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The association between hepatic steatosis, vitamin D status, and insulin resistance in adolescents with obesity

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ABSTRACT

Introduction: Epidemiological studies suggest an inverse relationship between circulating 25-hydroxy-vitamin D [25(OH)D] levels and insulin resistance (IR), yet interventional studies have yielded inconsistent findings. This study examined the relationship between changes in vitamin D status and markers of IR in adolescents, with a focus on the modifying effect of liver fat.

Methods: A post-hoc analysis was performed using data from 44 adolescents participating in a 6-month observational study evaluating biomarkers of hepatosteatosis. Participants were categorized into two groups based on vitamin D status at the end of the observation period: those whose vitamin D levels increased or remained sufficient (VDI, $n = 22$) and those whose levels decreased or remained insufficient/deficient (VDD, $n = 22$). Liver fat percentage was measured using magnetic resonance imaging (MRI) fat-fraction, and IR was assessed using the updated Homeostatic Model Assessment for Insulin Resistance (HOMA2-IR) and the triglyceride-to-high-density lipoprotein cholesterol ratio (TG/HDL).

Results: Across the cohort, liver fat was positively associated with HOMA2-IR ($\beta = 0.08$, $p = 0.023$). The association between changes in vitamin D status and HOMA2-IR trajectories was modified by liver fat but only in Hispanic adolescents ($\beta = -0.18$, $p < 0.001$). Among Hispanic adolescents in the VDD group, HOMA-IR worsened, particularly at higher levels of liver fat. In non-Hispanic adolescents, HOMA-IR increased in the VDD group ($\beta = 0.65$, $p = 0.033$) compared to the VDI group, independent of baseline liver fat. Across the cohort, changes in vitamin D status interacted with liver fat to influence TG/HDL trajectories ($\beta = 0.20$, $p = 0.034$).

Conclusions: The metabolic response to changes in vitamin D status in adolescents with IR may vary based on racial and ethnic differences and liver fat status. These findings underscore the importance of considering liver fat and racial/ethnic background in vitamin D and metabolic health studies. Future research with more extensive and diverse cohorts spanning the fatty liver disease spectrum is needed to clarify these relationships.

1. Introduction

Insulin resistance (IR) plays a central role in the development of obesity-associated metabolic complications [1,2]. The increasing prevalence of IR in youth mirrors the rise in obesity, with minority groups being disproportionately affected [3,4]. The prevalence of IR in children varies widely due to different definitions and measurement methods. In a population-based study of American adolescents, insulin resistance was assessed using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), revealing a prevalence of 52.1 % among those

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with obesity (95 % CI: 44.5–59.8) [4].

Metabolic dysfunction-associated steatotic liver disease (MASLD), considered a hepatic manifestation of IR, is the most common chronic liver disease in the United States [5]. Obesity is the leading risk factor for MASLD across all age groups. According to the National Health and Nutrition Examination Survey, 16.8 % of adolescents aged 12 to 17 are affected by some form of MASLD [6]. While MASLD is estimated to affect up to one-third of adolescents with obesity, it can also impact as much as 8 % of lean adolescents, which highlights the complexity of its pathogenesis [7,8]. Hepatosteatosis, marked by excessive liver fat accumulation without inflammation or fibrosis, is the initial stage of MASLD development. Its prevalence varies significantly across racial and ethnic groups, with Hispanics bearing the highest burden and Blacks the lowest [9–11]. In adults with type 2 diabetes, end-stage liver disease resulting from MASLD is 3.1 times more prevalent in Hispanics and 3.9 times less prevalent in Blacks compared to White Caucasians [9]. Among children, Hispanic ethnicity is associated with a 4-fold increased risk of hepatosteatosis, whereas Black children exhibit the lowest risk [10].

Emerging evidence in humans suggests that improving serum vitamin D levels may reduce insulin resistance in children with NAFLD [12]. In light with this, epidemiological studies suggest an inverse association between circulating total 25-hydroxy vitamin D (25(OH)D) levels and IR in children [13–15]. Consistent with these findings, animal models have shown that vitamin D deficiency worsens hepatic IR and promotes liver fat accumulation [16,17]. Vitamin D is hypothesized to enhance insulin secretion from pancreatic beta cells and upregulate insulin receptor expression in peripheral tissues. Acting as an epigenetic regulator, vitamin D modulates the transcription of genes linked to insulin sensitivity, improves insulin receptor responsiveness to insulin and glucose transport, and facilitates the conversion of proinsulin to insulin. Additionally, its antioxidant and anti-inflammatory properties may contribute to improved glycemic control [18,19]. However, despite these proposed benefits, interventional studies investigating the effects of vitamin D supplementation on IR and hepatosteatosis have yielded inconsistent results [18]. Variations in the severity and prevalence of IR and hepatosteatosis across populations.

