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Low serum 25-hydroxyvitamin D concentrations are associated with non-alcoholic fatty liver disease in adolescents independent of adiposity

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Abstract

Background and aims: Non-alcoholic fatty liver disease (NAFLD) and serum 25-hydroxyvitamin D (s25(OH)D) concentrations are both associated with adiposity and insulin resistance (IR) and thus may be pathogenically linked. We aimed to determine the prevalence of vitamin D deficiency in adolescents with NAFLD and to investigate the longitudinal and cross-sectional associations between s25(OH)D concentrations and NAFLD.

Methods: Participants in the population-based West Australian Pregnancy (Raine) Cohort had seasonally-adjusted s25(OH)D concentrations determined at ages 14 and then 17 years. NAFLD was diagnosed at 17 years using liver ultrasonography. Associations were examined after adjusting for potential confounders. Odds ratios (OR) and confidence intervals (CI) are reported per standard deviation in s25(OH)D concentrations.

Results: NAFLD was present in 16% (156/994) of adolescents. The majority of participants with NAFLD had either insufficient (51%) or deficient (17%) vitamin D status. Lower s25(OH)D concentrations at 17 years were significantly associated with increased risk of NAFLD (OR 0.74, 95%CI 0.56,0.97; p=0.029), after adjusting for sex, race, physical activity, television/computer viewing, body mass index and IR. The effect of s25(OH)D concentrations at 17 years was minimally affected after further adjusting for s25(OH)D concentrations at 14 years (OR 0.76, 95%CI 0.56,1.03; p=0.072). **Conclusions:** Lower s25(OH)D concentrations are significantly associated with NAFLD, independent of adiposity and IR. Screening for vitamin D deficiency in adolescents at risk of NAFLD is appropriate, and clinical trials investigating the effect of vitamin D supplementation in the prevention and treatment of NAFLD may be warranted.

Keywords: 25-hydroxyvitamin D; non-alcoholic fatty liver disease; obesity

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a pathological condition marked by excessive hepatic steatosis and may be associated with liver injury and progression to cirrhosis in some individuals[1]. Insulin resistance (IR) is considered a primary mechanism in the development of hepatic steatosis and well-documented risk factors for NAFLD include obesity, hyperlipidaemia, IR and type 2 diabetes mellitus[2]. In developed countries, NAFLD has been reported in approximately 20-35% of adults in the general population, increasing to 70-90% in obese individuals[6]. The prevalence of fatty liver in obese children in China, Italy, Japan, and the United States ranges from 10-77%[7].

Vitamin D deficiency is common worldwide in both adults[8-11] and adolescents[12, 13] and a growing body of evidence suggests that low serum 25-hydroxyvitamin D (s25(OH)D) concentrations are associated with IR, type 2 diabetes mellitus, cardiovascular disease and the metabolic syndrome[14]. Vitamin D receptors (VDR) regulate over 200 genes, including those involved in glucose and lipid metabolism[15] and are widely distributed throughout the liver where they regulate hepatic lipid metabolism[16]. The hydroxylated form of 25(OH)D (1,25-dihydroxyvitamin D) is capable of reducing free fatty acid-induced IR in peripheral tissues[17]. Furthermore, clinical trials have shown that vitamin D supplementation improves IR and insulin sensitivity compared to placebo[18].

Although a number of studies have examined the relationship between vitamin D levels and NAFLD[19-25], not all have included adequate adjustment for key potential confounders, such as adiposity, IR and physical activity. Furthermore, some studies have relied on alanine aminotransferase (ALT) as a biomarker for NAFLD, which is relatively insensitive and

nonspecific for NAFLD[26, 27]. Most studies have been conducted in adult populations and, to our knowledge, only one study has examined the association between s25(OH)D concentrations and NAFLD in adolescents[21]. In order to elucidate the relationship between vitamin D levels and NAFLD in adolescents, we examined s25(OH)D concentrations at 14 and 17 years and the presence of NAFLD identified by liver ultrasonography at 17 years, in a Western Australian population-based cohort.

