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The relationship between 25-hydroxyvitamin D concentration and liver enzymes in overweight or obese adults: Cross-sectional and interventional outcomes



Negar Naderpoor^{a,b}, Aya Mousa^a, Maximilian de Courten^c, Robert Scragg^d,
Barbora de Courten^{a,b,*}

^a Monash Centre for Health Research and Implementation, School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC 3168, Australia

^b Diabetes and Vascular Medicine Unit, Monash Health, Melbourne, VIC 3168, Australia

^c Centre for Chronic Disease, Victoria University, Melbourne, VIC 3021, Australia

^d School of Population Health, The University of Auckland, Auckland, New Zealand

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ABSTRACT

Vitamin D deficiency is prevalent in individuals with non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis. However, there is limited and inconsistent data on the effect of vitamin D supplementation on liver function. Hepatic enzymes have been used as surrogate markers for NAFLD and have been associated with metabolic syndrome. We examined the relationships between 25-hydroxyvitamin D (25(OH)D) and γ -glutamyl transferase (GGT), alanine aminotransferase (ALT), alkaline phosphatase (ALP) in 120 drug-naïve individuals with no history of liver disease. In addition, the effect of vitamin D supplementation (100,000 loading dose of cholecalciferol followed by 4000IU daily for 16 weeks) on hepatic enzymes was investigated in a subgroup of 54 vitamin D-deficient overweight or obese individuals (28 randomised to cholecalciferol and 26 to placebo). Hepatic enzymes, anthropometric parameters, lipid profile, insulin sensitivity (hyperinsulinaemic-euglycaemic clamp, M value) and high sensitivity C-reactive protein (hs-CRP) were measured before and after the intervention. In the cross-sectional study, levels of GGT and ALT were higher in men compared to women (both $p = 0.001$). There were no significant differences in GGT, ALT and ALP between vitamin D categories (25(OH)D < 25 nmol/L, 25–50 nmol/L, and > 50 nmol/L) and no relationships were found between the three enzymes and 25(OH)D before and after adjustment for age, sex, BMI, WHR, and insulin sensitivity (all $p > 0.5$). In the randomised trial, 25(OH)D concentrations increased in the vitamin D group (mean change 57.0 ± 21.3 nmol/L) compared to the placebo group (mean change 1.9 ± 15.1 nmol/L). Mean changes in GGT, ALT and ALP were not significantly different between vitamin D and placebo groups (all $p > 0.2$). Change in 25(OH)D concentration was not correlated with changes in GGT, ALT and ALP before and after adjustments for age and sex (all $p > 0.1$).

In summary, 25(OH)D concentrations were not related to hepatic enzymes in drug-naïve adults with no history of liver disease, and vitamin D supplementation had no effect on the serum levels of hepatic enzymes in vitamin D-deficient and overweight or obese, otherwise healthy individuals. Hence, vitamin D supplementation is unlikely to prevent incident NAFLD.

1. Introduction

Vitamin D deficiency is prevalent worldwide and is associated with obesity and metabolic syndrome [1,2], which are also risk factors for non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) [3,4]. Serum levels of the liver enzymes, alanine aminotransferase (ALT) and γ -glutamyl transferase (GGT), although not

very sensitive, have been used as biomarkers to help diagnose NAFLD and they are related to insulin resistance and risk of diabetes [5–7]. Similar to the trends for vitamin D deficiency, the prevalence of NAFLD has also increased significantly and it is now the most common chronic liver disease affecting about 30% of the general population in the US [8,9]. Serum levels of ALT and risk of NAFLD, have been shown to be inversely associated with serum vitamin D levels [10–13]; while

* Corresponding author at: Monash Centre for Health Research and Implementation (MCHRI), School of Public Health and Preventive Medicine, Monash University, Level 1, 43-51 Kanooka Grove, Clayton, Melbourne, VIC 3168, Australia.

E-mail addresses: barbora.decourten@monash.edu, bdcourten@mac.com (B. de Courten).

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decreased vitamin D concentrations are related to the severity of histopathological features of NAFLD on biopsy, even after adjustment for BMI and insulin resistance [12]. In animal studies, increasing vitamin D concentration by phototherapy or supplementation (1,25-dihydroxyvitamin D or combination of 25-hydroxyvitamin D and calcium), ameliorates development and progression of NAFLD and NASH [14–16]. Mechanisms suggested from observational and few intervention studies on how low vitamin D might contribute to NAFLD are through insulin resistance, lipid accumulation, oxidative stress and inflammation [8,17–20]. However, results from observational studies are often affected by confounders including comorbidities and concurrent use of medications, and the use of surrogate markers of adiposity and insulin resistance. Additionally, there is very limited data from the clinical trials examining the effects of vitamin D supplementation on liver enzymes as surrogate markers of NAFLD. If proven to be beneficial, vitamin D would be an affordable and easily accessible supplementation which is also well-tolerated. Hence, we investigated the relationship between vitamin D and liver enzymes, GGT, ALT and alkaline phosphatase (ALP), in a healthy drug naïve and well-characterised population. Furthermore, in a randomised double-blind clinical trial, we examined the effect of vitamin D supplementation on these liver enzymes in vitamin D-deficient overweight and obese, but otherwise healthy individuals.

