benefits observed when obtaining normal concentrations.

Interestingly, the changes in hepatic lipid fat content did not reflect body fat content, neither did they correlate with significant reductions in LFTs, even though the proportion of patients with elevated aminotransferases decreased with supplementation. Therefore, hepatic lipid storage appears to be a highly dynamic process amenable to short-term modulation. The mechanisms by which vitamin D supplementation reduces hepatic steatosis are likely multifactorial. The fact that vitamin D can reduce FFA circulation, thus subsequently decreasing lipid acumulation via PPAR-γ provides an interesting concept [12]. Moreover, vitamin D regulates hepatic inflammatory and oxidative stress genes via VDR, which are causally implicated in hepatic steatosis [35, 36].

In the only published study assessing the direct effects of vitamin D on hepatic steatosis, Sharifi et al. [37] recently showed an anti-inflammatory response in NALFD patients. In addition to a weight loss intervention in all patients recommending restriction of high-carbohydrate, high-fat foods and an increase in physical activity, 27 patients received 50,000 IU vitamin D3/biweekly for four months (and 26 received placebo). The vitamin D group displayed marked improvements in serum vitamin D and C-reactive protein (CRP) levels, as compared to the placebo group, in whom CRP levels increased. Despite weight loss, no improvements in hepatic steatosis assessed using ultrasonography or in LFTs were noted. However, ultrasonography has been reported to have markedly lower sensitivity when compared to CAP for detecting liver fat, particularly with < 30% liver fat infiltration [38, 39]. In contrast, the patients herein displayed reduced liver fat contents, despite no alterations in body composition during the six months. Nevertheless, given the fact that some observational studies have found no association between vitamin D and NAFLD, this general area warrants further investigation. For example, Li et al. [15] found no association between the presence of ultrasonography quantified NAFLD and vitamin D status in 1,248 Chinese participants. More recently, Bril et al. [14] assessed for associations between vitamin D deficiency and liver fat, in addition to the severity of NASH in 239 participants. Using magnetic resonance imaging and proton spectroscopy, the authors segregated patients based on their vitamin D status. They found no differences in the amount of accumulation of liver triglyceride between patients with normal vitamin D levels, versus those with vitamin D insufficiency or vitamin D deficiency (17 ± 2% vs. 19 ± 2% vs. 21 ± 1%, respectively), even after controlling for key metabolic variables such as insulin resistance.

The strength of the current study is its prospective nature and that only patients with inadequate vitamin D levels received supplementation. Additionally, we were able to frequently and non-invasively monitor hepatic steatosis [40, 41]. A lack of a control group, is however a limitation which means that the 'placebo effect' cannot be entirely eliminated. Therefore, these findings need to be replicated in adequately powered,

randomised controlled trials. Moreover, a second method to quantify hepatic steatosis such as the 'gold standard' liver biopsy, or any other comparable non-invasive method was not used.

CONCLUSION

The degree of hepatic steatosis significantly improved after only four weeks of vitamin D replacement therapy in the absence of concomitant weight loss in this six-month supplementation study. Hepatic steatosis, as assessed by CAP, is a dynamic process, which appears to be modulated by interventions such as vitamin D substitution. The use of non-invasive instruments such as CAP, afford the regular, short-term monitoring of such patients. This potential efficacious effect needs to be explored further in well powered and controlled trials. Finally, the molecular mechanisms underlying hepatocellular lipid remodelling by vitamin D in the presence of hepatic steatosis remain to be identified.

Funding and competing interests. The present study did not receive external funding. All authors declare that they have no conflicts of interest.

Authors contributions. C.S.S. and F.L. designed the study; I.P. collected the study data; C.S.S. drafted the manuscript; F.L. and C.S.S. edited the manuscript. All authors contributed to the analysis and interpretation of the data, the revision of the manuscript for important intellectual content, and all approved the final version. No writing assistance was provided for this manuscript.