Methods

Participants

The Western Australian Pregnancy Cohort (Raine) Study is a prospective, population-based, study [28]. In brief, a total of 2900 pregnant women from the public antenatal clinic at King Edward Memorial Hospital or surrounding private clinics in Perth, Western Australia, were recruited between May 1989 and November 1991, and gave birth to 2868 live children. These children underwent serial assessment at birth and throughout childhood and adolescence. Recruitment and all follow-ups were approved by the human research ethics committees of King Edward Memorial Hospital for Women and the Princess Margaret Hospital for Children, Perth, Western Australia. Informed and written consent was obtained from the participant and/or their primary caregiver for all follow-ups.

Assessment of NAFLD

Liver ultrasonography was performed at the 17 year follow-up by trained ultrasonographers with a Siemens Antares ultrasound machine with a CH 6-2 curved array probe (Sequoia,

Siemens Medical Solutions, Mountain View, CA) according to standardised protocol[30] which provides 92% sensitivity and 100% specificity for the histological diagnosis of fatty liver. Ultrasound images were interpreted by a single specialist radiologist, who was blinded to the clinical and laboratory characteristics of the participants. Scores of 0-3, 0-2 and 0-1 were determined from captured images for liver echotexture, deep attenuation and vessel blurring, respectively. The diagnosis of fatty liver required a total score of two or more, including an echotexture score of one or more. The intra-observer reliability (κ statistic) for fatty liver diagnosis in this cohort was previously reported as 0.78 (95% CI 0.73,0.88)[31]. Hepatic fatty infiltration (steatosis) severity was classified by the total fatty liver score as 0 to 1 (no fatty liver), 2 to 3 (mild fatty liver), or 4 to 6 (moderate to severe fatty liver).

Information on alcohol intake over the past 12 months was derived from a self-reported food frequency questionnaire developed by the CSIRO, Adelaide, Australia[32]. Based on diagnostic guidelines for NAFLD, adolescents with sonographic fatty liver were classified as having NAFLD if their self-reported weekly alcohol intake was less than 210 g and 140 g for males and females, respectively[6]. Medications and a comprehensive medical history were documented to exclude secondary causes of NAFLD and concomitant liver disease.

Assessment of s25(OH)D concentrations

Venous blood samples were taken from an antecubital vein after an overnight fast and stored at -80°C until analysis. Serum 25(OH)D is extremely stable in storage, providing useful and accurate samples even in long term epidemiologic studies[33]. s25(OH)D concentrations at 14 years were measured by enzyme immunoassay (Immunodiagnostic Systems Ltd, Scottsdale, Arizona, USA). At 17 years s25(OH)D_2 and s25(OH)D_3 concentrations were measured using

isotope-dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) (RDDT, Victoria, Australia). Twelve samples from the 14 year cohort were also measured by LC-MS/MS (RDDT, Victoria, Australia), according to published methodology[34]. Correlation between the enzyme immunoassay and LC-MS/MS was strong (r^2=0.933), confirming that there were no vitamin D metabolites interfering with the immunoassay[35]. The inter-assay coefficients of variations (CVs) for the enzyme immunoassay were: low standard (40.3 nmol/L) 4.6%; medium standard (72.0 nmol/L) 6.4%; high standard (132.0 nmol/L) 8.7%. For the LC-MS/MS, the CVs for s25(OH)D_3 were: low standard (27.1 nmol/L) 7.1%; medium standard (75.4 nmol/) 5.0%; high standard (163.8 nmol/L) 5.3%. The CVs for s25(OH)D_2 were: low standard (23.4 nmol/L) 8.8%; medium standard (66.0 nmol/) 6.7%; high standard (150.1 nmol/L) 6.7%.

Since the immunoassay at the 14 year follow-up did not differentiate between s25(OH)D_2 and 25(OH)D_3, analyses at both time points were performed on total s25(OH)D concentrations. A sinusoidal model incorporating month of blood collection was used to calculate deseasonalised s25(OH)D concentrations[36]. Vitamin D status was defined as sufficient when concentrations of s25(OH)D were ≥75 nmol/L, insufficient when they were 50-74.9 nmol/L and deficient when they were <50 nmol/L[37].

Serum biochemistry

Laboratory assessments at the 17 year follow-up were performed with venous blood samples taken from an antecubital vein after an overnight fast and stored at -80°C. Serum glucose, insulin, triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), ALT and gamma-glutamyl transpeptidase (GGT) were assayed. All assays were performed at an

accredited central laboratory (PathWest Laboratories, Perth, Western Australia). The homeostatic model assessment for IR (HOMA-IR) score was calculated as follows: HOMA-IR score=(fasting insulin (μU/mL) x fasting glucose (mmol/L)) / 22.5[38].