2. Methods

2.1. Study design and participants

This study comprised two parts; cross-sectional and interventional. For the cross-sectional part, we combined and performed secondary analyses of the baseline data from three separate studies [21–23] which investigated risk factors for cardiometabolic diseases. The study population consisted of 120 healthy volunteers (73 males and 47 females) with no history of liver disease who were recruited through advertisement from the community in Melbourne, Australia. The intervention part is an extension of our previously published randomised clinical trial (RCT) which was designed to investigate the effect of vitamin D supplementation on insulin sensitivity and secretion in 54 overweight or obese otherwise healthy adults (35 males and 19 females) with vitamin D deficiency [24]. The details of the RCT protocol and main outcomes have been published [22,25]. Inclusion criteria were as follows: aged 18–60 years, stable weight for at least 12 months (no weight change > 5 kg) and no intention to lose weight or change diet or physical activity for the trial duration, and no known comorbidities. For the vitamin D trial, additional inclusion criteria were BMI ≥ 25 kg/m² and 25(OH)D ≤ 50 nmol/L. Exclusion criteria included: smoking, alcohol consumption > 2 standard drinks/week for women and > 4 standard drinks/week for men, taking any medications or supplements, hypercalcaemia, diabetes (based on oral glucose tolerance test (OGTT)), active cancer within the preceding 5 years, pregnant, lactating, or perimenopausal women, or the presence of acute inflammation based on history or blood test. Participants were required not to have taken vitamin D supplements for at least three months prior to taking part in the study. Ethics approvals for the three trials were obtained from the Alfred Hospital and Monash University Human Research Ethics Committees, Melbourne, Australia (Protocol ID: CF13/3874-2013001988) and all participants provided written informed consent.

Participants in the clinical trial (n = 54) were randomised by a researcher not involved in the trial, to receive a bolus dose of 100,000IU cholecalciferol or matching placebo following by a daily dose of 4000IU of cholecalciferol or matching placebo for 16 weeks. All participants filled out validated questionnaires to self-report physical activity (International Physical Activity Questionnaire), diet (3-day food diary; Foodworks 8.0 Professional, Xyris Software, Australia), and sun exposure. Participants, investigators, and outcome assessors were blinded to intervention allocation until data analysis was complete.

2.2. Anthropometric measurements

All participants (n = 120) attended a medical screening and examination which involved measurements of body weight and height, waist and hip circumferences, and blood pressure. Body mass index (BMI) was calculated as weight (kg)/height squared (m²). Waist-to-hip ratio (WHR) was calculated to evaluate abdominal obesity. Body composition was assessed by total body dual energy X-ray absorptiometry (DEXA) (Lunar Radiation Corp., Madison, WI, USA).

2.3. Metabolic measurements

Average systolic and diastolic blood pressures were derived from three measurements using an automated sphygmomanometer (M6 Automatic BP monitor, Omron, Japan) after at least 5 min rest. Every participant had a two-hour 75-g OGTT after an overnight fast to determine the glucose tolerance status based on WHO criteria [26], and exclude diabetes.

Fasting venous blood samples were analysed for 25(OH)D concentrations (direct competitive chemiluminescent immunoassays (CLIA), DiaSorin Inc., MN, USA), inter- and intra-assay coefficients of variation (CVs) of < 10% and < 4%, glucose (the glucose oxidase method, YSI 2300 STAT, YSI Inc., OH, USA), insulin, renal and liver function tests, lipid profiles (all using commercial enzymatic immunoassays, Beckman Coulter, Australia), full blood counts (Beckman Coulter LH750) and high sensitivity C-reactive protein concentrations (hs-CRP) (sensitive near-infrared particle immunoassay rate methodology on a Synchron LX system Chemistry Analyser, Beckman Coulter, Australia).