To address these limitations, we **examined** the relationship between **changes in vitamin D status and the progression of surrogate markers of IR in adolescents**, with a specific focus on **determining whether liver fat modifies this association.** Data presented in this report were obtained from the adolescent subjects (age range 10–17 years) with obesity who participated in a 6-month clinical observational study at the Arkansas Children's Hospital Weight Management Clinic between 2017 and 2019 [20]. The objective of the primary study was to examine the association between serum fibroblast growth factor-21 to adiponectin ratio (FAR) and intrahepatic triglyceride (i.e., liver fat). Results demonstrated a positive association between percent change in FAR and liver fat content, thus suggesting that FAR could be used as a potential biomarker to monitor the liver change in those with MASLD.

Based on published evidence, we hypothesize that **improvements in vitamin D status will be associated with reductions in IR markers**, particularly among adolescents with **greater liver fat accumulation,** as hepatic insulin resistance may play a key role in this relationship.

2. Materials and methods

2.1. Study design and subject recruitment

This study was a secondary analysis of data collected during a 6-month observational study designed to evaluate the relationship between circulating biomarkers and hepatic steatosis [20]. Briefly, sixty adolescents with obesity were recruited from the Arkansas Children's Hospital Weight Management Clinic. Those with diabetes, known liver diseases, or any underlying medical problems known to affect liver fat metabolism were excluded. Liver magnetic resonance imaging (MRI) and fasting blood tests were performed in all participants at baseline and six months later. Each subject received standard-of-care counseling about lifestyle modifications from a registered dietitian and physical therapist. The pubertal stages of participants were assessed clinically by a pediatric endocrinologist using Tanner stages. The University of Arkansas for Medical Sciences Institutional Review Board approved the study (IRB Protocol No: 206278, approval date: March 10, 2017). Written informed consent from the parents and participant assent for those <18 years old were obtained as previously described [20]. Participants (n = 44) whose vitamin D results were available at baseline and 6 months were included in this post-hoc analysis (Fig. 1).

2.2. Classification of subjects based on changes in vitamin D status

At enrollment, 20 subjects presented with vitamin D deficiency (serum 25(OH)D level <20 ng/mL), 19 with vitamin D insufficiency (serum 25(OH)D level ≥20 but <30 ng/mL), and 5 with sufficient vitamin D levels (serum 25(OH)D level ≥30 ng/mL). Subjects with Vitamin D deficiency and insufficiency were treated with 50,000 units of cholecalciferol capsules weekly for three months, followed by 2000 units daily for the last three months of the study. Adherence to vitamin D treatment was not assessed due to the study's observational nature.

Subjects were categorized into two groups based on changes (or lack thereof) in their vitamin D status at six months compared to baseline: 1) The Vitamin D Increased or Remained Sufficient (VDI) group included those whose vitamin D status improved from deficient to insufficient, deficient to sufficient, or insufficient to sufficient, as well as those who were sufficient at both baseline and six months, or 2) The Vitamin D Decreased or Remained Deficient or Insufficient (VDD) group included those whose vitamin D status remained deficient or insufficient, declined from insufficient to deficient, from sufficient to insufficient, or from sufficient to deficient.

2.3. Measurements

2.3.1. Anthropometrics and body composition

Body weight (kg) and height (cm) were measured in the clinic using standard techniques and rounded to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI), sex and age-adjusted BMI percentiles, and standardized z-scores (BMIz) were calculated according to the Centers for Disease Control and Prevention guidelines. Total body fat (TBF) was estimated via the InBody® 570 body composition analyzer (InBody USA, Cerritos, CA). This method of TBF measurement has been shown to correlate well with Dual-energy X-ray absorptiometry (DXA)

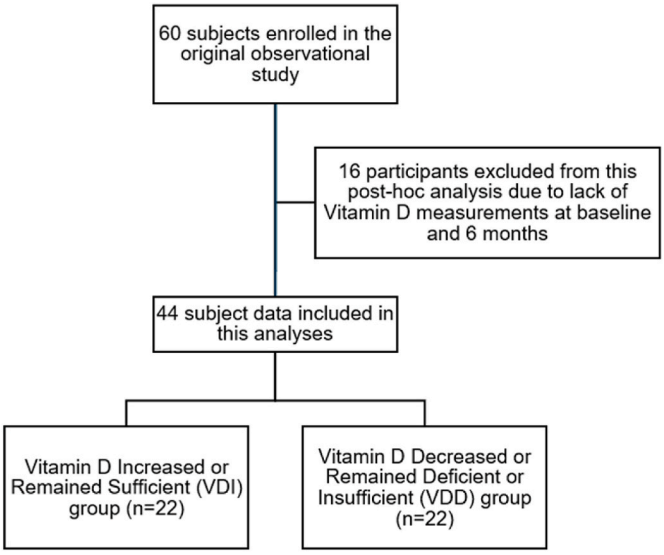

Fig. 1. Consort diagram.

scan [15]. Briefly, tissue impedance is measured over 60 s when a low-intensity current travels between the bare feet and hands of the subjects. Fat mass index [(FMI) = FM (kg)/height (m^2)] and FMI z scores (FMI-z) were calculated using reference standards [21].