Potential confounding variables

Participants were classified as Caucasian if both parents were Caucasian, or as non-Caucasian if one or both parents were of an alternate ethnicity. Participants were weighed to the nearest 100 g using a Wedderburn Digital Chair Scale and height was determined to the nearest 0.1 cm with a Holtain Stadiometer. BMI (kg/m^2) at 17 years was categorised using sex- and age-specific thresholds recommended by the International Obesity Task Force [39, 40]. Waist circumference was measured at the level of the umbilicus to the nearest 0.1 cm until two readings were within one centimetre of each other. Central obesity was defined as waist circumference ≥80 cm in females and ≥94 cm in males, consistent with International Diabetes Federation criteria[41]. Suprailiac skinfold measurements were obtained with a skinfold caliper (Holtain Tanner/Whitehouse skinfold caliper, Holtain, Crosswell, United Kingdom). Physical activity at 17 years was assessed using a self-reported questionnaire based on exercise outside of school hours per week, with exercise defined in three categories as activity causing breathlessness or sweating (≥4 times per week, 1-3 times per week and <once per week). Sedentary activity at 17 years was assessed in three categories by self-reported questionnaire based on the number of hours per day spent watching television or videos, or using the computer (<2 hours per day, 2-4 hours per day and >4 hours per day).

Statistical analysis

Analyses were performed using IBM SPSS Statistics Release Version 19.9.9.1 (IBM SPSS Inc., 2010, Chicago, IL). Statistical significance was defined as two-tailed $p<0.05$. Baseline characteristics of participants and presence of NAFLD at 17 years were compared using Pearson's chi-square tests for categorical variables, independent samples t-tests for parametric continuous variables and Mann-Whitney U tests for non-parametric continuous variables. The correlation between s25(OH)D concentrations at 14 and 17 years was examined using Pearson Correlation.

Odds ratios (OR), confidence intervals (CI) and β coefficients are reported per standard deviation of deseasonalised s25(OH)D concentrations, with the standard deviation based on the sample of 718 adolescents. We examined univariate associations between s25(OH)D concentrations at both 14 and 17 years and NAFLD at 17 years. Multiple logistic regression was applied to investigate associations between s25(OH)D concentrations at both 14 and 17 years and NAFLD at 17 years. Models were first adjusted for sex, race, physical activity and television/computer viewing. The model fit was similar using three measures of adiposity (BMI, waist circumference and suprailiac skinfold). Since more complete data were available for BMI, we used this measure of adiposity in the analyses. Models were then further adjusted for BMI and HOMA-IR. We investigated interactions between sex, BMI, physical activity and s25(OH)D concentrations. We also combined serum 25OHD concentrations from 14 and 17 years into one adjusted model. In addition, we conducted three models using s25(OH)D concentrations at 17 years as a binary variable for vitamin D insufficiency/deficiency (<75 nmol/L) and sufficiency (≥75 nmol/L), adjusted as above.

The association between s25(OH)D concentrations and severity of hepatic steatosis was explored using one-way ANOVA. We examined univariate associations between s25(OH)D

concentrations at both 14 and 17 years and aminotransaminases (ALT and GGT) at 17 years. Multivariate general linear models were used to investigate associations between s25(OH)D concentrations at 17 years and aminotransaminases at 17 years, adjusted first for sex, race, physical activity and television/computer viewing and then further adjusted for BMI and HOMA-IR.

Results

Characteristics of study participants and presence of NAFLD

Participants in the current study were more likely to be Caucasian, from families with a higher income during pregnancy, and from mothers with a higher age at birth, higher education and healthier body mass index (Supplementary Table 1). A total of 1754 adolescents participated in the 17 year follow-up between July 2006 and June 2009 (Supplementary Figure 1). Data on both s25(OH)D concentrations at 17 years and liver ultrasonography at 17 years were available for 994 participants. Complete data, including s25(OH)D concentrations at 14 and 17 years, and all confounding variables, were available for 718 participants.