Insulin sensitivity was measured using the hyperinsulinaemic euglycaemic clamp as per our protocol [22], and involved an initial intravenous insulin bolus (9 mU/kg) followed by a constant insulin infusion at a rate of 40 mU/m²/min for at least 120 min and concurrent adjustable glucose infusion to maintain blood glucose at about 5 mmol/L [22]. The rate of total insulin-stimulated glucose disposal (M-value) was calculated based on the glucose infusion rate for the last 30 min of the clamp and when a steady state was achieved. For RCT participants, all measurements except for OGTT were repeated at follow up.

2.4. Statistical analysis

Baseline characteristics of participants are presented as mean \pm standard deviation (SD) for normally distributed variables and median [interquartile range (IQR)] for non-normally distributed variables. Normality of distributions was assessed using visual inspection of frequency histograms and Shapiro-Wilk tests. Log transformation values were used to approximate normal distribution when required for parametric tests. To compare two different groups, independent-samples *t* tests, and for baseline and follow up comparisons, paired *t* tests were used. Pearson correlations (or Spearman correlations for skewed variables) were performed to assess associations between GGT, ALT, and ALP and other variables including 25(OH)D. Linear regression analyses were conducted with GGT, ALT, and ALP as dependent variables in separate models. Variables which were significantly associated with the dependant variables on Pearson or Spearman analyses were adjusted for in the multiple regression models, and we also adjusted for clinically relevant variable including age, sex, BMI, fat mass and insulin sensitivity (M). To examine the effect of vitamin D supplementation, we used mean changes (follow up minus baseline value) for each variable.

For subgroup analysis, participants were categorised based on 25(OH)D concentrations as: severely deficient (< 25 nmol/L), deficient (25–49.99 nmol/L) and non-deficient (≥ 50 nmol/L). A two-tailed *p*-value < 0.05 was considered statistically significant. Statistical analyses were conducted using SPSS (version 24).

Table 1
Baseline characteristic of participants in the cross-sectional study.

Variable	All participants (n = 120)	Males (n = 73, 60.8%)	Females (n = 47, 39.2%)	p-value
^a Age (year)	29.00 [15.0]	29.00 [14]	28.00 [18]	0.9
^a BMI (Kg/m ²)	29.67 [6.0]	29.14 [6.0]	30.15 [5.8]	0.5
^a Waist-to-hip ratio	0.91 [0.1]	0.95 [0.1]	0.88 [0.1]	< 0.001
% body fat	35.91 ± 10.2	31.74 ± 9.1	42.79 ± 7.9	< 0.001
^a Systolic blood pressure (mmHg)	122.00 [17.0]	124.00 [16.0]	114.30 [19.0]	0.001
Diastolic blood pressure (mmHg)	78.22 ± 9.8	78.87 ± 10.0	77.19 ± 9.6	0.4
^a M (mg/kg/min)	7.27 [4.8]	6.94 [4.6]	7.75 [5.8]	0.3
^a Total cholesterol (mmol/L)	4.62 [1.29]	4.70 [1.6]	4.48 [1.0]	0.1
^a Triglyceride (mmol/L)	1.20 [1.1]	1.50 [1.4]	1.00 [0.6]	< 0.001
HDL (mmol/L)	1.21 ± 0.3	1.15 ± 0.3	1.31 ± 0.3	0.004
^a LDL (mmol/L)	2.80 [1.1]	2.70 [1.3]	2.82 [0.8]	0.3
^a hs-CRP (mg/L)	1.40 [2.3]	1.05 [1.6]	2.20 [3.3]	0.01
^a GGT (U/L)	21.00 [14.0]	27.00 [15.5]	16.00 [8.0]	0.001
^a ALT (U/L)	23.00 [16.5]	27.00 [19.5]	17.00 [10.0]	0.001
^a ALP (U/L)	69.00 [28.0]	72.50 [27.3]	67.00 [28.0]	0.1
^a 25(OH)D (nmol/L)	36.50 [21.8]	38.00 [22.1]	33.60 [20.0]	0.6

ALT: alanine aminotransferase, ALP: alkaline phosphatase, BMI: body mass index, hs-CRP: high-sensitivity C-reactive protein, GGT: γ -glutamyl transferase, HDL: high-density lipoprotein, LDL: low-density lipoprotein, 25(OH)D: 25-hydroxyvitamin D.

^a Data are presented as median [IQR] due to non-normal distribution.

3. Results

3.1. Cross-sectional study

Baseline characteristics of participants are shown in Table 1. The study population (n = 102) included 73 males and 47 females aged 18–57 years. There were no significant differences in age, BMI, insulin sensitivity (M), ALP and 25(OH)D between males and females (all p > 0.1). Male participants had a higher systolic blood pressure (p = 0.001) and WHR (p < 0.001), lower% body fat (p < 0.001), less favourable lipid profiles with higher triglycerides (< 0.001) and lower high-density lipoprotein (HDL) (p = 0.004), and higher GGT (p = 0.001) and ALT (p = 0.001).