2.3.2. Blood analytes and estimates of insulin resistance

Blood samples were collected between November 2017 and July 2018 after an overnight fast. Serum glucose, liver enzymes, lipid profile, insulin, and 25(OH)D concentrations were measured via a clinical analyzer (Siemens Atellica, Malvern, PA) at the Arkansas Children's Hospital clinical laboratory.

Estimates of insulin sensitivity and resistance, including beta cell function (%β), insulin sensitivity (%S), and IR were calculated from fasting glucose and insulin concentrations using the University of Oxford Diabetes Trials Unit online HOMA2 calculator (https://www.dtu.ox.ac.uk/homacalculator/) [22]. The triglyceride to high-density lipoprotein cholesterol (TG/HDL) ratio was calculated as an additional surrogate marker of IR [23].

2.3.3. Quantification of liver fat

Liver fat percent was quantified via MRI fat-fraction (Dixon method) at baseline and six months using the same 1.5T scanner (Philips Healthcare, Best, The Netherlands) with the same imaging protocol as previously described [20]. Briefly, a multi-echo multi-slice gradient-echo pulse sequence was used to acquire in/out of phase images of the whole liver. Confounding effects of intrinsic T2/T1 relaxation were controlled using the triple-echo method [24]. Liver fat was reported as the percentage of total liver volume.

2.4. Statistical analysis

In this post-hoc analysis, data are presented as means (SD, standard deviation) or median (Q1, Q3) for continuous variables and count (percentages) for categorical variables. Comparative analyses between Vitamin D groups were assessed by two-sample *t*-test for normally distributed continuous variables, Wilcoxon Rank-sum test for non-normally distributed continuous variables, and Fisher's exact test or Chi-square test for categorical variables. The relationships between subject characteristics and trajectories of markers of IR (HOMA-IR and TG/HDL) were assessed. A significant time x ethnicity (Hispanic vs. non-Hispanic) interaction was observed in association with HOMA-IR and TG/HDL trajectories. Notably, HOMA-IR ($\beta = 5.58$, $p = 0.0100$) and TG/HDL ($\beta = 1.54$, $p = 0.0246$) levels increased over the observation period exclusively in Hispanics. Therefore, regression analysis modeling the trajectories of markers of IR was stratified by ethnicity. *Statistical analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA).* Our study has approximately 80 % power to detect an effect size of Cohen's f = 0.44 at a 5 % level of significance. This translates to Cohen's d = 0.88 for the two-group design employed, indicating a difference between groups equivalent to 0.88 standard deviation units. Our study was powered using the R Superpower package [25].

3. Results

3.1. Subject characteristics and body composition

Of the 61 adolescents enrolled in the original study, forty-four had serum 25(OH)D levels available at baseline and six months. As such, comparative analyses were performed on forty-four subjects. Descriptive characteristics of subjects in the VDI (n = 22) and VDD (n = 22) groups at baseline and six months are presented in Table 1. Subjects were 14 ± 2 years old. Fifty-two percent (n = 23) self-identified as non-Hispanic Black (NHB), 27 % (n = 12) as Hispanic White (HW), and 21 % (n = 9) as non-Hispanic White (NHW). The VDI and VDD groups did not differ in the distribution of age, sex, self-identified race, ethnicity, or body composition at baseline or six months (Table 1).

Table 1
Baseline and six-month characteristics of groups defined by changes in vitamin D status.