A total of 156/994 participants (16%) were identified by liver ultrasonography and self-reported alcohol intake as having NAFLD at 17 years. Of those identified with NAFLD, 62% were female and 39% were male ($p<0.001$) (Table 1). Central obesity, as determined by waist circumference, was also significantly more prevalent in females compared to males (38% v 12%, $p<0.001$, data not shown). As expected, participants with NAFLD, compared to those without NAFLD, had higher measures of adiposity (BMI, waist circumference, suprailiac

skinfold), dyslipidemia (serum fasting triglyceride, HDL-cholesterol), insulin resistance (serum insulin, HOMA-IR) and liver transaminases (ALT, GGT) (p<0.005 for all). Those with NAFLD were also less likely to be physically active compared to those without NAFLD (p=0.020).

Serum 25(OH)D concentrations and NAFLD

The prevalence of vitamin D deficiency and insufficiency at 17 years was higher in those with NAFLD compared to those without NAFLD (p<0.001) (Table 1). Of the participants who were vitamin D sufficient, 10% had NAFLD; in contrast, among those who were vitamin D deficient, 23% had NAFLD (Figure 1). Mean (± standard deviation) s25(OH)D concentrations at 17 years were significantly lower in those with NAFLD (67±22 nmol/L) compared to those without NAFLD (77±24 nmol/L, p<0.001). Similarly, s25(OH)D concentrations at 14 years were lower among individuals subsequently diagnosed with NAFLD at 17 years compared to those who had no NAFLD (80±23 nmol/L vs. 87±28 nmol/L, p=0.005).

Serum 25(OH)D concentrations at 14 years and risk of NAFLD at 17 years

In univariate analyses, s25(OH)D concentrations at 14 years were significantly associated with NAFLD at 17 years (OR 0.68, 95% CI 0.54,0.86; p=0.001, data not shown). After adjusting for sex, race, physical activity and television/computer viewing at 17 years, s25(OH)D concentrations at 14 years were significantly associated with NAFLD at 17 years (OR 0.69, 95% CI 0.54,0.89; p=0.004) (Table 2). However, when further adjusted for

HOMA-IR and BMI at 17 years, the association was attenuated. There were no significant interactions between sex, BMI or physical activity and s25(OH)D concentrations.

Serum 25(OH)D concentrations at 17 years and risk of NAFLD at 17 years

In univariate analyses, s25(OH)D concentrations at 17 years were significantly associated with NAFLD at 17 years (OR 0.61, 95% CI 0.49,0.76; $p<0.001$, data not shown). After adjusting for sex, race, physical activity and television/computer viewing, s25(OH)D concentrations at 17 years were significantly associated with NAFLD (OR 0.58, 95% CI 0.45,0.73; $p<0.001$) (Table 3). When further adjusted for HOMA-IR and BMI, the significant association remained (OR 0.74, 95% CI 0.56,0.97; $p=0.029$). There were no significant interactions between sex, BMI or s25(OH)D concentrations. The correlation between s25(OH)D concentrations at 14 and 17 years was 0.50 ($p<0.001$). When s25(OH)D concentrations at 14 and 17 years were combined in one adjusted model, there was minimal change in the effect of s25(OH)D concentrations at 17 years and NAFLD (Table 4).

The risk of NAFLD was nearly double for those with insufficient/deficient vitamin D status compared to those with sufficient status, after adjusting for sex, race, physical activity and television/computer viewing, BMI and HOMA-IR (OR 1.85, 95% CI 1.13,3.01; $p=0.014$) (Supplementary Table 2). When further adjusted for s25(OH)D concentrations at 14 years, the risk of NAFLD remained significantly higher in those with insufficient/deficient vitamin D status at 17 years compared to those who were sufficient in vitamin D (OR 1.76, 95% CI 1.05,2.96; $p=0.033$).

Serum 25(OH)D concentrations and sonographic severity of hepatic steatosis

There was a significant difference ($p < 0.001$) in s25(OH)D concentrations between levels of hepatic steatosis, with s25(OH)D concentrations decreasing as sonographic severity of steatosis increased ($n = 718$). Mean (95% CI) s25(OH)D concentrations were 78 (76, 80) nmol/L for those with no fatty liver, 70 (65, 74) nmol/L in those with mild fatty liver, and 59 (50, 69) nmol/L in those with moderate to severe fatty liver.