Acknowledgements. C.S.S. would like to thank the Association for the Study of the Liver (EASL) for a full bursary to present this study at the International Liver Congress 2015.
This manuscript has been presented, in part, at the Annual Meeting of the European Association for the Study of the Liver (EASL), Vienna, April 2015, and published in abstract form in the Journal of Hepatology 2015;62:S187-S212.

REFERENCES

1. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 2011; 140: 124-131. doi: 10.1053/j.gastro.2010.09.038
2. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA 2015; 313: 2263-2273. doi: 10.1001/jama.2015.5370
3. Bhala N, Angulo P, van der Poorten D, et al. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. Hepatology 2011; 54: 1208-1216. doi: 10.1002/hep.24491
4. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol 2015; 13: 643-654. doi: 10.1016/j.cgh.2014.04.014
5. Review Team, LaBrecque DR, Abbas Z, et al. World Gastroenterology Organisation global guidelines: Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. J Clin Gastroenterol 2014; 48: 467-473. doi: 10.1097/MCG.0000000000000116
6. Sung KC, Kim SH. Interrelationship between fatty liver and insulin resistance in the development of type 2 diabetes. J Clin Endocrinol Metab 2011; 96: 1093-1097. doi: 10.1210/jc.2010-2190
7. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology 2010; 52: 1836-1846. doi: 10.1002/hep.24001
8. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet 2015; 385: 956-965. doi: 10.1016/S0140-6736(14)61933-4
9. Nascimbeni F, Pais R, Bellentani S, et al. From NAFLD in clinical practice to answers from guidelines. J Hepatol 2013; 59: 859-871. doi: 10.1016/j.jhep.2013.05.044
10. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012; 55: 2005-2023. doi: 10.1002/hep.25762
11. Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. J Hepatol 2010; 53: 372-384. doi: 10.1016/j.jhep.2010.04.008
12. Barchetta I, Angelico F, Del Ben M, et al. Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. BMC Med 2011; 9: 85. doi: 10.1186/1741-7015-9-85
13. Wang DQ, Portincasa P, Neuschwander-Tetri BA. Steatosis in the liver. Compr Physiol 2013; 3: 1493-1532. doi: 10.1002/cphy.c130001
14. Bril F, Maximos M, Portillo-Sanchez P, et al. Relationship of vitamin D with insulin resistance and disease severity in non-alcoholic steatohepatitis. J Hepatol 2015; 62: 405-411. doi: 10.1016/j.jhep.2014.08.040
15. Li L, Zhang L, Pan S, Wu X, Yin X. No significant association between vitamin D and nonalcoholic fatty liver disease in a Chinese population. Dig Dis Sci 2013; 58: 2376-2382. doi: 10.1007/s10620-013-2658-1
16. Dasarathy J, Periyalwar P, Allampati S, et al. Hypovitaminosis D is associated with increased whole body fat mass and greater severity of non-alcoholic fatty liver disease. Liver Int 2014; 34: e118-e127. doi: 10.1111/liv.12312
17. Eliades M, Spyrou E, Agrawal N, et al. Meta-analysis: vitamin D and non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2013; 38: 246-254. doi: 10.1111/apt.12377
18. Targher G, Bertolini L, Scala L, et al. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 2007; 17: 517-524. doi: 10.1016/j.numecd.2006.04.002
19. Roth CL, Elfers CT, Figlewicz DP, et al. Vitamin D deficiency in obese rats exacerbates nonalcoholic fatty liver disease and increases hepatic resistin and Toll-like receptor activation. Hepatology 2012; 55: 1103-1111. doi: 10.1002/hep.24737
20. Han SS, Kim M, Lee SM, et al. Association between body fat and vitamin D status in Korean adults. Asia Pac J Clin Nutr 2014; 23: 65-75. doi: 10.6133/apjcn.2014.23.1.10
21. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000; 72: 690-693.
22. Nakano T, Cheng YF, Lai CY, et al. Impact of artificial sunlight therapy on the progress of non-alcoholic fatty liver disease in rats. J Hepatol 2011; 55: 415-425. doi: 10.1016/j.jhep.2010.11.028

23. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clinical Endocrin Metab 2011; 96: 1911-1930. doi: 10.1210/jc.2011-0385
24. Yilmaz Y, Ergelen R, Akin H, Imeryuz N. Noninvasive detection of hepatic steatosis in patients without ultrasonographic evidence of fatty liver using the controlled attenuation parameter evaluated with transient elastography. Eur J Gastroenterol Hepatol 2013; 25: 1330-1334. doi: 10.1097/MEG.0b013e3283623a16
25. Myers RP, Pollett A, Kirsch R, et al. Controlled Attenuation Parameter (CAP): a noninvasive method for the detection of hepatic steatosis based on transient elastography. Liver Int 2012; 32: 902-910. doi: 10.1111/j.1478-3231.2012.02781.x
26. Stokes CS, Grünhage F, Baus C, et al. Vitamin D supplementation reduces depressive symptoms in patients with chronic liver disease. Clin Nutr 2015. doi: 10.1016/j.clnu.2015.07.004
27. Babor TF, Higgins-Biddle JC, Saunders J B. The Alcohol Use Disorders Identification Test. Guidelines for use in Primary Health Care. Second Edition. Geneva: World Health Organisation: 2001.
28. Arslanow A, Stokes CS, Weber SN, Grünhage F, Lammert F, Krawczyk M. The common *PNPLA3* variant p.I148M is associated with liver fat contents as quantified by controlled attenuation parameter (CAP). Liver Int 2016; 36; 418-426. doi: 10.1111/liv.12937
29. Boursier J, Zarski JP, de Ledinghen V, et al. Determination of reliability criteria for liver stiffness evaluation by transient elastography. Hepatology 2013; 57: 1182-1191. doi: 10.1002/hep.25993
30. Banim PJ, Luben RN, Wareham NJ, Sharp SJ, Khaw KT, Hart AR. Physical activity reduces the risk of symptomatic gallstones: a prospective cohort study. Eur J Gastroenterol Hepatol 2010; 22: 983-988. doi: 10.1097/MEG.0b013e32833732c3
31. Wareham NJ, Jakes RW, Rennie KL, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2003; 6: 407-413. doi: 10.1079/PHN2002439
32. de Ledinghen V, Vergniol J, Foucher J, Merrouche W, le Bail B. Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography. Liver Int 2012; 32: 911-918. doi: 10.1111/j.1478-3231.2012.02820.x
33. Heaney RP. Vitamin D--baseline status and effective dose. N Engl J Med 2012; 367: 77-78. doi: 10.1056/NEJMe1206858
34. Hochrath K, Stokes CS, Geisel J, et al. Vitamin D modulates biliary fibrosis in ABCB4-deficient mice. Hepatol Int 2014; 8: 443-452. doi: 10.1007/s12072-014-9548-2
35. Eliades M, Spyrou E. Vitamin D: a new player in non-alcoholic fatty liver disease? World J Gastroenterol 2015; 21: 1718-1727. doi: 10.3748/wjg.v21.i6.1718
36. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 2009; 94: 26-34. doi: 10.1210/jc.2008-1454
37. Sharifi N, Amani R, Hajiani E, Cheraghian B. Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. Endocrine 2014; 47: 70-80. doi: 10.1007/s12020-014-0336-5
38. Ryan CK, Johnson LA, Germin BI, Marcos A. One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation. Liver Transpl 2002; 8: 1114-1122. doi: 10.1053/jlts.2002.36740
39. Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. Nat Rev Gastroenterol Hepatol 2013; 10: 666-675. doi: 10.1038/nrgastro.2013.175
40. Betzel B, Drenth JP. A new noninvasive technique for estimating hepatic triglyceride: will liver biopsy become redundant in diagnosing non-alcoholic fatty liver disease? BMC Med 2014; 12: 152. doi: 10.1186/s12916-014-0152-z
41. Lupsor-Platon M, Feier D, Stefanescu H, et al. Diagnostic accuracy of controlled attenuation parameter measured by transient elastography for the non-invasive assessment of liver steatosis: a prospective study. J Gastrointestin Liver Dis 2015; 24: 35-42. doi: 10.15403/jgld.2014.1121.mlp