Table 2 presents the bivariate correlations with GGT, ALT, and ALP. There were no correlations between 25(OH)D and any liver enzyme levels (all p > 0.6). ALT levels were positively and ALP levels were negatively associated with age (p = 0.02 and 0.009, respectively). The

Table 2
Correlations of 25(OH)D, anthropometric, metabolic and inflammatory markers with GGT, ALT and ALP.

	GGT r (p-value)	ALT r (p-value)	ALP r (p-value)
25(OH)D (nmol/L)	0.06 (0.5)	0.04 (0.7)	−0.01 (0.9)
Age (year)	0.09 (0.4)	0.22 (0.02)	−0.25 (0.009)
BMI (Kg/m ²)	0.26 (0.005)	0.37 (< 0.001)	0.17 (0.07)
Waist-to-hip ratio	0.38 (< 0.001)	0.58 (< 0.001)	0.25 (0.009)
% body fat	−0.12 (0.2)	−0.04 (0.7)	0.11 (0.3)
Systolic blood pressure (mmHg)	0.17 (0.08)	0.24 (0.01)	−0.01 (0.9)
Diastolic blood pressure (mmHg)	0.19 (0.047)	0.26 (0.005)	0.06 (0.3)
M (mg/kg/min)	−0.39 (< 0.001)	−0.037 (< 0.001)	−0.29 (0.002)
Total cholesterol (mmol/L)	0.28 (0.009)	0.41 (< 0.001)	0.21 (0.03)
Triglyceride (mmol/L)	0.40 (< 0.001)	0.49 (< 0.001)	0.21 (0.01)
HDL (mmol/L)	−0.20 (0.04)	−0.32 (0.001)	−0.13 (0.2)
LDL (mmol/L)	0.17 (0.08)	0.39 (< 0.001)	0.22 (0.02)
hs-CRP(mg/L)	0.23 (0.02)	0.05 (0.7)	0.20 (0.05)
GGT (U/L)		0.63 (< 0.001)	0.34 (< 0.001)
ALT (U/L)	0.63 (< 0.001)		0.22 (0.02)

ALT: alanine aminotransferase, ALP alkaline phosphatase, BMI: body mass index, hs-CRP: high-sensitivity C-reactive protein, GGT: γ -glutamyl transferase, HDL: high-density lipoprotein, LDL: low-density lipoprotein, 25(OH)D: 25-hydroxyvitamin D.

p-values < 0.05 are bold for easier visualisation of statistically significant correlations in the table.

three liver enzymes were positively related to each other and to WHR (all p < 0.03) and inversely related to insulin sensitivity (M) (all p < 0.003) (Table 2). GGT and ALT were also related to BMI (p < 0.006). There were no associations between any of the liver enzymes and % body fat (all p > 0.2). ALT was positively related to both systolic and diastolic blood pressure (both p < 0.02) and there was also a positive relationship between GGT and diastolic blood pressure (p = 0.047). GGT was the only enzyme related to hs-CRP (p = 0.02).

Regarding correlations with lipid profile, the liver enzyme levels were positively related to cholesterol and triglyceride levels (all p < 0.04). GGT and ALT were also inversely related to HDL (both p < 0.05) and there were positive relationships between ALT, ALP and LDL (both p < 0.02). The associations of ALT with components of lipid profile were stronger than GGT and ALP (Table 2).

In multiple linear regression analyses, GGT, ALT or ALP levels remained unrelated to 25(OH)D concentrations after adjustments for covariates including age, BMI, WHR, insulin sensitivity (M), diastolic BP, cholesterol (or HDL or LDL), triglycerides and hs-CRP (Tables 3a–3c) (all p > 0.3). In these models, GGT was related to sex (p < 0.001) and hs-CRP (p = 0.02) (Table 3a). ALT was associated with sex, BMI and WHR (all p < 0.05) (Table 3b) and ALP was related to age, cholesterol and LDL (all p < 0.01).

When categorised based on 25(OH)D concentrations, 26 participants had 25(OH)D < 25 nmol/L, 76 participants had 25(OH)D of 25–50 nmol/L, and 18 participants had 25(OH)D > 50 nmol/L. There were no significant differences in GGT, ALT or ALP across vitamin D categories (Kruskal-Wallis test, all p > 0.4).