Variables	Vit D increased (n = 22)		Vit D decreased (n = 22)	
	Baseline	6-month	Baseline	6-month
Age (years)	14.5 ± 1.8		14.0 ± 2.3	
Sex, Female, n (%)	13 (59)		13 (59)	
Race, n (%)				
White	13 (59)		8 (36)	
Black	9 (41)		14 (64)	
Ethnicity, n (%)				
Hispanic	6 [27]		6 [27]	
Non-Hispanic	16 (73)		16 (73)	
BMI (kg/m^2)	36.9 ± 7.4*	37.7 ± 7.1*	36.9 ± 5.3	37.3 ± 5.3
BMIz	2.38 ± 0.25	2.39 ± 0.24	2.44 ± 0.25	2.43 ± 0.28
Fat mass index (kg/m^2)	16.8 ± 5.8	17.3 ± 5.8	16.7 ± 4.1	16.7 ± 4.2
Fat mass index z-score	1.87 ± 0.42	1.89 ± 0.41	1.91 ± 0.35	1.88 ± 0.41
MRI liver fat-fraction (%)	6.61 ± 6.43	7.34 ± 7.72	7.02 ± 5.24	6.99 ± 5.69
Vitamin D (ng/mL)	22.1 ± 7.9*	29.7 ± 8.0*,#	21.6 ± 5.7	21.8 ± 4.9#
Vitamin D Status, n (%)				
Deficient	12 (55)	0	8 (36)	9 (41)
Insufficient	6 [27]	10 (45)	13 (59)	13 (59)
Sufficient	4 [18]	12 (55)	1 [5]	0
Glucose (mg/dL)	96 ± 9^	95 ± 8	90 ± 10^	91 ± 11
Insulin (μIU/mL)	31.9 ± 18.6	31.1 ± 26.0	28.8 ± 19.3	31.8 ± 23.9
C-peptide (ng/mL)	3.97 ± 1.44	3.76 ± 1.90	3.23 ± 1.34	3.64 ± 2.05
Triglycerides (mg/dL)	105 ± 45	118 ± 66	117 ± 72	121 ± 67
HDL cholesterol (mg/dL)	41 ± 7	41 ± 8	45 ± 9	45 ± 9
Total cholesterol (mg/dL)	147 ± 25	145 ± 25	154 ± 27	159 ± 31
LDL cholesterol (mg/dL)	85 ± 23	81 ± 21	86 ± 23	91 ± 30
ALT (U/L)	33 ± 12	36 ± 26	31 ± 11	33 ± 19
AST (U/L)	29 ± 18	27 ± 11	26 ± 7	29 ± 9
HbA1c (%)	5.4 ± 0.3	5.4 ± 0.4	5.4 ± 0.4	5.4 ± 0.4
HOMA-IR	7.6 ± 4.8	7.4 ± 6.8	6.5 ± 4.3	7.3 ± 5.9
HOMA2-β	155 ± 44	149 ± 54	152 ± 53	160 ± 55
HOMA2-%S	38 ± 16	42 ± 18	48 ± 18	45 ± 18
HOMA2-IR	3.03 ± 1.13	2.87 ± 1.48	2.43 ± 1.0	2.75 ± 1.6
Triglyceride/HDL	2.78 ± 1.64	3.25 ± 2.37	2.85 ± 2.30	2.96 ± 2.04

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; HDL = high-density lipoprotein; HOMA = Homeostatic Model Assessment; IR = Insulin Resistance; LDL = low-density lipoprotein; S = Sensitivity; MRI = Magnetic Resonance Imaging. * denotes differences between baseline and 6-month values within the groups ($p < 0.05$). ^ denotes differences between baseline values between groups ($p < 0.05$). # denotes differences in 6-month values between groups ($p < 0.05$).

Mean changes in body composition markers did not differ between groups over time (see Supplementary Table). The mean BMI (kg/m^2) in the VDI group at six months was significantly higher than at baseline ($p < 0.05$); however, there was no significant difference in BMIz. The liver fat fraction did not change over time in either group.

3.2. Serum 25(OH)D levels and vitamin D status

At baseline, the mean serum 25(OH)D level (VDI = 22.1 ± 7.9 ng/mL vs. VDD = 21.6 ± 5.7 ng/mL; $p = 0.46$) and distribution of vitamin D status were comparable between groups (Table 1). At six months, 25(OH)D levels in the VDI group were significantly higher than at baseline (Six months = 29.7 ± 8.0 vs. Baseline = 22.1 ± 7.9 ng/mL, $p < 0.01$) and exceeded those in the VDD group at six months (VDI = 29.7 ± 8.0

vs. VDD = 21.8 ± 4.9 ng/mL, p < 0.01). As per the study design, the distribution of vitamin D status differed between groups at six months. In the VDI group, none of the participants had vitamin D deficiency at six months, whereas at baseline, 55 % were classified as deficient. The proportion of adolescents with sufficient vitamin D status in this group increased from 18 % to 55 %.

3.3. Metabolic characteristics

At baseline, fasting serum glucose concentration was higher in the VDI than in the VDD group (VDI = 96 ± 9 mg/dL vs. VDD = 90 ± 10 mg/dL, p = 0.04). A similar trend was observed for serum C-peptide level (VDI = 1.3 ± 0.5 mmol/L vs. VDD = 1.1 ± 0.5 mmol/L, p = 0.06) and HOMA2-IR (VDI = 3.0 ± 1.1 vs. VDD = 2.4 ± 1.0 mmol/L, p = 0.05). Serum concentrations of insulin, lipids, and hepatic transaminase levels did not differ within or between groups at baseline and six months (Table 1). Furthermore, the mean changes in any measured metabolic markers, including surrogate markers for IR and insulin sensitivity, between baseline and six months were not different between groups. There was a trend for HOMA2-%β to decrease within the VDI and to increase within the VDD group over time, but the difference between groups did not reach statistical significance [Median (Q1, Q3) for change: VDI = −6.5 (−32.2, 16.7) vs. VDD = 18.1 (−5.6, 37.3), p = 0.07)].

