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Association between hemoglobin and non-alcoholic fatty liver disease (NAFLD) in United States adults: Results from NHANES 2017–2020

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD), a chronic liver condition of increasing prevalence, is closely related to various metabolic disorders. Hemoglobin, a protein that transports oxygen in red blood cells, is the focus of this study, which seeks to investigate its potential association with NAFLD.
Methods: We selected 6,516 eligible adult participants from the United States using the 2017–2020 National Health and Nutrition Examination Survey database for cross-sectional analyses. We analyzed the association of hemoglobin with NAFLD using weighted logistic regression models.
Results: The study performed a weighted logistic regression modeling analysis, which verified that hemoglobin levels were positively associated with NAFLD, especially in the higher hemoglobin quartile groups. Subgroup analyses revealed no significant interactions, demonstrating the robustness of the model. The analysis of mediation effects showed that Gamma-Glutamyl Transferase, Alanine Aminotransferase, and triglycerides were important mediating variables in the relationship between hemoglobin and NAFLD.
Conclusion: Increased hemoglobin levels were found to be significantly and independently associated with an increased NAFLD risk. This insight is crucial for the risk assessment and early detection of NAFLD, underscoring the need for heightened vigilance in individuals with higher hemoglobin levels.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), with a global prevalence of about 25 %, represents a major public health issue (Younossi et al., 2018). It is characterized by excessive fat accumulation in the liver, not caused by heavy alcohol use or other specific liver conditions. This widespread condition underscores the need for increased awareness and interventions to address its growing impact on global health (Han et al., 2023). NAFLD consists of two main subtypes: non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) (Singh et al., 2015). As the disease progresses, NASH can continue to go to liver fibrosis, cirrhosis, and even hepatocellular carcinoma, and may become a predominant cause of liver-related diseases (Shah et al., 2023; Thomas et al., 2024). The "multiple-hit hypothesis" posits that the development of NAFLD is influenced by various factors, including insulin resistance, inflammation, oxidative stress, genetics, and the gut microbiome (Buzzetti et al., 2016). This approach moves beyond the simplistic view of NAFLD being caused by a single fact or, acknowledging the complex interplay of multiple contributors to both the onset and progression of the disease (Mirrazavi and Behrouz, 2024). As NAFLD becomes a global health problem, an in-depth study of its risk factors is essential for preventing and managing the disorder.

Hemoglobin, an iron-containing protein that transports oxygen within red blood cells, has its levels determined by various factors indicative of the body's oxygenation status and metabolic function (Bellelli and Tame, 2022). Although widely acknowledged as a standard hematological marker, the broader potential of hemoglobin has yet to be fully recognized and explored. Recent studies have highlighted a close association between elevated hemoglobin levels and the body's metabolic activities, suggesting untapped value in diverse medical fields (Tapio et al., 2021). Another study revealed that lower hemoglobin levels serve as a strong and independent predictor of adverse cardiovascular events in patients experiencing acute coronary syndromes, highlighting its significance in clinical prognostics (Sabatine et al., 2005). Nevertheless, clinical studies exploring the association mechanisms between hemoglobin levels and NAFLD are scarce.

Leveraging data from the National Health and Nutrition Examination Survey (NHANES) for the period 2017–2020, this study aims to

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investigate the correlation between hemoglobin levels and NAFLD, and to thoroughly analyze the mechanism by which hemoglobin levels may act as a potential risk factor for NAFLD.

2. Methods

2.1. Study design and population

NHANES represents a pivotal health and nutrition survey conducted in the United States, overseen by the National Center for Health Statistics (NCHS), which falls under the umbrella of the Centers for Disease Control and Prevention (CDC). The primary goals of this survey are to assess the health and nutritional status of adults and children within the U.S. and to compile extensive health-related data. The dataset utilized in this study was sourced from NHANES for 2017–2020, encompassing demographic, examination, laboratory, and questionnaire data. The survey received approval from the Institutional Review Board of the CDC and adheres to guidelines for the protection of human subjects' safety and privacy. All participants signed an informed consent form. Exclusion criteria for this study:(1) Participants under 18 were excluded (n = 5867). (2) Participants with missing liver ultrasound transient elastography data were excluded (n = 1376). (3) Participants with hepatitis (positive serum hepatitis B surface antigen or positive serum hepatitis C antibody) (n = 128). (4) Participants with excessive alcohol consumption were excluded (n = 1368). (5) participants with missing hemoglobin data were excluded (n = 305). Based on these criteria, the final dataset contained 6516 eligible participants for analysis. (Fig. 1).