3.2. Randomised clinical trial

The results relevant to the primary outcomes of insulin sensitivity and secretion are previously published [24] and the Consort diagram of participants flow through the RCT is presented as Supplementary Fig. 1 in the online version at DOI: <http://dx.doi.org/10.1016/j.jsmb.2017.09.009>. After screening, 65 participants, aged 18 to 57 years, were randomised between September 2014 and July 2016 to receive vitamin D (n = 33) or placebo (n = 32). Nine participants dropped out, two were withdrawn due to protocol violation, and one participant had an adverse event. Fifty-four participants (28 in the vitamin D and 26 in the placebo group) completed the study. Baseline and follow up characteristics of the participants are presented in Table 4. The vitamin D group included 17 males and 11 females and the placebo group had 18 male and 8 female participants [24]. There were no significant differences in baseline characteristics between the vitamin D

Table 3a
Multiple linear regression analyses for GGT.

Dependent variable	Independent variables	Model 1		Model 2	
		β	p-value	β	p-value
GGT	25(OH)D	0.11	0.2	0.11	0.2
	Age	-0.01	0.9	-0.03	0.8
	Sex	-0.40	< 0.001	-0.38	< 0.001
	BMI	0.05	0.7	0.03	0.8
	WHR	-0.03	0.8	-0.02	0.9
	Insulin sensitivity (M)	-0.15	0.2	-0.15	0.2
	Diastolic BP	0.08	0.4	0.09	0.4
	Cholesterol	-0.06	0.6		
	Triglycerides	0.22	0.07	0.17	0.1
	HDL			-0.03	0.8
	hs-CRP	0.23	0.02	0.23	0.02
	R ² (p-value)	0.41		0.41	
		(< 0.001)		(< 0.001)	

Model 1: adjusted for age, sex, BMI, WHR, insulin sensitivity (M), Diastolic BP, Cholesterol, triglycerides, hs-CRP.

Model 2: adjusted for age, sex, BMI, WHR, insulin sensitivity (M), Diastolic BP, triglycerides, HDL hs-CRP.

BMI: body mass index, β: standardised beta, Diastolic BP: diastolic blood pressure, hs-CRP: high-sensitivity C-reactive protein, GGT: γ-glutamyl transferase, 25(OH)D: 25-hydroxyvitamin D, WHR: waist to hip ratio.

Table 3b
Multiple linear regression analyses for ALT.

Dependent variable	Independent variables	Model 1		Model 2	
		β	p-value	β	p-value
ALT	25(OH)D	0.01	0.9	0.03	0.7
	Age	0.04	0.6	0.05	0.6
	Sex	-0.30	0.001	-0.31	0.001
	BMI	0.22	0.04	0.22	0.04
	WHR	0.21	0.03	0.17	0.09
	Insulin sensitivity (M)	-0.02	0.9	-0.02	0.9
	Diastolic BP	0.00	0.9	-0.01	0.9
	Cholesterol	0.06	0.5		
	Triglycerides	0.17	0.09	0.18	0.05
	HDL			-0.04	0.6
	LDL			0.11	0.2
	R ² (p-value)	0.46		0.48	
		(< 0.001)		(< 0.001)	

Model 1: adjusted for age, sex, BMI, WHR, insulin sensitivity (M), Diastolic BP, Cholesterol, triglycerides.

Model 2: adjusted for age, sex, BMI, WHR, insulin sensitivity (M), Diastolic BP, triglycerides, HDL and LDL.

ALT: alanine aminotransferase, BMI: body mass index, β: standardised beta, Diastolic BP: diastolic blood pressure, hs-CRP: high-sensitivity C-reactive protein, 25(OH)D: 25-hydroxyvitamin D, WHR: waist to hip ratio.

and placebo groups (all p > 0.1) (Table 4).

Mean serum 25(OH)D concentration at follow up was significantly higher in the vitamin D group compared to baseline (88.4 ± 21.0 vs 31.4 ± 12.6 nmol/L, p = 0.02) and did not significantly change in the placebo group (36.1 ± 15.3 vs 34.2 ± 10.02 nmol/L, p = 0.9). Follow-up 25(OH)D concentration in the vitamin D group reached 70nmol/L and above in 82% of participants (23 out of 28).

There were no significant differences in mean change in GGT (1.0 ± 8.6 vs -1.3 ± 7.9), ALT (-0.1 ± 11.4 vs -3.8 ± 13.4) or ALP (-0.1 ± 15.2 vs 2.0 ± 11.1) between the vitamin D and placebo groups (all p > 0.2). In addition, baseline and follow-up values of GGT, ALT and ALP were not significantly different within each group (all p > 0.2) (Table 4). In linear regression analyses, change in GGT, ALT and ALP remained not associated with change in 25(OH)D

Table 3c
Multiple linear regression analyses for ALP.