3.4. Association of race/ethnicity with liver fat, body composition, vitamin D, and IR markers at baseline

In this group of adolescents, n = 12 were Hispanic-White (HW), n = 9 were non-Hispanic White (NHW), and n = 23 were non-Hispanic Black (NHB). HW subjects had higher baseline percent liver fat (11.7 ± 6.9 %; range = 2.4–23.1 %; p = 0.009) compared to NHB (4.5 ± 3.6 %; range = 1.0–14.6 % and NHW subjects (6.1 ± 5.2 %, range = 1.8–17.1 %). The mean percent liver fat was comparable between the NHB and NHW groups (p = 0.39). BMIz and FMIz were comparable between race-ethnicity groups (data not shown). Serum 25(OH)D concentration was lower in NHB subjects (19.0 ± 5.8 ng/mL, p = 0.02) than in HW (25.5 ± 6.5 ng/mL) and NHW (24.3 ± 7.1 ng/mL). The distribution of vitamin D status (deficient, insufficient, and sufficient) was comparable among race-ethnicity groups (p = 0.21, data not shown). HOMA2-IR was lower in NHB (2.2 ± 0.9, p < 0.01) than in NHW (3.2 ± 1.0) and HW (3.4 ± 1.0) subjects. TG/HDL was 1.4 times higher in HW (3.6 ± 1.4, p = 0.0166) compared to NHB (2.5 ± 2.3) and NHW (2.5 ± 1.6) subjects. Other markers of IR or insulin sensitivity did not differ between racial/ethnic groups.

3.5. Regression analysis showing associations of percent liver fat, vitamin D group, and trajectories of IR markers in Hispanic and non-Hispanic adolescents

In the HW group, the vitamin D group (VDI or VDD) modified the association between percent liver fat and HOMA2-IR trajectories (β = 0.15, p = 0.01, Table 2). Specifically, IR increased in the VDD group, but this effect was observed only at higher levels of liver fat (Fig. 2A). There was no interaction between the percent liver fat and vitamin- D-group in association with HOMA2-IR trajectories in non-Hispanic (NHW + NHB) adolescents (β = 0.06, p = 0.28, data not shown in the table, Fig. 2B). Instead, positive HOMA2-IR trajectories were observed in non-Hispanic (NHW + NHB) adolescents in the VDD (β = 0.65, p = 0.03) independently of percent liver fat. When covariates were considered, these associations remained significant in the final model (Table 2).

No association or interaction was observed between the percent liver fat and vitamin D group with respect to TG/HDL trajectory in the HW group (data not shown). In non-Hispanic children (Black and White), the association between TG/HDL and percent liver fat was modified by the Vitamin D group (β = 0.27, p = 0.0372, Table 3). The TG/HDL increased over 6 months only in the VDD group, but only at higher percentages of liver fat (Fig. 3).

Table 2
Final models evaluating the association of vitamin D group, liver fat at enrollment, and their interaction with HOMA2-IR trajectory in Hispanic and non-Hispanic subjects.

Hispanic subjects (HW)	β	SE	95 % CI		p-value
Liver fat	0.20	0.04	0.12	0.28	<0.0001
Vitamin D group					
VDI	2.22	0.73	0.79	3.66	0.0024
VDD (Reference)					
MRI fat-fraction x vitamin D group	−0.18	0.05	−0.27	−0.09	0.0001
Time	−0.19	0.55	−1.28	0.89	0.7260
MRI fat-fraction x time	0.11	0.03	0.06	0.17	0.0001
Vitamin D group x time	1.64	0.87	−0.06	3.33	0.0586
MRI fat-fraction x vitamin D group x time	−0.15	0.06	−0.26	−0.03	0.0110
Non-Hispanic subjects (NHW + NHB)					
Liver fat	0.05	0.02	0.01	0.09	0.0215
Vitamin D Group					
VDI	−0.80	0.30	−1.39	−0.20	0.0084
VDD (Reference)					
Time	−0.82	0.32	−1.45	−0.18	0.0118
Vitamin D Group x time	0.65	0.31	0.05	1.26	0.0332
Pubertal stage					
Tanner stages II-III	−0.07	0.27	−0.60	0.45	0.7797
Tanner stages IV-V (Reference)					
Pubertal stage x Time	0.83	0.26	0.31	1.34	0.0017
Sex					
Female					
Male (Reference)	0.57	0.15	0.27	0.86	0.0002

CI = confidence interval; HW = Hispanic-White; NHB = Non-Hispanic Black; NHW = Non-Hispanic White; MRI = Magnetic Resonance Imaging; SE = standard error; VDD = Vitamin D Decreased, VDI = Vitamin D Increased.