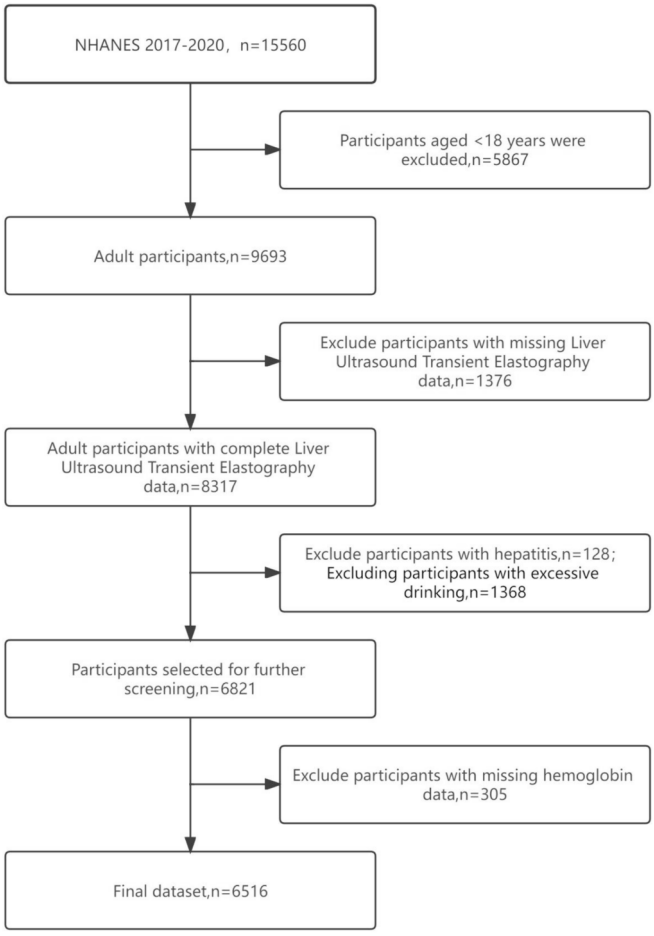

Fig. 1. Flowchart of participant selection from NHANES 2017–2020 among United States adults.

2.2. Exposure variable and covariates

The Beckman Coulter DxH 800 instrument at the NHANES Mobile Examination Center (MEC) was used to measure blood samples to obtain the independent variable hemoglobin data needed for this study. Demographic data were obtained on participants' age, gender, race, and Ratio of family income to poverty. NHANES employs FibroScan® to evaluate hepatic fibrosis and hepatic steatosis. This involves using ultrasound and vibration-controlled transient elastography (VCTE) to determine the liver's stiffness and to measure the Controlled Attenuation Parameter (CAP) to assess liver fat content. In Examination Data, you can access data for Liver Ultrasound Transient Elastography, Blood Pressure, and Body Measures. In Laboratory Data and Questionnaire Data, you can access data for High-Sensitivity C-Reactive Protein, Standard Biochemistry Profile, Glycohemoglobin, Alcohol Use, Blood Pressure & Cholesterol, and Diabetes.

2.3. Definition of variables

Although liver biopsy is regarded as the gold standard for diagnosing NAFLD, its invasive nature and high cost render it impractical for studies involving the general population (Henry et al., 2022). NAFLD is defined as having a CAP $\geq$ 285 dB/m (Heredia et al., 2023). Liver fibrosis is considered to be present if any of the following conditions are met: (1) VCTE $\geq$ 8.2 E/kpa; (2) NFS $>$ −1.45; (3) FIB4 >= 1.3 (Eddowes et al., 2019; Kjaergaard et al., 2023). The NAFLD Fibrosis Score (NFS) is a tool used to assess the risk of hepatic fibrosis in patients with NAFLD and is based on the following formula NFS = $-1.675 + 0.037 \times$ Age (years) + $0.094 \times$ BMI (kg/m^2) + $1.13 \times$ IFG/Diabetes (Yes = 1, No = 0) + $0.99 \times$ AST/ALT Ratio − $0.013 \times$ Platelets ($\times 10^9$/L) − $0.66 \times$ Albumin (g/dL) (Lu et al., 2023). The FIB-4 index, a non-invasive approach to evaluating liver fibrosis, is extensively utilized in the management of chronic liver diseases. It has demonstrated effectiveness in gauging the risk of liver fibrosis and cirrhosis in patients with NAFLD (Clinical Practice Guidelines, 2016). The formula for calculating the FIB-4 index is as follows: $\text{FIB}-4 = \frac{\text{Age (years)} \times \text{AST(U/L)}}{\text{Platelet Count } (10^9/L) \times \sqrt{\text{ALT(U/L)}}}$. Diabetes mellitus is classified as such when any one of the following conditions is satisfied: (1) a doctor's notification of diabetes mellitus, (2) glucose >= 7 (mmol/L), (3) diabetes medication, (4) insulin use, and (5) glycosylated hemoglobin >= 6.5 (%). Hepatitis patients were defined as seropositive for hepatitis B surface antigen or hepatitis C antibody. Excessive alcohol consumption is determined as $>$ 3 drinks/day for men and $>$ 2 drinks/day for women (Wang et al., 2023).

2.4. Statistical analysis

In our analysis, we incorporated sampling weight analysis to accurately reflect the demographic characteristics of the U.S. population. By using weighted analysis, we ensured fair comparisons among different groups, avoiding biases that could arise from unequal sample distributions. This approach enhances the validity and impartiality of our research findings. To examine the distribution of hemoglobin across different populations, we categorized hemoglobin levels into four groups based on weighted quartiles, facilitating a comparative analysis of baseline characteristics. For continuous variables, we reported the median along with its interquartile range (IQR) and employed the Wilcoxon rank-sum test tailored for complex survey samples. For categorical variables, we presented unweighted counts and weighted percentages (n (unweighted) (%)). In assessing the association between categorical variables, we utilized the chi-square test with a Rao & Scott second-order correction. This adjustment is crucial for accommodating the complexities of survey designs, such as stratification, weighting, and clustering, thereby ensuring the chi-square test's relevance and precision in analyzing this type of data.