Dependent variable	Independent variables	Model 1		Model 2	
		β	p-value	β	p-value
ALP	25(OH)D	0.12	0.2	0.13	0.2
	Age	-0.37	< 0.001	-0.37	< 0.001
	Sex	-0.05	0.6	-0.05	0.7
	BMI	-0.05	0.6	-0.06	0.6
	WHR	0.21	0.07	0.17	0.2
	Insulin sensitivity (M)	-0.22	0.07	-0.21	0.07
	Cholesterol	0.30	0.008		
	Triglycerides	-0.03	0.8	0.07	0.5
	LDL			0.27	0.009
	R ² (p-value)	0.26		0.26	
		(< 0.001)		(< 0.001)	

Model 1: adjusted for age, sex, BMI, WHR, insulin sensitivity (M), Cholesterol, triglycerides.

Model 2: adjusted for age, sex, BMI, WHR, insulin sensitivity (M), triglycerides, LDL.

ALP: alkaline phosphatase, BMI: body mass index, β: standardised beta, Diastolic BP: diastolic blood pressure, hs-CRP: high-sensitivity C-reactive protein, 25(OH)D: 25-hydroxyvitamin D, WHR: waist to hip ratio.

concentration after adjustments for change in BMI, WHR, lipids, insulin sensitivity and hs-CRP or change in physical activity, vitamin D intake, and sun exposure in different models (all p > 0.6). Changes in GGT and ALT were independently related to change in cholesterol (β = 0.43, p = 0.007 for relationship with change in GGT and β = 0.41, p = 0.01 for relationship with change in ALT).

3.3. Subgroup analysis

Fourteen participants (9 in vitamin D and 5 in placebo group) had severe vitamin D deficiency (25(OH)D < 25 nmol/L). Follow-up GGT, ALT, and ALP, as well as mean changes in each liver enzyme, were not significantly different between vitamin D and placebo groups among participants with severe vitamin D deficiency (data not shown). Furthermore, mean changes in GGT, ALT and ALP were not significantly different between those with < 25 and ≥ 25 nmol/L in the vitamin D group (all p > 0.2).

4. Discussion

We conducted a cross-sectional and a randomised clinical trial to examine the associations between 25(OH)D concentrations and liver enzymes and to determine whether liver enzymes would be improved with vitamin D supplementation. We found no association between 25(OH)D concentrations and serum liver enzymes (GGT, ALT and ALP) in healthy and drug-naïve adults, before and after adjustment for potential confounders including age, sex, markers of adiposity, insulin sensitivity, lipids and inflammation as characterised by hs-CRP. Liver enzymes were related to factors involved in the metabolic syndrome including BMI, WHR, insulin sensitivity (M), and lipid profile. Furthermore, vitamin D supplementation had no effect on these enzymes in a vitamin D-deficient overweight or obese otherwise healthy cohort despite a significant increase in 25(OH)D concentration in the vitamin D group.

Current evidence on the association between 25(OH)D concentrations and liver enzymes is inconsistent. A systematic review and meta-analysis of 17 cross-sectional and case-control studies in humans showed a lower 25(OH)D concentration in individuals with NAFLD compared to controls and individuals with NAFLD were 1.26 times more likely to be vitamin D deficient [27]. Three of the included studies used elevated liver enzymes (mainly ALT) to diagnose NAFLD after exclusion of other possible causes [10,28,29]. Similar to our findings,

Table 4
Baseline and follow up characteristics of RCT participants.