Serum 25(OH)D concentrations and aminotransamination

s25(OH)D concentrations at 14 years were not significantly associated with ALT or GGT at 17 years in univariate analyses; however, s25(OH)D concentrations at 17 years were inversely associated with both ALT (β -1.24, 95% CI -2.18,-0.30; p=0.010) and GGT (β -1.02, 95% CI -1.56,-0.48; p<0.001) at 17 years (n=718, data not shown). When adjusted for sex, race, physical activity and television/computer viewing, s25(OH)D concentrations at 17 years remained associated with serum ALT levels but the association was non-significant when further adjusted for HOMA-IR and BMI (Supplementary Table 3). Similarly, s25(OH)D concentrations at 17 years were not significantly associated with serum GGT levels after adjusting for HOMA-IR and BMI (Supplementary Table 4).

Discussion

To our knowledge, this is the first population-based study in adolescents investigating the relationship between s25(OH)D concentrations and NAFLD using liver ultrasonography. Our results suggest as association between s25(OH)D concentrations and NAFLD independent of adiposity, physical activity and IR. Furthermore, current s25(OH)D concentrations had a

stronger effect on the risk of NAFLD than prior s25(OH)D concentrations. However, since s25(OH)D concentrations at 14 and 17 years are correlated, low vitamin levels at 14 years do have some predictive ability in identifying adolescents at risk of NAFLD at 17 years.

A number of studies have found significant associations between vitamin D levels and NAFLD; however, these are mostly from specialised clinical centres. In 60 Italian adults, Targher and colleagues found that patients with biopsy-proven NAFLD had markedly lower s25(OH)D concentrations than matched controls [23]. Furthermore, in a study of 262 Italian adults, patients with NAFLD had reduced s25(OH)D concentrations compared to subjects without NAFLD and the association was not affected by BMI[22]. Similarly, in a retrospective case-control study of 1214 adult outpatients in the United States, NAFLD was associated with low 25(OH)D concentrations independently of BMI[42].

Several studies have shown no association between vitamin D levels and NAFLD. In a population-based cohort of adolescents in the United States (*n*=1630), s25(OH)D concentrations were not associated with suspected NAFLD, assessed by elevated ALT, after adjustment for obesity[21]. However, although serum ALT is commonly used in large-scale epidemiological studies as a biomarker for liver fat accumulation, it may not accurately represent NAFLD[26, 27]. We found that, although s25(OH)D concentrations were associated with the presence of NAFLD as assessed by liver ultrasonography, s25(OH)D concentrations were not associated with ALT in adjusted models.

There are a number of plausible explanations for the association between vitamin D deficiency and the development and progression of NAFLD. VDR are expressed throughout the liver where they regulate hepatic lipid metabolism, hepatic necroinflammation and

fibrosis[16]. Hepatic expression of VDR is negatively associated with the severity of liver histology in patients with nonalcoholic steatohepatitis (NASH) or chronic hepatitis C, indicating that VDR may play a role in the progression of chronic liver damage[43]. There is also a strong association between low vitamin D levels and IR, which leads to hepatic steatosis[44, 45]. Vitamin D depletion increased insulin resistance and up-regulated hepatic inflammatory and oxidative stress genes, exacerbating NAFLD[46]. In our human cohort study, however, we found no association between vitamin D and IR independent of NAFLD and were not able to assess the association between vitamin D and hepatic inflammation given the lack of liver histology.

It is possible that vitamin D may influence the development and progression of NAFLD in susceptible individuals with certain genetic polymorphisms. Genetic variations in vitamin D metabolism have been identified and may be associated with liver fibrosis[47]. In a genome-wide association study conducted in the Raine cohort[48] we observed that a common single nucleotide polymorphism (rs222054) in the group specific component gene (which encodes vitamin D binding protein) was associated with NAFLD. Furthermore, *GC* hepatic gene expression and serum protein levels were significantly altered in adult NAFLD subjects compared to controls, further suggesting that vitamin D metabolism was associated with a predisposition to NAFLD.

Although participants included in the current study were more likely to be from families with higher socioeconomic status relative to participants from the original cohort, the original Raine cohort slightly over-represented socially disadvantaged families, leaving an active cohort that is more representative of the general Western Australian population[29].A strength of this study included extensive characterization of a population-based cohort, allowing us to

assess the impact of various confounding factors. However, although we adjusted for physical activity and sedentary activity, the measure of assessment was not based on a validated questionnaire and may be subject to self-reporting bias. Furthermore, our assessment of physical activity did not differentiate between indoor and outdoor activity.