After conducting an initial data screening, the overall data

missingness rate is 1.9 %. The maximum missingness rate for single variable is 14 %, most other variables have missingness rates below 3 %. we utilized a multilevel multiple interpolation method specifically designed for survey data to address missing data and optimize the dataset. We employed the Jomo function, which performs interpolation by jointly modeling the data, to take full advantage of patterns in the existing data. The interpolation process involved 1000 Gibbs sampling burn-in period iterations to ensure model stabilization, and we saved the interpolation results in every 1000 iterations, resulting in 5 complete datasets. This method takes into account the data structure and correlation, and by not deleting missing samples, it reduces bias from missing data, thereby enhancing the reliability and accuracy of our estimates.

To explore the relationship between hemoglobin levels, NAFLD, and liver fibrosis, we employed weighted logistic regression analysis, adjusting for relevant covariates to construct a refined model. We conducted an extensive subgroup stratification analysis to assess the influence of various factors on the study's outcomes. Participants were categorized into subgroups based on several criteria, including body mass index (BMI), cholesterol levels, poverty income ratio (PIR), triglyceride levels, hypertension, diabetes mellitus, gender, age, glycosylated hemoglobin (HbA1c), and fibrosis status. We utilized forest plots to illustrate the outcome differences across these subgroups, providing a clear visual representation of the data. Additionally, we examined the interaction between the characteristics of different subgroups and the study outcomes by calculating p-values for interaction terms, aiming to uncover any significant modifying effects these factors might have on the relationship between hemoglobin levels and the conditions under study.

We conducted a mediation effect analysis to investigate the potential link between hemoglobin levels and NAFLD and identify potential mediating variables. In this analysis, NAFLD served as the dependent variable, hemoglobin levels as the independent variable, and levels of pertinent indicators such as age, BMI, cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and HbA1c were considered as mediating variables. This approach allowed us to elucidate the mechanisms through which hemoglobin levels may influence NAFLD, exploring these potential pathways and how they mediate the effect of hemoglobin on the risk and progression of NAFLD.

We performed all statistical analyses in R version 4.3.2 (R Core Team, Auckland, New Zealand) using the "survey" package for complex sampling statistics, and we considered two-tailed values of $P < 0.05$ to be statistically significant.

3. Result

3.1. Participant characteristics

Following rigorous selection criteria, our study incorporated 6,516 participants, who were subsequently categorized into four weighted quartiles based on their hemoglobin levels. The participants' median age was 50 years, with an equitable gender distribution observed. Our analysis identified significant disparities in racial composition, NAFLD prevalence, cholesterol levels, and liver function markers, including ALT, AST, and GGT, in addition to median HbA1c levels, all of which yielded P-values of less than 0.001. Conversely, no significant

Table 1
Baseline characteristics of individuals according to weighted hemoglobin quartiles among United States adults from NHANES 2017–2020.

		Hemoglobin (g/dL)				
Characteristic	Overall, N = 6516[1]	Q1, N = 2151 (27 %)	Q2, N = 1567 (24 %)	Q3, N = 1399 (24 %)	Q4, N = 1399 (25 %)	p-value[2]
Age (year), Median (IQR)	50 (34, 63)	51 (35, 67)	53 (36, 65)	49 (33, 62)	48 (32, 61)	<0.001
Gender, n (%)						<0.001
Male	3,125 (48)	387 (13)	537 (29)	937 (63)	1,264 (89)	
Female	3,391 (52)	1,764 (87)	1,030 (71)	462 (37)	135 (11)	
Race, n (%)						<0.001
Mexican_American	707 (7.5)	188 (7.3)	177 (7.4)	154 (6.5)	188 (8.9)	
Hispanic	666 (7.5)	205 (7.5)	168 (8.2)	141 (6.8)	152 (7.3)	
Non_Hispanic_White	2,248 (63)	566 (53)	543 (63)	550 (69)	589 (68)	
Non_Hispanic_Black	1,719 (11)	819 (20)	397 (11)	287 (7.8)	216 (6.2)	
Non_Hispanic_Asian	867 (6.5)	277 (8.0)	211 (6.5)	193 (5.7)	186 (5.7)	
Other	309 (3.8)	96 (4.1)	71 (3.4)	74 (4.2)	68 (3.6)	
NAFLD, n (%)						<0.001
No	4,119 (64)	1,496 (73)	1,025 (65)	872 (64)	726 (53)	
Yes	2,397 (36)	655 (27)	542 (35)	527 (36)	673 (47)	
FibrosisStatus, n (%)						0.3
No	4,285 (69)	1,384 (67)	1,053 (71)	907 (69)	941 (69)	
Yes	2,231 (31)	767 (33)	514 (29)	492 (31)	458 (31)	
PIR (%), Median (IQR)	3.34 (1.75, 5.00)	2.67 (1.39, 4.73)	3.53 (1.81, 5.00)	3.67 (2.00, 5.00)	3.42 (1.92, 5.00)	<0.001
BMI (kg/m2), Median (IQR)	28 (25, 33)	28 (23, 34)	28 (24, 33)	28 (25, 33)	29 (26, 33)	0.037
Cholesterol (mmol/L), Median (IQR)	4.76 (4.11, 5.48)	4.60 (4.00, 5.25)	4.86 (4.19, 5.61)	4.84 (4.16, 5.53)	4.76 (4.14, 5.51)	<0.001
Triglycerides (mmol/L), Median (IQR)	1.28 (0.89, 1.87)	1.12 (0.80, 1.64)	1.31 (0.89, 1.82)	1.31 (0.93, 1.94)	1.41 (1.03, 2.28)	<0.001
ALB (g/L), Median (IQR)	41.0 (39.0, 43.0)	40.0 (37.0, 42.0)	41.0 (39.0, 43.0)	42.0 (40.0, 44.0)	42.0 (40.0, 44.0)	<0.001
ALT (U/L), Median (IQR)	18 (13, 25)	14 (11, 19)	17 (13, 23)	19 (14, 26)	23 (17, 32)	<0.001
AST (U/L), Median (IQR)	19 (16, 23)	17 (15, 22)	18 (16, 22)	19 (16, 23)	21 (18, 26)	<0.001
GGT (IU/L), Median (IQR)	19 (13, 30)	16 (12, 25)	18 (13, 26)	20 (14, 31)	24 (17, 38)	<0.001
HbA1c (%), Median (IQR)	5.50 (5.20, 5.80)	5.50 (5.30, 5.90)	5.50 (5.30, 5.90)	5.50 (5.20, 5.80)	5.40 (5.10, 5.70)	0.001
Hypertension, n (%)						0.15
NO	3,786 (64)	1,120 (61)	933 (66)	855 (66)	878 (65)	
YES	2,730 (36)	1,031 (39)	634 (34)	544 (34)	521 (35)	
Diabetes, n (%)						0.4
NO	5,162 (84)	1,634 (82)	1,256 (85)	1,137 (85)	1,135 (84)	
YES	1,354 (16)	517 (18)	311 (15)	262 (15)	264 (16)	