4. Discussion

This study examined the associations between liver fat percentage, changes in vitamin D status, and trajectories of IR over six months in a diverse cohort of adolescents with obesity. The findings highlight the complex interplay between liver fat, vitamin D, and IR, with notable differences based on racial and ethnic backgrounds The primary findings were: i) Across all racial and ethnic groups, liver fat was directly associated with HOMA2-IR. ii) The effect modification of liver fat on the relationship between insulin resistance trajectories and vitamin D group varied depending on whether HOMA-IR or the TG/HDL ratio was used as an indirect measure of insulin resistance. iii) Changes in vitamin D status associated with differences in the relationship between liver fat and insulin resistance trajectories, with effects varying by ethnicity. Taken together, these data suggest that race/ethnicity and percent liver fat may play a role in the observed associations between vitamin D status and IR.

The relationship between vitamin D status and IR has been widely studied, with observational data suggesting a negative correlation between serum 25-hydroxyvitamin D [25(OH)D] levels and IR [13,26–29]. Vitamin D may regulate glucose metabolism through various mechanisms, including direct effects on insulin secretion from pancreatic beta-cells, increased insulin receptor expression at peripheral organs, and indirectly via its antioxidants and anti-inflammatory properties [18,19]. Moreover, emerging evidence suggests that vitamin D may influence adipose tissue function and distribution, which are critical factors in the development of IR [30]. However, clinical trials investigating vitamin D supplementation on IR have yielded inconsistent results. Possible explanations for this inconsistency may include the absence of data regarding subjects' liver fat status and the failure to stratify subjects based on their racial and ethnic backgrounds. Indeed, a cross-sectional study of 134 adolescent girls found that Hispanic girls were 4.2 times more likely to have hepatic steatosis (i.e., ALT >40 U/L) compared to non-Hispanic girls [10]. Consistently, our data showed that Hispanic

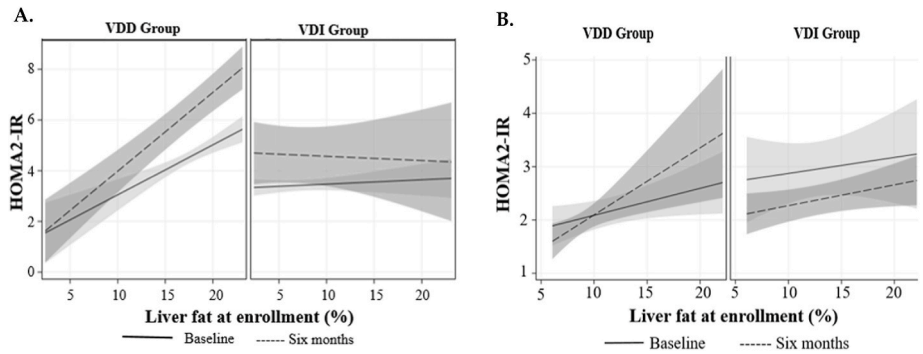

Fig. 2. Regression plots show the interaction between liver fat percentage at enrollment and vitamin D group.
Regression plots show the interaction between liver fat percentage at enrollment and vitamin D group in Hispanic-White subjects (Panel A), but no interaction was observed in non-Hispanic (Black and White) subjects (Panel B). HOMA2_IR, Homeostatic Model Assessment of Insulin Resistance; VDD = Vitamin D Decreased, VDI = Vitamin D Increased.

Table 3
Best fitted model evaluating the association of vitamin D group (VDI or VDD), liver fat at baseline, and their interaction with TG/HDL trajectory in non-Hispanic subjects with obesity.

Non-Hispanic (Black and White) Subjects	β	SE	95 % CI		p-value
Liver fat	0.31	0.06	0.20	0.43	<0.0001
Vitamin D group					
VDI	1.84	0.63	0.61	3.07	0.0034
VDD (Reference)					
MRI fat-fraction x vitamin D group	−0.27	0.10	−0.46	−0.08	0.0056
Time	1.97	1.17	−0.32	4.27	0.0920
MRI fat-fraction x time	−0.27	0.12	−0.50	−0.04	0.0225
Vitamin D group x time	−2.19	1.26	−4.67	0.28	0.0822
MRI fat-fraction x vitamin D group x time	0.27	0.13	0.02	0.52	0.0372

CI = confidence interval; NHB = Non-Hispanic Black; NHW = Non-Hispanic White; MRI = Magnetic Resonance Imaging; SE = standard error; VDD = Vitamin D Decreased, VDI = Vitamin D Increased.