A further limitation of our study was the use of liver ultrasonography to detect the presence of NAFLD, rather than the gold-standard of liver biopsy. Liver ultrasonography becomes less sensitive when detecting low levels of steatosis, and only liver biopsy can determine disease severity. However, liver ultrasound provides a useful non-invasive estimate of histological hepatic steatosis in children and adults, and is recommended for assessment in large population-based studies[49]. Although this study included s25(OH)D concentrations at two time points, the assessment of NAFLD was only conducted at 17 years; therefore, we cannot infer causality in the relationship between s25(OH)D concentrations and NAFLD.

Our results demonstrate that vitamin D insufficiency and deficiency are more common in adolescents with NAFLD than those without NALFD. The high prevalence of vitamin D insufficiency and deficiency in adolescents with NAFLD is concerning, since low vitamin D levels may contribute to other health problems beyond the risk of NAFLD. We found a significant association between s25(OH)D concentrations and the presence of NAFLD, independent of adiposity and IR. Given that NAFLD is considered the hepatic manifestation of the metabolic syndrome, prospective studies addressing vitamin D status and the metabolic syndrome are warranted. Screening for vitamin D deficiency in adolescents at risk of NAFLD, and clinical trials investigating the effect of vitamin D supplementation in the prevention and treatment of NAFLD, may be appropriate. Further studies are needed to elucidate the mechanisms that underpin the role of vitamin D in the development of NAFLD.

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Conflicts of interest: None

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Figure 1. Percentage of participants with and without NAFLD in relation to vitamin D status at 17 years (*n*=994)

Vitamin D status: sufficient, 25(OH)D ≥75 nmol/L; insufficient, 25(OH)D 50-74.9 nmol/L; deficient, 25(OH)D <50 nmol/L

Table 1. Characteristics of study participants and presence of NAFLD at 17 years (*n*=994)

	No NAFLD		NAFLD		*p* value
	n		*n*		
Sex[1]	*838*		*156*		<0.001*
Male		*457* (55)		*60* (38)	
Female		*381* (45)		*96* (62)	
Race[1]	*838*		*156*		0.680
Caucasian		*709* (85)		*134* (86)	
Non-Caucasian		*129* (15)		*22* (14)	
25(OH)D at 17 years (nmol/L)[3]	*838*	77 ± 24	*156*	67± 22	<0.001*
Vitamin D status[1]	*838*		*156*		
Sufficient		*429* (51)		*50* (32)	<0.001*
Insufficient		*316* (38)		*79* (51)	
Deficient		*93* (11)		*27* (17)	
25(OH)D at 14 years (nmol/L)[3]	*724*	87± 28	*137*	80 ± 23	0.005*
BMI (kg/m2)[2]	*838*	22 (20, 24)	*155*	27 (23, 33)	<0.001*
BMI categories[1]	*838*		*155*		<0.001*
Underweight		*58* (7)		*6* (4)	
Healthy weight		*647* (77)		*57* (37)	
Overweight		*104* (12)		*37* (24)	
Obese		*29* (4)		*55* (36)	
Waist circumference (cm)[2]	*815*	76 (72, 82)	*144*	87 (77, 102)	<0.001*
Waist circumference IDF[1]	*815*		*144*		<0.001*
<IDF threshold		*673* (83)		*53* (37)	
≥IDF threshold		*142* (17)		*91* (63)	
Suprailiac skinfold (cm)[2]	*801*	12 (8, 18)	*124*	25 (17, 33)	<0.001*
Total cholesterol (mmol/L)[3]	*812*	4.1 ± 0.7	*154*	4.2 ± 0.9	0.069
HDL (mmol/L)[3]	*812*	1.3 ± 0.3	*154*	1.2 ± 0.3	0.001
Triglycerides (mmol/L)[2]	*812*	0.9 (0.7, 1.2)	*154*	1.1 (0.8, 1.5)	<0.001*
Glucose (mmol/L)[2]	*811*	4.7 (4.5, 5)	*154*	4.7 (4.5, 5)	0.252
Insulin (mU/L)[2]	*812*	7.0 (4.7, 10.3)	*154*	9.8 (6.8, 15.9)	<0.001*
HOMA-IR[2]	*811*	1.5 (1.0, 2.2)	*154*	2.1 (1.4, 3.3)	<0.001*
ALT (IU/L)[3]	*812*	20 ± 10	*154*	27 ± 20	<0.001*
GGT (IU/L)[3]	*812*	14 ± 20	*154*	18 ± 11	<0.001*
Physical activity[1]	*712*		*140*		0.020*
≥4 times per week		*189* (27)		*23* (16)	
1-3 times per week		*381* (54)		*79* (56)	
<once per week		*142*(20)		*38* (27)	
Television/computer viewing[1]	*893*		*161*		0.347
<2 hours per day		*223* (29)		*34* (24)	
2-4 hours per day		*300* (40)		*56* (40)	
>4 hours per day		*236* (31)		*51* (36)	

NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; IDF, International Diabetes Federation; 25(OH)D, deseasonalised 25-hydroxyvitamin D; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; [1]Chi square test (n (%)); [2]Mann-

Whitney U test (median (IQR)); [3]t-test (mean ± standard deviation); *Significant at the $p<0.05$ level

Vitamin D status: sufficient, ≥75 nmol/L; insufficient 50 – 74.9 nmol/L; deficient,<50 nmol/L

Table 2. Adjusted logistic regression models of deseasonalised s25(OH)D concentrations at 14 years and risk of NAFLD at 17 years (*n*=718)

	Model 1[1]		Model 2[2]	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
25(OH)D (per SD) at 14 years	0.69 (0.54, 0.89)	0.004*	0.84 (0.64, 1.09)	0.191
Sex (female v male)	1.50 (0.98, 2.30)	0.059	1.75 (1.07, 2.86)	0.025*
Race (non-Caucasian v Caucasian)	0.68 (0.37, 1.23)	0.198	1.20 (0.61, 2.34)	0.600
Physical activity		0.105		0.112
≥4 times per week	-		-	
1-3 times per week	1.71 (0.97, 2.99)	0.063	1.48 (0.78, 2.80)	0.230
<once per week	1.96 (1.02, 3.73)	0.042*	2.17 (1.04, 4.52)	0.038*
Television/computer viewing		0.374		0.797
<2 hours per day	-		-	
2-4 hours per day	1.27 (0.77, 2.12)	0.350	1.19 (0.68, 2.10)	0.547
>4 hours per day	1.47 (0.86, 2.51)	0.163	1.20 (0.65, 2.21)	0.557
HOMA-IR			1.04 (0.90, 1.19)	0.629
BMI (kg/m^2)			1.32 (1.24, 1.40)	<0.001*

25(OH)D, deseasonalised serum 25-hydroxyvitamin D concentrations; SD, standard deviation; HOMA-IR, homeostatic model assessment for insulin resistance; BMI, body mass index

[1]Adjusted for sex, race, physical activity and television/computer viewing at 17 years; [2]Further adjusted for HOMA-IR and BMI at 17 years

*Significant at the $p<0.05$ level

Table 3. Adjusted logistic regression models of deseasonalised s25(OH)D concentrations at 17 years and risk of NAFLD at 17 years (*n*=718)

	Model 1[1]		Model 2[2]	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
25(OH)D (per SD) at 17 years	0.58 (0.45, 0.73)	<0.001*	0.74 (0.56, 0.97)	0.029*
Sex (female v male)	1.81 (1.18, 2.78)	0.006*	1.89 (1.16, 3.09)	0.011*
Race (non-Caucasian v Caucasian)	0.58 (0.31, 1.06)	0.077	1.08 (0.55, 2.13)	0.818
Physical activity		0.196		0.172
≥4 times per week	-		-	
1-3 times per week	1.67 (0.95, 2.93)	0.078	1.51 (0.80, 2.86)	0.209
<once per week	1.65 (0.85, 3.17)	0.137	2.03 (0.97, 4.24)	0.061
Television/computer viewing		0.286		0.743
<2 hours per day	-		-	
2-4 hours per day	1.32 (0.79, 2.20)	0.289	1.21 (0.69, 2.14)	0.505
>4 hours per day	1.54 (0.90, 2.65)	0.116	1.24 (0.67, 2.29)	0.488
HOMA-IR			1.03 (0.90, 1.19)	0.636
BMI (kg/m^2)			1.31 (1.23, 1.39)	<0.001*