[1]Median (IQR); n (unweighted) (%).
[2]Wilcoxon rank-sum test for complex survey samples; chi-squared test with Rao & Scott's second-order correction.
Hemoglobin: Hemoglobin; Age: Age; Gender: Gender; Race: Race; NAFLD: non-alcoholic fatty liver disease; FibrosisStatus: Liver Fibrosis Status; PIR: Poverty-Income Ratio; BMI: Body Mass Index; Cholesterol: Total Cholesterol; Triglycerides: Triglycerides; ALB: Albumin; ALT: Alanine Aminotransferase; GGT: Gamma-Glutamyl Transferase; HbA1c: Glycated Hemoglobin; Hypertension: Hypertension; Diabetes: Diabetes.

differences were detected regarding liver fibrosis status, hypertension, and diabetes mellitus across the quartiles, with P-values of 0.3, 0.15, and 0.4, respectively. These findings indicate a potential link between hemoglobin levels and several vital biomarkers.(Table 1).

3.2. NAFLD and baseline characteristics of liver fiber status

The group of patients with NAFLD exhibited significant differences in biomarkers and clinical characteristics compared to patients without NAFLD. It is important to note that these findings are objective and not based on subjective evaluations. Specifically, NAFLD patients were generally older and had higher BMI, hemoglobin, cholesterol, triglycerides, and liver function markers. All relevant markers had p-values less than 0.001. Furthermore, the prevalence of hypertension and diabetes mellitus was significantly higher in the group of patients with NAFLD compared to those without NAFLD. These results suggest that NAFLD patients are at an increased risk for metabolic abnormalities, indicating potential targets for future interventions. The group with fibrosis was significantly older (median age: 66 years, IQR: 56–74 years) compared to those without (median age: 42 years, IQR: 30–55 years) ($p < 0.001$). Additionally, the prevalence of hypertension and diabetes mellitus was significantly higher in the fibrosis group (55 % and 28 %, respectively) compared to the no-fibrosis group (27 % and 11 %, respectively) ($p < 0.001$). Significant differences were observed in the fibrosis group's median BMI, RPR, ALT, AST, GGT, and HbA1c. It is worth noting that hemoglobin levels may be associated with the development of NAFLD, but their predictive value for fibrosis status remains unclear.(Table 2).

3.3. A weighted logistic regression

Weighted logistic regression models were constructed to investigate the independent association between hemoglobin levels and NAFLD (Table 3). In all models, the risk of NAFLD significantly increased with each unit increase in hemoglobin. Specifically, the odds ratio (OR) between hemoglobin and NAFLD was 1.22 (95 % CI: 1.16–1.28, $p < 0.001$) in Model 1. With more model-adjusted variables, the OR was 1.17 (95 % CI: 1.09–1.25, $p < 0.001$) in Model 2 and 1.12 (95 % CI: 1.03–1.23, $p < 0.001$)in Model 3. Furthermore, subgroup analysis of hemoglobin levels by quartiles (Q1-Q4) indicates that the risk of NAFLD increases with higher hemoglobin quartiles. The odds ratio (OR) for individuals in the highest quartile (Q4) was significantly higher than those in the lowest quartile. Although the OR decreased slightly with an increase in the number of adjustment variables in the model, they remained statistically significant. In Model 1, individuals with hemoglobin at Q4 had a 2.36-fold (95 % CI: 1.92–2.91) risk of NAFLD. In Model 3, this risk was 1.96-fold (95 % CI: 1.11–3.43) even after adjusting for many potential confounders. The association between hemoglobin levels and NAFLD was confirmed in all models, and this positive association persisted and was statistically significant even after fully adjusting for multiple potential confounders.

3.4. Subgroup analysis and mediation analysis

We performed subgroup analyses with weighting, and the results indicated no significant interaction effects among the subgroups. This suggests that the impact of the variables on NAFLD was consistent across the analyzed subgroups, demonstrating the model's robustness. This consistency across subgroups highlights the reliability of the model's

Table 2
Baseline characteristics grouped by NAFLD and Fibrosis Status, weighted, among United States adults from NHANES 2017–2020.