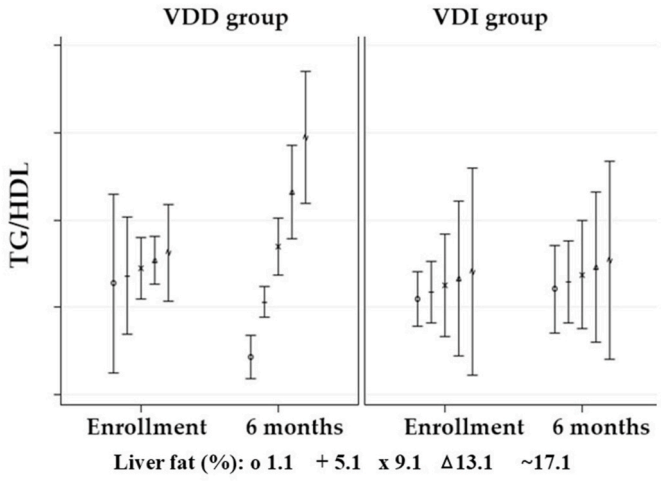

Fig. 3. Regression plot shows the interaction of liver fat (%) and Vitamin D group in association with TG/HDL trajectories.
TG/HDL: Triglyceride to High-Density Lipoprotein Ratio; VDD = Vitamin D Decreased; VDI = Vitamin D Increased.

adolescents had twice the liver fat percentage of their non-Hispanic counterparts. In the present study, the modifying effects of liver fat on the association between insulin resistance trajectories and vitamin D group varied depending on whether HOMA-IR or the TG/HDL ratio was used as an indirect measure of insulin resistance. Notably, differences between Hispanics vs. non-Hispanics were observed when HOMA-IR was used as markers of insulin resistance but not when the TG/HDL ratio was applied. Specifically, regardless of ethnic background, TG/HDL worsened in the VDD group compared to the VDI group. While our sample size of Hispanic adolescents was small, the higher risk of hepatic steatosis in this population is well documented. Future studies utilizing gold-standard measurements of insulin resistance in individuals from diverse backgrounds are needed to further clarify these associations. Finally, genetic polymorphisms in vitamin D metabolism should also be considered, as they may influence individual responses to supplementation and contribute to variability in outcomes [19]. Our findings align with studies demonstrating the metabolic significance of vitamin D but add a novel layer by incorporating liver fat and racial/ethnic stratification.

Rudolph et al. (2021) investigated the relationship between vitamin D deficiency and hepatosteatosis in 276 children, 87 % of whom were Hispanic, with elevated serum ALT levels (≥35 U/L). All participants underwent elastography, and 92 with persistently elevated ALT levels received liver biopsies. The study found no association between vitamin D deficiency and elastography results or liver architecture (e.g., ballooning, inflammation, fibrosis) in biopsy-confirmed MASLD. A trend was noted between serum 25(OH)D concentration and fasting insulin (rho = −0.11, p = 0.08) and HbA1c (rho = −0.11, p = 0.09) as surrogate markers of IR, but not with glucose or HOMA-IR [31]. The focus on children with elevated ALT may have obscured the potential benefits of vitamin D supplementation, as ALT elevation often indicates liver inflammation or advanced disease stages. By contrast, our study's use of MRI fat-fraction captured liver fat at earlier stages, offering broader insights into these relationships.

A randomized controlled clinical trial in Egyptian children with biopsy-confirmed MASLD (n = 100) reported improvements in hepatic steatosis, lobular inflammation, and IR in children treated with 2000 IU/day of 25(OH)D for six months compared to placebo [12]. However, participants in both study arms followed a hypocaloric diet, reducing total energy intake by 30 %. Notably, significant weight loss was observed only in the vitamin D treatment arm, raising the possibility that the observed metabolic benefits were driven more by changes in body composition than by vitamin D supplementation itself. In contrast, the subjects in our study showed no significant changes in standardized body composition markers between baseline and six months, providing a clearer view of vitamin D's direct effects on metabolic parameters. In a triple-masked randomized controlled study conducted in Sri Lankan children (n = 96) with obesity and vitamin D deficiency, researchers compared the effects of a treatment dose of vitamin D (50,000 IU/week), a supplementation dose (2500 IU/week), and placebo on body composition, liver enzymes, and markers of glucose homeostasis over 24 weeks

[32]. No differences were observed between groups in liver enzymes (ALT and AST) or markers of glucose metabolism (fasting glucose, insulin, HOMA-IR) at baseline or post-intervention. However, ALT levels significantly decreased post-intervention in both the treatment (39 ± 51 U/L vs. 21 ± 9 U/L; $p = 0.036$) and supplementation (33 ± 21 U/L vs. 25 ± 9 U/L; $p = 0.035$) arms. Interestingly, fasting glucose levels increased significantly in the treatment dose group (83 ± 5 mg/dL at baseline vs. 90 ± 8 mg/dL at week 24; $p < 0.001$), while no notable changes occurred in the supplementation or placebo groups. Changes in HOMA-IR did not differ between groups. Notably, the study did not include data on liver fat, limiting comparisons with our findings.