25(OH)D, deseasonalised serum 25-hydroxyvitamin D concentrations; SD, standard deviation; HOMA-IR, homeostatic model assessment for insulin resistance; BMI, body mass index

[1]Adjusted for sex, race, physical activity and television/computer viewing at 17 years; [2]Further adjusted for HOMA-IR and BMI at 17 years

*Significant at the $p<0.05$ level

Table 4. Adjusted logistic regression model combining deseasonalised s25(OH)D concentrations at 14 and 17 years and risk of NAFLD at 17 years (*n*=718)

	OR (95% CI)	*p* value
25(OH)D (per SD) at 17 years	0.76 (0.56, 1.03)	0.072
25(OH)D (per SD) at 14 years	0.94 (0.70, 1.26)	0.685
Sex (female v male)	1.86 (1.14, 3.06)	0.014*
Race (non-Caucasian v Caucasian)	1.07 (0.54, 2.11)	0.855
Physical activity		0.167
≥4 times per week	-	
1-3 times per week	1.50 (0.79, 2.85)	0.211
<once per week	2.04 (0.98, 4.27)	0.059
Television/computer viewing		0.763
<2 hours per day	-	
2-4 hours per day	1.20 (0.68, 2.13)	0.525
>4 hours per day	1.23 (0.67, 2.27)	0.509
HOMA-IR	1.03 (0.89, 1.19)	0.650
BMI (kg/m^2)	1.31 (1.23, 1.39)	<0.001*

25(OH)D, deseasonalised serum 25-hydroxyvitamin D concentrations; SD, standard deviation; HOMA-IR, homeostatic model assessment for insulin resistance; BMI, body mass index

*Significant at the $p<0.05$ level

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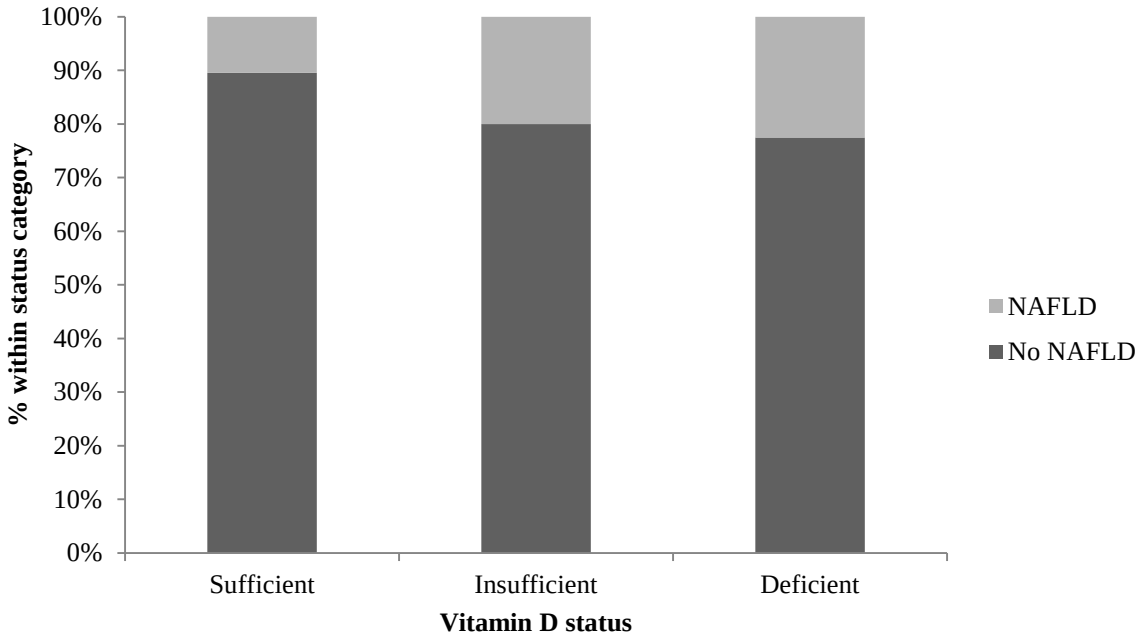

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