Characteristic	Overall, N = 6516[1]	NAFLD		p-value[2]	FibrosisStatus		p-value[2]
		No, N = 4119 (64 %)	Yes, N = 2397 (36 %)		No, N = 4285 (69 %)	Yes, N = 2231 (31 %)	
Age (year), Median (IQR)	50 (34, 63)	47 (31, 62)	55 (40, 66)	<0.001	42 (30, 55)	66 (56, 74)	<0.001
Gender, n (%)				<0.001			<0.001
Male	3,125 (48)	1,820 (44)	1,305 (55)		1,922 (46)	1,203 (52)	
Female	3,391 (52)	2,299 (56)	1,092 (45)		2,363 (54)	1,028 (48)	
Race, n (%)				<0.001			<0.001
Mexican_American	707 (7.5)	364 (6.1)	343 (10.0)		508 (8.6)	199 (5.1)	
Hispanic	666 (7.5)	429 (7.8)	237 (6.8)		465 (8.3)	201 (5.6)	
Non_Hispanic_White	2,248 (63)	1,357 (62)	891 (65)		1,303 (60)	945 (71)	
Non_Hispanic_Black	1,719 (11)	1,194 (13)	525 (9.0)		1,161 (12)	558 (9.9)	
Non_Hispanic_Asian	867 (6.5)	577 (6.8)	290 (6.0)		635 (7.1)	232 (5.2)	
Other	309 (3.8)	198 (3.9)	111 (3.6)		213 (4.0)	96 (3.4)	
Hemoglobin (g/dL), Median (IQR)	14.20 (13.30, 15.10)	14.10 (13.10, 15.00)	14.50 (13.50, 15.40)	<0.001	14.20 (13.30, 15.10)	14.20 (13.20, 15.10)	0.7
PIR (%), Median (IQR)	3.34 (1.75, 5.00)	3.43 (1.75, 5.00)	3.18 (1.74, 5.00)	0.2	3.37 (1.71, 5.00)	3.23 (1.85, 5.00)	>0.9
BMI (kg/m2), Median (IQR)	28 (25, 33)	26 (23, 30)	33 (29, 38)	<0.001	28 (24, 33)	29 (25, 35)	<0.001
Cholesterol (mmol/L), Median (IQR)	4.76 (4.11, 5.48)	4.71 (4.11, 5.46)	4.81 (4.14, 5.53)	0.13	4.78 (4.14, 5.48)	4.71 (4.03, 5.51)	0.2
Triglycerides (mmol/L), Median (IQR)	1.28 (0.89, 1.87)	1.08 (0.80, 1.58)	1.67 (1.22, 2.38)	<0.001	1.24 (0.86, 1.83)	1.36 (0.99, 1.96)	<0.001
ALB (g/L), Median (IQR)	41.0 (39.0, 43.0)	41.0 (39.0, 44.0)	41.0 (39.0, 43.0)	<0.001	41.0 (39.0, 43.0)	41.0 (39.0, 43.0)	<0.001
ALT (U/L), Median (IQR)	18 (13, 25)	16 (12, 22)	22 (16, 31)	<0.001	17 (13, 25)	19 (14, 27)	<0.001
AST (U/L), Median (IQR)	19 (16, 23)	19 (16, 22)	20 (17, 25)	<0.001	18 (15, 22)	21 (18, 27)	<0.001
GGT (IU/L), Median (IQR)	19 (13, 30)	16 (12, 25)	24 (18, 38)	<0.001	19 (13, 28)	21 (14, 32)	<0.001
HbA1c(%), Median (IQR)	5.50 (5.20, 5.80)	5.40 (5.20, 5.70)	5.70 (5.40, 6.20)	<0.001	5.40 (5.20, 5.70)	5.70 (5.40, 6.20)	<0.001
Hypertension, n (%)				<0.001			<0.001
NO	3,786 (64)	2,671 (73)	1,115 (50)		2,920 (73)	866 (45)	
YES	2,730 (36)	1,448 (27)	1,282 (50)		1,365 (27)	1,365 (55)	
Diabetes, n (%)				<0.001			<0.001
NO	5,162 (84)	3,590 (92)	1,572 (70)		3,626 (89)	1,536 (72)	
YES	1,354 (16)	529 (8.3)	825 (30)		659 (11)	695 (28)	
Hemoglobin quantile, n (%)				<0.001			0.3
Q1	2,151 (27)	1,496 (30)	655 (20)		1,384 (26)	767 (29)	
Q2	1,567 (24)	1,025 (25)	542 (23)		1,053 (25)	514 (22)	
Q3	1,399 (24)	872 (24)	527 (24)		907 (24)	492 (24)	
Q4	1,399 (25)	726 (20)	673 (32)		941 (25)	458 (25)	

Table 3
Association between hemoglobin levels and NAFLD, weighted, in United States adults from NHANES 2017–2020.

	Model 1			Model 2			Model 3		
Characteristic	OR[1]	95 % CI[1]	p-value	OR[1]	95 % CI[1]	p-value	OR[1]	95 % CI[1]	p-value
Hemoglobin	1.22	1.16, 1.28	**<0.001**	1.17	1.09, 1.25	**<0.001**	1.12	1.03, 1.23	**<0.001**
Hemoglobin_quantile			**<0.001**			**<0.001**			**<0.001**
Q1	—	—		—	—		—	—	
Q2	1.43	1.23, 1.66		1.38	1.17, 1.63		1.48	1.12, 1.96	
Q3	1.51	1.15, 1.99		1.41	1.01, 1.97		1.37	0.72, 2.60	
Q4	2.36	1.92, 2.91		2.13	1.55, 2.94		1.96	1.11, 3.43	

[1]OR = Odds Ratio, CI = Confidence Interval.
Model 1: Unadjusted.
Model 2: Adds Age, Gender, and Race to Model 1.
Model 3: Expands Model 2 by including Poverty Income Ratio (PIR), Body Mass Index (BMI), Cholesterol, Triglycerides, Albumin (ALB), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma Glutamyl Transferase (GGT), Glycated Hemoglobin (HbA1c), Hypertension, Diabetes and FibrosisStatus.

findings regarding the influence of variables on NAFLD, regardless of subgroup characteristics.(Fig. 2).