Variable	Vitamin D group			Placebo group			p-value
	Baseline	Follow up	Mean change	Baseline	Follow up	Mean change	
Age (year)	30.5 [10.0]			29.5 [16.3]			0.9
Sun exposure (index score)	3.2 [5.2]	3.9 [5.2]	0.3 [3.1]	4.4 [5.3]	3.3 [6.4]	0.7 [3.9]	0.6
Physical activity (IPAQ-METS)	1359.5 [3422.1]	2210.3 [2467.3]	58.8 [2165.8]	2493.0 [3443.0]	2376.0 [2787.0]	360.0 [2790.0]	0.7
Dietary vitamin D intake	92.9 [79.6]	76.1 [43.4]	-24.6 [100.9]	73.1 [49.8]	76.0 [61.5]	-6.1 [48.2]	0.4
BMI (Kg/m ²)	30.2 [7.1]	30.4 [6.0]	0.003 ± 0.9	29.8 [5.3]	29.2 [5.2]	-0.1 ± 1.2	0.7
Waist-to-hip ratio	0.9 ± 0.1	0.9 ± 0.1	-0.01 ± 0.04	0.9 ± 0.04	0.9 ± 0.04	0.001 ± 0.02	0.2
% body fat	40.3 ± 8.2	39.9 ± 8.1	-0.4 ± 1.6	38.8 ± 9.4	38.5 ± 9.2	-0.3 ± 1.7	0.9
Systolic blood pressure (mmHg)	117.2 ± 10.4	117.8 ± 11.4	0.6 ± 6.3	124.7 ± 14.2	121.0 ± 11.1	-3.7 ± 9.9	0.09
Diastolic blood pressure (mmHg)	78.8 ± 7.1	79.3 ± 9.2	0.5 ± 7.7	80.9 ± 10.0	81.9 ± 9.6	1.0 ± 7.7	0.9
M (mg/kg/min)	5.7 ± 2.5	5.8 ± 2.8	0.02 ± 2.0	7.7 ± 3.1	7.6 ± 3.3	-0.03 ± 2.8	0.9
Total cholesterol (mmol/L)	5.0 ± 1.0	4.9 ± 1.1	-0.1 ± 0.5	4.9 ± 0.8	4.7 ± 0.8	-0.2 ± 0.6	0.8
Triglyceride (mmol/L)	1.6 [1.0]	1.4 [1.5]	0.2 ± 0.6	1.4 [1.1]	1.5 [0.9]	-0.1 ± 0.9	0.3
HDL (mmol/L)	1.2 ± 0.2	1.1 ± 0.2	-0.04 ± 0.1	1.2 ± 0.3	1.1 ± 0.2	-0.05 ± 0.2	0.9
LDL (mmol/L)	3.1 ± 0.8	3.0 ± 0.8	-0.1 ± 0.5	3.0 ± 0.7	2.9 ± 0.6	-0.1 ± 0.5	0.8
hs-CRP (mg/l)	2.6 [3.7]	2.0 [4.5]	-0.1 ± 1.4	1.1 [2.4]	1.3 [2.2]	-0.4 ± 2.3	0.8
GGT	25.0 [15.3]	24.5 [17.0]	1.0 ± 8.6	21.5 [14.0]	22.0 [13.5]	-1.3 ± 7.9	0.7
ALT	25.0 [21.5]	30.5 [23.0]	-0.1 ± 11.4	25.5 [12.8]	22.5 [14.5]	-3.8 ± 13.4	0.5
ALP	80.3 ± 23.2	80.2 ± 20.1	-0.1 ± 15.2	74.3 ± 21.8	76.3 ± 18.8	2.0 ± 11.1	0.3
25(OH)D (nmol/L)	31.3 ± 12.6	88.4 ± 21.0	57.0 ± 21.3	34.2 ± 10.0	36.1 ± 15.3	1.9 ± 15.1	< 0.001

ALT: alanine aminotransferase, ALP: alkaline phosphatase, BMI: body mass index, hs-CRP: high-sensitivity C-reactive protein, GGT: γ -glutamyl transferase, HDL: high-density lipoprotein, LDL: low-density lipoprotein, 25(OH)D: 25-hydroxyvitamin D.

* p-values for Mann-Whitney *U* tests comparing change scores between vitamin D and placebo groups.

one study involving 1630 adolescents aged 12–19 years, defined suspected NAFLD as serum ALT > 30 U/L and showed no association between quartiles of 25(OH)D and suspected NAFLD based on increased ALT after adjusting for BMI [28]. A second study involving participants from the third National Health and Nutrition Examination Survey (NHANES III), showed no correlation between 25(OH)D concentrations and serum ALT levels before and after adjustment for insulin resistance based on HOMA-IR [10]. The findings of this study are consistent with our findings showing no association between ALT and 25(OH)D concentration after adjustment for insulin sensitivity based on hyperinsulinaemic euglycaemic clamp. However, this study showed a higher prevalence of unexplained elevated ALT in lower quartiles of 25(OH)D, as well as lower 25(OH)D concentrations in individuals with raised ALT levels ($n = 308$), compared to BMI-matched controls ($n = 976$) [10]. Notably, the study population included individuals with a variety of comorbidities including diabetes and hypertension, and did not adjust for medication use, which may have influenced their results. The third study included 654 individuals with risk factors for diabetes, aged ≥ 30 years, and reported an inverse association between 25(OH)D concentration and ALT after adjusting for components of metabolic syndrome including BMI, waist circumference, and lipids [29]. In contrast, a fourth study which also used NHANES III data for 12,155 participants with normal ALT levels, demonstrated a positive association between 25(OH)D concentrations and ALT levels, which persisted after adjustment for confounders including age, sex, race, and BMI, as well as history of diabetes, hypertension, smoking and alcohol use [30]. Notably, all individuals with serum ALT level > 39 U/L were excluded in this study. Hence, their finding may not be comparable with other studies which included high ALT levels, as higher ALT levels are likely to be associated with more severe liver injury and comorbidities. This NHANES study also cannot exclude possible effects of other confounders such as medications and insulin resistance. Our sample size was much smaller and participants were younger (median age = 29 in our sample vs mean age = 49.9 years in the NHANES study) which could be the reason for our finding of no relationship between 25(OH)D concentrations and ALT levels.