4.1. Limitations

This study is subject to several limitations. First, the relatively small sample size may limit the generalizability of the findings. Second, indirect measures of insulin resistance (e.g., HOMA2-IR and TG/HDL) were used instead of direct methods such as the euglycemic hyperinsulinemic clamp. Third, bioavailable (free) 25(OH)D and vitamin D binding protein concentrations were not measured, as these are not routinely obtained in clinical practice, potentially limiting the interpretation of our findings. Future research should consider incorporating these direct measures to provide a more precise assessment of insulin resistance and vitamin D status. Additionally, the follow-up period of six months may not have been sufficient to capture long-term metabolic changes or the sustainability of observed effects. While MRI fat-fraction provided precise liver fat quantification, it could not differentiate between disease stages, such as the presence of fibrosis. The complex roles of the liver in glucose and vitamin D metabolism make it challenging to draw definitive conclusions about the directionality of interactions among insulin resistance, metabolic dysfunction-associated steatotic liver disease (MASLD), and vitamin D status. Furthermore, seasonal variations in vitamin D levels, physical activity, and dietary intake were not accounted for, which may have influenced the results. Another consideration is that this study was observational in nature and represents a secondary analysis of existing data. As a result, adherence to vitamin D supplementation was not actively monitored, which likely affected vitamin D levels. However, the primary goal of this study was not to evaluate responsiveness to vitamin D supplementation but rather to examine the relationship between changes in vitamin D status and markers of insulin resistance in adolescents, with a specific focus on the modifying effect of liver fat. In this context, variability in adherence to supplementation did not directly impact our primary outcome of interest. Additionally, there appears to be dimorphism in the presentation of indirect markers of insulin resistance between Hispanic and non-Hispanic children, suggesting that these markers may vary in their expression or association with metabolic outcomes across racial and ethnic groups. This variability could have influenced the observed metabolic responses to vitamin D status and supplementation. While the study may not have been sufficiently sensitive to detect small or moderate effects, it remains adequately powered to detect large effects, which are often of practical significance in research. Therefore, the study's power is reasonable for the magnitude of effects hypothesized and explored in the analyses. Finally, although multiple relationships were observed, this was not an intervention trial, and observed changes in IR could be due to other factors, such as changes in diet and activity level rather than changes in vitamin D concentrations. Therefore, while these metabolic findings may imply potential mechanisms, this study does not establish causation. Future research involving larger sample sizes across the weight spectrum, diverse age groups stratified by vitamin D and IR status, and extended follow-up periods are needed to elucidate these relationships further. Investigating the molecular mechanisms by which vitamin D interacts with liver metabolism and insulin signaling pathways will also be crucial.

5. Conclusions

In conclusion, the modifying effect of liver fat on the relationship between IR trajectories and vitamin D status depended on the specific markers used (HOMA-IR vs. TG/HDL). Ethnic differences were seen when IR was measured by HOMA-IR but were not observed for the TG/HDL ratio. Furthermore, children who experienced a decline in vitamin D status over six months demonstrated worsening IR (as indicated by the TG/HDL ratio), particularly those adolescents with higher levels of liver fat. Future studies employing gold-standard methods to measure IR in adolescents with obesity from diverse racial and ethnic backgrounds are necessary to further clarify these complex interactions.

- Liver fat is correlated with insulin resistance in adolescents, though this relationship vary across racial and ethnic groups.
- Declining vitamin D levels may be associated with a stronger liver fat-IR relationship in Hispanic-White adolescents. In contrast, in non-Hispanic (Black and White) adolescents, vitamin D status is related to IR independently of liver fat.
- A higher triglyceride-to-HDL cholesterol ratio may be a marker of greater liver fat accumulation, particularly in adolescents with worsening vitamin D status.

Author contributions

The concept of the submission was by ET and ECD. Statistical analysis and data curation was performed by ET, DKW and ECD. Methodology by XO, EB, ECD. ET and ECD wrote the first draft. ET, AF, IL, RM, XO, DKW, EB, and ECD all reviewed, edited, and approved the final submission and publication.

Data availability

The original contributions presented in this study are included in the article material. Further inquiries can be directed to the corresponding author(s).

Ethnical statement

This research involved secondary analysis of deidentified data obtained between 2017 and 2019 from subjects who participated in an observational clinical study. The University of Arkansas for Medical Sciences Institutional Review Board approved the study (IRB Protocol No: 206278, approval date: March 10, 2017). Written informed consent from the parents and participant assent for those <18 years old were obtained.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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