The results of the mediating effects analysis indicate that ALT, GGT and triglycerides are significant mediating variables in the relationship between hemoglobin and NAFLD. Specifically, ALT explains 63.6 % of the effect proportion (IE = 0.006, 95 % CI = [0.002, 0.010], $p < 0.001$), GGT accounts for 31.1 % (IE = 0.003, 95 % CI = [0.000, 0.003], $p < 0.001$),and triglycerides account for 42.2 % of the mediating effect (IE = 0.004, 95 % CI = [0.001, 0.006], $p < 0.001$). In contrast, the mediating effect of AST was more minor but still statistically significant, accounting for 12.4 % (IE = 0.001, 95 % CI = [0.000, 0.002], $p < 0.001$). It is noteworthy that Age accounted for 14.6 % of the mediation effect (IE = −0.002, 95 % CI = [−0.002, −0.0002], $p < 0.001$). This suggests that Age may obscure the relationship between hemoglobinb and NAFLD. The statistical analysis revealed that the indirect effects (IE) of BMI, Cholesterol, and HbA1c were not significant. This suggests that these

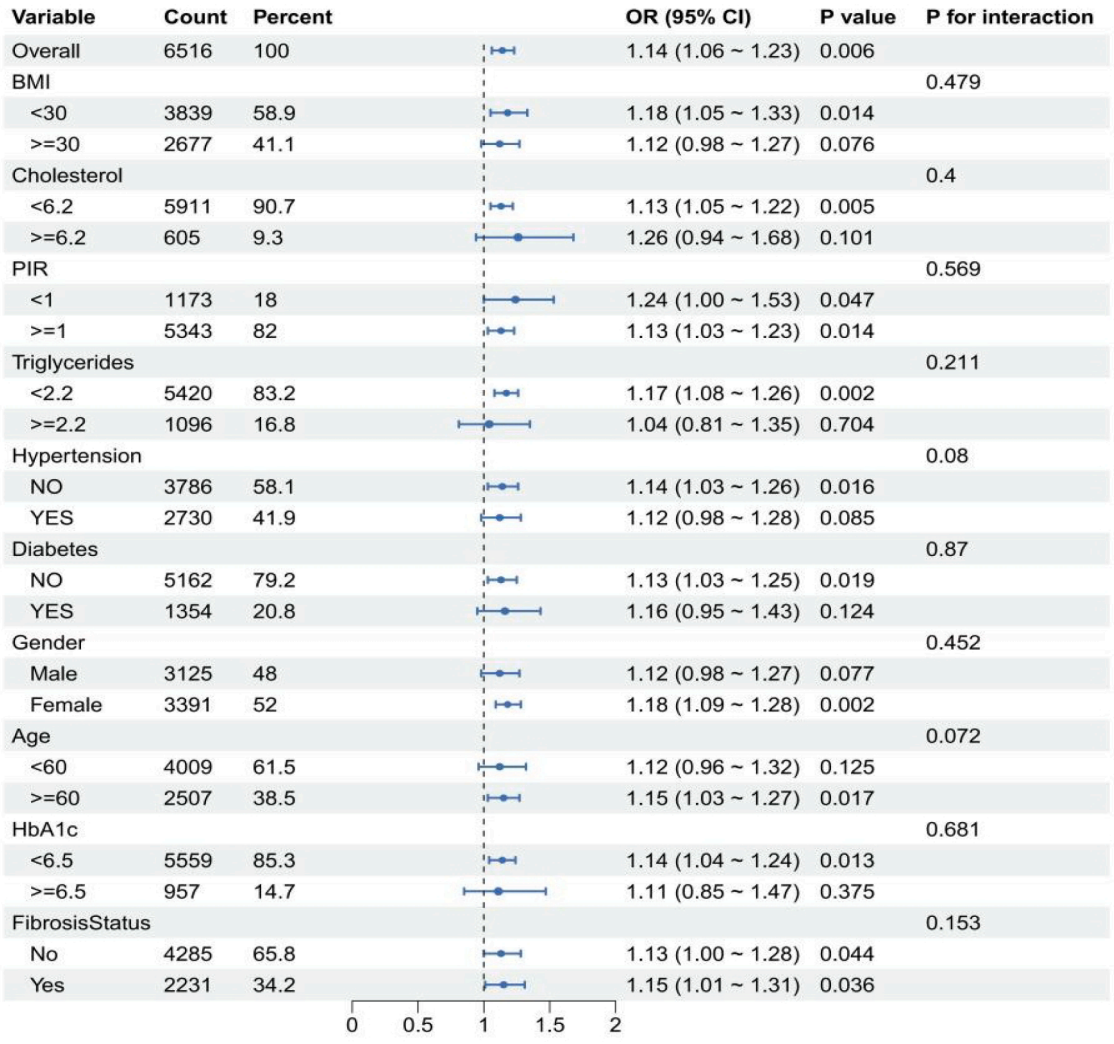

Fig. 2.W. Eighted logistic regression was used for subgroup analysis in united states adults from nhanes 2017–2020. Each stratification was adjusted for BMI, cholesterol, PIR, triglycerides, hypertension, diabetes, sex, age, HbA1c, fibrosis status, and other factors.OR:Odds ratio, CI:Confidence interval.

factors may not be the primary pathways by which hemoglobin affects NAFLD, as shown in Fig. 3.