There is limited literature on the effect of vitamin D supplementation on liver enzymes in drug-naïve individuals and the results of RCTs involving participants with NAFLD or NASH are inconclusive. Consistent with our findings, a randomised clinical trial involving 53

ultrasound-proven NAFLD patients with raised ALT levels (ALT > 19 U/L for women and > 30 U/L for men), showed no significant difference in ALT or ultrasound features after 4 months of vitamin D3 supplementation (50,000 IU fortnightly) compared to matching placebo [31]. Similarly, a study of 12 patients with biopsy-proven NASH, without cirrhosis, showed no effect of high dose vitamin D3 supplementation (25,000 IU weekly for 24 weeks) on ALT or NAFLD Activity Score based on biopsy [32]. In contrast, a randomised clinical trial involving 120 participants with confirmed NAFLD based on ultrasound, showed greater reductions in ALT levels after 12 weeks of diet + calcitriol + calcium supplementation compared to diet + calcitriol or diet + placebo [33]. The addition of calcium to the vitamin D supplementation may explain the different findings of this study. Furthermore, the effect of medications particularly statins can not be excluded which could have an impact on their results. In summary, a few studies which have investigated the effect of vitamin D on liver enzymes, are limited by the presence of comorbidities and use of medications. Here, we address these knowledge gaps by showing that correcting vitamin D deficiency by vitamin D supplementation in overweight or obese otherwise healthy and drug-naïve adults did not improve liver enzymes.

Our studies have some limitations. The effect of vitamin D on liver enzymes was not the primary outcome in either the cross-sectional study or RCT. The RCT was designed to assess the effect of vitamin D supplementation on insulin sensitivity and secretion [25]. Our study population included relatively young and healthy individuals and our findings may not be generalizable to populations with established liver disease. We did not measure AST, another marker of liver function and we did not perform ultrasounds, fibroscans, or liver biopsies which are more sensitive for detecting liver injury and steatosis compared to liver enzymes [34]. Our sample size for the RCT may not have been large enough to detect a small benefit from vitamin D supplementation, especially because almost all our participants had liver enzymes within the normal ranges. Despite these limitations, in our RCT, we addressed several gaps in the current literature by eliminating the confounding effects of comorbidities, medications, alcohol, and smoking. We were also able to adjust for a wide range of other confounding factors including insulin sensitivity based on the gold-standard hyperinsulinaemic euglycaemic clamp.

5. Conclusion

Evidence from some but not all observational studies indicate an association between 25(OH)D concentrations and liver enzymes even when these enzymes are within the normal ranges. In our study, there was no relationship between 25(OH)D concentrations and GGT, ALT or ALP levels in healthy drug-naïve individuals. We advance knowledge in the field by showing no effect of vitamin D supplementation, at a sufficient dose, in a well-designed double-blind randomised trial, not confounded by comorbidities or concurrent use of medications and after adjustments for multiple confounders. To our knowledge, this is the first study examining the effect of cholecalciferol supplementation on liver enzymes in drug-naïve adults at risk of NAFLD. Our findings suggest that vitamin D supplementation may not be beneficial for preventing the development of NAFLD in overweight or obese, otherwise healthy individuals. Future randomised trials with larger sample sizes, longer durations, and using more sensitive screening tools for liver injury are needed to further investigate the effects of vitamin D supplementation in overweight or obese individuals.

Author contributions

Dr. Negar Naderpoor wrote the first draft of the manuscript, conducted the data analysis and was involved in data acquisition for a part of the study. Ms. Aya Mousa contributed to writing and editing the manuscript and was involved in data acquisition for a part of the study. Prof Maximilian de Courten, and Prof Robert Scragg contributed to the study design and obtaining of funding and to writing and editing the manuscript. A/Prof Barbora de Courten is the principal investigator of the trials which collected the data used in this manuscripts, obtained funding for these trials, supervised all aspects of the studies and contributed to the planning, writing and editing the manuscript. All authors read and approved the final manuscript, and all authors meet the ICMJE criteria for authorship.

Conflict of interest

The authors declare no conflict of interest.

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