4. Discussion

Our study utilized nationally representative data to explore the correlation between hemoglobin levels and NAFLD and liver fibrosis in adults in the United States. Our findings indicated that hemoglobin levels were notably higher in patients diagnosed with NAFLD. This study suggests that hemoglobin levels represent an independent risk factor for NAFLD, while they are not significantly linked to liver fibrosis.

NAFLD is a common chronic liver disease typically characterized by macrovesicular steatosis, defined as more than 5 % of the liver parenchyma composed of hepatocytes with intracytoplasmic lipid droplets (Chalasani et al., 2018). The prevalence of NAFLD is increasing every year and is projected to reach a global prevalence of 55.4 % in 2040 (Le et al., 2022). NAFLD affects the health status of a wide range of populations and is independently associated with increased risk of all-cause

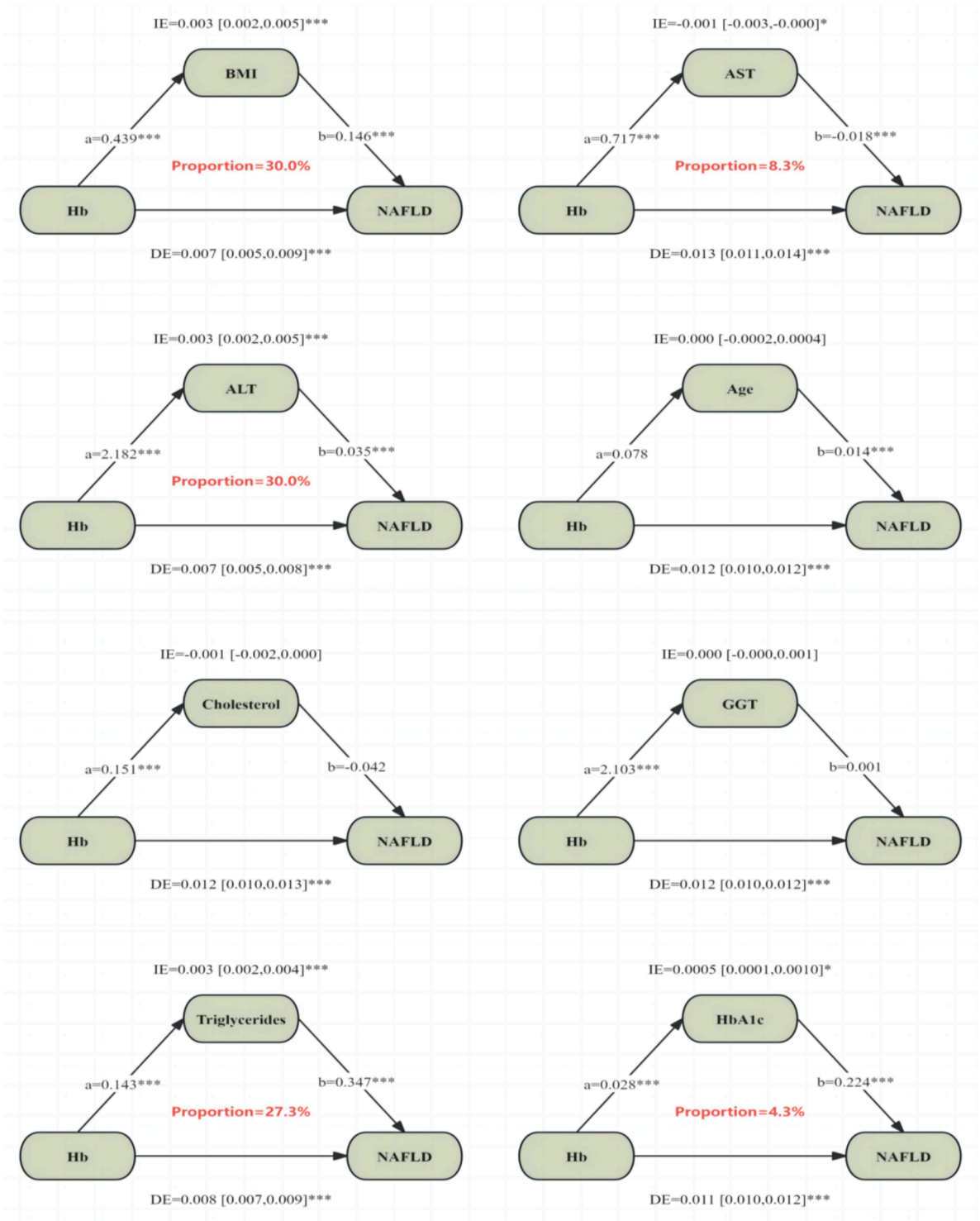

Fig. 3. Mediation analysis of hemoglobin and NAFLD, weighted, in United States adults from NHANES 2017–2020. IE: indirect effect; DE: direct effect; Proportion: proportion of mediation, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. $p < 0.05$ indicates significant differences.

mortality, heart disease, and cancer-related deaths (de Avila et al., 2023; Alvarez et al., 2020; Ko et al., 2023). Given that there are no specific drugs for NAFLD, timely diagnosis and lifestyle changes are ideal for treating the disease (Rong et al., 2023; Powell et al., 2021).

The significance of elevated hemoglobin as a routine test is often overlooked, and fewer studies have been conducted on hemoglobin and NAFLD, which may be closely related to the development of NAFLD (Yu et al., 2012; Chung et al., 2017). Hemoglobin is an essential product and participant in iron metabolism, and 70 % of the body's iron stores are heme iron, which is distributed in hemoglobin and myoglobin, while most of the rest is stored as ferritin or iron-containing hemoglobin in hepatocytes and reticuloendothelial cells (Li et al., 2018). The liver is an important organ for iron metabolism and is also strongly influenced by iron homeostasis (Yu et al., 2020). Ferritin is closely associated with NAFLD, and iron loading causes damage to the liver through oxidative stress and lipid peroxidation, and serum ferritin levels were positively correlated with the development of NAFLD in a study directed at a Korean population (Jung et al., 2019; Liu et al., 2022). A cross-sectional study suggests indicators of iron status may be potential biomarkers for NAFLD (Tan et al., 2023). In a model of NAFLD established in mice, elevated levels of ferrous iron were found to exacerbate oxidative stress further, leading to liver damage and the development of NAFLD (Zhang et al., 2022). Elevated hemoglobin may represent a high iron load that induces NAFLD.

Studies have shown a strong relationship between hemoglobin levels and insulin resistance and that high hemoglobin may be accompanied by an elevated risk of insulin resistance (Yang et al., 2019). Insulin resistance plays a pivotal role in the pathophysiology of NAFLD, primarily driving hepatocellular steatosis and hepatotoxicity (Bellucci et al., 2023). This condition is characterized by the release of proinflammatory cytokines or chemokines, leading to compromised inflammation and immune function and increased hepatic de novo lipogenesis. These processes contribute to the development of steatosis in individuals with NAFLD (Smith et al., 2020). Metabolic syndrome (MetS) is a collection of metabolic abnormalities associated with an increased risk of cardiovascular disease and diabetes mellitus, and it is often considered a multifactorial pathology that includes insulin resistance, hyperglycemia, dyslipidemia, hypertension, and central obesity (Ambroselli et al., 2023). The study found that participants with MetS had significantly higher hemoglobin levels, that high hemoglobin is a risk factor for MetS, and that NAFLD severity was strongly associated with MetS (He et al., 2021; Ahmadzadeh et al., 2018; Yang et al., 2016). The two conditions may cause a vicious cycle, with MetS leading to NAFLD and NAFLD worsening MetS (Hashimoto et al., 2015).

Previous studies have suggested that obstructive sleep apnoea (OSA) may be a manifestation of MetS. Obesity and insulin resistance may be responsible for sleep apnoea (Vgontzas et al., 2005). OSA is a prevalent sleep disorder characterized by recurrent episodes of upper airway narrowing or collapse during sleep, which can lead to recurrent awakenings and significant reductions in oxygen saturation (Lv et al., 2023; Martinez-Garcia et al., 2023). Therefore, elevated hemoglobin may be due to a compensatory increase in erythropoietin as a result of hepatic hypoxia (Nishimura et al., 2018). Several studies have shown that OSA leads to intermittent hypoxia and the production of reactive oxygen species (ROS), inducing oxidative stress, leading to liver injury and increased hepatic fat accumulation, and ultimately to the development of NAFLD (Li et al., 2005; Kim et al., 2022; Huang et al., 2023; Delli et al., 2021).

The mediating effect results in this study suggest that ALT, AST, GGT, Triglycerides and Age mediate the effect of hemoglobin on NAFLD, explaining to some extent the complex association between hemoglobin and NAFLD. The current study is unclear whether high hemoglobin levels in NAFLD are merely a consequence of disease severity or other potential associations and whether the possible mechanisms of elevated hemoglobin levels require more in-depth subsequent studies.

The strengths of this study are the investigation of the relationship between hemoglobin and NAFLD through nationally representative data; the use of a weighted logistic regression model and adjustment for confounders; the enhancement of the generalizability of the findings; and the exploration of potential influences on the association between hemoglobin and NAFLD. Of course, this study has some limitations that cannot be ignored. Firstly, this study is a cross-sectional study and, therefore, has limited ability to establish causality and can only reveal associations between variables. Second, based on the limitations of NAHNES, limited variables could be accessed to adjust the model for unmeasured confounders. Third, a biopsy is the gold standard for diagnosing NAFLD and liver fibrosis, and the definitions based on CAP and VCTE in this study may have resulted in some diagnostic inaccuracies.

5. Summary

This study found that elevated hemoglobin levels were significantly associated with the risk of NAFLD development in the U.S. population, which was an independent risk factor for NAFLD. Therefore, closer screening of individuals with higher hemoglobin levels may facilitate early detection of NAFLD, as well as provide an opportunity for early intervention.

6. Ethics statement

Research involving human subjects received approval from the NCHS Research Ethics Review Board. Participants in the study gave their consent through written informed consent forms.

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CRediT authorship contribution statement

Kang Yao: Writing – original draft, Data curation. **Zheng Chen:** Resources, Methodology, Investigation, Conceptualization. **Wei Zhou:** Resources, Methodology, Conceptualization. **Zhihua Liu:** Resources, Methodology, Conceptualization. **Wei Cui:** Conceptualization, Methodology, Resources.

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.

Data availability

Publicly available datasets were analyzed in this study. These datasets are available at: https://www.cdc.gov/nchs/nhanes.

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